2020

USP 43

THE UNITED STATES PHARMACOPEIA

NF 38

THE NATIONAL FORMULARY

Volume 4

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

Official from May 1, 2020

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

THE UNITED STATES PHARMACOPEIAL CONVENTION
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SIX-MONTH IMPLEMENTATION GUIDELINE

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

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

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USP 43–NF 38	November 1, 2019	May 1, 2020	May 1, 2021 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)
First Supplement to the USP 43–NF 38	February 1, 2020	August 1, 2020	May 1, 2021 (except as superseded by *Second Supplement, IRAs,* and *Revision Bulletins*)
Second Supplement to the USP 43–NF 38	June 1, 2020	December 1, 2020	May 1, 2021 (except as superseded by *IRAs* and *Revision Bulletins*)
USP-NF 2021 Issue 1	November 1, 2020	May 1, 2021	May 1, 2022 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)

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

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

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

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ISSN 0195-7996 (print)

ISSN 1930-2932 (online)

ISBN 978-1-936424-95-5

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

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GENERAL NOTICES AND REQUIREMENTS

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

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

1. TITLE AND REVISION

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

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

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

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

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

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

2. OFFICIAL STATUS AND LEGAL RECOGNITION

2.10. Official Text

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

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

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

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

2.20. Official Articles

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

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

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

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

2.30. Legal Recognition

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

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

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

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

3. CONFORMANCE TO STANDARDS

3.10. Applicability of Standards

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

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

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

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

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

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

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

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

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

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

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

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

3.10.30. Applicability of Standards to the Practice of Compounding

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

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

3.20. Indicating Conformance

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

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

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

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

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

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

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

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

4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. Monographs

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

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

4.10.10. Applicability of Test Procedures

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

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

4.10.20. Acceptance Criteria

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

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

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

4.20. General Chapters

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

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

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

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

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5. MONOGRAPH COMPONENTS

5.10. Molecular Formula

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

5.20. Added Substances

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

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

5.20.10. Added Substances in Official Substances

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

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

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

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

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

5.20.20.1. In Compounded Preparations

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

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

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

5.20.20.2. In Dietary Supplements

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

5.30. Description and Solubility

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

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

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

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

5.40. Identification

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

5.50. Assay

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

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5.50.10. Units of Potency (Biological)

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

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

5.60. Impurities and Foreign Substances

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

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

5.60.10. Other Impurities in USP and NF Articles

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

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

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

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

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

5.60.20. Residual Solvents in USP and NF Articles

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

5.60.30. Elemental Impurities in USP Drug Products and Dietary Supplements

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

5.70. Performance Tests

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

5.80. USP Reference Standards

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

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

6. TESTING PRACTICES AND PROCEDURES

6.10. Safe Laboratory Practices

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

6.20. Automated Procedures

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

6.30. Alternative and Harmonized Methods and Procedures

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

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

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

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

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

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

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

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

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

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

6.40.10. Ignite to Constant Weight

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

6.40.20. Dried to Constant Weight

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

6.50. Preparation of Solutions

6.50.10. Filtration

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

6.50.20. Solutions

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

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

6.50.20.1. Adjustments to Solutions

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

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

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

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

6.50.20.2. Test Solutions

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

6.50.20.3. Indicator Solutions

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

6.60. Units Necessary to Complete a Test

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

6.60.10. Tablets

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

6.60.20. Capsules

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

6.70. Reagents

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

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

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

6.80. Equipment

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

6.80.10. Apparatus for Measurement

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

6.80.10.1. Pipet/Pipette

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

6.80.10.2. Light Protection

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

6.80.20. Instrumental Apparatus

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

6.80.20.1. Chromatographic Tubes and Columns

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

6.80.20.2. Tubing

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

6.80.20.3. Steam Bath

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

6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

6.80.30. Temperature Reading Devices

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

7. TEST RESULTS

7.10. Interpretation of Requirements

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

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

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

7.10.5. Nominal Concentrations in Equations

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

7.10.10. Equivalence Statements in Titrimetric Procedures

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

7.20. Rounding Rules

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

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

8. TERMS AND DEFINITIONS

8.10. Abbreviations

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

8.20. About

"About" indicates a quantity within 10%.

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

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

8.30. Alcohol Content

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

8.40. Atomic Weights

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

8.50. Blank Determinations

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

8.60. Concomitantly

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

8.70. Desiccator

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

8.80. Logarithms

Logarithms are to the base 10.

8.90. Microbial Strain

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

8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

8.110. NLT/NMT

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

8.120. Odor

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

8.130. Percent

"Percent" used without qualification means:

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

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

8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

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

8.150. Pressure

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

8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

8.170. Specific Gravity

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

8.180. Temperatures

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

8.190. Time

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

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8.200. Transfer

"Transfer" indicates a quantitative manipulation.

8.210. Vacuum

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

8.220. Vacuum Desiccator

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

8.230. Water

8.230.10. Water as an Ingredient in an Official Product

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

8.230.20. Water in the Manufacture of Official Substances

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

8.230.30. Water in a Compendial Procedure

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

8.240. Weights and Measures

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

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

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

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

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

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

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

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

Selected SI Prefixes

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

9. PRESCRIBING AND DISPENSING

9.10. Use of Metric Units

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

9.20. Changes in Volume

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

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

10.10. Packaging and Storage

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

10.20. Labeling

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

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Guide to General Chapters

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

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Reagents, Indicators and Solutions

Introduction

1. SCOPE

Reagents required in the tests and assay for *U.S. Pharmacopeia* and *National Formulary* articles and those required only in determining the quality of other reagents are listed in this section, with specifications appropriate to their intended uses.

As stated in *General Notices, 6.70 Reagents*, listing of reagents, indicators, and solutions in the *U.S. Pharmacopeia* in no way implies that they have therapeutic utility; thus, any reference to *USP* or *NF* in their labeling shall include also the term "reagent" or "reagent grade".

Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a note or footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

1.1 ACS (American Chemical Society) Reagent Grade

Where it is directed to "Use ACS reagent grade", it is intended that a grade meeting the corresponding specifications of the current edition of *Reagent Chemicals*, published by the American Chemical Society (ACS), shall be used.

1.2 Suitable Grade

In the cases where no ACS reagent monograph exists or if the reagent is available in different quality grades, each one specific for a particular application, it is directed to "Use a suitable grade". The intent is that a suitable reagent grade available commercially shall be used.

Occasionally, additional test(s) augment the designation "suitable grade", as indicated in the text. Listed also are some, but not all, reagents that are required only in determining the quality of other reagents. For those reagents that are not listed, satisfactory specifications are available in standard reference publications.

1.3 *USP* or *NF* or *FCC* Grade

In the instances in which a reagent required in a *U.S. Pharmacopeia* or *National Formulary* test or assay meets the requirements in the monograph for that article appearing in this *U.S. Pharmacopeia* or the *National Formulary* or the current edition of the *Food Chemicals Codex* (*FCC*), it suffices to refer to the corresponding monograph in one of the these three compendia. In such cases it is to be understood that the specifications are minimum requirements and that any substance meeting more rigid specifications for chemical purity is suitable.

2. PACKAGING AND STORAGE

Reagents and solutions should be preserved in tight containers made of resistant glass or other suitable material. Directions for storage in light-resistant containers should be carefully observed.

Stoppers and stopcocks brought into contact with substances capable of attacking or penetrating their surfaces may be given a protective coating of a thin film of a suitable lubricant unless specifically interdicted.

3. METAL-ION STANDARD SOLUTIONS

Atomic absorption and flame photometry require the use of a number of metal-ion standard solutions. While the individual monographs usually provide directions for preparation of these solutions, use of commercially prepared standardized solutions of the appropriate ions is permissible, provided that the analyst confirms the suitability of the solutions and has data to support their use.

4. DEFINITIONS

4.1 Reagents: Reagents are substances used either as such or as constituents of solutions.

4.2 Indicators: Indicators are reagents used to determine the specified endpoint in a chemical reaction, to measure hydrogen-ion concentration (pH), or to indicate that a desired change in pH has been effected. They are listed together with indicator test papers.

4.3 Buffer Solutions: Buffer solutions resist changes in the activity of an ion on the addition of substances that are expected to change the activity of that ion.

4.4 Colorimetric Solutions (CS): Colorimetric solutions are solutions used in the preparation of colorimetric standards for comparison purposes.

4.5 Test Solutions (TS): Test solutions are solutions of reagents in such solvents and of such definite concentrations as to be suitable for the specified purposes.

4.6 Volumetric Solutions (VS): Volumetric solutions are solutions of reagents of known concentration intended primarily for use in quantitative determinations.

5. CHROMATOGRAPHIC SOLVENTS AND CARRIER GASES

The chromatographic procedures set forth in the *U.S. Pharmacopeia* may require use of solvents and gases that have been especially purified for such use. The purpose may be (a) to exclude certain impurities that interfere with the proper conduct of the test procedure, or (b) to extend the life of a column by reducing the buildup of impurities on the column. Where solvents and gases are called for in chromatographic procedures, it is the responsibility of the analyst to ensure the suitability of the solvent or gas for the specific use. Solvents and gases suitable for specific high-pressure or other chromatographic uses are available as specialty products from various reagent supply houses, although there is no assurance that similar products from different suppliers are of equivalent suitability in any given procedure.

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Reagents

1. DEFINITIONS

1.1 Blank

A blank consists of the same quantities of the same reagents treated in the same manner as the specimen under test.

1.2 Control

A control is a blank to which has been added the limiting quantity of the substance being tested for, or is a specified comparison solution prepared as directed in the particular test.

2. DESCRIPTION OF THE REAGENT ENTRY

See *Figure 1*.

Change to read:

3. VISUAL COMPARISONS

For color and turbidity comparisons, proceed as directed in ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019). The tubes used in this procedure are frequently called "Nessler tubes".

In making visual comparisons of the densities of turbid fluids, compensate for difference in color, if necessary, by viewing the turbidity through a column of water, the depth of which is determined by the volume specified in the individual reagent specification. Place the water in color-comparison tubes, and hold one of the tubes above the control tube and the other below the specimen tube.

4. RETAIN THE FILTRATE

"Retain the filtrate" is to be understood, unless otherwise indicated, that the washings of the residue are not to be added to the filtrate obtained.

5. EXPRESSION R_2O_3

The expression R_2O_3 is intended to indicate the residue on ignition from compounds precipitated upon the addition of ammonium hydroxide, such as iron oxide (Fe_2O_3) and aluminum oxide (Al_2O_3).

6. GENERAL TESTS FOR REAGENTS

The following general test methods are provided for the examination of reagents to determine their compliance with the specifications of the individual reagents and are to be used unless it is otherwise directed in such specifications.

6.1 Boiling or Distilling Range for Reagents

Use the following procedure for determining the boiling or distilling range of reagents, unless otherwise directed in the individual specifications:

APPARATUS: Use apparatus similar to that specified for *Distilling Range* ⟨721⟩, *Method I*, except that the distilling flask is to be of 250-mL capacity, to have a short neck, and to be connected to the condenser by means of a three-way connecting tube fitted with standard-taper ground joints.

PROCEDURE: Place the distilling flask in an upright position in the perforation in the asbestos board, and connect it to the condenser.

Measure 100 mL of the liquid to be tested in a graduated cylinder, and transfer to the boiling flask together with some device to prevent bumping. Use the cylinder as the receiver for the distillate. Insert the thermometer, and heat so as to distill at the rate of 3–5 mL/min. Make a preliminary trial, if necessary, to determine the adjustment for the proper rate of heating. Read the thermometer when about 20 drops have distilled and thereafter at volumes of distillate of 5, 10, 40, 50, 60, 90, and 95 mL. Continue the distillation until the dry point is reached.

The *Boiling or Distilling Range* is the interval between the temperatures when 1 mL and 95 mL, respectively, have distilled.

6.2 Amino Nitrogen Test in Reagents

Determine the percentage of loss on drying of the sample in appropriate conditions. Transfer about 500 mg of the sample to a 100-mL beaker. Add 20 mL of water. Adjust the pH potentiometrically with 0.1 N hydrochloric acid or 0.2 N sodium hydroxide to 6.0. Add 10 mL of formaldehyde solution. Titrate the solution potentiometrically with 0.2 N sodium hydroxide to a pH of 9.0. Calculate the percentage of amino nitrogen:

$$\% \text{ amino nitrogen} = \frac{V_{NaOH} \times N_{NaOH} \times 14 \times 100 \times f}{\text{mg sample} \times (1.0 - \%\text{LOD}/100)}$$

where f is the correction factor obtained in the standardization of 0.2 N sodium hydroxide and %LOD is the percentage of loss on drying.

6.3 Arsenic in Reagents

Select reagents for this test for a low arsenic content, so that a blank test results in either no stain or one that is barely perceptible.

APPARATUS: Prepare a generator by fitting a 1-hole rubber stopper into a wide-mouth bottle of about 60-mL capacity. Through the perforation insert a vertical exit tube about 12 cm in total length and 1 cm in diameter along the entire upper portion (for about 8 cm) and constricted at its lower extremity to a tube about 4 cm in length and about 5 mm in diameter. The smaller portion of the tube should extend to just slightly below the stopper. Place washed sand or a pledget of purified

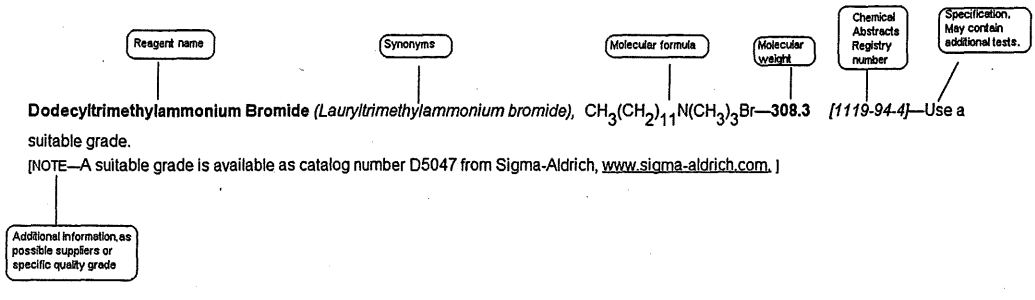


Figure 1. Components of the reagent entry.

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cotton in the upper portion to about 3 cm from the top of the tube. Moisten the sand or cotton uniformly with lead acetate TS, and remove any excess or adhering droplets of the latter from the walls of the tube. Into the upper end of this tube fit a second glass tube 12 cm in length, with an internal diameter of 2.5–3 mm, by means of a rubber stopper. Just before running the test, place a strip of mercuric bromide test paper (see *Indicator and Test Papers*) in this tube, crimping the upper end of the strip so that it will remain in position about 2 cm above the rubber stopper. Clean and dry the tube thoroughly each time it is used.

STANDARD ARSENIC SOLUTION: Use the *Standard Preparation*, prepared as directed in *Arsenic* ⟨211⟩.

TEST PREPARATION: Add 1 mL of sulfuric acid to 5 mL of a solution of the chemical substance (1 in 25), unless another quantity is directed in the individual reagent specification. Omit its addition entirely in the case of inorganic acids. Unless especially directed otherwise, add 10 mL of sulfurous acid. Evaporate the liquid in a small beaker, on a steam bath, until it is free from sulfurous acid and has been reduced to about 2 mL in volume. Dilute with water to 5 mL to obtain the *Test preparation*. Substances subjected to special treatments specified in the individual reagent specification may be used directly as the *Test preparation*. [NOTE—Solutions prepared by the dissolving of the chemical substances in dilute acids are not considered to have undergone special treatment.]

STANDARD STAIN: Place in the generator bottle 5 mL of potassium iodide TS, 2.0 mL of *Standard arsenic solution*, 5 mL of acid stannous chloride TS, and 28 mL of water. Add 1.5 g of granulated zinc (in No. 20 powder), and immediately insert the stopper containing the exit tube. Keep the generator bottle immersed in water at 25° during the period of the test to moderate the reaction so that the stain will take the form of a distinctive band to facilitate the comparison of color intensity. When evolution of hydrogen has continued for 1 h, remove the mercuric bromide test paper for comparison. This stain represents 2 μg of arsenic.

PROCEDURE: Pipet into the generator bottle 5 mL of potassium iodide TS and 5 mL of the *Test preparation*, and add 5 mL of acid stannous chloride TS. Set the apparatus aside at room temperature for a period of 10 min, then add 25 mL of water and 1.5 g of granulated zinc (in No. 20 powder), and proceed as directed for the *Standard stain*. Remove the mercuric bromide test paper, and compare the stain upon it with the *Standard stain*: the stain produced by the chemical tested does not exceed the *Standard stain* in length or in intensity of color, indicating NMT 10 parts of arsenic per million parts of the substance being tested. Because light, heat, and moisture cause the stain to fade rapidly, place the papers in clean, dry tubes, and make comparisons promptly.

INTERFERING CHEMICALS: *Antimony*, if present in the substance being tested, produces a gray stain. *Sulfites*, *sulfides*, *thiosulfates*, and other compounds that liberate hydrogen sulfide or sulfur dioxide when treated with sulfuric acid must be oxidized by means of nitric acid and then reduced by means of sulfur dioxide as directed for the *Test preparation* before they are placed in the apparatus. Certain *sulfur compounds*, as well as *phosphine*, give a bright yellow band on the test paper. If sulfur compounds are present, the lead acetate-moistened cotton or sand will darken. In that case, repeat the operation as directed for the *Test preparation* upon a fresh portion of the solution being tested, and use greater care in effecting the complete removal of the sulfurous acid. In testing hypophosphites, observe special care to oxidize completely the solution being tested as directed; otherwise the evolution of phosphine may result in a yellow stain that may be confused with the orange-yellow color produced by arsine. The stain produced by phosphine may be differentiated from that given by arsine by means of moistening it with 6 N ammonium hydroxide. A stain caused by arsine becomes dark when so treated, but a stain produced by phosphine does not materially change in color.

6.4 Chloride in Reagents

STANDARD CHLORIDE SOLUTION: Dissolve 165.0 mg of dried sodium chloride in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of chlorine (Cl) in each mL.

PROCEDURE: Neutralize, if alkaline, a solution of the quantity of the reagent indicated in the test in 25 mL of water, or a solution prepared as directed in the test, with nitric acid, litmus paper being used as the indicator, and add 3 mL more of nitric acid. Filter the solution, if necessary, through a filter paper previously washed with water until the paper is free from chloride, and add 1 mL of silver nitrate TS. Mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced in a control made with the same quantities of the same reagents as in the final test and a volume of *Standard chloride solution* equivalent to the quantity of chloride (Cl) permitted by the test. Adjust the two solutions with water to the same volume before adding the silver nitrate TS, and compare the turbidities.

In *testing barium salts*, neutralize, if alkaline, the solution containing the reagent, with nitric acid, and add only 3 drops more of nitric acid. Conduct the remainder of the test as described previously.

In *testing salts giving colored solutions*, dissolve 2 g of the reagent in 25 mL of water, and add 3 mL of nitric acid. Filter the solution, if necessary, through a filter paper previously washed with water, and divide the filtrate into two equal portions. Treat one portion with 1 mL of silver nitrate TS, allow to stand for 10 min, and, if any turbidity is produced, filter it through a washed filter paper until clear, and use the filtrate as a blank. Treat the other portion with 1 mL of silver nitrate TS, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity with that produced in the blank by the addition of a volume of *Standard chloride solution* equivalent to the quantity of chloride (Cl) permitted in the test, both solutions being adjusted with water to the same volume.

6.5 Flame Photometry for Reagents

The use of flame photometric procedures to determine traces of calcium, potassium, sodium, and strontium is called for in some of the reagent specifications. The suitability of such determinations depends upon the use of adequate apparatus, and several instruments of suitable selectivity are available. The preferred type of flame photometer is one that has a red-sensitive phototube, a multiplier phototube, a monochromator, an adjustable slit-width control, a selector switch, and a sensitivity control. Other types of photometers may be used, provided the operator has proved that the instrument will determine accurately the amount of impurities permitted in the reagent to be tested.

The flame photometric procedures depend upon the use of semi-internal standards, and thus require both a *Sample solution* and a *Control solution*. For the *Sample solution*, a specified weight of specimen is dissolved and diluted to a definite volume. For the *Control solution*, the same amount of specimen is dissolved, the limiting amounts of the suspected impurities are added, and the solution is then diluted to the same definite volume as the *Sample solution*. The flame photometer is set as directed in the general procedures and then adjusted to give an emission reading as near 100% transmittance as is possible with the *Control solution* at the wavelength specified for the particular impurity concerned. With the instrument settings left unchanged, the emission from the *Sample solution* is read at the same wavelength and at a specified background wavelength. The background reading is then used to correct the observed emission of the *Sample solution* for the emission due to the specimen and the

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solvent. The specimen being tested contains less than the specified limit of impurity if the difference between the observed background and total emissions for the *Sample solution* is less than the difference between the observed emissions for the *Control solution* and the *Sample solution* at the wavelength designated for the particular impurity.

CALCIUM IN REAGENTS

Standard calcium solution: Dissolve 250 mg of calcium carbonate in a mixture of 20 mL of water and 5 mL of diluted hydrochloric acid, and when solution is complete, dilute with water to 1 L. This solution contains 0.10 mg of calcium (Ca) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.03 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 422.7-nm calcium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 422.7 nm. Change the monochromator to the wavelength specified in the individual test procedure, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 422.7 nm and at the background wavelength is not greater than the difference between transmittances observed at 422.7 nm for the *Sample solution* and the *Control solution*.

POTASSIUM IN REAGENTS

Standard potassium solution: Dissolve 191 mg of potassium chloride in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of potassium (K) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure. [NOTE—In testing calcium salts, use an oxyhydrogen burner.]

Set the slit-width control of a suitable flame photometer equipped with a red-sensitive detector at 0.1 mm, unless otherwise directed, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 766.5-nm potassium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 766.5 nm. Change the monochromator to 750 nm, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 766.5 and 750 nm is not greater than the difference between transmittances observed at 766.5 nm for the *Sample solution* and the *Control solution*.

SODIUM IN REAGENTS

Standard sodium solution: Dissolve 254 mg of sodium chloride in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of sodium (Na) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.01 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 589-nm sodium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 589 nm. Change the monochromator to 580 nm, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 589 and 580 nm is not greater than the difference between transmittances observed at 589 nm for the *Sample solution* and the *Control solution*.

STRONTIUM IN REAGENTS

Standard strontium solution: Dissolve 242 mg of strontium nitrate in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of strontium (Sr) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.03 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 460.7-nm strontium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 460.7 nm. Change the monochromator to the wavelength specified in the individual test procedure, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 460.7 nm and at the background wavelength is not greater than the difference between transmittances observed at 460.7 nm for the *Sample solution* and the *Control solution*.

6.6 Heavy Metals in Reagents

STANDARD LEAD SOLUTION: Use standard lead solution TS. Each mL of this solution contains the equivalent of 0.01 mg of lead (Pb).

PROCEDURE: Unless otherwise directed, test for heavy metals as follows:

(*a*) If the heavy metals limit is 0.0005% (5 ppm), dissolve 6.0 g of the specimen in water to make 42 mL.

(*b*) If the heavy metals limit is 0.001% (10 ppm) or more, or in the event of limited solubility, use 4 g, and dissolve in water to make 40 mL, warming, if necessary, to aid solution.

For the control, transfer 7 mL of the solution from (*a*) to a color-comparison tube, and add a volume of *Standard lead solution* equivalent to the amount of lead permitted in 4 g of the reagent. Dilute with water to 35 mL, and add diluted acetic acid, or ammonia TS, until the pH is about 3.5, determined potentiometrically, then dilute with water to 40 mL, and mix. Transfer the remaining 35 mL of the solution from (*a*) to a color-comparison tube closely matching that used for the control, and add diluted acetic acid, or ammonia TS, until the pH is about 3.5, determined potentiometrically, then dilute with water to 40 mL, and mix. Then to each tube add 10 mL of hydrogen sulfide TS, mix, and compare the colors by viewing through the color-comparison tube downward against a white surface. The color in the test specimen is not darker than that of the control.

If the solution of the reagent is prepared as in (*b*), use for the control 10 mL of the solution, and add to it a volume of *Standard lead solution* equivalent to the amount of lead permitted in 2 g of the reagent. Dilute the remaining 30 mL of solution (*b*) with water to 35 mL, and proceed as directed in the preceding paragraph, beginning with "add diluted acetic acid, or ammonia TS," in the second sentence.

If the reagent to be tested for heavy metals is a salt of an aliphatic organic acid, substitute 1 N hydrochloric acid for the diluted acetic acid specified in the foregoing method.

6.7 Insoluble Matter in Reagents

Dissolve the quantity of reagent specified in the test in 100 mL of water, heat to boiling unless otherwise directed, in a covered beaker, and warm on a steam bath for 1 h. Filter the hot solution through a tared sintered-glass crucible of fine porosity. Wash the beaker and the filter thoroughly with hot water, dry at 105°, cool in a desiccator, and weigh.

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6.8 Loss on Drying for Reagents

Determine as directed in *Loss on Drying* ⟨731⟩.

6.9 Nitrate in Reagents

STANDARD NITRATE SOLUTION: Dissolve 163 mg of potassium nitrate in water, add water to make 100 mL, and dilute 10 mL of this solution with water to 1 L to obtain a solution containing the equivalent of 0.01 mg of nitrate (NO_3) per mL.

BRUCINE SULFATE SOLUTION: Dissolve 600 mg of brucine sulfate in 600 mL of nitrate-free, dilute sulfuric acid (2 in 3) that previously has been cooled to room temperature, and dilute with the acid to 1 L. [NOTE—Prepare the nitrate-free sulfuric acid by adding 4 parts of sulfuric acid to 1 part of water, heating the solution to dense fumes of sulfur trioxide, and cooling. Repeat the dilution and heating three or four times.]

SAMPLE SOLUTION: To the weight of sample specified in the individual reagent specification, dissolved in the designated volume of water, add *Brucine sulfate solution* to make 50 mL.

CONTROL SOLUTION: To a volume of *Standard nitrate solution* equivalent to the weight of nitrate (NO_3) specified in the individual reagent specification, add the weight of sample specified in the individual reagent specification, and then add *Brucine sulfate solution* to make 50 mL.

BLANK SOLUTION: Use 50 mL of *Brucine sulfate solution*.

PROCEDURE: Heat the *Sample solution*, *Control solution*, and *Blank solution* in a boiling water bath for 10 min, then cool rapidly in an ice bath to room temperature. Adjust a suitable spectrophotometer to zero absorbance at 410 nm with the *Blank solution*. Determine the absorbance of the *Sample solution*, note the result, and adjust the instrument to zero absorbance with the *Sample solution*. Determine the absorbance of the *Control solution*: the absorbance reading for the *Sample solution* does not exceed that for the *Control solution*.

6.10 Nitrogen Compounds in Reagents

PROCEDURE: Unless otherwise directed, test for nitrogen compounds as follows: dissolve the specified quantity of test specimen in 60 mL of ammonia-free water in a Kjeldahl flask connected through a spray trap to a condenser, the end of which dips below the surface of 10 mL of 0.1 N hydrochloric acid. Add 10 mL of freshly boiled sodium hydroxide solution (1 in 10) and 500 mg of aluminum wire, in small pieces, to the Kjeldahl flask, and allow to stand for 1 h, protected from loss of and exposure to ammonia. Distill 35 mL, and dilute the distillate with water to 50 mL. Add 2 mL of freshly boiled sodium hydroxide solution (1 in 10), mix, add 2 mL of alkaline mercuric–potassium iodide TS, and again mix: the color produced is not darker than that of a control containing the amount of added N (as ammonium chloride) specified in the individual test procedure.

6.11 Phosphate in Reagents

STANDARD PHOSPHATE SOLUTION: Dissolve 143.3 mg of dried monobasic potassium phosphate (KH_2PO_4) in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate (PO_4) in each mL.

PHOSPHATE REAGENT A: Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.

PHOSPHATE REAGENT B: Dissolve 200 mg of *p*-methylaminophenol sulfate in 100 mL of water, and add 20 g of sodium bisulfite. Store this reagent in well-filled, tightly stoppered bottles, and use within 1 month.

PROCEDURE: [NOTE—The tests with the specimen and the control are made preferably in matched color-comparison tubes.] Dissolve the quantity of the reagent specified in the test, or the residue obtained after the prescribed treatment, in 20 mL of water, by warming, if necessary, add 2.5 mL of dilute sulfuric acid (1 in 7), and dilute with water to 25 mL. (If preferable, the test specimen or the residue may be dissolved in 25 mL of approximately 0.5 N sulfuric acid.) Then add 1 mL each of *Phosphate reagents A* and *B*, mix, and allow to stand at room temperature for 2 h. Compare any blue color produced with that produced in a control made with the same quantities of the same reagents as in the test with the specimen, and a volume of *Standard phosphate solution* equivalent to the quantity of phosphate (PO_4) designated in the reagent specifications.

6.12 Residue on Ignition in Reagents

PROCEDURE: Unless otherwise directed, determine the residue on ignition as follows: accurately weigh 1–2 g of the substance to be tested in a suitable crucible that previously has been ignited, cooled, and weighed. Ignite the substance, gently and slowly at first and then at a more rapid rate, until it is thoroughly charred, if organic in nature, or until it is completely volatilized, if inorganic in nature. If the use of sulfuric acid is specified, cool the crucible, add the specified amount of acid, and ignite the crucible gently until fumes no longer are evolved. Then ignite the crucible at 800 ± 25°, cool in a suitable desiccator, and weigh. If the use of sulfuric acid is not specified, the crucible need not be cooled but can be ignited directly at 800 ± 25° once the charring or volatilization is complete. Continue the ignition until constant weight is attained, unless otherwise specified.

Conduct the ignition in a well-ventilated hood, but protected from air currents, and at as low a temperature as is possible to effect the complete combustion of the carbon. A muffle furnace may be used, if desired, and its use is recommended for the final ignition at 800 ± 25°.

6.13 Sulfate in Reagents

STANDARD SULFATE SOLUTION: Dissolve 181.4 mg of potassium sulfate (dried at 105° for 2 h) in water to make 1000 mL. This solution contains the equivalent of 0.10 mg of sulfate (SO_4) per mL.

PROCEDURE

Method I: Neutralize, if necessary, a solution of the quantity of the reagent or residue indicated in the test in 25 mL of water, or a solution prepared as directed in the test, with hydrochloric acid or with ammonia TS, litmus paper being used as the indicator, and add 1 mL of 1 N hydrochloric acid. Filter the solution, if necessary, through a filter paper previously washed with water, and add 2 mL of barium chloride TS. Mix, allow to stand for 10 min, and compare the turbidity, if any, with that produced in a control containing the same quantities of the same reagents used in the test and a quantity of *Standard sulfate solution* equivalent to the quantity of sulfate (SO_4) permitted in the test. Adjust the two solutions with water to the same volume before adding the barium chloride TS.

Method II: Heat the solution to boiling, prepared as directed in the individual test procedure, or the filtrate designated in the procedure, and add 5 mL of barium chloride TS. Then digest the solution on a steam bath for 2 h, and allow to stand overnight. If any precipitate is formed, filter the solution through paper, wash the residue with hot water, and transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Perform a blank determination concurrently with the test specimen determination, and subtract the weight of residue obtained from that obtained in the test specimen determination to obtain the weight of residue attributable to the sulfate content of the specimen.

REAGENT SPECIFICATIONS

Absolute Ether—See *Ethyl Ether, Anhydrous*.

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Absorbent Cotton—Use *Purified Cotton* (USP monograph).

Acetal, $C_6H_{14}O_2$**—118.2**—Use a suitable grade.

Acetaldehyde *(Ethanal; Acetic Aldehyde),* CH_3CHO**—44.05** [75-07-0]—Colorless liquid. Miscible with water and with alcohol. Use ACS reagent grade.

Acetaldehyde Ammonia Trimer Trihydrate *(Hexahydro-2,4,6-trimethyl-1,3,5-triazine trihydrate),* $C_6H_{15}N_3 \cdot 3H_2O$**—183.25** [58052-80-5]—Use a suitable grade with a content of NLT 96.0%.

Acetanilide *(Phenylacetamide; Antifebrin),* C_8H_9NO**—135.16** [103-84-4]—White, shiny crystals, usually in scales, or a white, crystalline powder. Is stable in air. Freely soluble in alcohol and in chloroform; soluble in boiling water, in ether, and in glycerin; slightly soluble in water.
Melting Range ⟨741⟩: between 114° and 116°.
Reaction: Its saturated solution is neutral to litmus.
Loss on Drying ⟨731⟩: Dry it over sulfuric acid for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition (Reagent test): not more than 0.05%.

Acetic Acid *(6 N Acetic Acid)*—Use *Acetic Acid* (NF monograph) or prepare a suitable dilution of glacial acetic acid in such a way as to obtain a final concentration of acetic acid between 36.0% and 37.0%, by weight.

Acetic Acid, Diluted *(1 N Acetic Acid)*—Dilute 60.0 mL of glacial acetic acid with water to make 1000 mL.
- **Residue on Evaporation:** Evaporate 50 mL on a steam bath, and dry the residue at 105° for 2 hours: the residue weighs not more than 1 mg (0.002%).
- **Chloride** (Reagent test): Five mL shows not more than 0.01 mg of Cl (2 ppm).
- **Sulfate** (Reagent test, *Method I*): Ten mL shows not more than 0.5 mg of SO_4 (50 ppm).
- **Heavy Metals** (Reagent test): Evaporate 20 mL on a steam bath to dryness. Add to the residue 2 mL of the acid, dilute with water to 25 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 0.04 mg of added Pb and 2 mL of the diluted acetic acid (2 ppm).

Acetic Acid, Glacial, CH_3COOH**—60.05** [64-19-7]—Use ACS reagent grade.

Acetic Anhydride *(Acetic Oxide; Acetyl Oxide),* $(CH_3CO)_2O$**—102.09** [108-24-7]—Use ACS reagent grade.

Acetone *(Propanone; Dimethylformaldehyde),* CH_3COCH_3**—58.08** [67-64-1]—Use ACS reagent grade.
[Note—For UV spectrophotometric determinations, use ACS reagent grade Acetone Suitable for Use in UV Spectrophotometry.]

Acetone, Anhydrous, CH_3COCH_3**—58.08**—Use ACS reagent grade Acetone.

Acetone, Neutralized—To a suitable quantity of acetone add 2 or 3 drops of phenolphthalein TS and a sufficient amount of 0.02 or 0.01 N sodium hydroxide to produce a faint pink color. Prepare neutralized acetone just prior to use.

Acetonitrile *(Methyl Cyanide; Cyanomethane),* CH_3CN**—41.05** [75-05-8]—Use ACS reagent grade.

Acetonitrile, Spectrophotometric—Use ACS reagent grade, which meets also the requirements of the following test.
Spectral Purity: Measure in a 1-cm cell between 250 nm and 280 nm, with a suitable spectrophotometer, against air as the blank: its absorbance is not more than 0.01.

Acetophenone *(Phenylethanone; Phenyl Methyl Ketone),* $CH_3COC_6H_5$**—120.15** [98-86-2]—Liquid. Slightly soluble in water, freely soluble in alcohol and in ether.
Melting Range ⟨741⟩: between 19° and 20°.
Refractive Index ⟨831⟩: about 1.534 at 20°.
Specific Gravity ⟨841⟩: about 1.03.

***p*-Acetotoluidide,** $C_9H_{11}NO$**—149.19** [103-89-9]—White to off-white powder.
- **Assay:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 230°; the detector temperature is maintained at 300°; and the column temperature is maintained at 130° and programmed to rise 10° per minute to 280°. The area of the $C_9H_{11}NO$ peak is not less than 98.5% of the total peak area.
- **Melting Range** ⟨741⟩: between 145° and 151°.

Acetylacetone *(2,4-Pentanedione; Diacetylmethane),* $C_5H_8O_2$**—100.12** [123-54-6]—Clear, colorless to slightly yellow, flammable liquid. Soluble in water; miscible with alcohol, with chloroform, with acetone, with ether, and with glacial acetic acid.
Assay: Not less than 98% of $C_5H_8O_2$, a suitable gas chromatograph equipped with a flame-ionization detector being used and helium being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 1.83-m stainless steel column containing 10% phase G43 on support S1A; the injection port and detector temperatures are maintained at 250° and 310°, respectively; the column temperature is programmed to rise 8° per minute, from 50° to 220°.
Refractive Index ⟨831⟩: between 1.4505 and 1.4525, at 20°.

Acetyl Chloride, CH_3COCl**—78.50** [75-36-5]—Clear, colorless liquid. Is decomposed by water and by alcohol. Miscible with benzene and with chloroform. Use ACS reagent grade.
Specific Gravity ⟨841⟩: about 1.1.

Acetylcholine Chloride *(Trimethylethanaminium Chloride; Acecoline),* $[CH_3COOCH_2CH_2N(CH_3)_3]Cl$**—181.66** [60-31-1]—White, crystalline powder. Very deliquescent; very soluble in water; freely soluble in alcohol.
Melting Range ⟨741⟩: When previously dried at 110° in a capillary tube for 1 hour, it melts between 149° and 152°.
Reaction: A solution (1 in 10) is neutral to litmus.
Residue on Ignition (Reagent test): negligible, from 200 mg.
Solubility in Alcohol: A solution of 500 mg in 5 mL of alcohol is complete and colorless.
Percent of Acetyl (CH_3CO): Weigh accurately about 400 mg, previously dried at 105° for 3 hours, and dissolve in 15 mL of water in a glass-stoppered conical flask. Add 40.0 mL of 0.1 N sodium hydroxide VS, and heat on a steam bath for 30 minutes. Insert the stopper, allow to cool, add phenolphthalein TS, and titrate the excess alkali with 0.1 N sulfuric acid VS. Determine the exact normality of the 0.1 N

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sodium hydroxide by titrating 40.0 mL after it has been treated in the same manner as in the test. Each mL of 0.1 N sodium hydroxide is equivalent to 4.305 mg of CH_3CO. Between 23.2% and 24.2% is found.

Percent of Chlorine (Cl): Weigh accurately about 400 mg, previously dried at 105° for 3 hours, and dissolve in 50 mL of water in a glass-stoppered, 125-mL flask. Add with agitation 30.0 mL of 0.1 N silver nitrate VS, then add 5 mL of nitric acid and 5 mL of nitrobenzene, shake, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS: each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 19.3% and 19.8% of Cl is found.

Add the following:

▲**2-Acetylresorcinol** *(2′,6′-Dihydroxyacetophenone),* $C_8H_8O_3$—**152.15** [699-83-2]—Use a suitable grade with a content of NLT 97%.▲ (USP 1-Aug-2019)

3-Acetylthio-2-methylpropanoic Acid, $C_6H_{10}O_3S$—**162.21**—Use a suitable grade.

[NOTE—A suitable grade is available as β-(Acetylmercapto) isobutyric Acid, catalog number 39059, from Senn Chemicals AG www.sennchem.com.]

***N*-Acetyl-L-tyrosine Ethyl Ester,** $C_{13}H_{17}NO_4$—**251.28**—Determine the suitability of the material as directed in the *Assay* under *Chymotrypsin* (USP monograph).

Acrylic Acid *(2-Propenoic Acid; Vinylformic Acid),* $C_3H_4O_2$—**72.06** [79-10-7]—Colorless liquid. Miscible with water, with alcohol, and with ether.

Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩), equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 150°; the detector temperature is maintained at 300°; and the column temperature is maintained at 50° and programmed to rise 10° per minute to 200°. The area of the $C_3H_4O_2$ peak is not less than 99% of the total peak area.

Refractive Index ⟨831⟩: between 1.419° and 1.423° at 20°.

Activated Alumina—See *Alumina, Activated.*

Activated Charcoal—See *Charcoal, Activated.*

Activated Magnesium Silicate—See *Magnesium Silicate, Activated.*

Adamantane, $C_{10}H_{16}$—**136.23** [281-23-2]

Melting Range ⟨741⟩: between 270° and 271°.

Adenine Sulfate, $(C_5H_5N_5)_2 \cdot H_2SO_4 \cdot 2H_2O$—**404.36**—White crystals or crystalline powder. Melts, after drying at 110°, at about 200° with some decomposition. One g dissolves in about 160 mL of water; less soluble in alcohol. Soluble in solutions of sodium hydroxide. It is not precipitated from solution by iodine TS or mercuric-potassium iodide TS, but a precipitate is produced with trinitrophenol TS.

Residue on Ignition (Reagent test): negligible, from 100 mg.

Water: Dry it at 105° to constant weight: it loses not more than 10.0% of its weight.

Adipic Acid *(Hexanedioic Acid; 1,4-Butanedicarboxylic Acid),* $C_6H_{10}O_4$—**146.14** [124-04-9]—Colorless to white, crystalline powder. Slightly soluble in water and in cyclohexane; soluble in alcohol, in methanol, and in acetone; practically insoluble in benzene and in petroleum benzin.

Assay: Weigh accurately about 0.3 g, and dissolve in 50 mL of alcohol. Add 25 mL of water, mix, and titrate with 0.5 N sodium hydroxide VS to a pH of 9.5. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 36.54 mg of $C_6H_{10}O_4$. Not less than 98% is found.

Melting Range ⟨741⟩: between 151° and 155°, but the range between beginning and end of melting does not exceed 2°.

Agar—Use *Agar* (NF monograph). When used for bacteriological purposes, it is to be dried to a water content of not more than 20%.

Agarose [9012-36-6]—Polysaccharide consisting of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose. Use a suitable grade.

Air–Helium Certified Standard—A mixture of 1.0% air in industrial grade helium. It is available from most suppliers of specialty gases.

Albumin Bovine Serum [9048-46-8]—Almost colorless to faintly yellow powder. Not less than 95% pure. Solubility, 40 mg in 1 mL of water. Molecular weight is approximately 66,000. Use a suitable grade. Store between 2° and 8°.

Alcohol, *Ethanol, Ethyl Alcohol,* C_2H_5OH—**46.07** [64-17-5]—Use a suitable grade with a content of NLT 92.3% and NMT 93.8%, by weight, corresponding to NLT 94.9% and NMT 96% by volume, at 15.56°.

Alcohol, 70 Percent, 80 Percent, and 90 Percent—Prepare by mixing alcohol and water in the proportions given, the measurements being made at 25°.

		Relative Proportions		
Percent by Volume of C_2H_5OH at 15.56°	Specific Gravity at 25°	Alcohol, mL	Water, mL	Volume in mL of Alcohol, 94.9% v/v, Required for 100 mL
70	0.884	38.6	15	73.7
80	0.857	45.5	9.5	84.3
90	0.827	51	3	94.8

The proportions of alcohol and water taken to prepare these or any other percentage (v/v) solutions may be determined as follows. Calculate the amount, in mL, of water to be mixed with 100 mL of alcohol taken by the formula:

$$[94.9(d/c) - 0.8096]100$$

in which 94.9 is the percentage (v/v) of C_2H_5OH in alcohol, 0.8096 is the specific gravity of 94.9% alcohol, d is the specific gravity, obtained from the Alcoholometric Table (see *Reference Tables*), of the solution containing c% (v/v) of C_2H_5OH, and 100 is the volume, in mL, of alcohol taken.

Alcohol, Absolute, C_2H_5OH—**46.07**—Use ACS reagent grade Ethyl Alcohol, Absolute.

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Alcohol, Aldehyde-free—Dissolve 2.5 g of lead acetate in 5 mL of water, add the solution to 1000 mL of alcohol contained in a glass-stoppered bottle, and mix. Dissolve 5 g of potassium hydroxide in 25 mL of warm alcohol, cool the solution, and add it slowly, without stirring, to the alcohol solution of lead acetate. After 1 hour shake the mixture vigorously, allow it to stand overnight, decant the clear liquid, and recover the alcohol by distillation.

Alcohol, Amyl—See *Amyl Alcohol.*

Alcohol, Dehydrated *(Absolute Alcohol),* C_2H_5OH—**46.07**—Use ACS reagent grade Ethyl Alcohol, Absolute.

Alcohol, Dehydrated Isopropyl—See *Isopropyl Alcohol, Dehydrated.*

Alcohol, Denaturated: It is ethyl alcohol to which has been added some substance or substances which, while allowing the use of the alcohol in most applications, renders it entirely unfit for consumption as a beverage. The most common denaturants used, either alone or in combination, are the following: methanol, camphor, aldehol, amyl alcohol, gasoline, isopropanol, terpineol, benzene, castor oil, acetone, nicotine, aniline dyes, ether, cadmium iodide, pyridine bases, sulfuric acid, kerosene, and diethyl phthalate. Use a suitable grade.

Alcohol, Diluted—Use *Diluted Alcohol* (NF monograph).

Alcohol, Isobutyl—See *Isobutyl Alcohol.*

Alcohol, Isopropyl—See *Isopropyl Alcohol.*

Alcohol, Methyl—See *Methanol.*

Alcohol, Neutralized—To a suitable quantity of alcohol add 2 or 3 drops of phenolphthalein TS and just sufficient 0.02 N or 0.1 N sodium hydroxide to produce a faint pink color. Prepare neutralized alcohol just prior to use.

Alcohol, *n*-Propyl—See *n-Propyl Alcohol.*

Alcohol, Secondary Butyl—See *Butyl Alcohol, Secondary.*

Alcohol, Tertiary Butyl—See *Butyl Alcohol, Tertiary.*

Aldehyde Dehydrogenase—A white powder. One mg contains not less than 2 enzyme activity units.

Assay: Transfer about 20 mg, accurately weighed, to a 200-mL volumetric flask, dissolve in 1 mL of water, dilute with an ice-cold solution of bovine serum albumin (1 in 100) to volume, and mix. Use this solution as the *Assay preparation.* Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol, and 40 mg of edetate disodium in 70 mL of water, adjust with citric acid monohydrate solution (2.1 in 10) to a pH of 9.0 ± 0.1, dilute with water to 100 mL, and mix to obtain a *pH 9.0 buffer.* Dissolve an accurately weighed quantity of β-nicotinamide adenine dinucleotide (β-NAD) in water to obtain a *β-NAD solution* having a known concentration of about 20 mg per mL. Pipet 0.1 mL of the *Assay preparation* into a 1-cm spectrophotometric cell. Pipet 0.1 mL of water into a second 1-cm spectrophotometric cell to provide the reagent blank. Add 2.5 mL of *pH 9.0 buffer,* 0.2 mL of *β-NAD solution,* and 0.1 mL of pyrazole solution (0.68 in 100) to each cell, and mix. Stopper the cells, and allow to stand for 2 minutes at 25 ± 1°. Add 0.01 mL of acetaldehyde solution (0.3 in 100) to each cell, and mix. Stopper the cells, and determine the absorbance of the solution obtained from the *Assay preparation* at a wavelength of 340 nm, using the solution obtained from the reagent blank as the reference. Calculate the change, ΔA, in absorbance per minute for the solution obtained from the *Assay preparation,* starting at the point when the absorbance and time relationship becomes linear. One enzyme activity unit is defined as the amount of enzyme that oxidizes 1 µmol of acetaldehyde per minute when the test is conducted under the conditions described herein. Calculate the enzyme activity units in each mg of aldehyde dehydrogenase taken by the formula:

$$[(2.91)(200)/(6.3)(0.1)(1000)](\Delta A/W)$$

in which ΔA is as defined above and W is the weight, in g, of aldehyde dehydrogenase taken.

Alizarin Complexone *(Alizarin-3-methyliminodiacetic Acid; Alizarin Fluorine Blue),* $C_{19}H_{15}NO_8$—**385.32** [3952-78-1]—Use a suitable grade.

Alkaline Phosphatase Enzyme—See *Phosphatase Enzyme, Alkaline.*

Alkylphenoxypolyethoxyethanol—A nonionic surfactant. Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Triton X-100" from Sigma-Aldrich, www.sigma-aldrich.com.]

Alpha-Chymotrypsin—25 kDa [9004-07-3]—Use a suitable salt-free grade for protein sequencing.

[NOTE—A suitable grade is available as catalog number 4423 from www.sigma-aldrich.com.]

Alpha-Cyclodextrin Hydrate *(Alpha-Schardinger Dextrin; Cyclohexaamylose),* $C_{36}H_{60}O_{30} \cdot xH_2O$[51211-51-9]—Use a suitable grade with a content of NLT 98%.

[NOTE—A suitable grade is available as catalog number 22729 from www.acros.com.]

Alpha-(2-(methylamino)ethyl)benzyl alcohol—Use a suitable grade.

Alphanaphthol—See *1-Naphthol.*

Alprenolol Hydrochloride, $C_{15}H_{23}NO_2 \cdot HCl$—**285.8** [13707-88-5]—Use a suitable grade.

Alum *(Ammonium Alum, Aluminum Ammonium Sulfate),* $AlNH_4(SO_4)_2 \cdot 12H_2O$—**453.33** [7784-26-1]—Large, colorless crystals or crystalline fragments or a white powder. Soluble in 7 parts of water and in about 0.5 part of boiling water; insoluble in alcohol. Use ACS reagent grade.

Ammonium Alum—See *Alum.*

Alumina—See *Aluminum Oxide, Acid-washed.*

Alumina, Activated *(Aluminum Oxide),* [1344-28-1]—Use a suitable grade.

Alumina, Anhydrous *(Aluminum Oxide; Alumina specially prepared for use in chromatographic analysis)* [1344-28-1]—A white or practically white powder, 80- to 200-mesh. It does not soften, swell, or decompose in water. It is not acid-washed. Store it in well-closed containers.

Aluminon *(Aurin Tricarboxylic Acid, [tri]Ammonium Salt),* $C_{22}H_{23}N_3O_9$—**473.43** [569-58-4]—Yellowish-brown, glassy powder. Freely soluble in water. Use ACS reagent grade.

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Aluminum, Al—**At. Wt. 26.98154** [7429-90-5]—Use ACS reagent grade, which also meets the requirements of the following test.

Arsenic: Place 750 mg in a generator bottle (see *Arsenic in Reagents* under *General Tests for Reagents*), omitting the pledget of cotton. Add 10 mL of water and 10 mL of sodium hydroxide solution (3 in 10), and allow the reaction to proceed for 30 minutes: not more than a barely perceptible stain is produced on the mercuric bromide test paper.

Aluminum Chloride, $AlCl_3$ **—133.34** [7446-70-0]—Use a suitable grade with a content of NLT 99.0%.

Add the following:

▲Aluminum Chloride Hexahydrate, $AlCl_3 \cdot 6H_2O$**—241.43** [7784-13-6]—Use a suitable grade with a content of NLT 99%. ▲ (USP 1-Dec-2019)

Aluminum Oxide, Acid-Washed *(Alumina specially prepared for use in chromatographic analysis)*[1344-28-1]—White or practically white powder or fine granules. Very hygroscopic. Store in tight containers.

pH of Slurry: The pH of a well-mixed slurry of 5 g in 150 mL of ammonia-free and carbon dioxide-free water, after 10 minutes' standing, is between 3.5 and 4.5.

Loss on Ignition: Weigh accurately about 1 g, and ignite, preferably in a muffle furnace at 800° to 825°, to constant weight: it loses not more than 5.0% of its weight.

Silica: Fuse 500 mg with 10 g of potassium bisulfate for 1 hour in a platinum crucible, cool, and dissolve in hot water: not more than a small amount of insoluble matter remains.

Suitability for Chromatographic Adsorption: Dissolve 50 mg of *o*-nitroaniline in benzene to make 50.0 mL. Dilute 10 mL of the resulting solution with benzene to 100.0 mL, and mix (*Solution A*).

Weigh quickly about 2 (±0.005) g of specimen in a glass-stoppered weighing bottle, and rapidly transfer it to a dry, glass-stoppered test tube. Add 20.0 mL of *Solution A*, insert the stopper, shake vigorously for 3 minutes, and allow to settle.

Pipet 10 mL of the clear supernatant into a 100-mL volumetric flask, dilute with benzene to volume, and mix (*Solution B*).

Determine the absorbances of *Solutions A* and *B* at 395 nm, with a suitable spectrophotometer, using benzene as the blank. Calculate the quantity, in mg, adsorbed per g of test specimen by the formula:

$$[2(1 - A_B/A_A)]/W$$

in which A_A and A_B are the absorbances of *Solutions A* and *B*, respectively; and W is the weight, in g, of the aluminum oxide. Not less than 0.3 mg of *o*-nitroaniline is adsorbed for each g of the aluminum oxide.

Add the following:

▲Aluminum Oxide Strips—Aluminum oxide coated with fluorescent indicator F254 deposited on aluminum plates or sheets for thin-layer chromatography (TLC). It can be cut in the appropriate size. [NOTE—A suitable material is available as catalog number 105550 from www.emdmillipore.com] ▲ 2S (USP41)

Aluminum Potassium Sulfate, $AlK(SO_4)_2 \cdot 12H_2O$**—474.39** [10042-67-1]—Use ACS reagent grade.

Amaranth, $C_{20}H_{11}N_2Na_3O_{10}S_3$**—604.48** [915-67-3]—A deep brown or dark reddish-brown fine powder. Use a suitable grade.

Aminoacetic Acid *(Glycine),* NH_2CH_2COOH**—75.07** [56-40-6]—White, crystalline powder. Very soluble in water; slightly soluble in alcohol.

- **NITROGEN CONTENT** (Reagent test): Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 18.4% and 18.8% of N is found, corresponding to not less than 98.5% of $C_2H_5NO_2$.
- **INSOLUBLE MATTER** (Reagent test): not more than 1 mg, from 10 g (0.01%).
- **RESIDUE ON IGNITION** (Reagent test): not more than 0.05%.
- **CHLORIDE** (Reagent test): One g shows not more than 0.1 mg of Cl (0.01%).
- **SULFATE** (Reagent test, *Method I*): Two g shows not more than 0.1 mg of SO_4 (0.005%).
- **HEAVY METALS** (Reagent test): 0.001%, 5 mL of 1 N hydrochloric acid VS being used to acidify the solution of the test specimen.
- **IRON** ⟨241⟩: One g, dissolved in 47 mL of water containing 3 mL of hydrochloric acid, shows not more than 0.01 mg of Fe (0.001%).

4-Aminoantipyrine, $C_{11}H_{13}N_3O$**—203.24** [83-07-8]—Light yellow, crystalline powder. A 500-mg portion dissolves completely in 30 mL of water and yields a clear solution.

Melting Range ⟨741⟩: between 108° and 110°.

***p*-Aminobenzoic Acid**—See *Para-aminobenzoic Acid.*

2-Aminobenzonitrile *(Anthranilonitrile),* $C_7H_6N_2$**—118.14** [1885-29-6]—Use 2-Aminobenzonitrile 98%.

Melting Range ⟨741⟩: between 49° and 52°.

4-Amino-6-chloro-1,3-benzenedisulfonamide, $C_6H_8ClN_3O_4S_2$**—285.73** [121-30-2]—White powder. Insoluble in water and in chloroform; soluble in ammonia TS.

Residue on Ignition (Reagent test): not more than 2 mg from 2 g (0.1%).

Absorbance: A 1 in 200,000 solution in methanol exhibits absorbance maxima at about 223 nm, 265 nm, and 312 nm. Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 265 nm is about 64.0.

4-Amino-2-chlorobenzoic Acid, $C_6H_3Cl(NH_2)(COOH)$**—171.58** [2457-76-3]—White crystals or white, crystalline powder.

Melting Range ⟨741⟩: between 208° and 212°.

2-Amino-5-chlorobenzophenone, $C_{13}H_{10}ClNO$**—231.68** [719-59-5]—Use USP 2-Amino-5-chlorobenzophenone RS.

7-Aminodesacetoxycephalosporanic Acid, $C_8H_{10}N_2O_3S$**—214.2**—Light yellow powder.

Ordinary Impurities ⟨466⟩

Test solution: 1 N ammonium hydroxide

Standard solution: 1 N ammonium hydroxide

Eluant: 0.5 N sodium chloride

Visualization: 1

2-Aminoethyl Diphenylborinate—See *Diphenylborinic Acid, Ethanolamine Ester.*

1-(2-Aminoethyl)piperazine, $C_6H_{15}N_3$**—129.20** [140-31-8]—Viscous, colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped

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with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G2. The injection port temperature is maintained at 280°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280° and held there for 10 minutes. The detector temperature is maintained at 300°. The area of the main peak is not less than 97% of the total peak area.
Refractive Index ⟨831⟩: between 1.4978 and 1.5010 at 20°.

Aminoguanidine Bicarbonate *(Aminoguanidine Hydrogen Carbonate)*, $CH_6N_4 \cdot H_2CO_3$—**136.11** [2582-30-1]—White powder.
Assay: Dissolve about 34 mg, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 13.61 mg of $CH_6N_4 \cdot H_2CO_3$. Not less than 98.5% is found.
Melting Point ⟨741⟩: about 170°, with decomposition.

2-Aminoheptane *(2-Heptylamine; 1-Methylhexylamine)*, $C_7H_{17}N$—**115.22** [123-82-0]—Use a suitable grade with a content of not less than 99%.

***N*-Aminohexamethyleneimine** *(N-Aminohomopiperidine, 1-Aminohomopiperidine)*, $C_6H_{14}N_2$—**114.19** [5906-35-4]—Colorless liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G2. The injection port temperature is maintained at 180°; the column temperature is maintained at 80° and programmed to rise 10° per minute to 230° and then maintained at 230° for 5 minutes. The detector temperature is maintained at 300°. The area of the main peak is not less than 95% of the total peak area.
Refractive Index ⟨831⟩: between 1.4840 and 1.4860 at 20°.

4-Amino-3-hydroxy-1-naphthalenesulfonic Acid *(1,2,4-Aminonaphtholsulfonic Acid; 1-Amino-2-naphthol-4-sulfonic Acid)*, $C_{10}H_9NO_4S$—**239.25** [116-63-2]—Light purple powder. Use ACS reagent grade.

1,2,4-Aminonaphtholsulfonic Acid: see 4-Amino-3-hydroxy-1-naphthalenesulfonic Acid.

2-Aminophenol *(o-Aminophenol; 2-Hydroxyaniline)*, C_6H_7NO—**109.13** [95-55-6]—Off-white powder. Use a suitable grade with a content of not less than 99%.

***m*-Aminophenol** *(3-Amino-1-Hydroxybenzene)*, C_6H_7NO—**109.13** [591-27-5]—Cream-colored to pale yellow flakes. Sparingly soluble in cold water; freely soluble in hot water, in alcohol, and in ether.
Assay: Dissolve about 1.5 g, accurately weighed, in about 400 mL of water in a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to an iodine flask, add 50.0 mL of 0.1 N bromine VS, dilute with 50 mL of water, add 5 mL of hydrochloric acid, and immediately insert the stopper in the flask. Shake for 1 minute, allow to stand for 2 minutes, and add 5 mL of potassium iodide TS through the slightly loosened stopper. Shake thoroughly, allow to stand for 5 minutes, remove the stopper, and rinse it and the neck of the flask with 20 mL of water, adding the rinsing to the flask. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. From the volume of 0.1 N sodium thiosulfate used, calculate the volume, in mL, of 0.1 N bromine consumed by the test specimen. Each mL of 0.1 N bromine is equivalent to 1.819 mg of C_6H_7NO: not less than 99.5% is found.
Melting Range ⟨741⟩: between 121° and 123°.
Loss on Drying ⟨731⟩: Dry it over calcium chloride for 4 hours: the loss in weight is negligible.
Residue on Ignition ⟨Reagent test⟩: negligible, from 2 g.

***p*-Aminophenol** *(p-Hydroxyaniline)*, C_6H_7NO—**109.13** [123-30-8]—Fine, yellowish, crystalline powder. Slightly soluble in water and in alcohol. Use a suitable grade with a content of not less than 99%.

3-Amino-1-propanol, $H_2N(CH_2)_3OH$—**75.11** [156-87-6]—Liquid.
Boiling Range ⟨Reagent test⟩: between 184° and 188°.
Refractive Index ⟨831⟩: between 1.461 and 1.463 at 20°.

3-Aminopropionic Acid *(β-Alanine)*, $NH_2CH_2CH_2COOH$—**89.09** [107-95-9]—Use a suitable grade.

3-Aminosalicylic Acid, $C_7H_7NO_3$—**153.14** [570-23-0]—Tan-grey powder. Use a suitable grade with a content of not less than 97%.

Ammonia Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator bromophenol blue.
Measuring Range: 5 to 70 ppm.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Ammonia Solution, Diluted—Use *Ammonia TS*.

Ammonia Water, 25 Percent [1336-21-6]—Use a suitable grade.

Ammonia Water, Stronger *(Ammonium Hydroxide)*— [1336-21-6]—Use ACS reagent grade Ammonium Hydroxide.

Ammonium Acetate, $NH_4C_2H_3O_2$—**77.08** [631-61-8]—Use ACS reagent grade.

Ammonium Bicarbonate ***(Ammonium Hydrogen Carbonate)***, NH_4HCO_3—**79.06** [1066-33-7]—Use a suitable grade with a content of NLT 99.0%.

Ammonium Bisulfate *(Ammonium Hydrogen Sulfate)*, NH_4HSO_4—**115.11** [7803-63-6]—White crystals. Freely soluble in water; practically insoluble in alcohol, in acetone, and in pyridine.
Assay: Dissolve about 300 mg, accurately weighed, in 50 mL of a mixture of water and alcohol (25:25). Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 11.51 mg of NH_4HSO_4. Not less than 98% is found.

Ammonium Bromide, NH_4Br—**97.94** [12124-97-9]—Use ACS reagent grade.

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Ammonium Carbonate *(Hartshorn Salt)* [506-87-6]—Use ACS reagent grade.

Ammonium Chloride *(Salmiac)*, NH_4Cl—**53.49** [12125-02-9]—Use ACS reagent grade.

Ammonium Citrate, Dibasic *(Citric Acid Diammonium Salt)*, $(NH_4)_2HC_6H_5O_7$—**226.18** [3012-65-5]—Use ACS reagent grade.

Ammonium Dihydrogen Phosphate—See *Ammonium Phosphate, Monobasic.*

Ammonium Fluoride, NH_4F—**37.04** [12125-01-8]—Use ACS reagent grade.

Ammonium Formate (*Formic Acid Ammonium Salt*), CH_5NO_2—**63.06** [540-69-2]—Use a suitable grade.

Ammonium Hydrogen Difluoride, $(NH_4)HF_2$ —**57.04** [1341-49-7]—Use a suitable grade.

Ammonium Hydroxide *(Ammonium Aqueous)*, [1336-21-6]—Use ACS reagent grade.

Ammonium Hydroxide, 6 N: Prepare by diluting 400 mL of Ammonia Water, Stronger (see *Reagents* section) with water to make 1000 mL.

Ammonium Molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$—**1235.86** [12054-85-2]—Use ACS reagent grade.

Ammonium Nitrate, NH_4NO_3—**80.04** [6484-52-2]—Use ACS reagent grade.

Ammonium Oxalate, $(NH_4)_2C_2O_4 \cdot H_2O$—**142.11** [6009-70-7]—Use ACS reagent grade.

Ammonium Persulfate *(Ammonium Peroxydisulfate)*, $(NH_4)_2S_2O_8$—**228.20** [7727-54-0]—Use ACS reagent grade Ammonium Peroxydisulfate.

Ammonium Phosphate, Dibasic *(Diammonium Hydrogen Phosphate)*, $(NH_4)_2HPO_4$—**132.06** [7783-28-0]—Use ACS reagent grade.

Ammonium Phosphate, Monobasic *(Ammonium Dihydrogen Phosphate)*, $NH_4H_2PO_4$—**115.03** [7722-76-1]—Use ACS reagent grade.

Ammonium Pyrrolidinedithiocarbamate *(1-pyrrolidinecarbodithioic acid, ammonium salt)*, $C_5H_{12}N_2S_2$—**164.29** [5108-96-3]—Use a suitable grade.

Ammonium Reineckate *(Reinecke Salt)*, $NH_4[Cr(NH_3)_2(SCN)_4] \cdot H_2O$—**354.44** [13573-16-5]—Dark red crystals or red, crystalline powder. Moderately soluble in cold water; more soluble in hot water. Gradually decomposes in solution.

Sensitiveness: Dissolve 50 mg in 10 mL of water. Add 0.2 mL of the solution to 1 mL of a solution of 10 mg of choline chloride in 20 mL of water, and shake gently: a distinct precipitate forms within 5 to 10 seconds.

Ammonium Sulfamate, $NH_4OSO_2NH_2$—**114.13** [7773-06-0]—Use ACS reagent grade.

Ammonium Sulfate, $(NH_4)_2SO_4$—**132.14** [7783-20-2]—Use ACS reagent grade.

Add the following:

▲**Ammonium Tartrate** *(Dibasic Ammonium Tartrate; L-(+)-Tartaric Acid Diammonium Salt; Diammonium Tartrate)*, $C_4H_{12}N_2O_6$—**184.15** *[3164-29-2]*—Use a suitable grade with a content of NLT 99%.▲ 2S (USP41)

Ammonium Thiocyanate *(Ammonium Rhodanide)*, NH_4SCN—**76.12** [1762-95-4]—Use ACS reagent grade.

Ammonium Vanadate *(Ammonium Metavanadate)*, NH_4VO_3—**116.98** [7803-55-6]—White, crystalline powder. Slightly soluble in cold water; soluble in hot water and in dilute ammonia TS.

Assay: Weigh accurately about 500 mg, transfer to a suitable container, add 30 mL of water and 2 mL of dilute sulfuric acid (1 in 4), swirl to dissolve, and pass sulfur dioxide gas through the solution until reduction is complete and the solution is bright blue in color. Boil gently while passing a stream of carbon dioxide through the solution to remove any excess sulfur dioxide, then cool, and titrate with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate consumed is equivalent to 11.7 mg of NH_4VO_3. Not less than 98.0% is found.

Solubility in Ammonium Hydroxide: Dissolve 1 g in a mixture of 3 mL of ammonium hydroxide and 50 mL of warm water: the solution is clear and colorless.

Carbonate: To 500 mg add 1 mL of water and 2 mL of diluted hydrochloric acid: no effervescence is produced.

Chloride: Dissolve 250 mg in 40 mL of hot water, add 2 mL of nitric acid, and allow to stand for 1 hour. Filter, and to the filtrate add 0.5 mL of silver nitrate TS: any turbidity produced does not exceed that of a blank containing 0.5 mg of added Cl (0.2%).

Sulfate: Dissolve 500 mg in 50 mL of hot water, and add 2 mL of diluted hydrochloric acid and 1.5 g of hydroxylamine hydrochloride. Heat at 60° for 3 minutes, filter, cool, and add to the filtrate 2 mL of barium chloride TS: no turbidity or precipitate is produced within 30 minutes.

Amyl Acetate *(Pentyl Acetate)*, $CH_3CO_2C_5H_{11}$—**130.18** [628-63-7]—Use a suitable grade with a content of NLT 99%.

Amyl Alcohol *(Isoamyl Alcohol)*, $C_5H_{11}OH$—**88.15** [123-51-3]—Use ACS reagent grade Isopentyl Alcohol.

***tert*-Amyl Alcohol,** $C_5H_{12}O$—**88.15** [75-85-4]—Clear, colorless, flammable, volatile liquid.

- **SPECIFIC GRAVITY** ⟨841⟩: about 0.81.
- **BOILING RANGE** (Reagent test): not less than 95%, between 100° and 103°.
- **RESIDUE ON EVAPORATION:** Evaporate 50 mL (40 g) on a steam bath, and dry at 105° for 1 hour: the residue weighs not more than 1.6 mg (0.004%).
- **ACIDS AND ESTERS:** Dilute 20 mL with 20 mL of alcohol, add 5.0 mL of 0.1 N sodium hydroxide VS, and reflux gently for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.1 N hydrochloric acid VS: not more than 0.75 mL of the 0.10 N sodium hydroxide is consumed, correction being made for the amount consumed in a blank (0.06% as amyl acetate).
- **ALDEHYDES:** Shake 5 mL with 5 mL of potassium hydroxide solution (30 in 100) in a glass-stoppered cylinder for 5 minutes, and allow to separate: no color develops in either layer.

α-Amylase—Use a suitable grade. It can be from vegetal or animal or microbiological origin.

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(*E*)-Anethole *(1-Methoxy-4-(1-propenyl)benzene)*, $C_{10}H_{12}O$—**148.20** [4180-23-8]—Use a suitable grade of *trans* isomer.

Anhydrous Alumina—See *Alumina, Anhydrous.*

Anhydrous Barium Chloride—See *Barium Chloride, Anhydrous.*

Anhydrous Calcium Chloride—See *Calcium Chloride, Anhydrous.*

Anhydrous Cupric Sulfate—See *Cupric Sulfate, Anhydrous.*

Anhydrous Dibasic Sodium Phosphate—See *Sodium Phosphate, Dibasic, Anhydrous.*

Anhydrous Magnesium Perchlorate—See *Magnesium Perchlorate, Anhydrous.*

Anhydrous Magnesium Sulfate—See *Magnesium Sulfate, Anhydrous.*

Anhydrous Methanol—See *Methanol, Anhydrous.*

Anhydrous Potassium Carbonate—See *Potassium Carbonate, Anhydrous.*

Anhydrous Sodium Acetate—See *Sodium Acetate, Anhydrous.*

Anhydrous Sodium Carbonate—See *Sodium Carbonate, Anhydrous.*

Anhydrous Sodium Sulfate—See *Sodium Sulfate, Anhydrous.*

Anhydrous Sodium Sulfite—See *Sodium Sulfite, Anhydrous.*

Aniline, $C_6H_5NH_2$**—93.13** [62-53-3]—Use ACS reagent grade.

Aniline Blue *(Certified Biological Aniline Blue)* [8004-91-9]—A water-soluble dye consisting of a mixture of the tri-sulfonates of triphenylpararosaniline and of diphenylrosaniline.

Aniline Sulfate, $C_{12}H_{14}N_2 \cdot H_2SO_4$**—284.33** [542-16-5]—Use a suitable grade.

Anion-Exchange Resin, Chloromethylated Polystyrene-Divinylbenzene—Strongly basic, cross-linked resin containing quaternary ammonium groups. It consists of small, moist, yellow beads having a characteristic amine odor. It is available in the chloride form which can be converted to the hydroxide form by regeneration with sodium hydroxide solution (1 in 4). For satisfactory regeneration a contact time of about 25 minutes is required, after which it must be washed with water until neutral. Suitable for use in column chromatography.

[NOTE—A suitable resin is "Amberlite IRA-400," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Anion-Exchange Resin, Strong, Lightly Cross-Linked, in the Chloride Form—Use a suitable grade.

[NOTE—A suitable resin is "AG 1-X4, catalog number 140-1331," produced by BioRad Laboratories, www.bio-rad.com.]

Anion-Exchange Resin, Styrene-Divinylbenzene: Strongly basic, cross-linked resin containing quaternary ammonium groups and about 8% of divinylbenzene. It is available in the chloride form in the 50- to 100-, 100- to 200-, and 200- to 400-mesh sizes. It can be converted to the hydroxide form by regeneration with a sodium hydroxide solution (5 in 100). Insoluble in water, in methanol, and in acetonitrile. Suitable for use in column chromatography.

[NOTE—A suitable resin is Dowex 1X8, produced by Dow Chemical Co. (www.dow.com) and available through Sigma-Aldrich (www.sigma-aldrich.com).]

Anion-Exchange Resin, 50- to 100-Mesh, Styrene-Divinylbenzene—Strongly basic, cross-linked resin containing quaternary ammonium groups and about 4% of divinylbenzene. It consists of tan-colored beads that may be relatively free flowing. It is available in the chloride form which can be converted to the hydroxide form by regeneration with a sodium hydroxide solution (5 in 100). For satisfactory regeneration a contact time of at least 30 minutes is required after which it must be washed free of excess alkali. Insoluble in water, in methanol, and in acetonitrile. Suitable for use in column chromatography.

[NOTE—A suitable resin is "Dowex 1X4," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Moisture Content of Fully Regenerated and **Expanded Resin:** Transfer 10 to 12 mL of the resin (as received) to a flask, and convert it completely to the chloride form by stirring with 150 mL of hydrochloric acid (5 in 100) for not less than 30 minutes. Decant the acid, and wash the resin in the same manner with distilled water until the wash water is neutral to litmus.

Transfer 5 to 7 mL of the regenerated resin to a glass filtering crucible, and remove only the excess surface water by very careful suction filtration. Transfer the conditioned, dried resin to a tared weighing bottle, and weigh. Dry in a vacuum oven at 100° to 105° and at a pressure of 50 mm of mercury for 16 hours. Transfer from the vacuum oven to a desiccator, and cool to room temperature. Reweigh. The loss in weight is between 50% and 65%.

Total New Volume Capacity: Transfer 2.5 to 3 mL of the conditioned, undried (See *Moisture Content*, above) resin to a 5-mL graduated cylinder, and fill it with water. Remove any air bubbles from the resin bed with a stainless steel wire, and settle the resin to its minimum volume by tapping the graduated cylinder. Record the volume of the resin.

Transfer the resin with 100 mL of water to a 250-mL flask. Add 2 mL of sulfuric acid, heat to 70° to 80°, and hold at that temperature for 5 minutes with occasional stirring (do not boil). Cool to room temperature, and add 2.5 mL of nitric acid (1 in 2), 2 mL of ferric ammonium sulfate TS, and 0.20 mL of 0.1 N ammonium thiocyanate. Titrate with 0.1 N silver nitrate VS until the solution turns colorless, and add a measured excess (1 to 5 mL). Heat to boiling to coagulate the silver chloride precipitate. Cool to room temperature, add 10 mL of nitrobenzene, shake vigorously, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS.

$$(\text{net mL } AgNO_3 \times N)/(\text{mL of resin}) = \text{mEq/mL}$$

The total exchange capacity of the regenerated, wet resin is more than 1.0 mEq per mL.

Wet Screen Analysis: The purpose of this test is to identify properly the mesh size of the resin. To obtain an accurate screen analysis requires special apparatus and technique.

Add 150 mL of resin to 200 mL of distilled water in an appropriate bottle, and allow it to stand at least 4 hours to completely swell the resin.

Transfer by means of a graduated cylinder 100 mL of settled and completely swollen resin to the top screen of a series (20-, 50-, 100-mesh) of 20.3-cm brass screens. Thoroughly wash the resin on each screen with a stream of distilled water until the resin is completely classified, collecting the wash water in a suitable container. Wash the

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beads remaining on the respective screens back into the 100-mL cylinder, and record the volume of settled resin on each screen: not less than 80% of the resin is between 50- and 100-mesh.

***p*-Anisaldehyde** *(4-Methoxybenzaldehyde)*, $C_8H_8O_2$—**136.15** [123-11-5]—Clear, colorless liquid.
Boiling Temperature: 248°
Density: between 1.119 and 1.123.
• **Refractive Index** ⟨831⟩: between 1.5725 and 1.5730 at 20°.

***p*-Anisidine**, C_7H_9NO—**123.06** [104-94-9]—Brown crystals. Use a suitable grade.

Anisole, $CH_3OC_6H_5$—**108.14** [100-66-3]—Colorless liquid.
Assay: Inject an appropriate specimen (about 0.5 µL) into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, nitrogen being used as the carrier gas. The following conditions have been found suitable: a 30-m capillary column is coated with phase G3; the injection port and detector temperatures are maintained at 140° and 300°, respectively; the column temperature is maintained at 70° and programmed to rise 10° per minute to 170°. The area of the anisole peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: 1.5160 at 20°.

Anthracene, $C_{14}H_{10}$—**178.23** [120-12-7]—White to off-white crystals or platelets. Darkens in sunlight. Insoluble in water; sparingly soluble in alcohol, in benzene, and in chloroform.
Melting Range ⟨741⟩: between 215° and 218°.

Anthrone, $C_{14}H_{10}O$—**194.23** [90-44-8]—Use ACS reagent grade.

Anti-D Reagent: The reagent can be monoclonal (low protein) or polyclonal (high-protein) and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results. Note that this reagent is different from Anti-D (Rh_o) Reagent. Consult manufacturer's package insert to ensure that the reagent is suitable for the Weak Anti-D test and does not contain other antibodies that will react when antihuman immunoglobulin is added.
[Note—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Anti-D (Rh_o) Reagent: The reagent can be monoclonal or polyclonal and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration for use in microplate tests. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results. Note that this reagent is different from Anti-D Reagent. Consult manufacturer's package insert to ensure that it is Anti-D (Rh_o) Reagent and not Anti-D Reagent.
[Note—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Antifoam Reagent—A 10% silicone–glycol emulsion, white in appearance. Nonionic but miscible with cool water.
[Note—A suitable grade is available as "Antifoam Reagent, "catalog number 2210, from Dow Corning Corporation, www.dowcorning.com.]

Antihuman Globulin Reagent: The reagent can be polyspecific or anti-immunoglobulin (Anti-IgG) and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results.
[Note—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Antimony Pentachloride, $SbCl_5$—**299.02** [7647-18-9]—Clear, reddish-yellow, oily, hygroscopic, caustic liquid. Fumes in moist air and solidifies by absorption of one molecule of water. Is decomposed by water; soluble in dilute hydrochloric acid and in chloroform. Boils at about 92° at a pressure of 30 mm of mercury and has a specific gravity of about 2.34 at 25°.
[**Caution**—Antimony pentachloride causes severe burns, and the vapor is hazardous.]
Assay ($SbCl_5$): Accurately weigh a glass-stoppered, 125-mL flask, quickly introduce about 0.3 mL of the test specimen, and reweigh. Dissolve with 20 mL of diluted hydrochloric acid (1 in 5), and add 10 mL of potassium iodide solution (1 in 10) and 1 mL of carbon disulfide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. The brown color will gradually disappear from the solution, and the last traces of free iodine will be collected in the carbon disulfide, giving a pink color. When this pink color disappears the endpoint has been reached. Each mL of 0.1 N sodium thiosulfate is equivalent to 14.95 mg of $SbCl_5$: not less than 99.0% of $SbCl_5$ is found.
Sulfate (Reagent test, ***Method II***): Dissolve 4.3 mL (10 g) in the minimum volume of hydrochloric acid, dilute with water to 150 mL, neutralize with ammonium hydroxide, and filter. To the filtrate add 2 mL of hydrochloric acid: the solution, 10 mL of barium chloride TS being used, yields not more than 1.3 mg of residue, correction being made for a complete blank test (0.005%).
Arsenic: Add 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid to 100 mg of specimen dissolved in 5 mL of hydrochloric acid. Mix, transfer to a color-comparison tube, and allow to stand for 30 minutes. Any color in the solution of the specimen should not be darker than that in a control containing 0.02 mg of arsenic (As), which has been treated in the same manner as the test specimen, when viewed downward over a white surface (0.02% of As).
Substances Not Precipitated By Hydrogen Sulfide (*as* SO_4): Dissolve 0.90 mL (2 g) in 5 mL of hydrochloric acid, and dilute with 95 mL of water. Precipitate the antimony completely with hydrogen sulfide, allow the precipitate to settle, and filter, being careful not to transfer much of the precipitate to the filter paper. (Retain the precipitate.) To 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate in a tared porcelain crucible to dryness, and ignite at 800 ± 25° for 15 minutes. (Retain the residue.) The weight of the ignited residue should not be more than

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0.0010 g greater than the weight obtained in a complete blank test (0.10%).

Iron ⟨241⟩: To the residue from the test for *Substances Not Precipitated By Hydrogen Sulfide* add 2 mL of hydrochloric acid and 5 drops of nitric acid, and evaporate on a steam bath to dryness. Take up the residue in 2 mL of hydrochloric acid, and dilute with water to 47 mL: the solution shows not more than 0.01 mg of Fe (0.001%).

Other Heavy Metals (as Pb): Dissolve the precipitate on the filter paper from the test for *Substances Not Precipitated By Hydrogen Sulfide,* with 75 mL of a solution containing 6 g of sodium sulfide and 4 g of sodium hydroxide dissolved in and diluted with water to 100 mL. Collect the filtrate in the original flask containing the remainder of the sulfide precipitate. Warm the solution to dissolve the soluble sulfides, and allow the insoluble sulfides to settle. Filter, wash thoroughly with hydrogen sulfide TS, and dissolve any precipitate remaining on the filter paper with 10 mL of hot diluted hydrochloric acid. Dilute the filtrate with water to 50 mL. Neutralize a 25-mL portion of this solution with 1 N sodium hydroxide, and add 1 mL of 1 N acetic acid and 10 mL of hydrogen sulfide TS. Any brown color should not exceed that produced by 0.05 mg of lead ion in an equal volume of solution containing 1 mL of 1 N acetic acid and 10 mL of hydrogen sulfide TS (0.005%).

Antimony Trichloride *(Antimonous Chloride),* $SbCl_3$—**228.12** [10025-91-9]—Use ACS reagent grade.

Antithrombin III: Antithrombin III human (heparin cofactor, factor II_a inhibitor, and factor X_a inhibitor) is a serine protease inhibitor. It is a glycoprotein having a molecular weight of 58,000 Da.

One Antithrombin III Unit is the amount found in 1 mL of normal human plasma. The potency of antithrombin III is not less than 4.0 Antithrombin III Units per mg of protein when tested in the presence of heparin. It exhibits 90% homogeneity when tested by SDS-PAGE.

Antithrombin III for test or assay purposes contains no detectable heparin. Test as follows. To a solution containing 1 Antithrombin III Unit per mL, add 1 µL of toluidine blue solution. In the presence of heparin, the color changes from blue to purple.

Aprobarbital, $C_{10}H_{14}N_2O_3$—**210.23** [77-02-1]—Fine, white crystalline powder. Slightly soluble in cold water; soluble in alcohol, in chloroform, and in ether.

Assay: Dissolve about 200 mg, previously dried at 105° for 2 hours and accurately weighed, in 20 mL of dimethylformamide in a 100-mL conical flask. Add 4 drops of thymol blue solution (1 in 200 in methanol), and titrate with 0.1 N lithium methoxide VS using a 10-mL buret, a magnetic stirrer, and a cover for the flask to protect against atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 21.02 mg of $C_{10}H_{14}N_2O_3$. Between 98.5% and 101.0% of $C_{10}H_{14}N_2O_3$ is found.

Melting Range ⟨741⟩: between 140° and 143°.

Add the following:

▲**Aqua Regia**—$HCl:HNO_3$ (3:1). Under a hood, carefully add, e.g., 5 mL of nitric acid to 15 mL of hydrochloric acid.

Must be prepared just before use and in just the amount necessary.

Do not store it.

Handle it and dispose of it with the appropriate safety procedures. ▲ (USP 1-Dec-2019)

Arsenazo III Acid, $C_{22}H_{18}As_2N_4O_{14}S_2$—**776.38** [1668-00-4]—Brown powder. Stable in air. Store at room temperature in a dry area.

Melting Temperature ⟨741⟩: greater than 320°.

Arsenic Trioxide, As_2O_3—**197.84** [1327-53-3]—Use ACS reagent grade.

[NOTE—Arsenic Trioxide of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Office of Standard Reference Materials, www.nist.gov, as standard sample No. 83.]

Ascorbic Acid *(Vitamin C),* $C_6H_8O_6$—**176.13** [50-81-7]—Use ACS reagent grade.

L-Asparagine *(L -2-Aminosuccinamic Acid),* $COOHCH(NH_2)CH_2CONH_2 \cdot H_2O$—**150.13** [70-47-3]—Colorless crystals. One g dissolves in 50 mL of water; soluble in acids and in alkalies; insoluble in alcohol and in ether. Its neutral or alkaline solutions are levorotatory; its acid solutions are dextrorotatory.

- **SPECIFIC ROTATION** ⟨781⟩: between +31° and +33°, determined in a solution in diluted hydrochloric acid containing the equivalent of 5 g (on the anhydrous basis, as determined by drying at 105° for 5 hours) in each 100 mL.
- **RESIDUE ON IGNITION** (Reagent test): not more than 0.1%.
- **CHLORIDE** (Reagent test): One g shows not more than 0.03 mg of Cl (0.003%).
- **SULFATE** (Reagent test, *Method I*): One g shows not more than 0.05 mg of SO_4 (0.005%).
- **HEAVY METALS** (Reagent test): 0.002%.
- **NITROGEN DETERMINATION** ⟨461⟩, *Method II*: between 18.4% and 18.8% of N is found.

L-Aspartic Acid, $C_4H_7NO_4$—**133.1** [56-84-8]—White to off-white powder. Use a suitable grade.

Azure A, $C_{14}H_{14}ClN_3S$—**291.80** [531-53-3]—Use a suitable grade.

Bacterial Alkaline Protease Preparation: Use a suitable grade.

[NOTE—A suitable grade is commercially available from www.bio-cat.com/alkaline-protease or as catalog number P3910 from www.sigma-aldrich.com.]

Barbital Sodium, $C_8H_{11}N_2NaO_3$—**206.2** [144-02-5]—White, crystalline powder or colorless crystals. Freely soluble in water; slightly soluble in alcohol. Use a suitable reagent grade.

Barbituric Acid, $C_4H_4N_2O_3$—**128.09** [65-52-7]—Faintly beige powder. Soluble in water, in alcohol, in chloroform, and in ether. Melts with decomposition at 251.6°. Use a suitable grade.

Barium Acetate, $C_4H_6BaO_4$—**255.43** [543-80-6]—Use ACS reagent grade.

Barium Chloride, $BaCl_2 \cdot 2H_2O$—**244.26** [10326-27-9]—Use ACS reagent grade.

Barium Chloride, Anhydrous, $BaCl_2$—**208.23** [10361-37-2]—This may be made by drying barium chloride in thin layers at 125° until the loss in weight between two successive, 3-hour drying periods does not exceed 1%.

Barium Chloride Dihydrate—Use *Barium Chloride.*

Barium Hydroxide, $Ba(OH)_2 \cdot 8H_2O$—**315.46** [12230-71-6]—Use ACS reagent grade.

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Barium Nitrate, $Ba(NO_3)_2$**—261.34** [10022-31-8]—Use ACS reagent grade.

Basic Fuchsin—See *Fuchsin, Basic.*

Beclomethasone $C_{22}H_{29}ClO_5$**—408.92** [4419-39-0]—Use a suitable grade with a content of not less than 99%.

Change to read:

Beef Extract—A fine beige to light-tan powder obtained by extraction from fresh lean beef by means of cooking with water and evaporating the broth at low temperature, usually under vacuum, and dried.
Loss on Drying ⟨731⟩: NMT 6.0%
Residue on Ignition ⟨281⟩: NMT 18.0%
Amino Nitrogen: 2.0%–2.3%
Nitrogen: 8.0%–18.0%
Chloride: NMT 7% of the total solids, calculated as sodium chloride
Microbial Content: ▲NMT▲ (ERR 1-May-2019) 10^4 cfu/g

Benzaldehyde, C_7H_6O**—106.12** [100-52-7]—Colorless, strongly refractive liquid. Soluble in water; miscible with alcohol, with ether, and with fixed and volatile oils.
Assay: Pipet about 1 mL into a tared, glass-stoppered weighing bottle, and weigh accurately. Loosen the stopper, and transfer both the weighing bottle and its contents to a 250-mL conical flask containing 25 mL of a hydro-alcoholic solution of hydroxylamine hydrochloride (prepared by dissolving 34.7 g of hydroxylamine hydrochloride in 160 mL of water, then adding alcohol to make 1000 mL, and neutralizing to bromophenol blue by the addition of sodium hydroxide TS). Using a graduated cylinder to measure the volume, rinse the sides of the flask with an additional 50 mL of this reagent solution. Allow the solution to stand for 10 minutes, add 1 mL of bromophenol blue TS, and titrate the liberated hydrochloric acid with 1 N sodium hydroxide VS. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 1 N sodium hydroxide consumed is equivalent to 106.1 mg of C_7H_6O. Not less than 98% is found.
Specific Gravity ⟨841⟩: between 1.041 and 1.046.
Refractive Index ⟨831⟩: between 1.5440 and 1.5465 at 20°.
Hydrocyanic Acid: Shake 0.5 mL with 5 mL of water, add 0.5 mL of sodium hydroxide TS and 0.1 mL of ferrous sulfate TS, and warm the mixture gently. Add a slight excess of hydrochloric acid: no greenish-blue color or blue precipitate is observed within 15 minutes.

Benzalkonium Chloride—Use *Benzalkonium Chloride* (NF monograph).

Benzamidine Hydrochloride Hydrate *(Benzenecarboximidamide Monohydrochloride, Hydrate),* $C_7H_8N_2 \cdot HCl \cdot xH_2O$**—156.6** [206752-36-5]—White to off-white powder. Use a suitable grade.
[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

Benzanilide, $C_{13}H_{11}NO$**—197.23** [93-98-1]—Off-white, light gray to grayish-green powder. Insoluble in water; sparingly soluble in alcohol; slightly soluble in ether.
Melting Range ⟨741⟩: between 162° and 165°.
Solubility in Acetone: A 1.0-g portion dissolves completely in 50 mL of acetone to yield a clear solution.

Benzene, C_6H_6**—78.11** [71-43-2]—Use ACS reagent grade.

Benzenesulfonamide, $C_6H_5SO_2NH_2$**—157.19** [98-10-2]—White to pale beige crystals.
Melting Range ⟨741⟩: between 150° and 153°.

Benzenesulfonyl Chloride, $C_6H_5SO_2Cl$**—176.62** [98-09-9]—Colorless, oily liquid. Insoluble in cold water; soluble in alcohol and in ether. Solidifies at 0°.
Melting Range ⟨741⟩: between 14° and 17°.
Boiling Range (Reagent test): between 251° and 252°.

Benzethonium Chloride—Use *Benzethonium Chloride* (USP monograph).

Benzhydrol *(α-Phenylbenzenemethanol),* $C_{13}H_{12}O$**—184.23** [91-01-0]—White to pale yellow crystals. Very slightly soluble in water; soluble in alcohol, in ether, and in chloroform.
Melting Range ⟨741⟩: between 65° and 67°, but the range between beginning and end of melting does not exceed 2°.

Benzil *(Dibenzoyl; Diphenylethanedione),* $C_{14}H_{10}O_2$ **—210.23** [134-81-6]—Use a suitable grade with a content of NLT 98%.

Benzoic Acid, C_6H_5COOH**—122.12** [65-85-0]—Use ACS reagent grade.
[NOTE—Benzoic Acid of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Office of Standard Reference Materials, www.nist.gov, as standard sample No. 350.]

Benzophenone, $(C_6H_5)_2CO$**—182.22** [119-61-9]—White, crystalline powder.
Melting Range ⟨741⟩: between 47° and 49°.

***p*-Benzoquinone** *(Quinone),* $C_6H_4O_2$**—108.09** [106-51-4]—Dark yellow powder having a green cast. Slightly soluble in water; soluble in alcohol, in ether, and in fixed alkali solutions. May darken on standing. Darkened material may be purified by sublimation in vacuum.
• **MELTING RANGE** ⟨741⟩: between 113° and 115°.

Benzoyl Chloride, C_6H_5COCl**—140.57** [98-88-4]—Use ACS reagent grade.

***N*-Benzoyl-L-arginine Ethyl Ester Hydrochloride,** $C_{15}H_{22}N_4O_3 \cdot HCl$**—342.82**—Determine suitability for use as a substrate as directed under *Crystallized Trypsin* (USP monograph).

3-Benzoylbenzoic Acid, $C_{14}H_{10}O_3$**—226.23** [579-18-0]—White to off-white powder.
Assay: Prepare a mixture of 1% trifluoroacetic acid in water and 1% trifluoroacetic acid in acetonitrile (55:45) for the mobile phase. Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The area of the $C_{14}H_{10}O_3$ peak is not less than 98.5% of the total peak area.

Benzoylformic Acid *(Phenylglyoxylic Acid),* $C_6H_5COCO_2H$**—150.14** [611-73-4]—Powder. Soluble in methanol.
Melting Range ⟨741⟩: between 62° and 67°.

Benzphetamine Hydrochloride, $C_{17}H_{21}N \cdot HCl$**—275.82** [5411-22-3]—White to off-white, crystalline powder. Freely soluble in water, in alcohol, and in chloroform; slightly soluble in ether.
Assay: Dissolve about 500 mg, accurately weighed, in a mixture of 50 mL of glacial acetic acid and 10 mL of mercuric

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acetate TS, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.58 mg of $C_{17}H_{21}N \cdot HCl$. Between 98.0% and 101.0%, calculated on the dried basis, is found.
Melting Range ⟨741⟩: between 152° and 158°.
Specific Rotation ⟨781⟩: between +22° and +26°, determined in a solution containing 200 mg in 10 mL, the specimen having been previously dried in vacuum at 60° for 3 hours.
Loss on Drying ⟨731⟩: Dry it in vacuum at 60° for 3 hours: it loses not more than 1% of its weight.
Residue on Ignition ⟨281⟩: not more than 0.2%.

Benzyl Ether *(Dibenzyl ether)*, $C_{14}H_{14}O$—**198.26** [103-50-4]—Use a suitable grade with a content of NLT 99%.

Add the following:

▲***N*-Benzylacetamide** *(N-Acetylbenzylamine)*, $C_9H_{11}NO$—**149.19** [588-46-5]—Use a suitable grade with a content of NLT 98%. [NOTE—A suitable grade is available as catalog number B0404 from www.tcichemicals.com.]▲ (USP 1-May-2019)

2-Benzylaminopyridine, $C_{12}H_{12}N_2$—**184.24** [6935-27-9]—Use a suitable grade.

1-Benzylimidazole, $C_{10}H_{10}N_2$—**158.20** [4238-71-5]—White crystals.
Assay: Transfer about 40 mg, accurately weighed, to a 100-mL beaker. Dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically using a combination calomel-platinum electrode. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.82 mg of $C_{10}H_{10}N_2$. Not less than 99% is found.

Benzyltrimethylammonium Chloride, $C_6H_5CH_2N(CH_3)_3Cl$—**185.69** [56-93-9]—Available as a 60% aqueous solution. Is clear and is colorless or not more than slightly yellow.
Assay: Pipet 2 mL into a 50-mL volumetric flask, and add water to volume. Pipet 20 mL of the solution into a 125-mL conical flask, add about 30 mL of water, then add 0.25 mL of dichlorofluorescein TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 18.57 mg of $C_6H_5CH_2N(CH_3)_3Cl$. Between 59.5% and 60.5% is found.

Beta-lactamase—Beta-lactamase is an enzyme produced by a variety of bacteria, but is usually obtained from culture filtrates of a strain of *Bacillus cereus*. It has the specific property of inactivating penicillins and cephalosporins by splitting the bond linking the nitrogen of the thiazolidine to the adjacent carbonyl carbon.
It occurs in the form of small, brown, easily pulverizable pieces or granules. Freely soluble in water, forming a slightly opalescent solution that is practically neutral to litmus paper. Is precipitated from its water solutions by acetone, by alcohol, and by dioxane, and is inactivated by contact with these solvents. Is rapidly inactivated by ethyl acetate and is irreversibly destroyed at a temperature of about 80°.
Beta-lactamase is assayed by a procedure depending upon a determination of the amount of penicillin G potassium or penicillin G sodium destroyed at a pH of 7.0 in a solution of such concentration that the inactivation proceeds as a zero-order reaction.

Betanaphthol—See *2-Naphthol*.

Bibenzyl *(Dibenzyl)*, $C_{14}H_{14}$—**182.26** [103-29-7]—Colorless crystals. Freely soluble in chloroform and in ether; sparingly soluble in alcohol; practically insoluble in water.
Melting Range ⟨741⟩: between 53° and 55°.

Bile Salts—A concentrate of beef bile, the principal constituent of which is sodium desoxycholate, determined as cholic acid. Soluble in water and in alcohol; the solutions foam strongly when shaken.
Insoluble Substances: Dissolve 5 g in 100 mL of dilute alcohol (84 in 100), warming if necessary to aid solution. Filter within 15 minutes through a tared filter, and wash with small portions of the dilute alcohol until the last washing is colorless or practically so, then dry the residue at 105° for 1 hour, and weigh: the weight of the residue does not exceed 0.1%.
Assay
Standard cholic acid solution: Dissolve 50.0 mg of cholic acid, accurately weighed, in dilute acetic acid (6 in 10) to make 100 mL, and mix. Store in a refrigerator.
Procedure: Dissolve 1.0 g, accurately weighed, in 50 mL of dilute acetic acid (6 in 10). Filter the solution, if necessary, into a 100-mL volumetric flask, wash the original container and the filter with small portions of dilute acetic acid (6 in 10), add the same acetic acid to volume, and mix. Dilute 10 mL of this solution, accurately measured, with dilute acetic acid (6 in 10) to make 100 mL, and mix.
Pipet 1 mL each of the *Standard cholic acid solution* and the solution of the Bile Salts into two matched test tubes. To each tube add 1 mL, accurately measured, of freshly prepared furfural solution (1 in 100), immediately place the tubes in an ice-bath for 5 minutes, then add to each tube 13 mL, accurately measured, of dilute sulfuric acid, made by cautiously mixing 50 mL of sulfuric acid with 65 mL of water. Mix the contents of the tubes, and place them in a water bath maintained at a temperature of 70° for 10 minutes. Immediately transfer the tubes to an ice-bath for 2 minutes, then determine the absorbance of each solution at the wavelength of maximum absorbance at about 670 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of cholic acid ($C_{24}H_{40}O_5$) in the weight of the Bile Salts taken by the formula:

$$500(A_U/A_S)$$

in which A_U and A_S are the absorbances of the solutions from the Bile Salts and the *Standard cholic acid solution*, respectively. Not less than 45% of cholic acid is found.

(*S*)-Binol *((S)-(–)-1,1′-Bi(2-naphthol); (–)-2,2′-Dihydroxy-1,1′-dinaphthyl)*, $C_{20}H_{14}O_2$—**286.32** [18531-99-2]—Use a suitable grade with a content of NLT 99%. [NOTE—A suitable grade is available as catalog number 246956 from www.sigmaaldrich.com.]

Biphenyl, $C_{12}H_{10}$—**154.21** [92-52-4]—Colorless to white crystals or crystalline powder. Insoluble in water; soluble in alcohol and in ether. Boils at about 254°.
Melting Range ⟨741⟩: between 68° and 72°.

2,2′-Bipyridine *(α,α′-Dipyridyl)*, $C_{10}H_8N_2$—**156.18** [366-18-7]—White or pink, crystalline powder. Soluble in water and in alcohol. Melts at about 69°, and boils at about 272°.
Sensitiveness: Prepare the following solutions: (*A*)—Dissolve 350 mg of ferrous ammonium sulfate in 50 mL of water containing 1 mL of sulfuric acid, and add 500 mg of hydrazine sulfate, then add water to make 500 mL. For use, dilute this solution with water in the ratio of 1 in 100 mL.

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(*B*)—Dissolve 8.3 g of sodium acetate and 12 mL of glacial acetic acid in water to make 100 mL. Add 1 mL of a solution of the specimen (1 in 1000) to a mixture of 10 mL of water and 1 mL of each of solutions *A* and *B:* a pink color results immediately.
Solubility: A 100-mg portion dissolves completely in 10 mL of water.
Residue on Ignition ⟨Reagent test⟩: not more than 0.2%.

4,4′-Bis(4-amino-1-naphthylazo)-2,2′-stilbenedisulfonic Acid, $C_{34}H_{26}N_6O_6S_2$**—678.74** [5463-64-9]—Use a suitable grade.
[NOTE—A suitable grade is available from TCI America, www.tciamerica.com.]

Bis(2-ethylhexyl) Maleate, $C_{20}H_{36}O_4$**—340.50** [142-16-5]—Colorless to pale yellow, clear liquid. Miscible with acetone and with alcohol. Specific gravity about 0.945.
Assay: Place about 2.5 g, accurately weighed, in a 250-mL flask, add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, and reflux for 45 minutes. Cool, add 0.5 mL of phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination at the same time, using the same amount of 0.5 N alcoholic potassium hydroxide (see *Residual Titrations* under *Titrimetry* ⟨541⟩). The difference, in mL, between the volumes of 0.5 N hydrochloric acid consumed in the test titration and blank titration, multiplied by 85.1, represents the quantity, in mg, of bis(2-ethylhexyl) maleate in the portion taken. Not less than 97% is found.

Bis(2-ethylhexyl) Phthalate, C_6H_4-1,2-$[COOCH_2(C_2H_5)CH(CH_2)_3CH_3]_2$**—390.56** [117-81-7]—Colorless to light yellow liquid.
Refractive Index ⟨831⟩: between 1.4855 and 1.4875, at 20°.

Bis(2-ethylhexyl) Sebacate *(Dioctyl Sebacate),* $C_8H_{17}OOC(CH_2)_8COOC_8H_{17}$**—426.67** [122-62-3]—Pale straw-colored liquid. Insoluble in water. Refractive index about 1.448. Suitable for use in gas chromatography.
Specific Gravity, 20°/20° ⟨841⟩: between 0.913 and 0.917.
Boiling Range: between 243° and 248° at 5 mm of mercury.
[NOTE—A suitable grade is "Dioctyl Sebacate," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Bis(2-ethylhexyl)phosphoric Acid *[Bis(2-ethylhexyl) Phosphate],* $[CH_3(CH_2)_3CH(C_2H_5)CH_2]_2HPO_4$**—322.42** [298-07-7]—Light yellow, viscous liquid. Insoluble in water; freely soluble in chloroform and in ethyl acetate. Refractive index: about 1.443. Specific gravity: about 0.997.
Assay: Dissolve about 250 mg, accurately weighed, in 50 mL of dimethylformamide, add 3 drops of a 1 in 100 solution of thymol blue TS in dimethylformamide, and titrate with 0.1 N sodium methoxide VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 32.24 mg of $(C_8H_{17})_2HPO_4$. Between 95% and 105% is found.
Solubility: One volume dissolves in 9 volumes of chloroform to yield a clear solution, and 1 volume dissolves in 9 volumes of ethyl acetate to yield a clear solution.
Color: A 1 in 100 solution in chloroform exhibits an absorptivity of not more than 0.03 at 420 nm.

Bis(4-sulfobutyl) Ether Disodium, $Na_2C_8H_{16}O_7S_2$**—334.32**—Use a suitable grade with a content of NLT 95%.
[NOTE—A suitable grade is available as catalog number RM-969-C50 from www.cydexpharma.com.]

Bismuth, Bi**—208.98** [7440-69-9]— Use a suitable grade with a content of NLT 99.999%.

Bismuth Nitrate Pentahydrate, $Bi(NO_3)_3 \cdot 5H_2O$**—485.07** [10035-06-0]—Use ACS reagent grade.

Bismuth Subnitrate *(Bismuth(III) Nitrate Basic),* $Bi_5O(OH)_9(NO_3)_4$**—1461.99** [1304-85-4]—Use *Bismuth Subnitrate* ⟨USP monograph⟩.

Bismuth Sulfite Agar—Use a suitable grade.

Bis(trimethylsilyl)acetamide *(N,O-Bis(trimethylsil yl) acetamide; BSA),* $CH_3CON[Si(CH_3)_3]_2$**—203.43** [10416-59-8]—Clear, colorless liquid. Readily hydrolyzes when exposed to moist air. Handle under nitrogen, and store in a cool place.
Assay: Not less than 90% of $CH_3CON[Si(CH_3)_3]_2$, a suitable gas chromatograph equipped with a thermal conductivity detector being used. The following conditions are suitable and provide a retention time of approximately 15 minutes.
Column: 3-mm × 1.83-m stainless steel containing 5% phase G1 on support S1A
Injection temperature: 160°
Column temperature: 90°, programmed to rise 4° per minute to 160°
Carrier gas: Helium
Refractive Index ⟨831⟩: between 1.4150 and 1.4170 at 20°.

Bis(trimethylsilyl)trifluoroacetamide *(N,O-Bis(trimethylsilyl) trifluoroacetamide; BSTFA),* $CF_3CON[Si(CH_3)_3]_2$**—257.40** [25561-30-2]—Clear, colorless liquid. Readily hydrolyzes when exposed to moist air. Store in a cool place.
• **ASSAY:** Not less than 98% of $CF_3CON[Si(CH_3)_3]_2$, a suitable gas chromatograph equipped with a thermal conductivity detector being used. The following conditions are suitable and provide a retention time of approximately 15 minutes.
Column: 3-mm × 1.83-m stainless steel containing 5% phase G1 on support S1A
Injection temperature: 170°
Column temperature: 70°, programmed to rise 4° per minute to 140°
Carrier gas: Helium
• **REFRACTIVE INDEX** ⟨831⟩: between 1.3820 and 1.3860 at 20°.

Bis(trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane[25561-30-2]—Use a suitable grade.
[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

Blood ⟨for carbon monoxide test in gases⟩—Use *oxalated* or *defibrinated blood* of dogs, sheep, cattle, or human beings within 24 hours after bleeding. Prepare *oxalated blood* by adding 10 mg of sodium oxalate to each mL of the freshly drawn blood.

Anti-A Blood Grouping Reagent, Anti-B Blood Grouping Reagent, and Anti-AB Blood Grouping Reagent: The reagents can be monoclonal or polyclonal and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration for use in microplate tests. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results. Generally, all three reagents are available as part of a kit.

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[NOTE—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Blue Tetrazolium *(3,3'-(3,3'-Dimethoxy[1,1'-biphenyl]-4,4'-diyl)bis[2,5-diphenyl-2H-tetrazolium]dichloride),* $C_{40}H_{32}Cl_2N_8O_2$ **—727.64** [1871-22-3]—Lemon-yellow crystals. Slightly soluble in water; freely soluble in chloroform and in methanol; insoluble in acetone and in ether.

Solubility in Methanol: Dissolve 1 g in 100 mL of methanol: complete solution results, and the solution is clear.

Color: Transfer a portion of the methanol solution obtained in the preceding test to a 1-cm cell, and determine its absorbance at 525 nm, against water as the blank: the absorbance does not exceed 0.20.

Ultraviolet-Visible Spectroscopy ⟨857⟩: Its molar absorptivity in methanol, at 252 nm, is not less than 50,000.

Suitability Test

Standard preparation: Dissolve in alcohol a suitable quantity of USP Hydrocortisone RS, previously dried at 105° for 3 hours and accurately weighed, and prepare by stepwise dilution a solution containing about 10 µg per mL.

Procedure: Pipet 10-, 15-, and 20-mL portions of *Standard Preparation* into separate, glass-stoppered, 50-mL conical flasks. Add 10 mL and 5 mL, respectively, of alcohol to the flasks containing the 10- and 15-mL portions of *Standard preparation,* and swirl to mix. To each of the flasks, and to a fourth flask containing 20 mL of alcohol, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of alcohol, mix, and then add 2.0 mL of a solution prepared by diluting 1 mL of tetramethylammonium hydroxide TS with alcohol to 10 mL. Mix, allow the flasks to stand in the dark for 90 minutes, and determine the absorbances of the three solutions of the steroid standard at 525 nm, with a suitable spectrophotometer, using the solution in the fourth flask as the blank. Plot the absorbances on the abscissa and the amount of hydrocortisone on the ordinate scale of arithmetic coordinate paper, and draw the curve of best fit: the absorbance of each solution is proportional to the concentration, and the absorbance of the solution containing 200 µg of hydrocortisone is not less than 0.50.

Boldine *(2,9-Dihydroxy-1,10-dimethoxyaporphine),* $C_{19}H_{21}NO_4$ **—327.37** [476-70-0]—Use a suitable grade.

Boric Acid, H_3BO_3**—61.83** [10043-35-3]—Use ACS reagent grade.

(–)-Bornyl Acetate *(1,7,7-Trimethylbicyclo[2,2,1]-heptan-2-ol acetate),* $C_{12}H_{20}O_2$**—196.29** [5655-61-8]—Use a suitable grade.

Boron Trifluoride, BF_3**—67.81** [7637-07-2]—Use a suitable grade.

14% Boron Trifluoride–Methanol [373-57-9]—Use a suitable grade.

Bovine Collagen: Use a suitable grade that contains less than 1 µg glycosaminoglycan per mg.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

7 Percent Bovine Serum Albumin Certified Standard—Available from the National Institute of Standards and Technology, www.nist.gov, as SRM 927.

Branched Polymeric Sucrose—about 400 kDa[26873-85-8] —White to off-white powder. Synthetic polymer made by the copolymerization of sucrose and epicholrohydrin. Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Ficoll" from Pharmacia Fine Chemicals, Inc., 800 Centennial Ave., Piscataway, NJ 08854.]

Brilliant Green *(Malachite Green G),* $C_{27}H_{34}N_2O_4S$**—482.64** [3051-11-4]—Glistening, golden-yellow crystals. Soluble in water and in alcohol. Absorption maximum: 623 nm.

Bromelain—[9001-00-7]

A glycoprotein that is highly active thiol proteinase. It is found in the leaves and stems of the pineapple plant. Yellowish-white to tan powder.

• **ACTIVITY DETERMINATION**

pH 4.5 water: Adjust with 0.1 N hydrochloric acid to a pH of 4.5.

Gelatin substrate: Dissolve 25 g of gelatin in 375 mL of hot water. Bring to a boil. Cool to 45°. Adjust the pH to 4.5 with 0.1 N hydrochloric acid. Dilute with *pH 4.5 water* to 500 mL. Keep it at 45°. This substrate should be prepared fresh daily.

Buffer solution: Add 150 g of sodium chloride to 700 mL of *pH 4.5 water,* stir to dissolve, then add 5.7 mL of acetic acid. Adjust with 50% sodium hydroxide to a pH of 4.5, if necessary. Dilute to 1 L.

3% Hydrogen peroxide solution: Transfer 2.5 mL of hydrogen peroxide to a 25-mL volumetric flask. Dilute with *pH 4.5 water* to volume.

pH 9.0 formaldehyde solution: Adjust a 100-mL formaldehyde solution to a pH of 9.0 with 0.1 N sodium hydroxide VS.

Bromelain preparation: Weigh 100 mg of bromelain with a theoretical activity of 2400 GDU/g. If the sample activity differs by more than 10% from 2400 GDU/g, determine the sample weight:

$$\text{mg of sample} = (2400 \times 100)/\text{theoretical activity}$$

Transfer the sample to a 50-mL volumetric flask. Add 8.3 mL of *Buffer solution.* Let stand for 30 min at room temperature. Dilute with *pH 4.5 water* to volume. Add a small stir bar and stir for 10–15 min.

Procedure: Transfer 25 mL of *Gelatin substrate* to each of two 100-mL beakers containing stir bars and place them in a water bath at 45° for 5 min. One beaker is for the *Test solution* and the other for the *Blank solution.*

Test solution: Add 1.0 mL of *Bromelain preparation* into the beaker, start timing, and swirl. After exactly 20 min of incubation at 45°, add 0.1 mL of *3% Hydrogen peroxide solution,* and swirl. Incubate for an additional 5 min. Remove the beaker from the water bath and, with constant stirring, insert the pH electrode. Record the pH after 10 s (initial pH). Adjust with 0.1 N sodium hydroxide VS to a pH of 6.0. [NOTE—When adjusting the pH to 6.0 be cautious at pH 5.8; the pH increases slowly but minute additions of sodium hydroxide at this point will significantly increase the pH.] Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring. Titrate to a pH of 9.0 with 0.1 N sodium hydroxide VS. Record the volume of titrant used. This is the test titer, *T.*

Blank solution: Run concurrently with the *Test solution* by starting the *Blank solution* determination 12 min after the *Test solution* is started. Add 0.1 mL of *3% Hydrogen peroxide solution* to the *Blank solution* beaker, and swirl. After exactly

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20 min of incubation at 45°, add 1.0 mL of *Bromelain preparation*, and swirl. Incubate for an additional 15 min. Remove the beaker from the water bath and, with constant stirring, insert the pH electrode. Record the pH after 10 s (initial pH). Adjust with 0.1 N sodium hydroxide to a pH of 6.0. See the *Note* under *Test solution*. Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring. Titrate to a pH of 9.0 with 0.1 N sodium hydroxide VS. Record the volume of titrant used. This is the blank titer, *B*.
Calculation: 1 Gelatin Digestion Unit (GDU) is the amount of enzyme that, after 20 min of digestion at 45°, will liberate 1 mg of amino nitrogen from a standard gelatin solution at a pH of 4.5.

$$GDU/g = (T - B) \times 14 \times N \times (50/W)$$

T = mL of 0.1 N sodium hydroxide used with the *Test solution*
B = mL of 0.1 N sodium hydroxide used with the *Blank solution*
N = actual normality of 0.1 N sodium hydroxide VS from standardization
W = weight of bromelain taken (g)

Bromine, Br—At. Wt. 79.904 [7726-95-6]—Use ACS reagent grade.

α-Bromo-2′-acetonaphthone *(Bromomethyl 2-naphthyl ketone)*, $C_{12}H_9BrO$—**249.10**—Tannish-pink crystals.
Melting Range ⟨741⟩: between 81° and 83°.

***p*-Bromoaniline**, C_6H_6BrN—**172.02** [106-40-1]—White to off-white crystals. Insoluble in water; soluble in alcohol and in ether.
- **ASSAY:** Transfer about 650 mg, accurately weighed, to a suitable container, and dissolve in 50 mL of glacial acetic acid TS. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 17.20 mg of C_6H_6BrN. Not less than 98% is found.
- **MELTING RANGE** ⟨741⟩: between 60° and 65°, within a 2° range.

Bromofluoromethane: Use a suitable grade.

***N*-Bromosuccinimide**, $C_4H_4BrNO_2$—**177.98** [128-08-5]—White to off-white crystals or powder. Freely soluble in water, in acetone, and in glacial acetic acid. [**CAUTION**—Highly irritating to eyes, skin, and mucous membranes.]
- **ASSAY:** Transfer 200 mg, accurately weighed, to a conical flask, add 25 mL of 0.5 N alcoholic potassium hydroxide, cover with a watch glass, heat to boiling, and boil for 5 minutes. Cool, transfer the solution to a beaker, rinsing the flask with water until the total volume of solution plus rinsings is about 100 mL, and add 10 mL of glacial acetic acid. Insert suitable electrodes, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 17.80 mg of $C_4H_4BrNO_2$. Not less than 98% is found.

Brucine Sulfate, $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$—**1013.11** [60583-39-3]—Use ACS reagent grade.

Butane-1,2-diol *(1,2-Butanediol)*, $C_4H_{10}O_2$—**90.12** [584-03-2]—Use a suitable grade with a content of NLT 97%.

Butane-1,4-diol *(1,4-Butanediol, 1,4-Butylene Glycol)*, $C_4H_{10}O_2$—**90.12** [110-63-4]—Use a suitable grade with a content of NLT 98%.

Butane-2,3-diol *(2,3-Butanediol, 2,3-Butylene Glycol)*, $C_4H_{10}O_2$—**90.12** [513-85-9]—Use a suitable grade with a content of NLT 97%.

1,3-Butanediol *(1,3-Butylene Glycol)*, $C_4H_{10}O_2$—**90.12** [107-88-0]—Viscous, colorless liquid. Very hygroscopic. Soluble in water, in alcohol, in acetone, and in methyl ethyl ketone; practically insoluble in aliphatic hydrocarbons, in benzene, and in toluene.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 1.8-m stainless steel column containing 20% phase G16 on support S1A; the injection port temperature is maintained at 265°; the column temperature is maintained at 150° and programmed to rise 8° per minute to 210°. The area of the butanediol peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: between 1.4390 and 1.4410 at 20°.

2,3-Butanedione *(Diacetyl)*, $CH_3COCOCH_3$—**86.09** [431-03-8]—Bright yellow to yellowish-green liquid. Soluble in water. Miscible with alcohol and with ether. Boils at about 88°.
Assay
Hydroxylamine hydrochloride solution: Dissolve 20 g of hydroxylamine hydrochloride in 40 mL of water, and dilute with alcohol to 400 mL. Add, with stirring, 300 mL of 0.5 N alcoholic potassium hydroxide VS, and filter. Discard after 2 days.
Procedure: Transfer about 1 g, accurately weighed, to a glass-stoppered, 250-mL flask, add 75.0 mL of *Hydroxylamine hydrochloride solution*, and insert the stopper in the flask. Reflux the mixture for 1 hour, then cool to room temperature. Add bromophenol blue TS, and titrate with 0.5 N hydrochloric acid VS to a greenish-yellow endpoint.
[NOTE—Alternatively, the solution may be titrated potentiometrically to a pH of 3.4.]
Perform a blank test with the same quantities of reagent used for the test specimen, and make any necessary correction. Each mL of 0.5 N hydrochloric acid is equivalent to 43.05 mg of $CH_3COCOCH_3$. Not less than 97% of $CH_3COCOCH_3$ is found.
Congealing Temperature ⟨651⟩: between −2.0° and −5.5°.
Refractive Index ⟨831⟩: between 1.3935 and 1.3965, at 20°.
Specific Gravity ⟨841⟩: about 0.98.

1-Butanesulfonic Acid Sodium Salt *(Sodium 1-butanesulfonate)*, $C_4H_9NaO_3S$—**160.16** [2386-54-1]—Use a suitable grade with a content of not less than 99.0%.

1,4-Butane Sultone *(4-Hydroxybutane-1-sulfonic Acid delta-sultone)*, $C_4H_8O_3S$—**136.17** [1633-83-6]—Use a suitable grade with a content of NLT 99%.

Butanol—See *Butyl Alcohol*.

Butyl Acetate, Normal, $CH_3COO(CH_2)_3CH_3$—**116.16** [123-86-4]—Use ACS reagent grade.

Butyl Alcohol *(1-Butanol; Normal Butyl Alcohol)*, $CH_3(CH_2)_2CH_2OH$—**74.12** [71-36-3]—Use ACS reagent grade.

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Butyl Alcohol, Normal—See *Butyl Alcohol.*

Butyl Alcohol, Secondary *(2-Butanol),* $CH_3CH_2CH(OH)CH_3$—**74.12** [78-92-2]—Use a suitable grade with a content of NLT 99%.

Butyl Alcohol, Tertiary, $(CH_3)_3COH$—**74.12** [75-65-0]—Use ACS reagent grade *tert*-Butyl Alcohol.

Butyl Benzoate, $C_{11}H_{14}O_2$—**178.23** [136-60-7]—Thick, oily, colorless to pale yellow liquid. Practically insoluble in water; soluble in alcohol and in ether.

Assay: When examined by gas-liquid chromatography, it shows a purity of not less than 98%. The following conditions have been found suitable for assaying it: use a 3-mm × 1.8-m stainless steel column packed with liquid phase G4 on support S1A. Helium is the carrier gas, the injection port temperature is maintained at 180°, the column temperature is maintained at 190°, and the flame-ionization detector is maintained at 280°. The retention time is about 15 minutes.

Refractive Index ⟨831⟩: between 1.4980 and 1.5000, at 20°.

***n*-Butyl Chloride** *(1-Chlorobutane),* C_4H_9Cl—**92.57** [109-69-3]—Clear, colorless, volatile liquid.

[**CAUTION**—Highly flammable.]

Practically insoluble in water. Miscible with alcohol and with ether. Use HPLC grade.

Butyl Ether *(n-Dibutyl Ether),* $C_8H_{18}O$—**130.23** [142-96-1]—Use a suitable grade.

Butyl Methacrylate, $C_8H_{14}O_2$—**142.20** [97-88-1]—Use a suitable grade.

***tert*-Butyl Methyl Ether,** $C_5H_{12}O$—**88.15** [1634-04-4]—Colorless liquid.

- **ASSAY:** Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 100°; the detector temperature is maintained at 300°; and the column temperature is maintained at ambient temperature and programmed to rise 10° per minute to 150°. The area of the $C_5H_{12}O$ peak is not less than 99.8% of the total peak area.
- **REFRACTIVE INDEX** ⟨831⟩: between 1.367 and 1.371 at 20°.

***n*-Butylamine,** $CH_3CH_2CH_2CH_2NH_2$—**73.14** [109-73-9]—Colorless to pale yellow, flammable liquid. Miscible with water, with alcohol, and with ether. Store it in tight containers. Specific gravity: about 0.740.

Distilling Range, *Method I* ⟨721⟩: Not less than 95% distills between 76° and 78°.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, determined by the *Titrimetric Method.*

Chloride (Reagent test): One g (1.5 mL) shows not more than 0.01 mg of Cl (0.001%).

Acidic Impurities: To 50 mL add 5 drops of a saturated solution of azo violet in benzene, and titrate quickly with 0.1 N sodium methoxide VS to a deep blue endpoint, observing precautions to prevent absorption of atmospheric carbon dioxide as by use of an atmosphere of nitrogen: not more than 1.0 mL of 0.1 N sodium methoxide is required for neutralization.

***tert*-Butylamine,** $C_3H_9CNH_2$—**73.14** [75-64-9]—Liquid.

- **ASSAY:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 230°; the detector temperature is maintained at 300°; the column temperature is maintained at 130° and programmed to rise 10° per minute to 280°. The area of the $C_3H_9CNH_2$ peak is not less than 99.5% of the total peak area.
- **REFRACTIVE INDEX** ⟨831⟩: between 1.3770 and 1.3790 at 20°.

4-(Butylamino)benzoic Acid $C_{11}H_5NO_2$—**193.25** [4740-24-3]—Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, Inc., P.O. Box 2060, Milwaukee, WI 53201; www.sigma-aldrich.com.]

Butylated Hydroxytoluene *(2,6-Di-tert-butyl-4-methylphenol; BHT; 2,6-Di-tert-butyl-p-cresol),* $C_{15}H_{24}O$—**220.35** [128-37-0]—Use a suitable grade.

***n*-Butylboronic Acid** *(1-Butaneboronic Acid),* $C_4H_9B(OH)_2$—**101.94** [4426-47-5]—Use a suitable grade.

[NOTE—This reagent is usually shipped and stored under water. Before use, remove any excess water by light vacuum filtration. A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

***tert*-Butyldimethylchlorosilane in *N*-Methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide, (1 in 100)**—Use a suitable grade.

[NOTE—A suitable grade is available as 99% MTBSTFA, 1% TBDMCS from Regis Chemical Company, 8210 Austin Ave., P.O. Box 519, Morton Grove, IL 60053.]

Butylparaben—Use *Butylparaben* (NF monograph).

4-*tert*-Butylphenol, $C_{10}H_{14}O$—**150.22** [98-54-4]—White, crystalline flakes or needles. Practically insoluble in water; soluble in alcohol and in ether.

- **MELTING RANGE** ⟨741⟩: between 98° and 101°.

***t*-Butylthiol** *(tert-Butylthiol; tert-Butyl Mercaptan; 2-Methyl-2-propanethiol; TBM),* $(CH_3)_3CSH$—**90.19** [75-66-1]—Use a suitable grade with a content of NLT 98.0%.

[NOTE—A suitable grade is available as catalog number 20230 at www.sigma-aldrich.com.]

Butyraldehyde *(Butanal),* C_4H_8O—**72.11** [123-72-8]—Use a suitable grade, purified by redistillation, with a content of not less than 99.5%.

Butyric Acid, $C_4H_8O_2$—**88.11** [107-92-6]—Clear, colorless to faint yellow liquid. Miscible with water and with methanol.

Assay: Weigh accurately about 500 mg, transfer to a suitable container, add 30 mL of water, and mix. Add 40 mL of water, and mix. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 8.81 mg of $C_4H_8O_2$: not less than 99.0% of $C_4H_8O_2$ is found.

Refractive Index ⟨831⟩: about 1.398 at 20°.

Butyrolactone *(Dihydro-2-(3H)-furanone, γ-butyrolactone)*—**86.1** [96-48-0]—Clear, colorless to practically colorless, oily liquid. Miscible with water. Soluble in methanol and in ether.

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Boiling Range ⟨721⟩: between 193° and 208°.
Refractive Index ⟨831⟩: about 1.435, at 20°.
Specific Gravity ⟨841⟩: between 1.128 and 1.135.

Butyrophenone *(Phenyl Propyl Ketone)*, $C_{10}H_{12}O$—**148.21** [495-40-9]—Use a suitable grade with a content of not less than 98.0%.

Cadmium Acetate, $C_4H_6CdO_4 \cdot 2H_2O$—**266.53** [543-90-8]—Colorless, transparent to translucent crystals. Freely soluble in water; soluble in alcohol.
- **Insoluble Matter** (Reagent test): not more than 1 mg, from 20 g (0.005%).
- **Chloride** (Reagent test): One g shows not more than 0.01 mg of Cl (0.001%).
- **Sulfate** (Reagent test, *Method II*): Dissolve 10 g in 100 mL of water, add 1 mL of hydrochloric acid, and filter: the residue weighs not more than 1.2 mg more than the residue obtained in a complete blank test (0.005%).
- **Substances Not Precipitated By Hydrogen Sulfide:** Dissolve 2 g in a mixture of 135 mL of water and 15 mL of 1 N sulfuric acid, heat to boiling, and pass a rapid stream of hydrogen sulfide through the solution as it cools. Filter, and to 75 mL of the clear filtrate add 0.25 mL of sulfuric acid, then evaporate to dryness, and ignite gently: the residue weighs not more than 1 mg (0.1%).

Cadmium Nitrate $Cd(NO_3)_2 \cdot 4H_2O$—**308.48** [10022-68-1]—Use ACS reagent grade Cadmium Nitrate Tetrahydrate.

Calcium Acetate, $Ca(C_2H_3O_2)_2 \cdot H_2O$—**176.18** [5743-26-0]—White, crystalline granules or powder. Soluble in about 3 parts of water; slightly soluble in alcohol. Use ACS reagent grade.

Calcium Carbonate, $CaCO_3$—**100.09** [471-34-1]—Use ACS reagent grade.

[Note—Calcium Carbonate of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Office of Standard Reference Materials, www.nist.gov, as standard sample No. 915.]

Calcium Carbonate, Chelometric Standard, $CaCO_3$—**100.09** [471-34-1]—Use ACS reagent grade.

Calcium Caseinate—White or slightly yellow, nearly odorless, powder. Insoluble in cold water, but forms a milky solution when suspended in water, stirred, and heated.

Residue on ignition (Reagent test): Ignite 5 g at 550°: the residue weighs between 150 and 300 mg (3.0% to 6.0%).
Calcium: Treat the residue from the preceding test with 10 mL of diluted hydrochloric acid, filter, and to the clear filtrate add 5 mL of ammonium oxalate TS: it shows a white precipitate upon standing.
Loss on Drying ⟨731⟩: Dry it in vacuum at 70° to constant weight: it loses not more than 7.0% of its weight.
Fat: Suspend 1.0 g in 5 mL of alcohol in a Mojonnier flask, add 0.8 mL of stronger ammonia water and 9 mL of water, and shake. Add a second 5-mL portion of alcohol, then add successive portions of 25 mL each of ether and solvent hexane, shaking after each addition by inverting the flask 30 times. Centrifuge, decant the solvent layer, evaporate it at a low temperature, and dry on a steam bath: the residue weighs not more than 20 mg (2.0%).
Nitrogen Determination ⟨461⟩, *Method I*: Between 12.5% and 14.3% of N is found, calculated on the anhydrous basis.
Suspensibility in Water: Place 2 g in a beaker, and add cool water slowly with stirring to form a thin, smooth paste. Add additional water to make a total of 100 mL. Stir, and heat to 80°: a milky suspension is formed that does not settle after standing for 2 hours.

Calcium Chloride, $CaCl_2 \cdot 2H_2O$—**147.01** [10035-04-8]—Use ACS reagent grade Calcium Chloride Dihydrate.

Calcium Chloride, Anhydrous *(for drying)*, $CaCl_2$—**110.98** [10043-52-4]—Use ACS reagent grade Calcium Chloride Desiccant.

Calcium Citrate, $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$—**570.49** [813-94-5]—A white, crystalline powder. Freely soluble in 3 N hydrochloric acid and in 2 N nitric acid; slightly soluble in water; insoluble in alcohol. To 15 mL of hot 2 N sulfuric acid add, in small portions and with stirring, about 500 mg of calcium citrate. Boil the mixture for 5 min, and filter while hot: the cooled filtrate responds to the identification test for *Identification Tests—General, Citrate* ⟨191⟩.
- **Assay:** Accurately weigh about 400 mg of the salt, previously dried at 150° to constant weight, and transfer to a 250-mL beaker. Dissolve the test specimen in 150 mL of water containing 2 mL of 3 N hydrochloric acid, add 15 mL of 1 N sodium hydroxide and 250 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS until the solution turns deep blue. Each mL of 0.05 M edetate disodium is equivalent to 8.307 mg of calcium citrate $[Ca_3(C_6H_5O_7)_2]$: between 97.5% and 101% is found.
- **Calcium Oxide and Carbonate:** Triturate 1 g of calcium citrate with 5 mL of water for 1 min: the mixture does not turn red litmus blue. Then add 5 mL of warm 3 N hydrochloric acid: only a few isolated bubbles escape.
- **Hydrochloric Acid-Insoluble Matter:** Dissolve 5 g by heating with a mixture of 10 mL of hydrochloric acid and 50 mL of water for 30 min: not more than 2.5 mg of insoluble residue remains (0.05%).
- **Loss on Drying** ⟨731⟩: Dry it at 150° to constant weight: it loses between 12.2% and 13.3% of its weight.
- **Arsenic** ⟨211⟩: Proceed with 0.50 g as directed for organic compounds (6 ppm of As).
- **Heavy Metals** (reagent test): 0.002%.

Calcium Hydroxide [1305-62-0]—Use ACS reagent grade.

Calcium Lactate, $(CH_3CHOHCOO)_2Ca \cdot 5H_2O$—**308.29** [814-80-2]—White granules or powder. Is somewhat efflorescent and at 120° becomes anhydrous. One g dissolves in 20 mL of water; practically insoluble in alcohol. Store it in tight containers.

Assay: Accurately weigh about 500 mg, previously dried at 120° for 4 hours, transfer to a suitable container, and dissolve in 150 mL of water containing 2 mL of diluted hydrochloric acid. Add 15 mL of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 10.91 mg of $C_6H_{10}CaO_6$. Not less than 98% is found.
Loss on Drying ⟨731⟩: Dry it at 120° for 4 hours: it loses between 25.0% and 30.0% of its weight.
Acidity: Add phenolphthalein TS to 20 mL of a 1 in 20 solution, and titrate with 0.10 N sodium hydroxide: not more than 0.50 mL is required to produce a pink color.
Heavy Metals (Reagent test): Dissolve 1 g in 2.5 mL of diluted hydrochloric acid, dilute with water to 40 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 0.02 mg of added Pb (0.002%).
Magnesium and Alkali Salts: Mix 1 g with 40 mL of water, carefully add 5 mL of hydrochloric acid, heat the solution, boil for 1 minute, and add rapidly 40 mL of oxalic acid TS.

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Add immediately to the warm mixture 2 drops of methyl red TS, then add ammonia TS dropwise, from a buret, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 hours or overnight. Filter, and transfer to a platinum dish 50 mL of the clear filtrate, to which has been added 0.5 mL of sulfuric acid. Evaporate the mixture on a steam bath to a small bulk. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally ignite the residue at 800 ± 25° for 15 minutes: the residue weighs not more than 5 mg (1%).
Volatile Fatty Acid: Stir about 500 mg with 1 mL of sulfuric acid, and warm: the mixture does not emit an odor of volatile fatty acid.

Calcium Nitrate, $Ca(NO_3)_2 \cdot 4H_2O$**—236.15** [13780-06-8]—Use ACS reagent grade.

Calcium Pantothenate, Dextro—Use *Calcium Pantothenate* (USP monograph).

Calcium Sulfate—See *Calcium Sulfate Dihydrate.*

Calcium Sulfate Dihydrate, $CaSO_4 \cdot 2H_2O$**—172.17** [10101-41-4]—Use ACS reagent grade.

Calconcarboxylic Acid *(2-Naphthalenecarboxylic acid, 3-hydroxy-4-[(2-hydroxy-4-sulfo-1-naphthalenyl)azo]; Calcon-3-carboxylic Acid; Cal-Red),* $C_{21}H_{14}N_2O_7S$**—438.42** [3737-95-9]—Use a suitable grade.

Calconcarboxylic Acid Triturate: Mix 1 part of calconcarboxylic acid with 99 parts of sodium chloride.
Test for Sensitivity: Dissolve 50 mg of calconcarboxylic acid triturate in a mixture of 2 mL 10 N sodium hydroxide and 100 mL of water. The solution is blue but becomes violet on addition of 1 mL of a 10 g per L solution of magnesium sulfate and 0.1 mL of a 1.5 g per L solution of calcium chloride and turns pure blue on addition of 0.15 mL of 0.01 M sodium edetate.

Calf Thymus DNA—Use a suitable grade. [NOTE—A suitable grade is commercially available from Worthington Biochemical Corp., www.worthington-biochem.com.]

***dl*-Camphene,** $C_{10}H_{16}$**—136.24** [79-92-5]—Use a suitable grade.
[NOTE—A suitable grade is available as camphene, 95%, catalog number 45,606-5, from Sigma-Aldrich, www.sigma-aldrich.com.]

***d*-10-Camphorsulfonic Acid** *[(1S)-(+)-10-Camphorsulfonic acid; (1S)-Camphor-10-sulfonic acid; (+)-Camphor-10-sulfonic acid (β)],* $C_{10}H_{16}O_4S$**—232.30** [3144-16-9]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number C2107 from www.sigma-aldrich.com.]

***dl*-10-Camphorsulfonic Acid,** *[Camphor-10-sulfonic acid (β)]; [(RS)-10-Camphorsulfonic acid],* $C_{10}H_{16}O_4S$**—232.30** [5872-08-2]— Is optically inactive.
Use a suitable grade.
[NOTE—A suitable grade is available as catalog number 147923 from www.sigma-aldrich.com.]

Canada Balsam [8007-47-4]—A natural product derived from the resin of *Abies balsamea.* Use a suitable grade.

Canola Oil [120962-03-0]—Use a suitable grade.

Capric Acid *(Decanoic Acid),* $C_{10}H_{20}O_2$**—172.26** [334-48-5]—White, solidified melt or fragments. Soluble in alcohol, in chloroform, and in ether; practically insoluble in water.
Assay: Inject an appropriate sample dissolved in acetone into a gas chromatograph (see *Chromatography* ⟨621⟩) that is equipped with a flame-ionization detector and contains a 0.53-mm × 30-m capillary column coated with a layer of phase G25. The carrier gas is helium, flowing at a rate of 9 mL per minute. The chromatograph is programmed as follows. Initially the column temperature is equilibrated at 150°, then the temperature is increased at a rate of 10° per minute to 250°. The injection port temperature is maintained at 240°, and the detector temperature at 265°. The area of the capric acid peak is not less than 98.5% of the total peak area.
Melting Range ⟨741⟩: between 30° and 33°.

Carbazole, $C_{12}H_9N$**—167.21** [86-74-8]—Off-white to tan powder.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; and the column temperature is maintained at 280°. The area of the $C_{12}H_9N$ peak is not less than 95.5% of the total peak area.

Carbon Dioxide Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it. Contains suitable absorbing filters and support media for the indicators hydrazine and crystal violet.
Measuring Range: 0.01 to 0.3 Vol.–%.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Carbon Disulfide, Chromatographic—Use a suitable grade.

Change to read:

Carbon Disulfide, CS▲2▲ (ERR 1-May-2018)[75-15-0]—Use ACS reagent grade.

Carbon Monoxide Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it. Contains suitable absorbing filters and support media for the indicators iodine pentoxide and selenium dioxide and fuming sulfuric acid.
Measuring Range: 5 to 150 ppm.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Carbon Tetrachloride, CCl_4**—153.82** [56-23-5]—Use a suitable grade with a content of NLT 99.9% by gas chromatography.

Carboxylate (Sodium Form) Cation-exchange Resin (50- to 100-mesh)—See *Cation-Exchange Resin, Carboxylate (Sodium Form) (50- to 100-mesh).*

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Carboxymethoxylamine Hemihydrochloride, $2(C_2H_5NO_3) \cdot HCl$—**218.59** [2921-14-4]—White, crystalline powder. Use a suitable grade.

Carmine *(Alum Lake of Carminic Acid),* $C_{22}H_{20}O_{13} \cdot x$Al[1390-65-4]—Red powder. Use a suitable grade.

(*R*)-(–)-Carvone *(2-Methyl-5-(1-methylethenyl)-2-cyclohexene-1-one),* $C_{10}H_{14}O$—**150.22** [6485-40-1]—Use a suitable grade.

Casein [9000-71-9]—White or slightly yellow, granular powder. Insoluble in water and in other neutral solvents; readily dissolved by ammonia TS and by solutions of alkali hydroxides, usually forming a cloudy solution.

Residue on Ignition ⟨Reagent test⟩: Ignite 2 g: the residue weighs not more than 20 mg (1.0%).

Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 10.0% of its weight.

Alkalinity: Shake 1 g with 20 mL of water for 10 minutes, and filter: the filtrate is not alkaline to red litmus paper.

Soluble Substances: When the filtrate from the *Alkalinity* test is evaporated and dried at 105°, the residue weighs not more than 1 mg (0.1%).

Fats: Dissolve 1 g in a mixture of 10 mL of water and 5 mL of alcoholic ammonia TS, and shake out with two 20-mL portions of solvent hexane. Evaporate the hexane at a low temperature, and dry at 80°: the weight of the residue does not exceed 5 mg (0.5%).

Nitrogen Determination ⟨461⟩, *Method I*: between 15.2% and 16.0% of N is found, on the anhydrous basis.

Where vitamin-free casein is required, use casein that has been rendered free from the fat-soluble vitamins by continuous extraction with hot alcohol for 48 hours followed by air-drying to remove the solvent.

Casein, Hammarsten [9000-71-9]—Use a suitable grade.

Catechol *(o-Dihydroxybenzene; Pyrocathecol),* $C_6H_4(OH)_2$—**110.11** [120-80-9]—White crystals, which become discolored on exposure to air and light. Readily soluble in water, in alcohol, in benzene, in ether, in chloroform, and in pyridine, forming clear solutions.

Use a suitable grade with a content of not less than 99%.

Cation-Exchange Resin—Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Dowex 50-W-X8-100," from Sigma-Aldrich, www.sigma-aldrich.com.]

Cation-Exchange Resin, Carboxylate (Sodium Form) (50- to 100-mesh)—Use a suitable grade.

[NOTE—A suitable grade is available as "Bio-Rex 70" from BioRad Laboratories, www.bio-rad.com.]

Cation-Exchange Resin, Polystyrene—Use a suitable grade.

[NOTE—A suitable grade is available as Dowex-50X2-100, from Sigma-Aldrich, www.sigma-aldrich.com.]

Cation-Exchange Resin, Styrene-Divinylbenzene—A strongly acidic, cross-linked sulfonated resin containing about 2% of divinylbenzene. It is available in the hydrogen form in the 50- to 100-, 100- to 200-, and 200- to 400-mesh sizes. It can be regenerated to the hydrogen form by treating with a hydrochloric acid solution (5 in 100). For satisfactory regeneration, a contact time of at least 30 minutes is required after which it must be washed free of excess acid. It is insoluble in water, in methanol, and in acetonitrile. Suitable for use in column chromatography.

Moisture Content of Fully Regenerated and Expanded Resin: Transfer 10 to 12 mL of the resin (as received) to a flask, and convert it completely to the hydrogen form by stirring with 150 mL of hydrochloric acid solution (5 in 100) for not less than 30 minutes. Decant the acid, and wash the resin in the same manner with water until the wash water is neutral to litmus (pH 3.5).

Transfer 5 to 7 mL of the regenerated resin to a glass filtering crucible, and remove only the excess surface water by very careful suction filtration. Transfer the conditioned resin to a tared weighing bottle, and weigh. Dry in a vacuum oven at a pressure of 50 mm of mercury at 100° to 105° for 16 hours. Transfer from the vacuum oven to a desiccator, cool to room temperature, and weigh again. The loss in weight is between 75% and 83%.

Total Wet Volume Capacity: Transfer 3 to 5 mL of the regenerated, undried (See *Moisture Content* above) resin to a 5-mL graduated cylinder, and fill it with water. Remove any air bubbles from the resin bed with a stainless steel wire, and settle the resin to its minimum volume by tapping the graduated cylinder. Record the volume of the resin.

Transfer the resin to a 400-mL beaker. Add about 5 g of sodium chloride, and titrate, stirring well, with 0.1 N sodium hydroxide to the blue endpoint of bromothymol blue (pH 7.0).

$$(\text{net mL NaOH} \times \text{N})/(\text{mL of resin}) = \text{mEq/mL}$$

The total wet volume capacity of the resin is more than 0.6 mEq per mL.

Wet Screen Analysis: The purpose of this test is to properly identify the mesh size of the resin. To obtain an accurate screen analysis would require a special apparatus and technique.

Add 150 mL of resin to 200 mL of water in an appropriate bottle, and allow it to stand at least 4 hours to completely swell the resin.

Transfer, by means of a graduated cylinder, 100 mL of settled and completely swollen resin to the top screen of a series of the designated U.S. Standard 20.3-cm brass screens. Thoroughly wash the resin on each screen with a stream of water until the resin is completely classified, collecting the wash water in a suitable container. Wash the beads remaining on the respective screens back into the 100-mL graduate, and record the volume of settled resin on each screen. At least 70% of the resin will be within the specific mesh size.

[NOTE—A suitable resin is Dowex 50WX2, produced by Dow Chemical Co. (www.dow.com) and also available through Sigma-Aldrich (www.sigma-aldrich.com).]

Cation-Exchange Resin, Styrene-Divinylbenzene, Strongly Acidic—Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Dowex 50-W- X8-100," from Sigma-Aldrich, www.sigma-aldrich.com.]

Cation-Exchange Resin, Sulfonic Acid—Use a suitable grade.

[NOTE—A suitable grade is available commercially as "Amberlyst 15" or as "Dowex 50-W-X2" from Sigma- Aldrich, www.sigma-aldrich.com.]

Cedar Oil (for clearing microscopic sections) [8000-27-9]—A selected, distilled oil from the wood of the red cedar, *Juniperus virginiana* Linné (Fam. Pinaceae), should be used for this purpose. Refractive index: about 1.504 at 20°. For use with homogeneous immersion lenses, a specially prepared oil having a refractive index of 1.5150 ± 0.0002 at 20° is required.

Cellulose, Chromatographic—Use a suitable grade.

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[NOTE—A suitable grade is available from EMD Chemicals, www.emdchemicals.com.]

Add the following:

▲**Cellulose Diethylaminoethyl** *(Cellulose DEAE)* —It is deposited on plastic or aluminum sheets for thin-layer chromatography (TLC). It can be cut in the appropriate size. [NOTE—A suitable material is available as catalog number 4477 from www.avantorinc.com.]▲ 2S (USP41)

Cellulose, Microcrystalline—Use Cellulose, Microcrystalline, FCC.

Cellulose Mixture, Chromatographic—Use a suitable grade. [NOTE—A suitable grade is available commercially, in precoated plate form, with fluorescent indicator, from EMD Chemicals, www.emdchemicals.com.]

Ceric Ammonium Nitrate, $Ce(NO_3)_4 \cdot 2NH_4NO_3$—**548.22**—Use ACS reagent grade.

Ceric Ammonium Sulfate, $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$—**632.55**—Yellow to yellowish–orange crystals. Dissolves slowly in water, but more rapidly when mineral acids are present. Use ACS reagent grade.

Ceric Sulfate, $Ce(SO_4)_2$ with a variable amount of water—**(anhydrous) 332.24** [13590-82-4]—It may also contain sulfates of other associated rare earth elements. Yellow to orange-yellow crystals or crystalline powder. Practically insoluble in cold water; slowly soluble in cold dilute mineral acids, but more readily soluble when heated with these solvents.

- **ASSAY:** Weigh accurately about 800 mg, transfer to a flask, add 25 mL of water and 3 mL of sulfuric acid, and warm until dissolved. Cool, and add 60 mL of a mixture of 1 volume of phosphoric acid and 20 volumes of water. Add 25 mL of potassium iodide solution (1 in 10), insert the stopper in the flask, and allow to stand for 15 minutes. Replace the air over the solution with carbon dioxide, and while continuing the flow of carbon dioxide into the flask, titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 33.22 mg of $Ce(SO_4)_2$. Not less than 80.0% is found.
- **CHLORIDE** (Reagent test): Dissolve 1 g in a mixture of 5 mL of nitric acid and 4 mL of water. Filter, if necessary, and dilute with water to 20 mL. To 10 mL of the dilution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter until clear. To the remaining 10 mL of test solution add 1 mL of silver nitrate TS: any turbidity produced does not exceed that in a control prepared by adding 0.05 mg of Cl to the filtrate obtained from the first 10 mL of test solution (0.01%).
- **HEAVY METALS:** Heat 500 mg with a mixture of 10 mL of water and 0.5 mL of sulfuric acid until solution is complete. Cool, dilute with water to 50 mL, and bubble hydrogen sulfide gas through the solution until it is saturated: the precipitate that is formed is white or not darker than pale yellow.
- **IRON:** Dissolve 100 mg in a mixture of 5 mL of water and 2 mL of hydrochloric acid, warming if necessary, and cool. Transfer to a glass-stoppered cylinder, dilute with water to 25 mL, and add 5 mL of ammonium thiocyanate TS and 25 mL of ether. Shake gently, but well, and allow the layers to separate: any pink color in the ether layer is not darker than that of a control, similarly prepared, containing 0.02 mg of added Fe (0.02%).

Cesium Chloride, CsCl—**168.36** [7647-17-8]—A white powder. Very soluble in water; freely soluble in methanol; practically insoluble in acetone. Use a suitable grade.

Cetrimide—See *Cetyltrimethylammonium Bromide.*

Cetyltrimethylammonium Bromide *(CTAB; Cetrimide; Hexadecyltrimethylammonium Bromide),* $C_{19}H_{42}BrN$—**364.46** [57-09-0]—Use a suitable grade. For any chromatographic application, use a suitable grade with a content of not less than 99.0%.

Cetyltrimethylammonium Chloride, 25 Percent in Water, $C_{19}H_{42}ClN$—**320.00**—Use a suitable grade.

Charcoal, Activated *(Activated Carbon; Decolorizing Carbon)* C—**12.01** [7440-44-0]—A fine, black powder, which is the residue from the destructive distillation of various organic materials, treated to increase its high capacity for adsorbing organic coloring substances, as well as nitrogenous bases. Use *Activated Charcoal* (USP monograph).

Add the following:

▲**Chelating Ion-Exchange Resin**—Weak cation-exchange resin composed of styrene divinylbenzene copolymer containing paired iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions. [NOTE—A suitable grade is available as Chelex® 100 Resin from www.bio-rad.com.▲ (USP 1-Dec-2019)]

Chenodeoxycholic Acid, $C_{24}H_{40}O_4$—**392.57** [474-25-9]—White to off-white powder. Use a suitable grade with a content of NLT 95%.

Chloramine T *(Sodium p-Toluenesulfonchloramide),* $C_7H_7ClNNaO_2S \cdot 3H_2O$—**281.69** [7080-50-4]—Use ACS reagent grade.

Chlorine, Cl_2—**70.9** [7782-50-5]—Greenish-yellow gas. High-purity grade available from most suppliers of specialty gases.

Chlorine Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator *o*-tolidine.

Measuring Range: 0.2 to 3 ppm.

[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

***m*-Chloroacetanilide,** C_8H_8ClNO—**169.61**—Off-white to beige granules.

- **ASSAY**

Mobile phase: Prepare a mixture of acetonitrile and water (22:3).

Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The area of the C_8H_8ClNO peak is not less than 99.9% of the total peak area.

- **MELTING RANGE** ⟨741⟩: between 79° and 80°.

***p*-Chloroacetanilide,** C_8H_8ClNO—**169.61**—White or pale yellow, needle-shaped crystals or crystalline powder. Insoluble in water; soluble in alcohol and in ether.

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Solubility: One g dissolves in 30 mL of alcohol to form a clear solution.
- **MELTING RANGE** ⟨741⟩: between 178° and 181°.
- **RESIDUE ON IGNITION** (Reagent test): not more than 0.1%.

1-Chloroadamantane, $C_{10}H_{15}Cl$**—170.68** [935-56-8]—White crystalline solid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the $C_{10}H_{15}Cl$ peak is not less than 97.5% of the total peak area.

2-Chloro-4-aminobenzoic Acid—See *4-Amino-2-chlorobenzoic Acid*.

5-Chloro-2-aminobenzophenone—See *2-Amino-5-chlorobenzophenone*.

3-Chloroaniline, C_6H_6ClN**—127.57** [108-42-9]—Colorless to light brown liquid. Soluble in acid and in most organic solvents; practically insoluble in water.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the C_6H_6ClN peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.592 and 1.596 at 20°.

***p*-Chloroaniline,** *(4-Chloroaniline),* C_6H_6ClN**—127.57** [106-47-8]—Use a suitable grade.

Chlorobenzene, C_6H_5Cl**—112.56** [108-90-7]—Clear, colorless liquid. Insoluble in water; soluble in alcohol, in benzene, in chloroform, and in ether. Use ACS reagent grade.

4-Chlorobenzoic Acid, ClC_6H_4COOH**—156.57** [74-11-3]—White, crystalline solid.
Assay: Dissolve about 700 mg, accurately weighed, in a mixture of 100 mL of hot alcohol and 50 mL of water. Titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 78.28 mg of ClC_6H_4COOH. Not less than 98% is found.
Solubility: One g dissolved in 25 mL of 0.5 N sodium hydroxide yields a clear and complete solution.

***m*-Chlorobenzoic Acid** *(3-Chlorobenzoic Acid),* $C_7H_5ClO_2$**—156.57** [535-80-8]—Use a suitable grade.

2-Chlorobenzophenone $C_{13}H_9ClO$**—216.66** [5162-03-8]—Use a suitable grade with a content of NLT 99%.

4-Chlorobenzophenone, $C_{13}H_9ClO$**—216.66** [134-85-0]—Use a suitable grade.

1-Chlorobutane—See *n-Butyl Chloride*.

2-Chloroethanol *(Ethylene Chlorohydrin),* C_2H_5ClO**—80.51** [107-07-3]—Use a suitable grade with a content of NLT 99%.

2-Chloroethylamine Monohydrochloride *(2-Chloroethylamine Hydrochloride; 2-Aminoethyl Chloride Hydrochloride),* $C_2H_6ClN \cdot HCl$**—115.99**—Off-white powder.
Assay: The area of the 2-chloroethylamine monohydrochloride ($C_2H_6ClN \cdot HCl$) peak is NLT 99% of the total peak area.
Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩). The following conditions have been found suitable.
Detector: Flame ionization
Column: 0.25-mm × 30-m capillary; phase G46
Temperatures
Injection port: 150°
Column: 50°, programmed to rise 10°/min to 200°
Detector: 300°
Carrier gas: Helium

Chloroform, $CHCl_3$**—119.38** [67-66-3]—Use ACS reagent grade.

Chloroform, Alcohol-Free—Use a suitable grade that does not contain alcohol as a stabilizer.

Chloroform, Methyl—See *Methyl Chloroform*.

Chlorogenic Acid, $C_{16}H_{18}O_9$**—354.31** [327-97-9]—White to off-white powder. Use a suitable grade.
Assay: When tested by thin-layer chromatography (see *Chromatography* ⟨621⟩) with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of butyl alcohol, water, and acetic acid (60:25:15), and examined under short-wavelength UV light, a single spot is exhibited, with trace impurities.

Chloromethylated Polystyrene-Divinylbenzene Anion-exchange Resin—See *Anion-exchange Resin, Chloromethylated Polystyrene-Divinylbenzene*.

1-Chloronaphthalene *(α-Chloronaphthalene),* $C_{10}H_7Cl$**—162.62** [90-13-1]—Colorless to light yellow liquid.
Assay: Use a gas chromatograph equipped with a flame-ionization detector. The following conditions have been found suitable: a 3.2-mm × 1.83-m stainless steel column is packed with 7% phase G2 on support S1A; the injection port temperature is maintained at 250° and the detector temperature at 310°; and the column temperature is programmed to increase at a rate of 10° per minute from 50° to 250°. Not less than 90% of $C_{10}H_7Cl$ is found, of which not more than 10% is 2-chloronaphthalene.
Refractive Index ⟨831⟩: between 1.6320 and 1.6340, at 20°.

4-Chloro-1-naphthol, $C_{10}H_7ClO$**—178.6** [604-44-4]—A white to off-white powder, with a melting point between 118° and 120°. Use a suitable grade. Store below 0°.

2-Chloronicotinic Acid, $C_6H_4ClNO_2$**—157.55** [2942-59-8]—Off-white powder.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is

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maintained at 280°; the detector temperature is maintained at 300°; and the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_6H_4ClNO_2$ peak is not less than 98% of the total peak area.

2-Chloro-4-nitroaniline, 99%, $C_6H_5ClN_2O_2$**—172.57** [121-87-9]—White to off-white powder.
Melting Range ⟨741⟩: between 107° and 109°.

Chloroplatinic Acid, $H_2PtCl_6 \cdot 6H_2O$**—517.90** [18497-13-7]—Use ACS reagent grade Chloroplatinic Acid Hexahydrate.

3-Chloropropane-1,2-diol *[(±)-3-Chloro-1,2-propanediol],* $C_3H_7ClO_2$**—110.54** [96-24-2]—Use a suitable grade with a content of NLT 97.5%.
[NOTE—A suitable grade is available as catalog number 107271 from www.sigma-aldrich.com.]

5-Chlorosalicylic Acid, $C_7H_5ClO_3$**—172.57** [321-14-2]—White to off-white powder.
Assay: When tested by thin-layer chromatography (see *Chromatography* ⟨621⟩) with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of cyclohexane, chloroform, and acetic acid (14:4:2), and examined visually and under long-wavelength UV light or iodine spray, a single spot is exhibited.
Melting Range ⟨741⟩: between 172° and 178°.

1-Chloro-2,2,2-trifluoroethylchlorodifluoromethyl Ether—Use a suitable grade.

Chlorotrimethylsilane *(Trimethylsylyl Chloride),* C_3H_9ClSi**—108.64** [75-77-4]—Clear, colorless to light yellow liquid. Fumes when exposed to moist air.
[**CAUTION**—It reacts vigorously with water, alcohols, and other hydrogen donors. Store in tight glass containers.]
Refractive Index ⟨831⟩: between 1.3850 and 1.3890 at 20°.

Chlortetracycline Hydrochloride—Use *Chlortetracycline Hydrochloride* (USP monograph).

Cholestane, $C_{27}H_{48}$**—372.67** [481-21-0]—Use a suitable grade.

Cholestanol *(β-Cholestanol, Dihydrocholesterol, Zymostanol, Cholestan-3β-ol, 3β-Hydroxy-5α-Cholestance, Dihydrocholesterin),* $C_{27}H_{48}O$**—388.67** [80-97-7]—Use a suitable grade with a content of NLT 95%. Store at −20°.
[NOTE—A suitable grade is available from www.sigmaaldrich.com or www.avantilipids.com.]

Cholesterol, $C_{27}H_{46}O$**—386.66** [57-88-5]—Use a suitable grade (*NF* monograph).

Cholesteryl Benzoate, $C_{34}H_{50}O_2$**—490.76** [604-32-0]—Use a suitable grade.

Cholesteryl *n*-Heptylate—Use a suitable grade.

Choline Chloride, $HOCH_2CH_2N(CH_3)_3Cl$**—139.62** [67-48-1]—White crystals or crystalline powder. Very soluble in water. Is hygroscopic. Store in tight containers.
Assay: Transfer about 100 mg, previously dried at 105° for 2 hours and accurately weighed, to a beaker, add 20 mL of water and 1 drop of aluminum chloride solution (1 in 10), and mix. Add slowly 20 mL of a freshly prepared, filtered sodium tetraphenylborate solution (1 in 50), and allow the mixture to stand for 30 minutes with occasional swirling. Pass through a medium-porosity, sintered-glass filter, and wash the beaker and the precipitate with four 10-mL portions of water. The weight of the precipitate, determined after drying at 105° for 2 hours, and multiplied by 0.3298, gives the equivalent weight of $C_5H_{14}ClNO$. Not less than 99.5% is found.
Residue on Ignition ⟨281⟩: not more than 0.1%.

Chromatographic Fuller's Earth—See *Fuller's Earth, Chromatographic.*

Chromatographic *n*-Heptane—See *n-Heptane, Chromatographic.*

Chromatographic Magnesium Oxide—See *Magnesium Oxide, Chromatographic.*

Chromatographic Reagents: See *Reagents, Chromatographic Reagents.*
[NOTE—Listings of the numerical designations for phases (G), packings (L), and supports (S), together with corresponding brand names, are published periodically in *Pharmacopeial Forum* as a guide for the chromatographer.]

Chromatographic Silica Gel—See *Silica Gel, Chromatographic.*

Chromatographic Silica Gel Mixture—See *Silica Gel Mixture, Chromatographic.*

Chromatographic Siliceous Earth—See *Siliceous Earth, Chromatographic.*

Chromatographic Siliceous Earth, Silanized—See *Siliceous Earth, Chromatographic, Silanized.*

Chromatographic Solvent Hexane—See *Hexane, Solvent, Chromatographic.*

Chromium Potassium Sulfate Dodecahydrate, $CrK(SO_4)_2 \cdot 12H_2O$**—499.40** [10279-63-7]—Use ACS reagent grade.

Chromium Trioxide, CrO_3**—99.99** [1333-82-0]—Use ACS reagent grade.

Chromogenic Substrate for Amidolytic Test—Synthetic molecules consisting of tripeptides or tetrapeptides coupled to a chromophore. The terminal amino acid is specific for the protease utilized. The synthetic peptides mimic the peptide sequence (specific to the activated coagulation factor) of the active site on the natural substrate. The coagulation factor catalyzes the splitting of the chromophore (*p*-nitroaniline) from the peptide. The amount of release can be measured directly in a spectrophotometer, because the maximum absorbance spectra of the bound and free chromophores differ. The released chromophore is a colored compound; the complete substrate itself is colorless.
Molecular weights of the various substrates range from about 600 to 750 Da. Solubility in aqueous solutions can vary. Not all substrates are of equal sensitivity, and incubation periods may have to be extended.

Chromotrope 2R, $C_{16}H_{10}N_2Na_2O_8S_2$**—468.4** [4197-07-3]—Red powder or crystals. Use a suitable grade.

Chromotropic Acid *(4,5-Dihydroxy-2,7-naphthalenedisulfonic Acid),* $C_{10}H_8O_8S_2 \cdot 2H_2O$**—356.33** [148-25-4], for the anhydrous form—Use a suitable grade.

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Chromotropic Acid Disodium Salt *(4,5-Dihydroxy-2,7-naphthalenedisulfonic Acid, Disodium Salt)*, $C_{10}H_6O_8Na_2S_2 \cdot 2H_2O$—**400.29** [5808-22-0]—Use ACS reagent grade.

Cinchonidine, $C_{19}H_{22}N_2O$—**294.39** [485-71-2]—White crystals, crystalline or granular powder. Soluble in alcohol and in chloroform; practically insoluble in water.

Assay: Dissolve about 125 mg, accurately weighed, in 50 mL of glacial acetic acid. Add a few drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.72 mg of $C_{19}H_{22}N_2O$. Not less than 99.0% is found.

- **Loss on Drying** ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.
- **Melting Range** ⟨741⟩: between 200° and 205°.
- **Specific Rotation** ⟨781⟩: between −105° and −115°, calculated on the dried basis, determined in a solution in alcohol containing 10 mg per mL.

Cinchonine, $C_{19}H_{22}N_2O$—**294.39** [118-10-5]—White crystals, crystalline or granular powder. Slightly soluble in chloroform, sparingly soluble in alcohol, and practically insoluble in water.

Assay: Dissolve about 125 mg, accurately weighed, in 50 mL of glacial acetic acid. Add a few drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.72 mg of $C_{19}H_{22}N_2O$. Not less than 99.0% is found.

Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.

Melting Range ⟨741⟩: between 255° and 261°.

Specific Rotation ⟨781⟩: between +219° and +229°, calculated on the dried basis, determined in a solution in alcohol containing 50 mg per 10 mL.

Citric Acid, $C_6H_8O_7 \cdot H_2O$—**210.14** [5949-29-1]—Use ACS reagent grade.

Citric Acid, Anhydrous, $C_6H_8O_7$—**192.13** [77-92-9]—Use ACS reagent grade.

Add the following:

▲**Clozapine *N*-Oxide** *(8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine N-oxide)*, $C_{18}H_{19}ClN_4O$—**342.82** [34233-69-7]—Use a suitable grade with a content of NLT 98%. ▲ (USP 1-May-2019)

Cobalt Chloride *(Cobaltous Chloride)*, $CoCl_2 \cdot 6H_2O$—**237.93** [7791-13-1]—Use ACS reagent grade.

Cobalt Nitrate, $Co(NO_3)_2 \cdot 6H_2O$—**291.03** [10026-22-9]—Use ACS reagent grade.

Cobaltous Acetate *(Cobalt Acetate)*, $Co(C_2H_3O_2)_2 \cdot 4H_2O$—**249.08** [6147-53-1]—Red, needlelike crystals. Soluble in water and in alcohol. Use ACS reagent grade.

Cobaltous Chloride—See *Cobalt Chloride.*

Coenzyme Q9 *(Ubiquinone 45)*, $C_{54}H_{82}O_4$—**795.2** [303-97-9]—Yellow to yellow-orange powder. Clear yellow solution, at a concentration of 1 mg per mL in a mixture of chloroform and ethanol (9:1).

Absorptivity: Its absorptivity at 275 nm, in alcohol solution, is about 1700.

Collagen: Use a suitable grade.

[NOTE—A suitable grade is acid-soluble Collagen Type I from calf skin, and is commercially available from Sigma-Aldrich Corp., www.sigma-aldrich.com; catalog number C3511.]

Rat Tail Collagen—Use a suitable grade.

[NOTE—A suitable grade is available from BD Biosciences, www.bdbiosciences.com.]

Collagenase: Use a suitable grade.

[NOTE—A suitable grade is commercially available as Collagenase Type 2, CLS-2, from Worthington Biochemical Corp., www.worthington-biochem.com.]

Compactin, $C_{23}H_{34}O_5$—**390.52** [73573-88-3]—Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

Congo Red, $C_{32}H_{22}N_6Na_2O_6S_2$—**696.67** [573-58-0]—A dark red or reddish-brown powder. Decomposes on exposure to acid fumes. Its solutions have a pH of about 8 to 9.5. One g dissolves in about 30 mL of water. Is slightly soluble in alcohol.

- **Loss on Drying** ⟨731⟩: Dry it at 105° for 4 hours: it loses not more than 3.0% of its weight.
- **Residue on Ignition:** Accurately weigh about 1 g, previously dried at 105° for 4 hours, and place it in a porcelain dish or crucible. Carefully ignite until well charred, cool, add 2 mL of sulfuric acid, and carefully ignite until the residue is white or practically so. Cool, add 0.5 mL of sulfuric acid and 1 mL of nitric acid, evaporate, and again ignite to constant weight: the weight of the sodium sulfate so obtained is between 20.0% and 24.0% of the weight of the dried specimen taken.
- **Sensitiveness:** To 50 mL of carbon dioxide-free water add 0.1 mL of congo red solution (1 in 1000). The red color of the solution is changed to violet by the addition of 0.05 mL of 0.10 N hydrochloric acid and is restored by the subsequent addition of 0.05 mL of 0.10 N sodium hydroxide.

Coomassie Blue G-250 ***(Coomassie Brilliant Blue G-250, Serva Blue G)***, $C_{47}H_{48}N_3O_7S_2Na$—**854.0** [6104-58-1]—A dark blue powder. Soluble in water. Use a suitable grade. Store between 15° and 30°.

Coomassie Brilliant Blue R-250, $C_{45}H_{44}N_3O_7S_2Na$—**825.97** [6104-58-1]—Brown powder.

Copper, Cu—**At. Wt. 63.546** [7440-50-8]—Use ACS reagent grade.

Copper Sulfate Pentahydrate *(Cupric Sulfate Pentahydrate)*, $CuSO_4 \cdot 5H_2O$—**249.68** [7758-99-8]—Use ACS Cupric Sulfate Pentahydrate reagent grade.

Cortisone, $C_{21}H_{28}O_5$—**360.44** [53-06-5]—White, crystalline powder. Practically insoluble in water; sparingly soluble in alcohol and in acetone. Melts at about 220°, with decomposition.

Absorption Maximum: The UV absorption spectrum of a 1 in 100,000 solution in alcohol shows a maximum at about 238 nm.

Specific Rotation ⟨781⟩: about +209°, determined in a 1 in 100 solution in alcohol.

Cotton, Absorbent—Use *Purified Cotton* (USP monograph).

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***m*-Cresol Purple,** $C_{21}H_{18}O_5S$—**382.43** [2303-01-7]—Use a suitable grade.

Cupric Acetate, $Cu(C_2H_3O_2)_2 \cdot H_2O$—**199.65** [6046-93-1]—Use ACS reagent grade.

Cupric Chloride, $CuCl_2 \cdot 2H_2O$—**170.48** [7447-39-4]—Bluish-green deliquescent crystals. Freely soluble in water; soluble in alcohol; slightly soluble in ether. Use ACS reagent grade.

Cupric Citrate *([Citrato(4-)]dicopper),* $Cu_2C_6H_4O_7$—**315.18** [866-82-0]—Use a suitable grade.

Cupric Nitrate Hydrate, $Cu(NO_3)_2 \cdot 2.5H_2O$—**232.59** [19004-19-4]

$Cu(NO_3)_2 \cdot 3H_2O$—**241.60** [10031-43-3]—Use ACS reagent grade.

[NOTE—This reagent is available containing either 2.5 or 3 molecules of water of hydration.]

Cupric Sulfate, $CuSO_4 \cdot 5H_2O$—**249.69**—Use ACS reagent grade.

Cupric Sulfate, Anhydrous, $CuSO_4$—**159.61** [7758-98-7]—A white or grayish-white powder free from a blue tinge. Upon the addition of a small quantity of water, it becomes blue. Soluble in water. Store in tight containers.

Chloride (Reagent test): One g shows not more than 0.02 mg of Cl (0.002%).

Substances Not Precipitated By Hydrogen Sulfide: Determine as directed for ACS reagent grade of Cupric Acetate: the residue weighs not more than 6 mg (0.15%).

Cupriethylenediamine Hydroxide Solution, 1.0 M—Use a suitable grade.

[NOTE—A suitable grade is available from GFS Chemicals, www.gfschemicals.com.]

Cyanoacetic Acid, $C_3H_3NO_2$—**85.06** [372-09-8]—White to light yellow, crystalline solid. Very soluble in water.

Assay: Dissolve about 300 mg, accurately weighed, in 25 mL of water and 25 mL of alcohol. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary corrections. Each mL of 0.1 N sodium hydroxide is equivalent to 85.06 mg of $C_3H_3NO_2$. Not less than 99% is found.

Cyanogen Bromide, BrCN—**105.92** [506-68-3]—Colorless crystals. Volatilizes at room temperature. Its vapors are highly irritating and *very toxic.* Melts at about 52°. Freely soluble in water and in alcohol. Store in tight containers in a cold place.

Solubility: Separate 1-g portions dissolve completely in 10 mL of water and in 10 mL of alcohol, respectively, to yield colorless solutions.

4-Cyanophenol *(4-Hydroxybenzonitrile),* C_6H_4CNOH—**119.12** [767-00-0]—Use 95 percent reagent.

4-Cyanopyridine *(4-Pyridinecarbonitrile; Isonicotinic Acid Nitrile; Isonicotinonitrile),* $C_6H_4N_2$—**104.11** [100-48-1]—Use a suitable grade with a content of NLT 98%.

Cyclam *(1,4,8,11-Tetraazacyclotetradecane),* $C_{10}H_{24}N_4$—**200.33** [295-37-4]—Use 98 percent reagent.

1,1-Cyclobutanedicarboxylic Acid *(Cyclobutane-1,1-dicarboxylic acid; 1,1-Dicarboxycyclobutane),* $C_6H_8O_4$—**144.13** [5445-51-2]—Use a suitable grade with a content of NLT 99%.

[NOTE—A suitable grade is available as catalog number C95803 from www.sigmaaldrich.com.]

α-Cyclodextrin *(Cyclomaltohexaose; Schardinger α-Dextrin),* $C_{36}H_{60}O_{30}$—**972.86** [10016-20-3]—Use a suitable grade with a content of not less than 98.0%.

[NOTE—A suitable grade is available from Fluka, www.sigma-aldrich.com, catalog number 28705.]

β-Cyclodextrin *(β-Schardinger dextrin; Cycloheptaamylose),* $C_{42}H_{70}O_{35} \cdot xH_2O$—**1134.98 (anhydrous)** [68168-23-0]—Use a suitable hydrate grade.

Cyclohexane, C_6H_{12}—**84.16** [110-82-7]—Use ACS reagent grade.

Cyclohexanol, $C_6H_{12}O$—**100.16** [108-93-0]—A clear liquid. Freely soluble in water. Miscible with alcohol, with ethyl acetate, and with aromatic hydrocarbons.

Assay: When examined by gas-liquid chromatography, using suitable gas chromatographic apparatus and conditions, it shows a purity of not less than 98%.

Melting Temperature: about 23°.

Specific Gravity: about 0.962, at 20°.

(1,2-Cyclohexylenedinitrilo)tetraacetic Acid *(trans-1,2-Diaminocyclohexane-N,N,N′,N′-tetraacetic Acid),* $C_{14}H_{22}N_2O_8 \cdot H_2O$—**364.35**—Use ACS reagent grade.

Cyclohexylmethanol, $C_7H_{14}O$—**114.19**—Use a suitable grade.

3-Cyclohexylpropionic Acid *(3-Cyclohexanepropionic Acid),* $C_9H_{16}O_2$—**156.22** [701-97-3]—Use a suitable grade with a content of NLT 99%.

L-Cystine, $HOOC(NH_2)CHCH_2S—SCH_2CH(NH_2)COOH$—**240.30** [56-89-3]

• **SPECIFIC ROTATION** ⟨781⟩

Sample solution: 20 mg/mL of L-cystine, previously dried over silica gel for 4 h

Temperature: 20°

Acceptance criteria: −215° to −225°

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over silica gel for 4 h.

Acceptance criteria: NMT 0.2%

• **RESIDUE ON IGNITION** (Reagent test): NMT 0.1%

DEAE-Agarose [57407-08-6]—Agarose beads chemically bonded with diethylaminoethane and suspended in a 20% ethanol solution in water.

[NOTE—Commercially available as DEAE-Sepharose.]

Decanol *(n-Decyl Alcohol),* $C_{10}H_{22}O$—**158.28** [112-30-1]—A clear, viscous liquid. Specific gravity: about 0.83 at 20°. Solidifies at about 6.5°. Insoluble in water; soluble in alcohol and in ether.

Assay: When examined by gas-liquid chromatography, using suitable gas chromatographic apparatus and conditions, it shows a purity of not less than 99%.

Decyl Sodium Sulfate, $C_{10}H_{21}NaO_4S$—**260.33**—White, crystalline solid.

Assay: Transfer about 1 g, accurately weighed, to a suitable, tared crucible, moisten with a few drops of sulfuric acid, and ignite gently to constant weight. Each mg of residue is

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equivalent to 3.662 mg of $C_{10}H_{21}NaO_4S$. Not less than 95% is found.

Dehydrated Alcohol—See *Alcohol, Dehydrated.*

Deoxyadenosine Triphosphate, $C_{10}H_{16}N_5O_{12}P_3$**—491.18** [1927-31-7]—Use a suitable grade.
[NOTE—A suitable grade is available from either BD Bio sciences, www.bdbiosciences.com or Applied Biosystems, www.appliedbiosystems.com.]

Deoxycytidine Triphosphate, $C_9H_{16}N_3O_{13}P_3$**—467.16** [2056-98-6]—Use a suitable grade.
[NOTE—A suitable grade is available from either BD Biosciences, www.bdbiosciences.com or Applied Biosystems, www.appliedbiosystems.com.]

Deoxyguanosine Triphosphate, $C_{10}H_{16}N_5O_{13}P_3$**—507.18** [2564-35-4]—Use a suitable grade.
[NOTE—A suitable grade is available from either BD Biosciences, www.bdbiosciences.com, or Applied Biosystems, www.appliedbiosystems.com.]

Deoxyribonucleic Acid Polymerase: Thermostable, recombinant DNA polymerase. Use a suitable grade.
[NOTE—A suitable grade is available from Applied Biosystems, www.appliedbiosystems.com.]

Deoxythymidine Triphosphate, $C_{10}H_{17}N_2O_{14}P_3$**—482.17**—Use a suitable grade.
[NOTE—A suitable grade is available from either BD Biosciences, www.bdbiosciences.com or Applied Biosystems, www.appliedbiosystems.com.]

Deschloroclotrimazole *(1-(Triphenylmethyl)imidazole; 1-Tritylimidazole), —Use a suitable grade with a content of NLT 97%.*
[NOTE—*A suitable grade is available as catalog number 524891 from* www.sigmaaldrich.com.]
$C_{22}H_{18}N_2$ **—310.39** [15469-97-3]

Desmosterol *(3β-Hydroxy-5,24-cholestadiene; 24-Dehydrocholesterol; 5,24-Cholestadien-3β-ol),* $C_{27}H_{44}O$**—384.64** [313-04-2]—Use a suitable grade with a content of NLT 94%.
[NOTE—A suitable grade is available as catalog number 700060 from www.avantilipids.com or as catalog number 190190 from www.mpbio.com.]

Desoxycorticosterone Acetate *(11-Desoxycorticosterone Acetate),* $C_{23}H_{32}O_4$**—372.5** [56-47-3]—Use a suitable grade.

Add the following:

▲**Deuterated Ethanol,** C_2DH_5O**—47.07** [925-93-9]—Use a suitable grade with NLT 99.5% of deuteration.▲ (USP 1-May-2019)

Deuterated Methanol *(Methanol-* ^{12} *C-d* $_4$ *, Methyl-* ^{12} *C-d* $_3$ *alcohol-d* $_1$ *)* **—36.1** [811-98-3]—The degree of deuteration is not less than 99.8%. Is a clear colorless liquid miscible with water, with alcohol, and with methylene chloride; density at 20°: 0.888 g/mL; refractive index at 20° (D-line): 1.326; boiling point 65.4° (760 mm Hg).

Deuterated Water—See *Deuterium Oxide.*

Deuterium Chloride ***(Deutero Hydrochloric Acid),*** DCl**—37.47** [7698-05-7]—Toxic gas. Use a suitable grade with a degree of deuteration of NLT 99%.

Add the following:

▲**Deuterium Chloride Solution** *(Hydrochloric Acid-D Solution),* DCl**—37.47** [7698-05-7]—Use a suitable grade with a concentration of NLT 35 w/% in heavy water (D_2O).▲ (USP 1-May-2019)

Change to read:

Deuterium Oxide ▲*(Heavy Water; Deuterated Water),*▲ (USP 1-May-2019) D_2O**—20.032** [7789-20-0]—Use a suitable grade having a minimum isotopic purity of 99.8 atom % of deuterium.

Deuterochloroform, $CDCl_3$**—120.38**—Use a suitable grade.

Devarda's Alloy *(Devarda's Metal)* [8049-11-4]—A gray powder composed of 50 parts of copper, 45 parts of aluminum, and 5 parts of zinc.

Dextran, High Molecular Weight [9004-54-0]—A dextran molecular weight standard having a weight-average molecular weight, M_W, of 1 to 2 × 10^6 Da and a weight-average molecular weight to number-average molecular weight ratio, M_W/M_N, of 1.0 to 1.8.
[NOTE—A suitable grade is available from American Polymer Standards Corporation, www.ampolymer.com.]

Dextrin, $(C_6H_{10}O_5)_n \cdot xH_2O$ [9004-53-9]—A white amorphous powder. Slowly soluble in cold water; more readily soluble in hot water; insoluble in alcohol.
- **INSOLUBLE MATTER:** Boil 1 g with 30 mL of water in a small flask: the solution is colorless and clear, or not more than opalescent.
- **LOSS ON DRYING** ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 10.0% of its weight.
- **RESIDUE ON IGNITION** (Reagent test): Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 5 mg (0.5%).
- **CHLORIDE** (Reagent test): Dissolve 3 g in 75 mL of boiling water, cool, dilute with water to 75 mL, and filter if necessary. To 25 mL of the filtrate add 2 mL of nitric acid and 1 mL of silver nitrate TS, and allow to stand for 5 minutes: any turbidity produced is not greater than that of a control containing 0.02 mg of added Cl (0.002%).
- **SULFATE** (Reagent test, *Method I*): To a 25-mL portion of the filtrate from the preceding test add 0.5 mL of diluted hydrochloric acid and 2 mL of barium chloride TS, and allow to stand for 10 minutes: any turbidity produced is not greater than that of a control containing 0.2 mg of added SO_4 (0.02%).
- **ALCOHOL-SOLUBLE SUBSTANCES:** Boil 1 g with 20 mL of alcohol for 5 minutes under a reflux condenser, and filter while hot. Evaporate 10 mL of the filtrate on a steam bath, and dry at 105°: the residue weighs not more than 5 mg (1%).
- **REDUCING SUGARS:** Shake 2 g with 100 mL of water for 10 minutes, and filter until clear. To 50 mL of the filtrate add 50 mL of alkaline cupric tartrate TS, and boil for 3 minutes. Filter through a tared filtering crucible, wash with water, then with alcohol, and finally with ether, and dry at 105° for 2 hours: the precipitate of cuprous oxide weighs not more than 115 mg (corresponding to about 5% of reducing sugars as dextrose).

Dextro Calcium Pantothenate—Use *Calcium Pantothenate* (USP monograph).

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Dextrose, Anhydrous, $C_6H_{12}O_6$—**180.16**—Use ACS reagent grade D-Glucose, Anhydrous.

Diacetyl—See 2,3-Butanedione.

3,3′-Diaminobenzidine Hydrochloride, $(NH_2)_2C_6H_3C_6H_3(NH_2)_2 \cdot 4HCl$—**360.11** [7411-49-6]—White to yellowish-tan (occasionally purple), needle-shaped crystals. Soluble in water. Stable in organic solvents but unstable in aqueous solution at room temperature. Store aqueous solutions in a refrigerator.

Insoluble Matter: Dissolve 2 g in 100 mL of water, without heating, and filter immediately: the insoluble residue does not exceed 1 mg (0.05%).

Residue on Ignition ⟨Reagent test⟩: not more than 1 mg, from 2 g (0.05%).

Suitability Test for Detection of Selenium: Dissolve 1.633 g of selenious acid (H_2SeO_3) in water, and dilute with water to 1 L. Dilute 10 mL of this solution with water to 1 L, to make a solution containing 0.010 mg of Se per mL. Place 1 mL of the resulting solution in a 100-mL beaker, add 2 mL of formic acid solution (1 in 7), and dilute with water to 50 mL. Add 2 mL of 3,3′-diaminobenzidine hydrochloride solution (1 in 200), and allow to stand for 30 to 50 minutes. Adjust with 6 N ammonium hydroxide to a pH between 6 and 7. Transfer to a 125-mL separator, add 10.0 mL of toluene, and shake vigorously for 30 seconds: a distinct yellow color is produced in the toluene layer. A blank containing diaminobenzidine hydrochloride but no selenium standard, treated in the same manner, shows no color in the toluene layer.

2,6-Diaminopyridine, $C_5H_7N_3$—**109.13** [141-86-6]—Use a suitable grade with a content of NLT 98%.

[NOTE—A suitable grade is available as catalog number D24404 from www.sigma-aldrich.com.]

2,3-Diaminonaphthalene, $C_{10}H_{10}N_2$—**158.20** [771-97-1]—Use a suitable grade.

Diatomaceous Earth [91053-39-3]—Use a suitable grade. [NOTE—A suitable grade is available as Celite 545-AW.]

Diatomaceous Earth, Flux-Calcined[91053-39-3]—Use a suitable grade.

[NOTE—A suitable grade is "Chromosorb W, AW-DMCS".]

Diatomaceous Earth, Silanized [91053-39-3]—Use a suitable grade.

[NOTE—Suitable grades are available commercially as "Anachrome Q," "Gas-Chrom Q," and "Varaport 30."]

Diatomaceous Silica, Calcined [68855-54-9]—A form of silica (SiO_2) consisting of fused frustules and fragments of diatoms. It is an amorphous, fine, light pink or white powder. Insoluble in water, in acids, and in dilute solutions of alkali hydroxides.

Loss on Ignition: Accurately weigh about 4 g, and ignite to constant weight: it loses not more than 10.0% of its weight.

Organic Impurities: It does not darken appreciably upon ignition.

Loss on Drying ⟨731⟩: Dry it at 110° for 2 hours: it loses not more than 2.0% of its weight.

[NOTE—Suitable grades are "Chromosorb P" and "Chromosorb W," available from Grace, www.grace.com.]

Diaveridine *(5-([3,4-Dimethoxyphenyl]methyl)-2,4-pyrimidinediamine),* $C_{13}H_{16}N_4O_2$—**260.3** [5355-16-8]—Use a suitable grade.

[NOTE—A suitable grade is available as catalog number 46174 from www.sigma-aldrich.com.]

Dibasic Ammonium Citrate—See *Ammonium Citrate, Dibasic.*

Dibasic Ammonium Phosphate—See *Ammonium Phosphate, Dibasic.*

Dibasic Potassium Phosphate—See *Potassium Phosphate, Dibasic.*

Dibenzyl—See *Bibenzyl.*

2,6-Dibromoquinone-chlorimide *(2,6-Dibromo-N-chloro-p-benzoquinone Imine; DBQ Reagent),* $O{:}C_6H_2Br_2{:}NCl$—**299.35** [537-45-1]—A yellow, crystalline powder. Insoluble in water; soluble in alcohol and in dilute alkali hydroxide solutions.

Melting Range ⟨741⟩: between 82° and 84°.

Solubility in Alcohol: A solution of 100 mg in 10 mL of alcohol is not more than faintly turbid.

Residue on Ignition ⟨Reagent test⟩: Ignite 500 mg with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.2%).

Sensitiveness: To 10 mL of a water solution containing 0.01 mg of phenol add 0.3 mL of a sodium borate buffer (made by dissolving 2.84 g of crystallized sodium borate in 90 mL of warm water, adding 8.2 mL of 1 N sodium hydroxide, and diluting with water to 100 mL) and 0.1 mL of a solution of 10 mg of the test specimen in 20 mL of alcohol: a distinct blue color develops within 10 minutes.

Dibutyl Phthalate, $C_{16}H_{22}O_4$—**278.34** [84-74-2]—Clear, colorless liquid.

Assay: Accurately weigh about 2 g into a suitable flask, add 25.0 mL of 1 N sodium hydroxide and 30 mL of isopropyl alcohol, and mix. Digest the mixture at a temperature near boiling for 30 minutes, then cool in a water bath to room temperature. Add phenolphthalein TS, and titrate with 1 N sulfuric acid VS to the disappearance of the pink color. Perform a complete blank determination, and make any necessary correction. Each mL of 1 N sulfuric acid consumed is equivalent to 139.2 mg of $C_{16}H_{22}O_4$. Not less than 98% is found.

Refractive Index ⟨831⟩: between 1.491 and 1.493 at 20°.

Acid Content: Accurately weigh about 10 g, and dissolve in 100 mL of an alcohol-ether mixture (1:1). Add phenolphthalein TS, and titrate immediately with 0.05 N alcoholic potassium hydroxide VS. Each mL of 0.05 N alcoholic potassium hydroxide is equivalent to 4.15 mg of phthalic acid: not more than 0.02% is found.

Dibutylamine, $C_8H_{19}N$—**129.24** [111-92-2]—Colorless liquid.

Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 200°; the detector temperature is maintained at 300°; and the column temperature is maintained at 100° and programmed to rise 10° per minute to 200°. The area of the $C_8H_{19}N$ peak is not less than 99% of the total peak area.

Refractive Index ⟨831⟩: between 1.415 and 1.419 at 20°.

Dibutylammonium Phosphate: Use a suitable grade.

[NOTE—A suitable grade is available as PIC Reagent D4 from Waters Corporation, www.waters.com.]

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1,3-Dicaffeoylquinic Acid ***(Cynarin; (1R,3R,4S,5R)-1,3-Bis[[3-(3,4-dihydroxyphenyl)propenoyl]oxy]-4,5-dihydroxycyclohexanecarboxylic Acid),*** $C_{25}H_{24}O_{12}$—**516.45** [30964-13-7]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number 3991 from www.chromadex.com.]

Dichloroacetic Acid, $C_2H_2Cl_2O_2$—**128.9** [79-43-6]—Colorless liquid. Miscible with water, with alcohol, and with ether. Use a suitable grade.

2,5-Dichloroaniline, $Cl_2C_6H_3NH_2$—**162.02** [95-82-9]—White, needle-like crystals. Slightly soluble in water; soluble in alcohol and in ether.
Melting Range, *Class I* ⟨741⟩: between 49° and 50°.

2,6-Dichloroaniline, $C_6H_5Cl_2N$—**162.02** [608-31-1]—Off-white powder.
Melting Range ⟨741⟩: between 38° and 41°.

***o*-Dichlorobenzene,** $C_6H_4Cl_2$—**147.00** [95-50-1]—Clear liquid, having a light yellowish-brown tint (about APHA 20). Practically insoluble in water. Miscible with alcohol and with ether. Boils at about 180°.
Assay: When examined by gas–liquid chromatography, with the use of suitable apparatus and conditions, it shows a purity of not less than 98%.
Density: between 1.299 and 1.301.
Refractive Index ⟨831⟩: between 1.548 and 1.550 at 25°.
Residue on Evaporation: Evaporate 80 mL on a steam bath, and dry at 105° for 1 hour: the residue weighs not more than 50 mg (0.005%).
Acidity: Add phenolphthalein TS to 25 mL of methanol, and titrate with 0.02 N alcoholic potassium hydroxide VS until a faint pink color persists for 15 seconds. Pipet 25 mL of test specimen into the solution; mix, avoiding exposure to the atmosphere; and titrate with 0.02 N alcoholic potassium hydroxide VS: not more than 2.2 mL is required to restore the pink color (about 0.005%).

1,2-Dichloroethane—See *Ethylene Dichloride.*

Dichlorofluorescein, $C_{20}H_{10}Cl_2O_5$—**401.20** [76-54-0]—
[NOTE—This specification covers both the 4,5- and 2,7-isomers of dichlorofluorescein, either of which is suitable for the preparation of dichlorofluorescein TS.]
A weak orange-colored, crystalline powder. Sparingly soluble in water; soluble in alcohol and in solutions of alkali hydroxides.
Residue on Ignition (Reagent test): Ignite 200 mg with 5 drops of sulfuric acid: the residue weighs not more than 1 mg (0.5%).
Sensitiveness: Dissolve 100 mg in 60 mL of alcohol, add 2.5 mL of 0.1 N sodium hydroxide, and dilute with water to 100 mL. Add 1 mL of this solution to a solution of potassium iodide prepared by dissolving 100 mg of potassium iodide, previously dried at 105° to constant weight and accurately weighed, in 50 mL of water containing 1 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS until the color of the precipitate changes from pale yellowish-orange to pink. The volume of 0.1 N silver nitrate consumed is not more than 0.10 mL greater than the calculated volume, the calculated volume being based upon the KI content of the dried specimen as determined in the *Assay* under *Potassium Iodide* (USP monograph).

Dichlorofluoromethane, $CHCl_2F$—**102.92** [75-43-4]—Colorless gas.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal-conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.53-mm × 30-m capillary column coated with a 5-µm layer of phase G2; the injection port temperature is maintained at 200°; the detector temperature is maintained at 200°; and the column temperature is maintained at 0° and programmed to rise 5° per minute to 40°, and then to rise 10° per minute to 180°. The area of the $CHCl_2F$ peak is not less than 98% of the total peak area.

2,6-Dichloroindophenol Sodium—See *2,6-Dichlorophenol-indophenol Sodium.*

Dichloromethane—Use *Methylene Chloride.*

1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethane ***(p,p'-DDD),*** $C_{14}H_{10}C_{l4}$—**320.03** [72-54-8]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number B0132 at www.tciamerica.com.]

2,4-Dichloro-1-naphthol, $C_{10}H_6OCl_2$—**213.06** [2050-76-2]—Light tan powder.
Melting Range ⟨741⟩: between 103° and 107°, but the range between beginning and end of melting does not exceed 2°.

2,6-Dichlorophenol-indophenol Sodium ***(2,6-Dichloro-indophenol Sodium),*** $O{:}C_6H_2Cl_2{:}NC_6H_4ONa$ with about $2H_2O$—**290.08 (anhydrous)** [620-45-1]—Use ACS reagent grade.

2,6-Dichlorophenylacetic Acid, $C_8H_6Cl_2O_2$—**205.04** [6575-24-2]—White powder.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the $C_8H_6Cl_2O_2$ peak is not less than 97% of the total peak area.

2,6-Dichloroquinone-chlorimide ***(2,6-Dichloro-N-chloro-p-benzoquinone Imine),*** $O{:}C_6H_2Cl_2{:}NCl$—**210.44**—Pale yellow, crystalline powder. Insoluble in water; soluble in alcohol and in dilute alkali hydroxide solutions.
Solubility in Alcohol: A solution of 100 mg in 10 mL of alcohol is complete and clear.
Residue on Ignition: Ignite 500 mg with 0.5 mL of sulfuric acid; the residue weighs not more than 1 mg (0.2%).
Sensitiveness: To 10 mL of a water solution containing 0.01 mg of phenol, add 0.3 mL of a sodium borate buffer (made by dissolving 2.84 g of sodium borate in 90 mL of warm water, adding 8.2 mL of 1 N sodium hydroxide, and diluting with water to 100 mL) and 0.1 mL of a solution of 10 mg of the test specimen in 20 mL of alcohol; a distinct blue color develops within 10 min.
[NOTE—A suitable grade is available as catalog number D2746 from www.spectrumchemical.com.]

Dicyclohexyl, *(Bicyclohexyl)* $C_{12}H_{22}$—**166.31** [92-51-3]—Use a suitable grade.

Dicyclohexyl Phthalate, $C_{20}H_{26}O_4$—**330.42** [84-61-7]
Melting Range ⟨741⟩: between 62° and 66°.

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Dicyclohexylamine, $(C_6H_{11})_2NH$—**181.32** [101-83-7]—Clear, strongly alkaline liquid. Sparingly soluble in water. Miscible with common organic solvents. Use a suitable grade with a content of not less than 98%.

Diethylamine, $(C_2H_5)_2NH$—**73.14** [109-89-7]—Colorless, flammable, strongly alkaline liquid. Miscible with water and with alcohol. Forms a hydrate with water. *May be irritating to skin and mucous membranes.* Store in well-closed containers. Use ACS reagent grade.

Diethylamine Phosphate *(Phosphoric Acid: Diethylamine),* $C_4H_{11}N \cdot H_3PO_4$—**171.13** [68109-72-8]—Use a suitable grade.

[NOTE—A suitable grade is available from www.richmanchemical.com.]

***N,N*-Diethylaniline,** $C_6H_5N(C_2H_5)_2$—**149.23** [91-66-7]—Light yellow to amber liquid.

Assay: Inject an appropriate specimen (about 0.2 µL) into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas flowing at about 40 mL per minute. The following conditions have been found suitable: a 3-mm × 1.8-m stainless steel column containing 20% phase G16 on support S1A; the injection port temperature is maintained at 250°; the column temperature is maintained at 140° and programmed to rise 6° per minute to 200°. The detector temperature is maintained at 310°. The area of the *N,N*-diethylaniline peak having a retention time of about 4.9 minutes is not less than 99% of the total peak area.

Refractive Index ⟨831⟩: between 1.5405 and 1.5425 at 20°.

Diethylene Glycol, *(Bis(2-hydroxyethyl) Ether; Diglycol; 2-Hydroxyethyl Ether; 2,2′-Oxydiethanol),* $C_4H_{10}O_3$—**106.12** [111-46-6]—A colorless to faintly yellow, viscous, hygroscopic liquid. Miscible with water, with alcohol, with ether, and with acetone. Insoluble in benzene and in carbon tetrachloride. Use a suitable grade with a content of not less than 99.0%.

Di(ethylene glycol) Methyl Ether *(2-(2-Methoxyeth oxy) ethanol; Methyldiglycol),* $C_5H_{12}O_3$—**120.15** [111-77-3]—Use a suitable grade.

Diethylene Glycol Succinate Polyester, $(OCH_2CH_2OCH_2CH_2OOCCH_2CH_2COO)_n$[26183-02-8]—Clear, viscous liquid. Soluble in chloroform. Is stabilized by modification of the diethylene glycol succinate polyester, to render it suitable for use in gas–liquid chromatography to a temperature of 200°.

[NOTE—A suitable grade is available from Alltech, www.alltechweb.com.]

Diethylenetriamine, $C_4H_{13}N_3$—**103.17** [111-40-0]—Colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G2. The injection port temperature is maintained at 200°; the column temperature is maintained at 100° and programmed to rise 10° per minute to 250° and held there for 5 minutes; and the detector temperature is maintained at 300°. The area of the main peak is not less than 95% of the total peak area.

Refractive Index ⟨831⟩: between 1.4815 and 1.4845 at 20°.

Di(2-ethylhexyl)phthalate *[Bis (2-ethylhexyl) phthalate],* $C_{24}H_{38}O_4$—**390.56** [117-81-7]—Use a suitable grade.

Diethylpyrocarbonate, $C_6H_{10}O_5$—**162.14** [1609-47-8]—Clear, colorless liquid. Use a suitable grade.

Add the following:

▲Diethyl Phthalate, $C_{12}H_{14}O_4$—**222.24** [*84-66-2*]—Use a suitable grade with a content of NLT 99.5%.▲ 2S (USP41)

Diethyl Sulfone ***(Ethyl Sulfone),*** $(C_2H_5)_2SO_2$—**122.19** [597-35-3]—Use a suitable grade with NLT 97%.

Digitonin, $C_{56}H_{92}O_{29}$—**1229.31** [11024-24-1]—White, crystalline powder. Almost insoluble in water; soluble in warm alcohol, and in glacial acetic acid and in 75% acetic acid; insoluble in chloroform and in ether. Melts at about 230°, with decomposition.

Specific Rotation ⟨781⟩: between −47° and −49°, determined in a solution in 75% acetic acid containing 100 mg per mL.

Solubility in Alcohol: A solution of 500 mg in 20 mL of warm alcohol is colorless and complete.

Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 6% of its weight.

Residue on Ignition (Reagent test): not more than 0.3%.

Digoxigenin *(3β,12β,14β,21-Tetrahydroxy-20(22)-norcholenic Acid Lactone; 3β,12β,14-Trihydroxy-5β,20(22)-cardenolide; 5β,20(22)-Cardenolide-3β,12β,14-triol; Lanadigenin),* $C_{23}H_{34}O_5$—**390.51** [1672-46-4]—Use a suitable grade.

Change to read:

▲7,8-Dihydrofolic Acid ▲*(L-Glutamic Acid, N-[4-[[(2-Amino-3,4,7,8-tetrahydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-)* ,▲ (ERR 1-Mar-2019) $C_{19}H_{21}N_7O_6$—**443.41** [*4033-27-6*]—Use a suitable grade with a content of NLT 90%.▲ 2S (USP41)

24,25-Dihydrolanosterol *(Lanostenol, Lanost-8-en-3β-ol, 5α-Lanost-8-en-3β-ol, DHL),* $C_{30}H_{52}O$—**428.73** [911660-54-3]—Use a suitable grade with a content of NLT 99%. Store at −20°.

[NOTE—A suitable grade is available from www.avantilipids.com.]

Dihydroquinidine Hydrochloride, $C_{20}H_{27}ClN_2O_2$—**362.89** [1476-98-8]—Rhombic plates. Freely soluble in methanol and in chloroform.

• **ASSAY**

Mobile phase: Prepare a mixture of water, acetonitrile, diethylamine, and methanesulfonic acid (860:100:20:20).

Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 235-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The area of the $C_{20}H_{27}ClN_2O_2$ peak is not less than 97.5% of the total peak area.

Dihydroquinine *(Hydroquinine),* $C_{20}H_{26}N_2O_2$—**326.43** [522-66-7]—Freely soluble in acetone, in alcohol, and in chloroform; almost insoluble in water.

• **ASSAY**

Mobile phase: Prepare a mixture of water, acetonitrile, diethylamine, and methanesulfonic acid (860:100:20:20) and methanol (82:18).

Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 235-nm detector and a 4.6-mm × 15-cm column that

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contains packing L1. The flow rate is about 1.5 mL per minute. The area of the $C_{20}H_{26}N_2O_2$ peak is not less than 97.5% of the total peak area.

Dihydroxybenzaldehyde *(3,4-Dihydroxybenzaldehyde; Protocatechualdehyde),* $C_7H_6O_3$**—138.12** [139-85-5]—Use a suitable grade with a content of NLT 97%.

2,5-Dihydroxybenzoic Acid, $C_7H_6O_4$**—154.12** [303-07-1]—Off-white powder. Freely soluble in alcohol yielding a clear, very pale yellow solution.

Assay: Dissolve about 75 mg, accurately weighed, in 30 mL of methanol. Slowly add 40 mL of water. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 15.41 mg of $C_7H_6O_4$. Not less than 99% is found.

Melting Range ⟨741⟩: about 207°, with decomposition.

2,7-Dihydroxynaphthalene—See 2,7-Naphthalenediol.

Dihydroxyphenylacetone *(3,4-Dihydroxyphenylacetone),* $C_9H_{10}O_3$**—166.17** [2503-44-8]—Use a suitable grade with a content of NLT 95%.

Diiodofluorescein, $C_{20}H_{10}I_2O_5$**—584.10** [31395-16-1]—Orange-red powder. Slightly soluble in water; soluble in alcohol and in solutions of alkali hydroxides.

Residue on Ignition: Ignite 200 mg with 5 drops of sulfuric acid: the weight of the residue does not exceed 1.0 mg (0.5%).

Sensitiveness: Accurately weigh about 100 mg of potassium iodide, previously dried at 105° to constant weight, and dissolve it in 50 mL of water. Add 1 mL of diiodofluorescein TS prepared from the test specimen and 1 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS until the color of the precipitate changes from brownish-red to a bluish-red. The volume of 0.1 N silver nitrate consumed is not in excess of 0.10 mL over the calculated volume, based on the KI content of the dried potassium iodide determined as follows. Dissolve about 500 mg of potassium iodide, accurately weighed, in about 10 mL of water, and add 35 mL of hydrochloric acid and 5 mL of chloroform. Titrate with 0.05 M potassium iodate VS until the purple color of iodine disappears from the chloroform. Add the last portions of the iodate solution dropwise, agitating vigorously and continuously. After the chloroform has been decolorized, allow the mixture to stand for 5 minutes. If the chloroform develops a purple color, titrate further with the iodate solution. Each mL of 0.05 M potassium iodate is equivalent to 16.60 mg of KI.

Diisodecyl Phthalate *[Bis(isodecyl)phthalate],* $C_{28}H_{46}O_4$**—446.66** [26761-40-0]—Use a suitable grade.

Diisopropyl Ether *(Isopropyl Ether),* $[(CH_3)_2CH]_2O$**—102.17** [108-20-3]—Colorless, mobile liquid. Slightly soluble in water. Miscible with alcohol and with ether.

[**CAUTION**—It is highly flammable. Do not use where it may be ignited. Do not evaporate to the point of near dryness, since it tends to form explosive peroxides.]

Specific Gravity: between 0.716 and 0.720.

Distilling Range, *Method II* ⟨721⟩: Not less than 95% distills between 65° and 70°.

Peroxides: To 10 mL, contained in a clean, glass-stoppered cylinder previously rinsed with a portion of the ether under examination, add 1 mL of freshly prepared potassium iodide solution (1 in 10). Shake, and allow to stand for 1 minute: no yellow color is observed in either layer (about 0.001% as H_2O_2).

Residue on Evaporation:

[NOTE—If peroxide is present, do not carry out this procedure.]

Evaporate 14 mL (10 g) from a tared shallow dish, and dry at 105° for 1 hour: the residue weighs not more than 1 mg (0.01%).

Acidity: Add 2 drops of bromothymol blue TS to 10 mL of water in a glass-stoppered, 50-mL flask, and titrate with 0.010 N sodium hydroxide until a blue color persists after vigorous shaking. Add 5 mL of diisopropyl ether, and titrate with 0.010 N sodium hydroxide: not more than 0.30 mL is required to restore the blue color (0.005% as CH_3COOH).

[NOTE—For spectrophotometric determinations, use diisopropyl ether that meets the following additional requirement:]

Absorbance: Its absorbance at 255 nm, in a 10-mm quartz cell, does not exceed 0.2, water being used as the blank.

Diisopropylamine, $[(CH_3)_2CH]_2NH$**—101.19** [108-18-9]—Colorless liquid.

Assay: Not less than 98% of $C_6H_{15}N$ is found, a suitable gas chromatograph equipped with a flame-ionization detector being used. The following conditions have been found suitable: a 3.2-mm × 1.83-m stainless steel column is packed with a cross-linked polystyrene support; the injection port temperature is maintained at 250° and the detector temperature at 310°; the column temperature is programmed to rise at 10° per minute from 50° to 220°.

Refractive Index ⟨831⟩: between 1.3915 and 1.3935, at 20°.

Diisopropylethylamine *(N,N-Diisopropylethylamine),* $C_8H_{19}N$**—129.24** [7087-68-5]—Clear, colorless liquid. Soluble in glacial acetic acid.

Assay: Accurately weigh about 500 mg, dissolve in 50 mL of glacial acetic acid, mix, add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Each mL of 0.1 N perchloric acid is equivalent to 12.92 mg of $C_8H_{19}N$. Not less than 98% is found.

Refractive Index ⟨831⟩: between 1.4125 and 1.4145 at 20°.

***N,N′*-Diisopropylethylenediamine,** $C_8H_{20}N_2$**—144.26** [4013-94-9]—Use a suitable grade with a content of NLT 99%.

1,2-Dilinoleoyl-3-oleoyl-rac-glycerol, $C_{57}H_{100}O_6$**—881.4** [2190-21-8]—Use a suitable grade.

1,2-Dilinoleoyl-3-palmitoyl-rac-glycerol, $C_{55}H_{98}O_6$**—855.4** [2190-15-0]—Use a suitable grade.

Diluted Acetic Acid—See *Acetic Acid, Diluted.*

Diluted Alcohol—Use *Diluted Alcohol* (NF monograph).

Diluted Hydrochloric Acid—See *Hydrochloric Acid, Diluted.*

Diluted Nitric Acid—See *Nitric Acid, Diluted.*

Diluted Sulfuric Acid—See *Sulfuric Acid, Diluted.*

Dimethicone, viscosity 500 centistokes *(Poly(dimethylsiloxane), viscosity 500 centistokes),* $[-Si(CH_3)_2O-]_n$[63148-62-9]—Use a suitable grade.

2,5-Dimethoxybenzaldehyde, $C_9H_{10}O_3$**—166.17** [93-02-7]—Off-white crystals.

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Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, nitrogen being used as the carrier gas. The following conditions have been found suitable: a 0.3-mm × 30-m capillary column coated with phase G1; the injection port temperature is maintained at 270°; the detector temperature is maintained at 300°; the column temperature is maintained at 150° and programmed to rise 10° per minute to 270°. The area of the main peak is not less than 97% of the total peak area.
Melting Range ⟨741⟩: between 50° and 52°.

1,2-Dimethoxyethane, $C_4H_{10}O_2$—**90.12** [110-71-4]—Clear, colorless liquid. Miscible with water and with alcohol. Soluble in hydrocarbon solvents. *May form peroxides on standing.*
- **Boiling Range** (Reagent test): Not less than 95% distills between 83° and 86°.
- **Refractive Index** ⟨831⟩: between 1.379 and 1.381, at 20°.
- **Acidity:** To 20 mL add bromophenol blue TS, and titrate with 0.020 N sodium hydroxide: not more than 2.0 mL is consumed (about 0.015% as CH_3COOH).
- **Water Determination,** *Method I* ⟨921⟩: not more than 0.2%.

Dimethoxymethane *(Formaldehyde Dimethyl Acetal, Methylal),* $C_3H_8O_2$—**76.10** [109-87-5]—Use a suitable grade.

(3,4-Dimethoxyphenyl)acetonitrile *(Homoveratronitrile),* $C_{10}H_{11}NO_2$—**177.20** [93-17-4]—Off-white fibers.
Melting Range ⟨741⟩: between 65° and 67°.

Dimethyl Phthalate, $C_{10}H_{10}O_4$—**194.19** [131-11-3]—Viscous, colorless liquid.
Assay
Mobile phase: Prepare a filtered and degassed mixture of chromatographic *n*-heptane and *n*-propyl alcohol (HPLC grade) (97:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution: Dissolve a suitable quantity of dimethyl phthalate in *Mobile phase* to obtain a solution having a known concentration of about 0.26 mg per mL.
Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.0 mL per minute. The area of the dimethyl phthalate peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.514 and 1.518 at 20°.

Dimethyl Sulfone *(Methyl Sulfone),* $(CH_3)_2SO_2$—**94.13** [67-71-0]—White crystals.
Melting Range ⟨741⟩: between 109° and 111°.

Dimethyl Sulfoxide *(Methyl Sulfoxide),* $(CH_3)_2SO$—**78.13**—Use ACS reagent grade methyl sulfoxide.

Dimethyl Sulfoxide, Spectrophotometric Grade [67-68-5]—Use methyl sulfoxide ACS spectrophotometric reagent grade.

***N,N*-Dimethylacetamide,** C_4H_9NO—**87.12** [127-19-5]—Clear, colorless liquid. Miscible with water and with many organic solvents. Use a suitable HPLC or spectroscopic grade.

***p*-Dimethylaminoazobenzene** *(Methyl Yellow, Butter Yellow),* $C_6H_5N{:}NC_6H_4N(CH_3)_2$—**225.29** [60-11-7]—Yellow leaflets or yellow, crystalline powder.
Solubility: Insoluble in water; sparingly soluble in chloroform, in ether, or in fatty oils. Dissolve 100 mg in 20 mL of alcohol: the solution is complete or practically so and clear.
Melting Range ⟨741⟩: between 115° and 117°.
Residue on Ignition ⟨281⟩: not more than 0.1%.
Sensitiveness: Add 0.05 mL of an alcohol solution (1 in 200) and 2 g of ammonium chloride to 25 mL of carbon dioxide-free water: the lemon-yellow color of the solution is changed to orange by the addition of 0.05 mL of 0.1 N hydrochloric acid and restored on the subsequent addition of 0.05 mL of 0.1 N sodium hydroxide.

***p*-Dimethylaminobenzaldehyde,** $(CH_3)_2NC_6H_4CHO$—**149.19** [100-10-7]—Use ACS reagent grade.

***p*-Dimethylaminocinnamaldehyde,** $(CH_3)_2NC_6H_4CH{:}CHCHO$—**175.23**—Orange-yellow powder. Soluble in acetone, in alcohol, and in benzene.
Melting range ⟨741⟩: between 132° and 136°.

2-Dimethylaminoethyl Methacrylate, $C_8H_{15}NO_2$——**157.2** [2867-47-2]—Use a suitable grade.

Dimethylaminophenol *(meta isomer),* $C_8H_{11}NO$—**137.18**—Purplish-black, gray, or tan-colored, crystalline solid.
Melting Range ⟨741⟩: between 83° and 85°.

2,6-Dimethylaniline, $C_8H_{11}N$—**121.18** [87-62-7]—Yellow liquid.
Refractive Index ⟨831⟩: about 1.5609 at 20°.

***N,N*-Dimethylaniline,** $C_6H_5N(CH_3)_2$—**121.18** [121-69-7]—Light yellow liquid. Clear, colorless liquid when freshly distilled, but acquiring a reddish to reddish-brown color. Specific gravity: about 0.960. Freezing point about 2°. Insoluble in water; soluble in alcohol, in chloroform, in ether, and in dilute mineral acids.
Assay: Inject an appropriate specimen (about 0.2 µL) into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas flowing at about 40 mL per minute. The following conditions have been found suitable: a 3-mm × 1.8-m stainless steel column containing 20% phase G16 on support S1A; the injection port temperature is maintained at 250°; the column temperature is maintained at 50° and programmed to rise 10° per minute to 200°. The detector temperature is maintained at 310°. The area of the *N,N*-dimethylaniline peak having a retention time of about 11.5 minutes is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.5571 and 1.5591 at 20°.
Boiling Range (Reagent test): Distill 100 mL: the difference between the temperatures observed, when 1 mL and 95 mL have distilled, is not more than 2.5°. Its boiling temperature at a pressure of 760 mm of mercury is 194.2°.
Hydrocarbons: Dissolve 5 mL in a mixture of 10 mL of hydrochloric acid and 15 mL of water: a clear solution results and it remains clear on cooling to about 10°.
Aniline or Monomethylaniline: Place 5 mL in a glass-stoppered flask, add 5 mL of a solution of acetic anhydride in benzene (1 in 10), mix, and allow to stand for 30 minutes. Add 30.0 mL of 0.5 N sodium hydroxide VS, shake the mixture, add phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Not more than 0.30 mL of 0.5 N sodium hydroxide is consumed by the test specimen.

3,4-Dimethylbenzophenone, $C_{15}H_{14}O$—**210.27** [2571-39-3]—White chunks melting at about 45°.

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Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with phase G1: the detector temperature and the injection port temperature are maintained at 300°; the column temperature is maintained at 180° and programmed to rise at the rate of 10° per minute to 280° and held at that temperature for 10 minutes. The area of the main peak is not less than 99% of the total peak area.

Dimethylcarbonate *(Carbonic Acid Dimethyl Ester)*, $C_3H_6O_3$ — **90.08** [616-38-6]—Use a suitable grade with a content of NLT 99%.

5,5-Dimethyl-1,3-cyclohexanedione, $C_8H_{12}O_2$—**140.18** [126-81-8]—White, crystalline solid. Slightly soluble in water; soluble in alcohol, in methanol, in chloroform, and in acetic acid.

Melting Range ⟨741⟩: between 148° and 150°.

***N,N*-Dimethyldecylamine** *(1-(Dimethylamino)decane)*, C_4H_9NO—**185.35** [1120-24-7]—Use a suitable grade with a content of not less than 98%.

1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide *(Polybrene)*, [28728-55-4]—This is a positively charged polymer. It is available as an off-white, crystalline solid or powder and is extremely hygroscopic. Use a suitable reagent grade.

[NOTE—Commercially available as Polybrene.]

***N,N*-Dimethyldodecylamine-*N*-oxide** *(Lauryldimeth ylamine-N-oxide)*, $CH_3(CH_2)_{11}NO(CH_3)_2$—**229.40** [1643-20-5]—Use a suitable grade.

[NOTE—*N,N*-Dimethyldodecylamine-*N*-oxide, 0.1 N in Water and *N,N*-Dimethyldodecylamine-*N*-oxide, 30 Percent are suitable grades as well.]

Dimethylethyl(3-hydroxyphenyl)ammonium Chloride—See *Edrophonium Chloride* (USP Monograph).

1,1-Dimethylethylamine—See *tert-Butylamine*.

Dimethylformamide *(N,N-Dimethylformamide)*, $HCON(CH_3)_2$ —**73.09** [68-12-2]—Use ACS reagent grade.

***N,N*- Dimethylformamide Diethyl Acetal—147.22** [1188-33-6]—Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

1,3-Dimethyl-2-imidazolidinone, $C_5H_{10}N_2O$—**114.15** [80-73-9]—Use a suitable grade.

1,9-Dimethyl-methylene Blue, $C_{36}H_{46}Cl_4N_6OS_2Zn$—**850.1** [23481-50-7]—Dark green powder. Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

***N,N*-Dimethyl-1-naphthylamine**, $C_{12}H_{13}N$—**171.24** [86-56-6]—Pale yellow to yellow, aromatic liquid. Soluble in alcohol and in ether.

Assay: Transfer about 250 mg, accurately weighed, to a suitable beaker, add 100 mL of glacial acetic acid, and dissolve by stirring. When solution is complete, titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 17.12 mg of $C_{12}H_{13}N$. Not less than 98% is found.

Refractive Index ⟨831⟩: between 1.6210° and 1.6230° at 20°, sodium light being used.

Sulfanilamide Test: Dissolve 20 mg of USP Sulfanilamide RS in 100 mL of water to obtain the *Sulfanilamide solution*. Into two 150-mL beakers pipet 1.0 mL and 2.5 mL of the *Sulfanilamide solution*, respectively. Dilute with water to 90 mL. To provide a blank, place 90 mL of water in a third beaker. To each beaker add 8.0 mL of trichloroacetic acid solution (3 in 20) and 1.0 mL of sodium nitrite solution (1 in 1000). Stir the solutions for 5 minutes, then add 10 mL of acetate buffer TS, and 1.0 mL of a 1 in 1000 solution of *N,N*-dimethyl-1-naphthylamine in alcohol. The pH is about 5 to 6, using pH paper. Stir for an additional 5 minutes, then add 20 mL of glacial acetic acid. The pH is about 3 to 4, using pH paper. In comparison with the blank, the beaker containing 1.0 mL of the *Sulfanilamide solution* shows a pink color, while the other beaker shows a deep pink to red color.

***N,N*-Dimethyloctylamine**, $C_{10}H_{23}N$—**157.30** [7378-99-6] —Colorless liquid.

Refractive Index ⟨831⟩: 1.4243 at 20°.

2,5-Dimethylphenol, $C_8H_{10}O$—**122.16** [95-87-4]—Use a suitable grade.

2,6-Dimethylphenol, $(CH_3)_2C_6H_3OH$—**122.16** [576-26-1] —White to pale yellow, crystalline solid.

Assay: Inject a 1 in 3 solution of it in xylene into a suitable gas chromatograph equipped with a flame-ionization detector, helium being used as the carrier gas at a flow rate of about 40 mL per minute. The following conditions have been found suitable: a 3.2-mm × 1.83-m stainless steel column packed with 10% phase G25 on support S1A; the injection port temperature is maintained at about 250° and the detector temperature at about 310°; the column temperature is programmed to rise at 8° per minute from 100° to 200°. Similarly inject a specimen of xylene. The area of the $C_8H_{10}O$ peak is not less than 98% of the total peak area corrected for xylene.

Melting Range ⟨741⟩: between 44° and 46°.

3,5-Dimethylphenol, $C_8H_{10}O$—**122.16** [108-68-9]—Use a suitable grade.

[NOTE—A suitable grade is available as product number 5600 from www.alfa.com.]

***N,N*-Dimethyl-*p*-phenylenediamine Dihydrochloride**, $(CH_3)_2NC_6H_4NH_2 \cdot 2HCl$—**209.12** [99-89-9]—Nearly white, fine, crystalline, hygroscopic solid that may have a pinkish cast. Freely soluble in water; soluble in alcohol.

Assay: Transfer about 400 mg, accurately weighed, to a 250-mL beaker, and dissolve in about 75 mL of water. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Each mL of 0.1 N sodium hydroxide is equivalent to 10.46 mg of $C_8H_{12}N_2 \cdot 2HCl$. Not less than 98% is found.

Solubility: A solution of 1 g in 10 mL of water produces not more than a slight haze.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide, $C_{18}H_{16}N_5SBr$—**414.3** [298-93-1]—Yellow to orange powder. Use a suitable grade.

Dimethyltin Dibromide, $C_2H_6Br_2Sn$—**308.59** [2767-47-7] —Use a suitable grade.

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Dimidium Bromide *(3,8-Diamino-5-methyl-6-phenylphenanthridinium bromide; Trypadine)*, $C_{20}H_{18}BrN_3$ — **380.28** [518-67-2]—Use a suitable grade with a content of NLT 95%.

***m*-Dinitrobenzene**, $C_6H_4(NO_2)_2$—**168.11** [99-65-0]—Pale yellow crystals or crystalline powder. Almost insoluble in cold water; slightly soluble in hot water. Soluble in chloroform and in benzene; sparingly soluble in alcohol. Is volatile in steam.
• **MELTING RANGE** ⟨741⟩: between 89° and 92°.
• **RESIDUE ON IGNITION** (Reagent test): not more than 0.5%.

3,5-Dinitrobenzoyl Chloride, $C_7H_3ClN_2O_5$—**230.56** [99-33-2]—Pale yellow, crystalline powder. Freely soluble in dilute sodium hydroxide solutions; soluble in alcohol.
[**CAUTION**—Corrosive, moisture-sensitive, lachrymator, and possible mutagen. Store under nitrogen.]
Melting Range ⟨741⟩: between 69° and 71°.
Solubility in Sodium Hydroxide: A solution of 500 mg in 25 mL of 1 N sodium hydroxide is clear or not more than faintly turbid.
Residue on Ignition: Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

2,4-Dinitrochlorobenzene, $C_6H_3(NO_2)_2Cl$—**202.55** [97-00-7]—Yellow to brownish-yellow crystals. Insoluble in water; soluble in hot alcohol, in ether, and in benzene.
Melting Range ⟨741⟩: between 51° and 53°.
Residue on Ignition: Ignite 500 mg with 5 drops of sulfuric acid: the residue weighs not more than 1 mg (0.2%).

2,4-Dinitrofluorobenzene *(1-Fluoro-2,4-dinitrobenzene)*, $C_6H_3FN_2O_4$—**186.10** [70-34-8]—Light yellow solid. Use a suitable grade.

2,4-Dinitrophenylhydrazine, 2,4-$C_6H_3(NO_2)_2NHNH_2$—**198.14** [119-26-6]—Orange-red crystals, which under the microscope appear individually to be lemon-yellow, lath-like needles. Very slightly soluble in water; slightly soluble in alcohol; moderately soluble in dilute inorganic acids. Use a suitable grade with a content of not less than 97%.

Dioctyl Sodium Sulfosuccinate—Use *Docusate Sodium* (USP monograph).

Dioxane *(Diethylene Dioxide; 1,4-Dioxane)*, $C_4H_8O_2$—**88.11** [123-91-1]—Use ACS reagent grade.

Diphenyl Ether *(Phenyl Ether)*, $(C_6H_5)_2O$—**170.21** [101-84-8]—A colorless liquid. Insoluble in water; soluble in glacial acetic acid and in most organic solvents. Boils at about 259°.
• **MELTING RANGE** ⟨741⟩: between 26° and 28°.

Diphenylamine, $(C_6H_5)_2NH$—**169.22** [122-39-4]—Use ACS reagent grade.

Diphenylborinic Acid, Ethanolamine Ester, *(2-Aminoethyl Diphenylborinate)*, $C_{14}H_{16}BNO$—**225.09**—White, crystalline powder. Use a suitable grade.
Melting Range ⟨741⟩: between 192° and 194°.

Diphenylcarbazide, $(C_6H_5NHNH)_2CO$—**242.28** [140-22-7]—Use ACS reagent grade 1,5-Diphenylcarbohydrazide.

Diphenylcarbazone *[Diphenylcarbazone compd. with s-Diphenylcarbazide (1:1)]*, $C_6H_5NHNHCON{:}NC_6H_5 \cdot C_6H_5NHNHCONHNHC_6H_5$—**482.54** [538-62-5]—Use ACS reagent grade Diphenylcarbazone Compound with *s*-Diphenylcarbazide (1:1).

2,2-Diphenylglycine, $C_{14}H_{13}NO_2$—**227.26** [3060-50-2]—Off-white powder. Melts at about 244°, with decomposition.
Assay: Dissolve about 115 mg, accurately weighed, in 30 mL of methanol. Slowly add about 20 mL of water, heating slightly if necessary for complete solution. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 22.73 mg of $C_{14}H_{13}NO_2$. Not less than 98.0% is found.

Dipicolinic Acid *(2,6-Pyridinedicarboxylic Acid)*, $C_7H_5NO_4$—**167.12** [499-83-2]—Use a suitable grade with a content of NLT 99%.

Dipicrylamine—See *Hexanitrodiphenylamine*.

Dipropyl Phthalate, $C_{14}H_{18}O_4$—**250.29** [131-16-8]—Viscous, colorless liquid.
Assay
Mobile phase: Prepare a mixture of acetonitrile and water (52:48).
Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. The area of the $C_{14}H_{18}O_4$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.495 and 1.499 at 20°.

4,4′-Dipyridyl, $C_{10}H_8N_2$—**156.18** [553-26-4]—Use a suitable grade. [NOTE—A suitable grade is available from Sigma-Aldrich Catalog number 289426.]

α,α′-Dipyridyl—See *2,2′-Bipyridine*.

Disodium Chromotropate *(4,5-Dihydroxy-2,7-naphthalenedisulfonic Acid, Disodium Salt)*, $C_{10}H_6O_8S_2Na_2 \cdot 2H_2O$—**400.29**—Use ACS reagent grade Chromotropic Acid Disodium Salt.

Disodium Ethylenediaminetetraacetate—See *Edetate Disodium*.

5,5′-Dithiobis(2-nitrobenzoic Acid) *(3-Carboxy-4-nitrophenyl disulfide; Ellman's reagent)*, $C_{14}H_8N_2O_8S_2$—**396.35** [69-78-3]—Yellow powder, melting at about 242°. Sparingly soluble in alcohol.

Dithiothreitol *(Cleland's Reagent; Threo-1,4-dimercapto-2,3-butanediol; DTT)*, $HSCH_2CH(OH)CH(OH)CH_2SH$—**154.25** [3483-12-3]—Slightly hygroscopic needles from ether. Freely soluble in water, in alcohol, in acetone, in ethyl acetate, and in ether.
Melting Range ⟨741⟩: between 42° and 44°.

Dithizone *(Diphenylthiocarbazone; Phenylazothioformic Acid 2-Phenylhydrazide)*, $C_6H_5N{:}NCSNHNHC_6H_5$—**256.33** [60-10-6]—Use ACS reagent grade.

1-Dodecanol *(Dodecyl Alcohol)*, $CH_3(CH_2)_{11}OH$—**186.33** [112-53-8]—A clear, colorless liquid. Crystallizes as leaflets from dilute alcohol solution. Use ACS reagent grade.

Dodecyl Alcohol—See *1-Dodecanol*.

Dodecyl Lithium Sulfate ***(Lithium Dodecyl Sulfate, Lithium Lauryl Sulfate)***, $C_{12}H_{25}LiO_4S$—**272.3** [2044-56-6]—White to off-white powder, clear to slightly hazy, colorless to faint

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yellow solution in water at 50 mg per mL at ambient temperature. The UV absorbance of a 0.1 M solution is less than 0.05 at both 260 and 280 nm. The pH of a 0.1 M solution in water is 7.0 ± 0.5. Use a suitable grade.

***t*-Dodecyl Mercaptan Ethoxylate** *(Poly(oxy-1,2-ethanediyl), α-[2-(tert-Dodecylthio)ethyl]-ω-hydroxy-),* $(C_2H_4O)_nC_{14}H_{30}OS$ [9004-83-5]—Commercially available as a 1% or 2% aqueous solution. Use a suitable grade.

[NOTE—A suitable grade is available as catalog number 805716 from www.fishersci.com or as catalog number R8057000 from www.riccachemical.com.]

Dodecyl Sodium Sulfonate *(Sodium 1-Dodecanesulfonate; 1-Dodecanesulfonic Acid, Sodium Salt),* $C_{12}H_{25}SO_3Na$**—272.38** [2386-53-0]—White, powdery solid. One g dissolves in 50 mL of warm water to yield a clear, colorless solution. Use a suitable grade.

Melting point ⟨741⟩: higher than 300°.

3-(Dodecyldimethylammonio)propanesulfonate ***(Lauryl sulfobetaine, N,N-dimethyl-N-dodecyl-N-(3-sulfopropyl) ammonium betaine),*** $C_{17}H_{37}NO_3S$**—335.54** [14933-08-5]—Use a suitable grade.

Dodecyltriethylammonium Phosphate, 0.5 M, $[C_{12}H_{25}N \cdot (C_2H_5)_3]_3PO_4$**—906.52**—Use a suitable grade.

[NOTE—A suitable grade is available as Ion Pair Cocktail Q12 (catalogue number 404031) from Regis Technologies, Inc., www.registech.com.]

Dodecyltrimethylammonium Bromide ***(Lauryltrimethylammonium bromide),*** $CH_3(CH_2)_{11}N(CH_3)_3Br$**—308.3** [1119-94-4]—Use a suitable grade.

[NOTE—A suitable grade is available as catalog number D5047 from Sigma-Aldrich, www.sigma-aldrich.com.]

Drabkin's Reagent: The reagent consists of 100 parts of sodium bicarbonate, 20 parts of potassium ferricyanide, and 5 parts of potassium cyanide.

[**CAUTION**—The reagent is HIGHLY TOXIC. Very toxic by inhalation, in contact with skin, and if swallowed, and there is a risk of serious damage to eyes. Wear suitable protective clothing, gloves, and eye and face protection. Do not mix with acids. Contact with acids liberates a very toxic gas. If ingested, perform gastric lavage, and call a physician.]

[NOTE—The reagent can be obtained from many manufacturers and suppliers. Some examples of manufacturers or suppliers are the following: Sigma Chemical Co., St. Louis, MO; and CIMA Scientific, Dallas, TX.]

Dried Peptone—See *Peptone, Dried.*

Earth, Chromatographic, Silanized, Acid-Base Washed—Use a suitable grade.

[NOTE—A suitable chromatographic grade is "Gas-Chrom Q," available from Alltech, www.alltechweb.com.]

Edetate Disodium *(Disodium Ethylenediaminetetraacetate)* $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$**—372.24**—Use ACS reagent grade (Ethylenedinitrilo)tetraacetic Acid Disodium Salt Dihydrate.

Edetic Acid *(EDTA; Edathamil; Ethylenediaminetetraacetic Acid; [Ethylenedinitrilo]tetraacetic Acid),* $C_{10}H_{16}N_2O_8$**—292.24** [60-00-4]—Use ACS (Ethylenedinitrilo)tetraacetic Acid reagent grade.

***n*-Eicosane,** $C_{20}H_{42}$**—282.55** [112-95-8]—White, crystalline solid.

- **MELTING RANGE** ⟨741⟩: between 37° and 39°.

Eicosanol [629-96-9]—Use a suitable grade.

Eosin Y (Eosin Yellowish Y) *(Certified Biological Eosin Y; Sodium Tetrabromofluorescein),* $C_{20}H_6Br_4Na_2O_5$**—691.85** [17372-87-1]—Red to brownish-red pieces or powder. Use ACS reagent grade.

Epiandrosterone *(trans-Androsterone, 5α-Androstan-3β-ol-17-one),* $C_{19}H_{30}O_2$**—290.44** [481-29-8]—Use a suitable grade.

[NOTE—A suitable grade is available as catalog number E3375 from www.sigma-aldrich.com.]

15-Epicarboprost *(15(R)-Methyl prostaglandin F2α),* $C_{21}H_{36}O_5$**—368.5** [35864-81-4]—Use a suitable grade with a content of NLT 98%. Store at –20°.

[NOTE—A suitable grade is available as catalog number 16730 from www.caymanchem.com.]

Equilenin, $C_{18}H_{18}O_2$**—266.33** [517-09-9]—Colorless or white crystals or crystalline powder. Insoluble in water; soluble in chloroform and in dioxane; moderately soluble in alcohol.

- **MELTING RANGE,** *Class II* ⟨741⟩: between 256° and 260°.
- **SPECIFIC ROTATION** ⟨781⟩: between +85° and +88°, determined in a solution in dioxane containing 75 mg of equilenin in each 10 mL.
- **ABSORPTION MAXIMA:** An alcohol solution exhibits absorption maxima at 231, 282, 325, and 340 nm.

α-Ergocryptine, $C_{32}H_{41}N_5O_5$**—575.70** [511-09-1]—Use a suitable grade.

Eriochrome Cyanine R, $C_{23}H_{15}Na_3O_9S$**—536.40** [3564-18-9]—Dark, red-brown powder. Freely soluble in water; insoluble in alcohol.

Solubility: 200 mg in 100 mL of water yields a solution that remains clear and free from undissolved matter for 30 minutes.

Loss on Drying ⟨731⟩: Dry it in vacuum over silica gel to constant weight: it loses not more than 2% of its weight.

Residue on Ignition (Reagent test): 0.5 g, treated with 1 mL of sulfuric acid and 2 mL of nitric acid, yields between 42.0% and 44.0% of the dry weight (theoretical yield is 42.9% of Na_2SO_4).

Sensitiveness: Add 2 mL of a solution (1 in 1000) to 1 mL of aluminum sulfate solution (1 in 10,000), heat at 37 ± 3° for 5 minutes, cool, and add 1 mL of sodium acetate TS: a strong red to red-violet color is produced in not more than 5 minutes.

Escin [6805-41-0]—Use a suitable grade.

Ethanesulfonic Acid, $C_2H_5SO_3H$**—110.13** [594-45-6]—Colorless to light yellow liquid. Soluble in water.

Assay: Accurately weigh about 300 mg, dissolve in 30 mL of water, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 11.013 mg of $C_2H_5SO_3H$: between 94.0% and 106.0% is found.

Refractive Index ⟨831⟩: between 1.432 and 1.436 at 20°.

Ether—See *Ethyl Ether.*

Ether, Absolute—See *Ethyl Ether, Anhydrous.*

Ether, Diphenyl—See *Diphenyl Ether.*

Ether, Isopropyl—See *Diisopropyl Ether.*

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Ether, Nonyl Phenyl Polyethylene Glycol—See *(p-tert-Octylphenoxy)nonaethoxyethanol.*

Ether, Peroxide-Free *(Diethyl Ether; Ether)*, $(C_2H_5)_2O$—**74.12**—Use ACS reagent grade.
Peroxide: Transfer 8 mL of potassium iodide and starch TS to a 12-mL ground glass-stoppered cylinder about 15 mm in diameter. Fill completely with the substance under test, mix, and allow to stand protected from light for 5 min. No color develops [NMT 10 ppm (10 mg/L)]. Alternatively, peroxide test strips may be used. [NOTE—Suitable peroxide test strips can be obtained from EMD Millipore, www.emdmillipore.com.]

Ethidium Bromide, $C_{21}H_{20}N_3Br$—**394.3** [1239-45-8]—Purple to purple-red powder. Use a suitable grade.

4′-Ethoxyacetophenone, $C_{10}H_{12}O_2$—**164.20** [1676-63-7]—White to tan crystals.
Assay: Dissolve about 50 mg in 1 mL of ether. Inject about 1 µL of this solution into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector and a 0.25-mm × 30-m capillary column containing stationary phase G1. The carrier gas is helium. The chromatograph is programmed as follows. Initially, the column temperature is equilibrated at 180°, then the temperature is increased at a rate of 10° per minute to 280°, and maintained at 280° for 10 minutes. The injection port temperature is maintained at 280°, and the detector is maintained at 300°. The area of the 4′-ethoxyacetophenone peak is not less than 97.5% of the total peak area.
Melting Range ⟨741⟩: between 34° and 39°.

2-Ethoxyethanol *(Ethylene Glycol Monoethyl Ether)*, $C_4H_{10}O_2$—**90.12** [110-80-5]—Clear, colorless liquid. Miscible with water, with alcohol, with ether, and with acetone.
Specific Gravity ⟨841⟩: about 0.93.
Boiling Range (Reagent test): Not less than 95% distills between 133° and 135°.

Ethyl Acetate, $CH_3COOC_2H_5$—**88.11** [141-78-6]—Use ACS reagent grade.

Ethyl Acrylate [140-88-5]—Use a suitable grade.

Ethyl Alcohol *(Alcohol; Ethanol)*, C_2H_5OH—**46.07**—Use *Alcohol.*

Ethyl Arachidate, $C_{22}H_{44}O_2$—**340.6** [18281-05-5]—Use a suitable grade.

Ethyl Benzoate, $C_9H_{10}O_2$—**150.17** [93-89-0]—Clear, colorless liquid. Practically insoluble in water; miscible with alcohol, with chloroform, and with ether.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩), helium being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 2.4-m stainless steel column containing 20% phase G16 on support S1A; the injection port, column, and detector temperatures are maintained at 180°, 195°, and 250°, respectively. The area of the ethyl benzoate peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: between 1.5048 and 1.5058 at 20°.

Ethyl Cyanoacetate, $CNCH_2COOC_2H_5$—**113.11** [105-56-6]—Colorless to pale yellow liquid. Slightly soluble in water. Miscible with alcohol and with ether. At atmospheric pressure it boils between 205° and 209°, with decomposition. At a pressure of 10 mm of mercury it distills at about 90°.
Specific Gravity ⟨841⟩: between 1.057 and 1.062.
Acidity: Dissolve 2 mL in 25 mL of neutralized alcohol, add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 1.5 mL is required to produce a pink color.

Ethyl Ether *(Diethyl Ether; Ether)*, $(C_2H_5)_2O$—**74.12** [60-29-7]—Use ACS reagent grade.

Ethyl Ether, Anhydrous *(Diethyl Ether, Anhydrous; Ether, Absolute)*, $(C_2H_5)_2O$—**74.12** [60-29-7]—Use ACS reagent grade.

Ethyl Salicylate, $C_9H_{10}O_3$—**166.17** [118-61-6]—Colorless liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 10-m capillary column coated with a 1-µm layer of methylsilicone; the injection port temperature is maintained at 240°; the detector temperature is maintained at 300°; the column temperature is maintained at 150° and programmed to rise 10° per minute to 250°. The area of the ethyl salicylate peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.5216 and 1.5236 at 20°.

Add the following:

▲**Ethyl *p*-Toluenesulfonate** *(Ethyl Tosylate; 4-Methylbenzenesulfonic Acid Ethyl Ester)*, $C_9H_{12}O_3S$—**200.25** *[80-40-0]*—Use a suitable grade with a content of NLT 98%. ▲ 2S (USP41)

2-Ethylaminopropiophenone Hydrochloride, $C_6H_5COCH(CH_3)NHC_2H_5 \cdot HCl$—**213.70** [51553-17-4]—Use a suitable grade.

4-Ethylbenzaldehyde, $C_2H_5C_6H_4CHO$—**134.18** [4748-78-1]—Colorless to pale yellow liquid.
Assay: Dissolve about 600 mg, accurately weighed, in a mixture of 100 mL of alcohol and 25 mL of 1 M hydroxylamine hydrochloride in a beaker. Cover the beaker with a watch glass. Heat gently until condensate begins to form on the watch glass. Allow to cool for about 30 minutes. Titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 67.09 mg of $C_2H_5C_6H_4CHO$. Not less than 98% is found.

Ethylbenzene, C_8H_{10}—**106.17** [100-41-4]—Not less than 99.5%.

Ethylene Dichloride *(1,2-Dichloroethane)*, $C_2H_4Cl_2$—**98.96** [107-06-2]—Use ACS reagent grade 1,2-Dichloroethane.

Ethylene Glycol, $HOCH_2CH_2OH$—**62.07** [107-21-1]—Clear, colorless, slightly viscous, hygroscopic liquid. Slightly soluble in ether; practically insoluble in benzene. Miscible with water and with alcohol.
- **SPECIFIC GRAVITY** ⟨841⟩: about 1.11.
- **BOILING RANGE** (Reagent test): between 194° and 200°.
- **RESIDUE ON IGNITION:** Evaporate 100 mL (110 g) in a tared evaporating dish over a flame until the vapors continue to burn after the flame is removed. Allow the vapors to burn until the specimen is consumed. Ignite at 800 ± 25° for

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1 hour, cool, and weigh: the residue weighs not more than 5.5 mg (0.005%).
- **ACIDITY:** Add 0.2 mL of phenol red TS to 50 mL of water, and titrate with 0.1 N sodium hydroxide to a red endpoint. Add 50 mL (55 g) of ethylene glycol, and titrate with 0.1 N sodium hydroxide: not more than 1 mL is required to restore the red color (0.01% as CH_3COOH).
- **CHLORIDE** (Reagent test): A 4.5-mL (5-g) portion shows not more than 0.025 mg of Cl (5 ppm).
- **WATER DETERMINATION,** *Method I* ⟨921⟩: not more than 0.20%.

Ethylene Glycol Monoethyl Ether—See *2-Ethoxyethanol.*

Ethylene Oxide in Methylene Chloride (50 mg/mL): Use a suitable grade.
[NOTE—A suitable grade is available from Sigma Aldrich Corporation, www.sigma-aldrich.com.]

Ethylenediamine *(1,2-Diaminoethane)* $C_2H_8N_2$—**60.10** [107-15-3]—Use a suitable grade with a content of not less than 99%.

2-Ethylhexanoic Acid *(2-Ethylcaproic Acid),* $C_8H_{16}O_2$ — **144.21** [149-57-5]—Use a suitable grade with a content of NLT 99%.

***N*-Ethylmaleimide** *(1-Ethyl-1H-pyrrole 2,5-Dione),* $C_6H_7NO_2$— **125.12** [128-53-0]—Use a suitable grade.
- **MELTING RANGE** ⟨741⟩: between 43° and 47°.

2-Ethyl-2-methylsuccinic Acid, $C_7H_{12}O_4$—**160.17**—White crystals.
Assay: Transfer about 80 mg, accurately weighed, to a suitable beaker, add 30 mL of methanol, and dissolve by stirring. Slowly add 40 mL of deionized water.
[NOTE—If cloudiness appears when adding the deionized water, dissolve in 50 mL of methanol only.] When solution is complete, titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank titration, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 16.02 mg of $C_7H_{12}O_4$. Not less than 98.5% of $C_7H_{12}O_4$ is found.
Melting Point ⟨741⟩: between 101° and 103°.

Ethylparaben—Use *Ethylparaben* (NF monograph).

1-Ethylquinaldinium Iodide, $C_{12}H_{14}IN$—**299.15** [606-55-3] —Yellow-green solid. Sparingly soluble in water.
Assay: Dissolve about 290 mg, accurately weighed, in 100 mL of water, and add 10 mL of glacial acetic acid. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-ion selective electrode and a calomel reference electrode containing 1 M potassium nitrate. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 29.92 mg of $C_{12}H_{14}IN$: not less than 97.0% is found.

Factor X_a (Activated Factor X) for Anti-Factor X_a Test— Factor X_a is the proteolytic enzyme derived from bovine plasma, and it cleaves prothrombin to form thrombin. It is a glycoprotein having a molecular weight of 40,000. One Factor X_a Unit is the amount of activated endogenous Factor X (55,000 Da when nonactivated) that is contained in 1 mL of normal plasma. Russell's viper venom is used to activate the enzyme, and is then removed. The preparation is stabilized and lyophilized.

Factor X_a is free of thrombin at the concentration used in the test. When tested against pure fibrinogen, no clotting takes place within 24 hours. It has not less than 40 Factor X_a Units per mg of protein, and exhibits 90% homogeneity when tested by disk electrophoresis.

One Factor X_a Unit per mL gives a 15-second clotting time when tested in the following manner. Mix 0.1 mL of a saturated solution of cephalin (derived from rabbit brain-acetone powder or from an equivalent amount of rabbit brain thromboplastin) in 0.1 mL of citrated bovine plasma and 0.1 mL of 0.025 M calcium chloride. Immediately add 0.1 mL of a solution of factor X_a (1 in 10), and incubate at 37°.

Fast Blue B Salt, $C_{14}H_{12}N_4O_2 \cdot ZnCl_4$—**475.47** [91-91-8]— Green powder.
Loss on Drying ⟨731⟩: Dry it in vacuum at 110° for 1 hour: it loses not more than 5.0% of its weight.
Absorbance: Dissolve 50 mg in 100 mL of water. In a second container dissolve 100 mg of 2-naphthol in 100 mL of 2-methoxyethanol. Pipet 5 mL of the test solution and 10 mL of the 2-naphthol solution into a 100-mL volumetric flask, and dilute with acetone to volume. For the blank, pipet 5 mL of water and 10 mL of 2-naphthol solution into a second 100-mL volumetric flask, and dilute with acetone to volume. Determine the absorbance of the test solution in a 1-cm cell at the wavelength of maximum absorbance at about 545 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance is not less than 0.80.

Fast Blue BB Salt, $(C_{17}H_{18}ClN_3O_3)_2 \cdot ZnCl_2$—**831.89** [15710-69-7]—Yellow powder melting at about 162°, with decomposition. Sparingly soluble in water.
Chloride: Transfer about 80 mg, accurately weighed, to a suitable beaker. Add 25 mL of acetone, 25 mL of water, and 500 mg of sodium nitrate. Stir until solution is complete. Titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Not less than 15.0% of chloride is found.

Fast Green FCF, $C_{37}H_{34}N_2Na_2O_{10}S_3$—**808.86** [2353-45-9]— Red to brown-violet powder or crystals. Soluble in water; sparingly soluble in alcohol. Use a suitable grade.

FD&C Blue No. 1 (*Brilliant Blue*), $C_{37}H_{34}N_2O_9S_3Na_2$—**792.86** [3844-45-9]—Use a suitable grade.

Ferric Ammonium Citrate—Thin, transparent, garnet-red scales or granules or brownish-yellow powder. Is deliquescent and is affected by light. Very soluble in water; insoluble in alcohol.
Assay: Accurately weigh about 1 g, dissolve in 25 mL of water in a glass-stoppered flask, add 5 mL of hydrochloric acid and 4 g of potassium iodide, insert the stopper in the flask, and allow to stand in the dark for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe: between 16.5% and 18.5% is found.
Ferric Citrate: To 250 mg dissolved in 25 mL of water add 1 mL of potassium ferrocyanide TS: no blue precipitate is formed.
Tartrate: Dissolve 1 g in 10 mL of water, add 1 mL of potassium hydroxide TS, boil to coagulate the ferric hydroxide, adding more potassium hydroxide TS, if necessary, to precipitate all of the iron, filter, and slightly

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acidify the filtrate with glacial acetic acid. Add 2 mL of glacial acetic acid, and allow to stand for 24 hours: no crystalline white precipitate is formed.
Lead ⟨251⟩: Dissolve 1.0 g in 30 mL of water, add 5 mL of dilute nitric acid (1 in 21), boil gently for 5 minutes, cool, and dilute with water to 50 mL: 20 mL of the solution shows not more than 0.008 mg of Pb (0.002%).

Ferric Ammonium Sulfate, $FeNH_4(SO_4)_2 \cdot 12H_2O$—**482.19**—Use ACS reagent grade.

Ferric Chloride, $FeCl_3 \cdot 6H_2O$—**270.29** [10025-77-1]—Use ACS reagent grade.

Change to read:

Ferric Nitrate, $Fe(NO_3)_3 \cdot 9H_2O$—**404.00** [▲7782-61-8▲ (ERR 1-May-2019)]—Use ACS reagent grade.

Ferric Sulfate, $Fe_2(SO_4)_3 \cdot xH_2O$ [15244-10-7]—Grayish-white, hygroscopic powder, or fawn-colored pearls, slowly soluble in water.
Assay: Accurately weigh about 700 mg, and dissolve it in a mixture of 50 mL of water and 3 mL of hydrochloric acid in a glass-stoppered flask. Add 3 g of potassium iodide, and allow to stand in the dark for 30 minutes. Then dilute with 100 mL of water, and titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe: not less than 21.0% and not more than 23.0% is found.
Insoluble matter (Reagent test): A 10-g portion, dissolved in a mixture of 100 mL of water and 5 mL of sulfuric acid, shows not more than 2 mg of insoluble matter (0.02%).
Chloride: Dissolve 1 g by warming with a mixture of 10 mL of water and 1 mL of nitric acid, add 4 mL of additional nitric acid, and dilute with water to 50 mL. To 25 mL add 1 mL of phosphoric acid and 1 mL of silver nitrate TS. Any turbidity does not exceed that produced in a control containing 0.01 mg of chloride ion (Cl), 1 mL of nitric acid, 1 mL of phosphoric acid, and 1 mL of silver nitrate TS (0.002%).
Ferrous iron: Dissolve 4 g by warming with 50 mL of dilute sulfuric acid (1 in 10), cool, and titrate with 0.1 N potassium permanganate: not more than 0.16 mL is required to produce a permanent pink color (0.02% as Fe+2).
[NOTE—Because the reagents used in the tests for *Copper* and *Zinc* may contain excessive amounts of copper and zinc, they should first be purified by extracting with *Dithizone Extraction Solution* (see *Lead* ⟨251⟩).]
Copper: Dissolve 1.2 g in 100 mL of water. To 10 mL add 50 mL of a solution containing 5 g of ammonium tartrate and 5 mL of ammonium hydroxide. Add 10 mL of *Standard Dithizone Solution* (see *Lead* ⟨251⟩), shake for 2 minutes, draw off the dithizone layer, and compare the pink color with that in a control containing 6 µg of copper ion (Cu) and treated exactly as the 10-mL portion of test solution. If the color in the test solution is less than that in the control, then the test specimen contains less than the limit of both *Copper* and *Zinc*. If the color in the test solution is more than that in the control, add 15 mL of dilute hydrochloric acid (1 in 250), and shake for 2 minutes. Draw off the dithizone solution, and shake with a second 15 mL of dilute hydrochloric acid (1 in 250) for 2 minutes. Draw off the dithizone, combine the two acid extracts, and reserve for the *Zinc* test. Any pink color in the dithizone solution is not darker than that in the control solution treated exactly as the test solution (0.005%).
Zinc: To the combined acid extracts saved from the *Copper* test, add 0.5 M sodium acetate to bring the pH between 5.0 and 5.5, and then add 1 mL of 0.1 N sodium thiosulfate. Add 10 mL of *Standard Dithizone Solution* (see *Lead* ⟨251⟩), shake for 2 minutes, and allow the layers to separate. Draw off the dithizone, and discard the water layer. Any pink color is not greater than that in a control prepared by adding 0.006 mg of zinc ion (Zn) to the combined acid extracts from the control used in the test for *Copper* (0.005%).
Nitrate: Dissolve 10 g in 100 mL of dilute sulfuric acid (1 in 100), heat to boiling, and pour, slowly, into a mixture of 140 mL of water and 50 mL of stronger ammonia TS. Filter through a folded filter while still hot, wash with hot water until the volume of the filtrate is 300 mL, mix, and cool. To 15 mL of this solution add 1 mL of sodium chloride solution (1 in 200), 0.10 mL of indigo carmine TS, and 15 mL of sulfuric acid. The blue color is not completely discharged at the end of 5 minutes (0.01%).
Substances not precipitated by ammonia: Evaporate to dryness 30 mL of the filtrate obtained in the test for *Nitrate*, and ignite gently: the weight of residue does not exceed 1 mg (0.10%).

Ferrocyphen *(Dicyano-bis (1,10-phenantroline) iron (II) Complex),* $(C_{12}H_8N_2)_2Fe(CN)_2$—**468.3**—Brown to black powder. Dark red solution at 10 mg per mL in glacial acetic acid. Soluble in chloroform and in water at 5 mg per mL, yielding a clear purple solution in chloroform and a clear orange solution in water.
• **WATER DETERMINATION,** *Method I* ⟨921⟩ : not more than 10%.

Ferrous Chloride Tetrahydrate *(Iron (II) Chloride Tetrahydrate),* $FeCl_2 \cdot 4H_2O$—**198.81** [13478-10-9]—Use a suitable grade with a content of NLT 99.0%.

Ferrous Sulfate, $FeSO_4 \cdot 7H_2O$—**278.02** [7782-63-0]—Use ACS reagent grade.

Ferrous Ammonium Sulfate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$—**392.14**—Use ACS reagent grade.

Ferulic Acid *(4-Hydroxy-3-methoxycinnamic acid),* $C_{10}H_{10}O_4$—**194.19** [1135-24-6]—Use a suitable grade.

Fibroblast Growth Factor-2: Use a suitable grade.
[NOTE—A suitable grade can be obtained from Roche Diagnostics Corporation, www.roche-diagnostics.com.]

Fluorene, $C_{13}H_{10}$—**166.22** [86-73-7]—White to off-white crystals or powder. Soluble in benzene, in carbon disulfide, in ether, and in hot alcohol; freely soluble in glacial acetic acid.
Solubility Test: One g dissolves in 10 mL of acetone to yield a clear and complete solution.
Melting Range ⟨741⟩: between 113° and 117°, within a 2° range.

(±)-1-(9-Fluorenyl)ethanol *(α-Methylfluorene-9-methanol),* $C_{15}H_{14}O$—**210.27** [3023-49-2]—Use a suitable grade with a content of NLT 97.0%.

(+)-1-(9-Fluorenyl)ethyl Chloroformate Solution *((+)-FLEC),* $C_{16}H_{13}ClO_2$ —**272.73** [107474-79-3]—Use a suitable grade with a concentration of NLT 18 mM in acetone.
• **ENANTIOMERIC PURITY**
Mobile phase: Acetonitrile and water (1:1)
Standard solution: 30 µg/mL of (±)-1-(9-fluorenyl)ethanol in alcohol
Sample solution: 18 mM solution of (+)-1-(9-fluorenyl) ethyl chloroformate in acetone

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Carbonate buffer solution: Transfer 338 mg of sodium carbonate and 152 mg of sodium bicarbonate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.
Sodium hydroxide solution: Transfer 30 g of sodium hydroxide to a 100-mL volumetric flask, add 50 mL of water to dissolve, and cool and dilute with water to volume.
Acetate buffer solution: Transfer 0.3 mL of glacial acetic acid to a 100-mL volumetric flask, add 90 mL of water, adjust with *Sodium hydroxide solution* to a pH of 4.2, and dilute with water to volume.
Blank: Water
Procedure: Transfer 20.0 µL each of *Sample solution* and *Blank* to separate 10-mL test tubes. Add 80 µL of *Carbonate buffer solution* to each test tube and cap the tubes tightly. Let each solution react for 24 h at 45° over a water bath. Cool to room temperature, add 5.0 mL of *Acetate buffer solution*, mix by vortex, and transfer to separate vials.
Chromatographic System
(See *Chromatography* ⟨621⟩, *System Suitability*)
Mode: LC
Detector: Fluorescence
Excitation wavelength: 260 nm
Emission wavelength: 315 nm
Column: 4.6-mm × 15-cm; 3.0-µm packing L40
Column temperature: 25°
Flow rate: 0.5 mL/min
Injection volume: 5 µL
Run time: 2 times the retention time of (±)-1-(9-fluorenyl)ethanol
System Suitability
Samples: *Standard solution*, hydrolyzed *Sample solution*, and hydrolyzed *Blank*
Suitability requirements
Relative retention times: About 0.95 for hydrolysate of (–)-1-(9-fluorenyl)ethyl chloroformate and 1.0 for hydrolysate of (+)-1-(9-fluorenyl)ethyl chloroformate
Resolution: NLT 1.5 between hydrolysate of (–)-1-(9-fluorenyl)ethyl chloroformate and hydrolysate of (+)-1-(9-fluorenyl)ethyl chloroformate. Absence of peaks at the retention times for hydrolysate of (–)-1-(9-fluorenyl)ethyl chloroformate and hydrolysate of (+)-1-(9-fluorenyl)ethyl chloroformate in the hydrolyzed *Blank*.
Analysis
Samples: *Standard solution* and hydrolyzed *Sample solution*
Integrate only peaks at the retention times of (–)-1-(9-fluorenyl)ethanol and (+)-1-(9-fluorenyl)ethanol. Disregard any extraneous peaks in the chromatogram of hydrolyzed *Sample solution*.
Calculate the percentage of (+)-1-(9-fluorenyl)ethyl chloroformate (P_A) in the portion of (+)-1-(9-fluorenyl) ethyl chloroformate taken:

$$\text{Result} = r_A/(r_A + r_B) \times 100$$

r_A = peak response of hydrolysate of (+)-1-(9-fluorenyl)ethyl chloroformate from the *Sample solution*
r_B = peak response of hydrolysate of (–)-1-(9-fluorenyl)ethyl chloroformate from the *Sample solution*

Calculate the percentage of (–)-1-(9-fluorenyl)ethyl chloroformate (P_B) in the portion of (+)-1-(9-fluorenyl) ethyl chloroformate taken:

$$\text{Result} = 100 - P_A$$

Acceptance criteria: NLT 99.5% of (+)-1-(9-fluorenyl)ethyl chloroformate and NMT 0.5% of (–)-1-(9-fluorenyl)ethyl chloroformate

9-Fluorenylmethyl Chloroformate, $C_{15}H_{11}ClO_2$**—258.70** [28920-43-6]—Clear, colorless solid. Melts at about 62°.

Fluorescamine, $C_{17}H_{10}O_4$**—278.26** [38183-12-9]—White to off-white powder. Very slightly soluble in water; freely soluble in methylene chloride; soluble in alcohol; slightly soluble in chloroform.
Assay: Dissolve about 600 mg in 75 mL of dimethylformamide, and titrate with 0.1 N lithium methoxide to a blue endpoint, using 1% thymol blue in dimethylformamide as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 27.83 mg of $C_{17}H_{10}O_4$. Not less than 99% is found.
Loss on Drying ⟨731⟩: Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

4′-Fluoroacetophenone, $FC_6H_4COCH_3$**—138.14** [403-42-9]—Colorless liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 200°; the detector temperature is maintained at 250°; the column temperature is maintained at 100° and programmed to rise 10° per minute to 250°. The area of the $FC_6H_4COCH_3$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: 1.510 at 20°.

Fluoroandrostadiene Carboxylic Acid *(Dexamethasone Acid),* $C_{21}H_{27}FO_5$ **—378.43** [37927-01-8.]—Use a suitable grade.
[NOTE—A suitable grade is available from www.scbt.com or as catalog number D298805 from www.trc-canada.com.]

Formaldehyde Solution, HCHO**—(30.03) and water**—Use ACS reagent grade.

Formamide, $HCONH_2$**—45.04** [75-12-7]—Use ACS reagent grade.
Preparation for Digitoxin Assay: To ensure freedom from ammonia, treat Formamide as follows. Shake a suitable quantity of formamide with about 10% of its weight of anhydrous potassium carbonate for 15 minutes, and filter. Distill the filtrate in an all-glass apparatus under vacuum at a pressure of about 25 mm of mercury or less. Reject the first portion of distillate containing water, and collect the fraction that boils at about 115° at a pressure of 25 mm of mercury or at 101° at a pressure of 12 mm of mercury. Store in tight containers, protected from light.

Formamide, Anhydrous, $HCONH_2$**—45.04** [75-12-7]—Use formamide that has a water content of less than 0.1%.

Formic Acid, HCOOH**—46.03** [64-18-6]—Use ACS reagent grade Formic Acid, 88 Percent.

Formic Acid, 96 Percent, HCOOH**—46.03** [64-18-6]—Use ACS reagent grade Formic Acid, 96 Percent.

Formic Acid, 98 Percent, HCOOH**—46.03** [64-18-6]—Use a suitable grade with a content of NLT 98%.

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Formic Acid, Anhydrous—Use ACS reagent grade Formic Acid, 96 Percent.

Fuchsin, Basic *(Basic Red 9, Parafuchsin Hydrochloride)*, $C_{19}H_{17}N_3 \cdot HCl$—**323.82** [569-61-9]—Use a suitable grade.

Fuller's Earth, Chromatographic—*(Very Fine and Moderately Coarse)*—Gray or grayish-white powder or granules consisting mainly of hydrous aluminum-magnesium silicate.
Powder Fineness: see *Powder Fineness* ⟨811⟩.
Soluble Matter: Twenty g, treated with 50 mL of cold water and filtered, yields not more than 60 mg of residue upon evaporation of the filtrate (0.3%). A second 20-g portion, treated with 50 mL of cold alcohol and filtered, yields not more than 14 mg upon evaporation of the filtrate (0.07%).
Loss on Drying ⟨731⟩: Dry it at 105° for 6 hours: it loses between 7.0% and 10.0% of its weight.
[NOTE—Adjust the water content, if necessary, by drying in vacuum at room temperature, restoring the water required, and equilibrating by shaking for 2 hours.]

Fuming Nitric Acid—See *Nitric Acid, Fuming*.

Fuming Sulfuric Acid—See *Sulfuric Acid, Fuming*.

Furfural *(2-Furancarboxyaldehyde; 2-Furaldehyde)*, C_4H_3OCHO—**96.08** [98-01-1]—Use ACS reagent grade.

G Designations—See phases for gas chromatography under *Reagents, Chromatographic Columns*.

Gadolinium (Gd III) Acetate Hydrate, $(CH_3CO_2)_3Gd \cdot xH_2O$—**334.38** [100587-93-7]—White, crystalline, hygroscopic powder. Irritant. Use a suitable grade.

Gadolinium Sulfate, $Gd_2(SO_4)_3 \cdot 8H_2O$—**746.83** [13450-87-8]—Use a suitable grade with a content of NLT 99.9%.
[NOTE—A suitable grade is available as catalog number 41111 from www.gfschemicals.com.]

Gelatin—Use *Gelatin* (*NF* monograph).

Geneticin *(G418; O-2-Amino-2,7-dideoxy- D -glycero-alpha- D -glucoheptopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-beta- L -arabinopyranosyl-(1-6)- D -streptamine)*, $C_{20}H_{40}N_4O_{10}$—**496.55** [49863-47-0]—Use a suitable grade, cell culture tested.

Girard Reagent T—See *Trimethylacethydrazide Ammonium Chloride*.

Gitoxin, $C_{41}H_{64}O_{14}$—**780.94** [4562-36-1]—White, crystalline powder. Practically insoluble in water, in chloroform, and in ether; slightly soluble in pyridine and in diluted alcohol. Melts at about 250°, with decomposition.
Specific Rotation ⟨781⟩: between +3.8° and +4.8°, determined in a solution of pyridine containing 10 mg per mL, with the use of a mercury light at 546.1 nm; between +21° and +25°, determined in a solution of equal parts of chloroform and methanol containing 5 mg per mL, with the use of sodium light.
Suitability: Dissolve 10 mg each of *USP Digitoxin RS*, previously dried, *USP Digoxin RS*, previously dried, and gitoxin, respectively, in separate 5-mL portions of a mixture of 2 parts of chloroform and 1 part of methanol, and dilute each with additional solvent mixture to 10 mL. Then proceed as directed in the *Identification test* under *Digoxin*. The chromatogram of gitoxin shows one fluorescent spot, located between the digoxin and digitoxin spots.

Glacial Acetic Acid—See *Acetic Acid, Glacial*.

Glass Wool—Fine threads of glass.
Acid-Soluble Substances: Boil 1 g for 30 minutes with 30 mL of diluted hydrochloric acid, and filter. Evaporate the filtrate, and dry the residue at 105° to constant weight: the residue weighs not more than 5 mg (0.5%).
Heavy Metals: Boil 2 g with a mixture of 25 mL each of diluted nitric acid and water for 5 minutes, and filter. Evaporate one-half of the filtrate to dryness, dissolve the residue in 10 mL of water to which 3 drops of hydrochloric acid have been added, filter if necessary, and add an equal volume of hydrogen sulfide TS to the filtrate: no darkening is produced.

Add the following:

▲**Glucoamylase** *(Glucan, 1-4-α-glucosidase; Amyloglucosidase; γ-Amylase)*, [9032-08-0]—Use a suitable grade.▲ (USP 1-May-2019)

D-Gluconic Acid, 50 Percent in Water, $C_6H_{12}O_7$—**196.16** [526-95-4]—Pale yellow liquid.
- **ASSAY:** Dilute about 200 mg of the solution, accurately weighed, with 30 mL of water. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 19.62 mg of $C_6H_{12}O_7$. Not less than 49.0% is found.
- **REFRACTIVE INDEX** ⟨831⟩: between 1.4160 and 1.4180 at 20°.
- **SPECIFIC ROTATION** ⟨781⟩: between +9.9° and +11.9°, determined as is, at 20°.

Glucose, $C_6H_{12}O_6$—**180.2** [50-99-7]—Use a suitable grade. A white, crystalline powder. Freely soluble in water; sparingly soluble in alcohol.

Add the following:

▲**Glucose Oxidase** *(β-D-Glucose:oxygen 1-oxidoreductase)*, [9001-37-0]—Use a suitable grade Type II from *Aspergillus niger*.▲ (USP 1-May-2019)

D-Glucuronolactone, $C_6H_8O_6$—**176.12** [32449-92-6]—Use a suitable grade.

Glutamic Acid, $C_5H_9NO_4$—**147.13** [56-86-0]—Use a suitable grade.

L-Glutamic Acid, $C_5H_9NO_4$—**147.1** [56-86-0]—White powder or white powder with a faint yellow cast. Use a suitable grade.

L-Glutamine, $C_5H_{10}N_2O_3$—**146.15** [56-85-9]—White, crystalline powder. Use a suitable grade.

Glycerin *(Glycerol)* [56-81-5]—Use ACS reagent grade Glycerol.

Glycerol—See *Glycerin*.

Add the following:

▲**Glyceryl Monostearate**—Use *Glyceryl Monostearate (FCC* monograph).▲ (USP 1-May-2020)

Glycolic Acid, $C_2H_4O_3$—**76.05** [79-14-1]—White crystalline powder or chunks.

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Assay

Silylating reagent: Pyridine, hexamethyldisilazane, chlorotrimethylsilane (9:3:1)

Sample preparation: Weigh approximately 25 mg (about 3 drops) of the sample into a test tube. Add 2 mL of the *Silylating reagent* and cap the test tube. Mix well and allow to incubate at room temperature for a minimum of 2 h. A white precipitate of ammonium chloride will form. Centrifuge and use the clear supernatant for injection.

Procedure: Inject an appropriate volume of the *Sample preparation* into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2. The injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; the column temperature is maintained at 100° and programmed to rise 10° per min to 250°. The area of the peak corresponding to $C_2H_4O_3$ is NLT 98.5% of the total peak area.

Add the following:

▲Glyoxal Solution, $C_2H_2O_2$**—58.04** [107-22-2]—Use a suitable grade with a content of 38.0%–42.0%.▲ (USP 1-May-2019)

Gold Chloride *(Chlorauric Acid),* $HAuCl_4 \cdot 3H_2O$**—393.83** [16903-35-8]—Use ACS reagent grade.

Guaiacol *(o-Methoxyphenol),* $C_7H_8O_2$**—124.14** [95-05-1]—Colorless to yellowish, refractive liquid. Soluble in about 65 parts of water; soluble in sodium hydroxide solution; miscible with alcohol, with chloroform, with ether, and with glacial acetic acid.

Assay: When examined by gas–liquid chromatography, it shows a purity of not less than 98%. The following conditions have been found suitable for assaying it: a 3-mm × 1.8-m stainless steel column containing liquid phase G16 on 60- to 80-mesh support S1A. Helium is the carrier gas, the injection port temperature is maintained at 180°, the column temperature is maintained at 200°, and the flame-ionization detector is maintained at 280°. The retention time is about 8 minutes.

Refractive Index ⟨831⟩: between 1.5430 and 1.5450, at 20°.

Guaifenesin—Use *Guaifenesin* (USP monograph).

Guanidine Hydrochloride, *(Aminoformamidine Hydrochloride; Aminomethanamidine Hydrochloride),* $CH_5N_3 \cdot HCl$**—95.53** [50-01-1]—White, crystalline powder. Freely soluble in water and in alcohol. Use a suitable grade with a content not less than 99%.

Guanidine Isothiocyanate, $C_2H_6N_4S$**—118.2** [593-84-0]—White powder or colorless crystals. Use a suitable grade.

Guanine Hydrochloride, $C_5H_5N_5O \cdot HCl \cdot H_2O$**—205.60** [635-39-2]—White, crystalline powder. Melts above 250°, with decomposition. Slightly soluble in water and in alcohol; soluble in acidulated water and in sodium hydroxide TS. Its solutions are not precipitated by iodine TS or by mercuric–potassium iodide TS, but form a precipitate with trinitrophenol TS.

Residue on Ignition (Reagent test): negligible, from 100 mg.

Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 10.0% of its weight.

Hematein, $C_{16}H_{12}O_6$**—300.26** [475-25-2]—Prepared from logwood extract or from hematoxylin by treatment with ammonia and exposure to air. Reddish-brown crystals with a yellowish-green metallic luster. Very slightly soluble in water (about 1 in 1700); slightly soluble in alcohol and in ether; insoluble in benzene and in chloroform; freely soluble in diluted ammonia solution to form a solution of dusky purplish-red color and in an aqueous solution of sodium hydroxide (1 in 50), to form a solution of bright red color, viewed in each case through a layer 1 cm in depth. Melts at a temperature above 200° and tends to decompose at 250°.

Hematoxylin *(Hydroxybrasilin),* $C_{16}H_{14}O_6 \cdot 3H_2O$**—356.32** [517-28-2]—A crystalline substance derived from the heartwood of *Haematoxylon campechianum* Linné (Fam. Leguminosae). Colorless to yellow prisms. Very slightly soluble in cold water and in ether; rapidly soluble in hot water and in hot alcohol. When exposed to light, it acquires a red color and yields a yellow solution. Dissolves in ammonia TS and in solutions of alkali hydroxides and carbonates. When dissolved in solutions of the following salts, it develops the colors indicated: in alum solution, a red color; in stannous chloride solution, a rose color; and in solutions of cupric salts, a greenish-gray color. It gradually turns black in potassium dichromate solution. Store hematoxylin and its solutions protected from light and air.

Hemoglobin, Bovine [9008-02-0]—Use a suitable grade.

[NOTE—A suitable grade is available as Bovine Hemoglobin substrate powder from Sigma-Aldrich, www.sigma-aldrich.com.]

1-Heptadecanol, $C_{17}H_{36}O$**—256.48** [1454-85-9]—Use a suitable grade.

Heptafluorobutyric Acid, $C_4F_7O_2H$**—214.04** [375-22-4]—Use a suitable grade.

Heptakis-(2,6-di- *O*-methyl)-β-cyclodextrin *(2,6-Di-O-methyl-β-cyclodextrin; Dimethyl-β-cyclodextrin),* $C_{56}H_{98}O_{35}$**—1331.36** [51166-71-3]—Use a suitable grade.

***n*-Heptane**—Use *n-Heptane, Chromatographic.*

***n*-Heptane, Chromatographic,** C_7H_{16}**—100.21** [142-82-5]—Clear, colorless, volatile, flammable liquid consisting essentially of C_7H_{16}. Practically insoluble in water; soluble in absolute alcohol. Miscible with ether, with chloroform, with benzene, and with most fixed and volatile oils. Use a suitable grade, chromatographic or HPLC, with a content of not less than 99%.

Heptyl *p*-Hydroxybenzoate *(Heptyl 4-Hydroxybenzoate; N-Heptyl 4-Hydroxybenzoate; Benzoic Acid, 4-Hydroxy-, Heptyl Ester),* $C_{14}H_{20}O_3$**—236.31** [1085-12-7]—Use a suitable grade with a content of NLT 98%.

Hexadecyl Hexadecanoate *(Hexadecyl Palmitate; Cetyl Palmitate),* $C_{32}H_{64}O_2$**—480.85** [540-10-3]—Use a suitable grade.

[NOTE—Suitable grades are available commercially as Hexadecyl Palmitate and Palmitic Acid Palmityl Ester from Sigma-Aldrich, www.sigma-aldrich.com, and Cetyl Palmitate, Catalog number C1203, from Spectrum Chemical Mfg. Corp., www.spectrumchemical.com.]

Hexadecyltrimethylammonium Bromide: See *Cetyltrimethylammonium Bromide.*

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Hexadimethrine Bromide, $(C_{13}H_{30}Br_2N_2)_n$ [28728-55-4]—White to off-white powder, hygroscopic, amorphous polymer. Soluble in water up to 10% to give a colorless to light yellow solution. Use a suitable grade.

Hexamethyldisilazane, $C_6H_{19}NSi_2$—**161.39** [999-97-3]—Clear, colorless liquid.
Assay: When examined by gas–liquid chromatography, it shows a purity of not less than 95%. The following conditions have been found suitable for assaying the article: A 2-mm × 1.8-m glass column packed with phase G3 on support S1. Helium, flowing at a rate of about 40 mL per minute, is the carrier gas; the detector temperature is about 310°; the injection port temperature is about 100°; and the column temperature is programmed to start at 35°, hold for 5 minutes, then rise at a rate of 8° per minute to 200°. A flame-ionization detector is employed.
Residue After Evaporation: Transfer 200 g to a tared dish, and evaporate on a steam bath to dryness. Dry the residue at 105° for 1 hour, cool, and weigh: not more than 0.0025% of residue is found.

Hexamethyleneimine *(Homopiperidine),* $C_6H_{12}NH$—**99.17** [111-49-9]—Colorless to nearly colorless liquid.
Refractive Index ⟨831⟩: between 1.4640 and 1.4660 at 20°.

Hexamethylenetetramine—See Methenamine.

***n*-Hexane,** C_6H_{14}—**86.18** [110-54-3](for use in spectrophotometry)—Use *Hexanes*.

Hexane, Solvent *(Petroleum Benzin; Petroleum Ether, Ligroin)* [8032-32-4]—Clear, volatile liquid. Practically insoluble in water; soluble in absolute alcohol. Miscible with ether, with chloroform, with benzene, and with most fixed and volatile oils.
[**CAUTION**—It is dangerously flammable. Keep it away from flames, and store in tight containers in a cool place.]
Use ACS reagent grade Petroleum Ether.

Hexane, Solvent, Chromatographic: Use ACS HPLC reagent grade.

Hexanes (suitable for use in UV spectrophotometry); usually a mixture of several isomers of hexane (C_6H_{14}), predominantly *n*-hexane, and methylcyclopentane (C_6H_{12})—Use ACS spectrophotometric reagent grade.

Hexanitrodiphenylamine *(Dipicrylamine),* $C_{12}H_5N_7O_{12}$—**439.21** [131-73-7]—Yellow-gold powder or prisms. *Explosive.* Usually contains about 15% of water as a safety precaution. Insoluble in water, in alcohol, in acetone, and in ether; soluble in glacial acetic acid and in alkalies.
Water Determination, *Method I* ⟨921⟩: not more than 16%.

Hexanophenone, $C_{12}H_{16}O$—**176.25** [942-92-7]—Yellow liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-μm layer of phase G3; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{12}H_{16}O$ peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: 1.511 ± 0.002 at 20°.

Hexylamine *(1-Aminohexane),* $C_6H_{15}N$—**101.19** [111-26-2]—Use a suitable grade with a content of not less than 99%.

Histamine Dihydrochloride, $C_5H_9N_3 \cdot 2HCl$—**184.07**—Use *USP Histamine Dihydrochloride RS*.

L-Histidine Hydrochloride Monohydrate, $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$—**209.63** [5934-29-2]—Use a suitable grade.

Horseradish Peroxidase Conjugated to Goat Anti-Mouse IgG: Affinity purified polyclonal antibody to Mouse Immune globulin (IgG) heavy and light chains (whole IgG) produced in Goat and labeled with horseradish peroxidase. Available either as a lyophilized powder or as a solution in a suitable buffer, generally 10 mM sodium phosphate, pH 7.4, containing a suitable preservative, such as 0.01% thimerosal, and an inactive protein(s) to prevent adsorption on the surface of the container. Use a suitable grade. Store at –20°.

Hydrazine Dihydrochloride, $(NH_2)_2 \cdot 2HCl$—**104.97** [5341-61-7]—White powder.
Assay: Dissolve about 34 mg, accurately weighed, in 50 mL of water. Add carefully while stirring, 1 g of sodium bicarbonate. [**CAUTION**—There may be a rapid evolution of carbon dioxide.] Titrate with 0.1 N iodine solution, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N iodine solution is equivalent to 2.63 mg of $(NH_2)_2 \cdot 2HCl$. Not less than 98% is found.

Hydrazine Hydrate, 85% in Water, $(NH_2)_2 \cdot H_2O$—**50.06** [7803-57-8]—Colorless liquid.
Assay: Transfer 600 mg, accurately weighed, to a 100-mL volumetric flask. Dilute with water to volume, and mix. Pipet 10 mL into a suitable beaker, and add 1.0 g of sodium bicarbonate and 50.0 mL of 0.1 N iodine VS. Titrate the excess iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N iodine is equivalent to 1.252 mg of $(NH_2)_2 \cdot H_2O$. Not less than 83% is found.

Hydrazine Sulfate, $(NH_2)_2 \cdot H_2SO_4$—**130.12** [10034-93-2]—Use ACS reagent grade. [**CAUTION**—Great care should be taken in handling hydrazine sulfate because it is a suspected carcinogen.]

Hydrindantin *(2,2′-Dihydroxy-2,2′-biindan-1,1′,3,3′-tet rone),* $C_{18}H_{10}O_6$—**322.27** [5103-42-4]—Sparingly soluble in hot water; soluble in methoxyethanol. When heated above 200°, it becomes reddish brown.
Melting Range ⟨741⟩: between 249° and 254°.

Hydriodic Acid, HI—**127.91** [10034-85-2]—Use ACS reagent grade (containing not less than 47.0% of HI).
[NOTE—For methoxy determination (see *Methoxy Determination* ⟨431⟩), use hydriodic acid ACS reagent grade 55%. Use this grade also for alkoxyl determinations in assays in the individual monographs.]

Hydrobromic Acid, HBr—**80.91** [10035-10-6]—Use ACS reagent grade.

Hydrochloric Acid, HCl—**36.46** [7647-01-0]—Use ACS reagent grade.

Hydrochloric Acid, Diluted (10 percent) [7647-01-0]—Prepare by mixing 226 mL of hydrochloric acid with sufficient water to make 1000 mL.

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25% Hydrochloric Acid—Transfer 56.4 mL of *hydrochloric acid* to a 100-mL volumetric flask containing about 40 mL of water. Cool and dilute with water to volume.

Hydrocodone Diol, $C_{18}H_{23}NO_4$**—317.4**—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number 1584 from www.mallinckrodt.com.]

Hydrofluoric Acid, HF**—20.01** [7664-39-3]—Use ACS reagent grade.

Hydrogen Peroxide, H_2O_2**—34.01** [7722-84-1]—Use ACS reagent grade with an assay content between 29.0% and 32.0%.

Hydrogen Peroxide, 10 Percent, H_2O_2**—34.01**—Dilute 30 mL of 30 percent hydrogen peroxide with water to 100 mL.

Hydrogen Peroxide, 30 Percent, Unstabilized H_2O_2**—34.01** [7722-84-1]—Use ACS reagent grade, with an assay content between 29.0% and 32.0%, without an added stabilizer.

Hydrogen Peroxide, 50 Percent in Water, H_2O_2**—34.01** [7722-84-1]—Use a suitable grade.

Hydrogen Peroxide Solution—Use *Hydrogen Peroxide Topical Solution* (USP monograph).

Hydrogen Sulfide, H_2S**—34.08** [7783-06-4]—Colorless, poisonous gas, heavier than air. Soluble in water. Is generated by treating ferrous sulfide with diluted sulfuric or diluted hydrochloric acid. Other sulfides yielding hydrogen sulfide with diluted acids may be used. Is also available in compressed form in cylinders.

Hydrogen Sulfide Detector Tube: A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator, the latter consisting of a suitable lead salt.
Measuring Range: 1 to 20 ppm.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Hydroquinone, $C_6H_4(OH)_2$**—110.11** [123-31-9]—Fine, colorless or white, needle crystals. Darkens on exposure to air and light. Soluble in water, in alcohol, and in ether.
Assay: Accurately weigh about 250 mg, and dissolve in a mixture of 100 mL of water and 10 mL of 0.1 N sulfuric acid in a 250-mL conical flask. Add 3 drops of a 1 in 100 solution of diphenylamine in sulfuric acid, and titrate with 0.1 N ceric sulfate VS until the solution is red-violet in color. Each mL of 0.1 N ceric sulfate is equivalent to 5.506 mg of $C_6H_4(OH)_2$. Not less than 99% is found.
Melting Range ⟨741⟩: between 172° and 174°.

4-Hydroxy-2-butanone *(2-Hydroxyethyl Methyl Ketone),* $C_4H_8O_2$**—88.11** [590-90-9]—Use a suitable grade with a content of NLT 94%.

4-Hydroxy-4-phenylpiperidine *(4-Phenyl-4-piperidinol),* $C_{11}H_{15}NO$**—177.24** [40807-61-2]—Use a suitable grade with a content of NLT 99%.

Hydroxy Naphthol Blue *(1-(2-Naphtholazo-3,6-disulfonic Acid)-2-naphthol-4-sulfonic Acid, Disodium Salt),* $C_{20}H_{12}N_2O_{11}S_3Na_2$**—598.50** [165660-27-5]—Deposited on crystals of sodium chloride in the concentration of about 1%. Use ACS reagent grade.

3′-Hydroxyacetophenone, $C_8H_8O_2$**—136.15** [121-71-1]—Light brown powder chips and chunks. Melts at about 96°. Sparingly soluble in chloroform, yielding a clear, light yellow solution.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G1; the detector and the injection port temperatures are maintained at 300°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280° and held at that temperature for 10 minutes. The area of the main peak is not less than 97% of the total peak area.

4′-Hydroxyacetophenone, $HOC_6H_4COCH_3$**—136.15** [99-93-4]—Gray powder, melting at about 109°.

***p*-Hydroxybenzoic Acid,** $C_7H_6O_3$**—138.12** [99-96-7]—White crystals.
Assay: Transfer about 700 mg, accurately weighed, to a suitable container, and dissolve in 50 mL of acetone. Add 100 mL of water, mix, and titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 69.06 mg of $C_7H_6O_3$: not less than 97% is found.
Melting Range ⟨741⟩: over a range of 2° that includes 216°.

4-Hydroxybenzoic Acid Isopropyl Ester, $HOC_6H_4COOCH(CH_2)_2$**—180.18** [4191-73-5]—Use a suitable grade.
[NOTE—A suitable grade is available from TCI America, www.tciamerica.com.]
Melting Range ⟨741⟩: between 84° and 87°.

2-Hydroxybenzyl Alcohol, $C_7H_8O_2$**—124.14** [90-01-7]—Off-white flakes. Very soluble in alcohol, in chloroform, and in ether; soluble in 15 parts water and in benzene.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩), equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the $C_7H_8O_2$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 83° and 85°.

4-Hydroxybutane-1-sulfonic Acid *(4-Hydroxy-1-butanesulfonic Acid),* $C_4H_{10}O_4S$**—154.19** [26978-64-3]—Use a suitable grade with a content of NLT 95%. [NOTE—A suitable grade is available as catalog number RM-967-C50 from www.anaxlab.com.]

***N*-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid),** $C_8H_{18}N_2O_4S$**—238.3** [7365-45-9]—Use a suitable grade.

Hydroxylamine Hydrochloride, $NH_2OH \cdot HCl$**—69.49** [5470-11-1]—Use ACS reagent grade.

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5-Hydroxymethylfurfural, $C_6H_6O_3$**—126.11** [67-47-0]—Use a suitable grade with a content of NLT 99.0%.

10β-Hydroxynorandrostenedione *(10β-Hydroxy-19-norandrost-4-ene-3,17-dione),* $C_{18}H_{24}O_3$**—288.38**—Use a suitable grade.

4-(4-Hydroxyphenyl)-2-butanone, $C_{10}H_{12}O_2$**—164.20** [5471-51-2]—White powder.

Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G43; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{10}H_{12}O_2$ peak is not less than 98.5% of the total peak area.

Melting Range ⟨741⟩: between 81° and 87°.

3-Hydroxyphenyldimethylethyl Ammonium Chloride [*Dimethylethyl(3-hydroxyphenyl)ammonium Chloride*]—Use *Edrophonium Chloride.*

D-α-4-Hydroxyphenylglycine, $C_8H_9NO_3$**—167.16** [22818-40-2]—Shiny leaflets. Sparingly soluble in water, in alcohol, in acetone, in ether, in chloroform, in ethyl acetate, in benzene, and in glacial acetic acid; soluble in alkalies and in mineral acids; freely soluble in warm 20% v/v hydrochloric acid.

- **MELTING RANGE** ⟨741⟩: between 220° and 247°, with decomposition.

2′-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole Trihydrochloride Pentahydrate—623.97 [23491-44-3]—Dark yellow to tan powder with a green cast. Use a suitable grade.

Hydroxypropyl-β-cyclodextrin *(Hydroxypropylbetadex),* $C_{42}H_{70}O_{35}(C_3H_6O)_x$ with $x = 7$ molar substitution [128446-35-5]—Use a suitable grade with a substitution degree between 0.40 and 1.50.

Hydroxypropyl Cellulose—Average molecular weight approximately 100,000 [9004-64-2]

Viscosity: Dissolve 5.0 g of Hydroxypropyl Cellulose with 95.0 mL of water by stirring. If necessary, centrifuge the solution to expel any entrapped air bubbles. Determine the viscosity of the solution as directed in *Viscosity—Rotational Methods* ⟨912⟩ at 25 ± 0.1°. The viscosity is between 75 and 150 centipoises.

8-Hydroxyquinoline *(Oxine),* C_9H_7NO**—145.16** [148-24-3]—Use ACS reagent grade 8-Quinolinol.

Hypophosphorous Acid, 50 Percent *(Hypophosphorous Acid),* HPH_2O_2**—66.00** [6303-21-5]—A colorless to faintly yellow liquid. Miscible with water and with alcohol.

- **ASSAY:** Accurately weigh about 4 mL, dilute with 25 mL of water, add methyl red TS, and titrate with 1 N sodium hydroxide VS: each mL of 1 N sodium hydroxide is equivalent to 66.00 mg of HPH_2O_2. Not less than 48% is found.
- **CHLORIDE:** Add 0.2 mL to a mixture of 10 mL of silver nitrate TS and 5 mL of nitric acid, and heat until brown fumes are no longer evolved: any white, insoluble residue remaining is negligible.
- **PHOSPHATE:** Dilute 1 mL with water to 50 mL, render alkaline with ammonia TS, filter if a precipitate is formed, and add to the filtrate 5 mL of magnesia mixture TS: not more than a slight precipitate is formed within 5 minutes.
- **SULFATE** (Reagent test, *Method I*): Dilute 1 mL with water to 50 mL: 20 mL of the solution shows not more than 0.2 mg of SO_4.

Hypoxanthine, $C_5H_4N_4O$**—136.11** [68-94-0]—White to yellowish-white powder. Soluble in 1 N sodium hydroxide. Use a suitable grade.

IgG-Coated Red Cells: Red cells coated with human immunoglobulin (IgG). The reagent must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results.

[NOTE—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Imidazole, $C_3H_4N_2$**—68.08** [288-32-4]—White to light yellow crystals. Freely soluble in water. Use ACS reagent grade.

Iminodiacetic Acid, $C_4H_7NO_4$**—133.10** [142-73-4]—Use a suitable grade with a content of NLT 98.0%.

Indene, C_9H_8**—116.16** [95-13-6]—Colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 10-m capillary column coated with a 1-µm layer of methylsilicone; the injection port temperature is maintained at 200°; the detector temperature is maintained at 300°; the column temperature is maintained at 100° and programmed to rise 10° per minute to 250°. The area of the indene peak is not less than 99% of the total peak area.

Refractive Index ⟨831⟩: between 1.5749 and 1.5769 at 20°.

Indicators—See separate subsection.

Change to read:

Indigo Carmine—Use ▲ACS reagent grade.▲ 2S (USP41)

Indole (*2,3-Benzopyrrole*), C_8H_7N**—117.14** [120-72-9]—Use a suitable grade.

Indole-3-carboxylic Acid, $C_9H_7NO_2$**—161.2** [771-50-6]—Use a suitable grade.

Inosine, $C_{10}H_{12}N_4O_5$**—268.23** [58-63-9]—White, crystalline powder.

Melting Point ⟨741⟩: about 90°.

Inositol *(Hexahydroxycyclohexane),* $C_6H_6(OH)_6$**—180.16** [87-89-8]—Fine, white crystals or a white, crystalline powder; stable in air. Its solutions are neutral to litmus. Optically inactive. One g dissolves in 5.7 mL of water. Slightly soluble in alcohol; insoluble in ether and in chloroform. Store in well-closed containers.

Melting Range ⟨741⟩: between 223° and 226°.

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Loss on Drying ⟨731⟩: Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on Ignition (Reagent test): not more than 0.1%.

Iobenguane Sulfate *(m-Iodobenzylguanidine Hemisulfate Salt)*, $C_8H_{10}IN_3 \cdot ½H_2SO_4$—**324.1**—White powder. Freely soluble in methanol.
Assay: When tested by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture, a developing system consisting of a mixture of butyl alcohol, water, and acetic acid (60:25:15), and examined under short-wavelength UV light, not more than a single impurity spot of not more than 0.5% is observed.

Iodic Acid, HIO_3—**175.91** [7782-68-5]—Use ACS reagent grade.

Iodine, I_2—**253.81** [7553-56-2]—Use ACS reagent grade.

Iodine Monobromide, IBr—**206.81** [7789-33-5]—Black, gray, or blue-purple crystals, crystalline needles, or crystalline chunks.
Assay: Place about 100 mL of acetic acid in a 150-mL beaker. Separately dissolve 2 g of potassium iodide in a minimum volume of water, add this solution to the acetic acid, and mix. Transfer about 200 mg of Iodine Monobromide, accurately weighed, to the beaker containing the potassium iodide and acetic acid mixture, and stir to dissolve. Titrate immediately with 0.1 N sodium thiosulfate VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 20.681 mg of IBr. Not less than 97.5% is found.

Iodine Monochloride, ICl—**162.36** [7790-99-0]—Use ACS reagent grade.

Iodoethane, C_2H_5I—**155.9** [75-03-6]—Use a suitable grade.

***p*-Iodonitrotetrazolium Violet,** *(2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride)*, $C_{19}H_{13}ClIN_5O_2$—**505.70**—Light yellow powder.
- **Assay:** When tested by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of amyl alcohol, formic acid, and water (8:1:1), sprayed with 0.1% sodium thiosulfate solution, and examined under short-wavelength UV light, a single spot is exhibited, with trace impurities.
- **Melting Point** ⟨741⟩: 240°, with decomposition.

Ion-Exchange Resin—An intimate mixture of 4 parts of a strongly acidic cation-exchanger in the hydrogen form (produced by sulfonation of a styrene-divinylbenzene copolymer, representing 8 to 10% divinylbenzene) and 6 parts of a strongly basic anion-exchanger in the hydroxyl form (produced by amination with trimethylamine of a chloromethylated styrene-divinylbenzene copolymer, representing 3 to 5% divinylbenzene).
[Note—A suitable resin is "Amberlite MB-150," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Iron (Powder), Fe—**55.85** [7439-89-6]—Use a suitable grade with a content of NLT 99.9%.

Iron Wire, Fe—**At. Wt. 55.847**—Use a suitable grade.

Isoamyl Alcohol—Use *Amyl Alcohol*.

Isobutyl Acetate, $C_6H_{12}O_2$—**116.16** [110-19-0]—Clear, colorless liquid. Slightly soluble in water. Miscible with alcohol.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G2. The injection port temperature is maintained at 130°; the column temperature is maintained at 30° and programmed to rise 10° per minute to 180° and held there for 10 minutes. The detector temperature is maintained at 300°. The area of the main peak is not less than 99% of the total peak area.
Specific Gravity ⟨841⟩: between 0.863 and 0.868.
Refractive Index ⟨831⟩: between 1.3900 and 1.3920 at 20°.

Isobutyl Alcohol *(2-Methyl-1-propanol)*, $(CH_3)_2CHCH_2OH$—**74.12** [78-83-1]—Use ACS reagent grade.

Add the following:

▲**Isobutyl Chloroformate** *(Chloroformic Acid Isobutyl Ester; IBCF)*, $C_5H_9ClO_2$—**136.58** [543-27-1]—Use a suitable grade with a content of NLT 98%.▲ (USP 1-May-2019)

4-Isobutylacetophenone, $C_{12}H_{16}O$—**176**—Pale yellow liquid. Soluble in chloroform, in glycerols, in alcohols, in ether, and in fatty oils; insoluble in water. Use a suitable grade.
[Note—A suitable grade is available from TCI America, www.tciamerica.com.]

***N*-Isobutylpiperidone,** $C_9H_{17}NO$—**155.24**—Use a suitable grade.

Isoflupredone Acetate *(9-α-Fluoroprednisolone Acetate)*, $C_{23}H_{29}FO_6$—**420.47**—Use *Isoflupredone Acetate* (USP monograph).

L-Isoleucine *(2S,3S)-2-Amino-3-methylpentanoic Acid*, $C_6H_{13}NO_2$—**131.17** [73-32-5]—Use a suitable grade.
[Note—A suitable grade is available as catalog number I2752 from www.sigma-aldrich.com.]

Isomaltotriose
(α-D-Glucosyl-(1-6)-α-D-glucosyl-(1-6)-α-D-glucose), $C_{18}H_{32}O_{16}$—**504.4** [3371-50-4]—White lyophilized powder. Use a suitable grade.

2-Isoniazid *(2-Pyridinecarboxylic acid hydrazide; Picolinic acid hydrazide; 2-Picolinyl hydrazide)*, $C_6H_7N_3O$—**137.14** [1452-63-7]—Use a suitable grade with a content of NLT 98.0%.
[Note—A suitable grade is available as catalog number P0426 from www.tcichemicals.com.]

Isonicotinamide *(Pyridine-4-carboxylic Acid Amide)*, $C_6H_6N_2O$—**122.12** [1453-82-3]—Use a suitable grade with a content of NLT 99%.

Isonicotinic Acid, $C_6H_5NO_2$—**123.11** [55-22-1]—Use a suitable grade.

Isonicotinic Acid Hydrazide—Use *Isoniazid* (USP monograph).

Isooctane—See *2,2,4-Trimethylpentane*.

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Isopropyl Acetate, $C_5H_{10}O_2$**—102.13** [108-21-4]—Use a suitable grade.

Isopropyl Alcohol *(2-Propanol),* $(CH_3)_2CHOH$**—60.10** [67-63-0]—Use ACS reagent grade.

[NOTE—For use in assays and tests involving UV spectrophotometry, use ACS reagent grade Isopropyl Alcohol Suitable for Use in UV Spectrophotometry.]

Isopropyl Alcohol, Dehydrated [67-63-0]—Use Isopropyl Alcohol that previously has been dried by being shaken with a suitable molecular sieve capable of adsorbing water, and filtered.

Isopropyl Ether—See *Diisopropyl Ether.*

Isopropyl Iodide *(2-Iodopropane),* C_3H_7I**—169.99** [75-30-9]—Use a suitable grade.

Isopropyl Myristate, $C_{17}H_{34}O_2$**—270.45** [110-27-0]—Use *Isopropyl Myristate* (NF monograph). For use as a solvent in sterility test procedures, Isopropyl Myristate conforms to the following additional specification:

• **pH OF WATER EXTRACT:** Transfer 100 mL to a 250-mL centrifuge bottle, add 10 mL of twice-distilled water, close the bottle with a suitable closure, and shake vigorously for 60 minutes. Centrifuge the mixture at 1800 rpm for 20 minutes, aspirate the upper (isopropyl myristate) layer, and determine the pH of the residual water layer: the pH is not less than 6.5.

Isopropyl Myristate not conforming to the test for *pH of Water Extract* may be rendered suitable for use in sterility test procedures as follows:

Using a 20-mm × 20-cm glass column, add activated alumina, and tamp down to a height of 15 cm. Pass 500 mL of the isopropyl myristate through the column, using a slight positive pressure to maintain an even flow, and use the eluate collected directly in the sterility test procedure.

Isopropyl Salicylate, $C_6H_4OHCOOCH(CH_3)_2$**—180.20**—Colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2-mm × 1.8-m glass column packed with 7% phase G2 on support S1A; the injection port temperature is maintained at 250°; the detector temperature is maintained at 310°; the column temperature is maintained at 50° and programmed to rise 10° per minute to 250°. The area of the main peak is not less than 97% of the total peak area.

Isopropyl Stearate [112-10-7]—A mixture containing isopropyl myristate, isopropyl palmitate, and isopropyl stearate.

[NOTE—A suitable grade is available from www.intlab.org or www.bocsci.com.]

Isopropylamine *(2-Aminopropane),* $C_3H_7NH_2$**—59.11** [75-31-0]—Clear, colorless, flammable liquid. Miscible with water, with alcohol, and with ether.

Assay: Transfer about 0.2 g, accurately weighed, to a suitable container, add 50 mL of water, and mix. Titrate with 0.1 N hydrochloric acid VS, using a mixture of bromocresol green TS and methyl red TS (5:1) as indicator. Each mL of 0.1 N hydrochloric acid is equivalent to 59.11 mg of C_3H_9N. Not less than 98% is found.

Boiling Range (Reagent test): Not less than 95% distills between 31° and 33°.

Refractive Index ⟨831⟩: between 1.3743 and 1.3753, at 20°.

Isorhamnetin, $C_{16}H_{12}O_7$**—316.27** [418-19-3]—Yellow powder.

Melting Point ⟨741⟩: greater than 300°.

Isostearic Acid *(16-Methylheptadecanoic Acid),* $C_{18}H_{36}O_2$ — **284.48** [2724-58-5]—Use a suitable grade with a content of NLT 97%.

Isovaleric Acid *(3-Methylbutanoic Acid, Isovalerianic Acid, Isopropylacetic Acid),* $C_5H_{10}O_2$**—102.13** [503-74-2]—Use a suitable grade.

Kaempferol, $C_{15}H_{10}O_6$**—286.24** [520-18-3]—Light yellow to yellow powder. It is a clear, bright yellow solution in alcohol.

Kerosene [8008-20-6]—A mixture of hydrocarbons, chiefly of the methane series. A clear, colorless liquid. Specific gravity: about 0.80. Distills between 180° and 300°.

L Designations—See packings for high-pressure liquid chromatography under *Reagents, Chromatographic Columns.*

Lactic Acid—Use *Lactic Acid* (USP monograph).

β-Lactoglobulin [9045-23-2]—Use β-Lactoglobulin from bovine milk with a content of NLT 90% (by polyacrylamide electrophoresis).

β-Lactoglobulin A—18,363 Da by calculation [50863-92-8]—Use a suitable grade from bovine milk with a content of NLT 90% (by electrophoresis).

Lactose, $C_{12}H_{22}O_{11} \cdot H_2O$**—360.31** [64-42-3]—Use ACS reagent grade.

Alpha Lactose Monohydrate *(α-D-Lactose Monohydrate),* $C_{12}H_{22}O_{11} \cdot H_2O$**—360.31**—White powder. The β-D-lactose content is less than 3%.

Assay: Inject an appropriate derivatized specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 280°; the column temperature is maintained at 230° and programmed to rise 4° per minute to 280°. The area of the $C_{12}H_{22}O_{11} \cdot H_2O$ peak is not less than 97% of the total peak area.

Beta Lactose *(β-D-Lactose),* $C_{12}H_{22}O_{11}$**—342.30**—White to faint yellow powder. The α-D-lactose content is not more than 35%.

Assay: Inject an appropriate derivatized specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G43; the injection port temperature is maintained at 250°; the detector temperature is maintained at 250°; the column temperature is maintained at 20° and programmed to rise 8° per minute to 280°. The area of the $C_{12}H_{22}O_{11}$ peak is not less than 99% of the total peak area.

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Lanthanum Alizarin Complexan Mixture—Dissolve 47.9 mg of alizarin complexone in a mixture of 0.1 mL of ammonium hydroxide, 1.0 mL of 20% (w/v) ammonium acetate, and a few mL of water. Filter the solution into a 200-mL volumetric flask containing 8.2 g of anhydrous sodium acetate, 6.0 mL of glacial acetic acid, and enough water to dissolve the solids. Wash the filter with a small volume of water. Slowly add 100 mL of acetone while swirling. Dissolve 140.45 mg of lanthanum nitrate hexahydrate in 2.5 mL of 2 M hydrochloric acid, warming gently to aid dissolution. Transfer this solution to the 200-mL volumetric flask containing the aqueous acetone solution. Dilute with water to volume. Mix well, and readjust the volume after 30 min. This reagent is stable for one week.

Lanthanum Chloride, $LaCl_3 \cdot (6–7)H_2O$ [10025-84-0]—This reagent is available in degrees of hydration ranging from 6 to 7 molecules of water. Use ACS reagent grade.

Lanthanum Nitrate Hexahydrate, $La(NO_3)_3 \cdot 6H_2O$—**433.01** [10277-43-7]—Use a suitable grade with a content of NLT 99.9%.

Lanthanum Oxide, La_2O_3—**325.82** [1312-81-8]—An almost white, amorphous powder, practically insoluble in water. It dissolves in dilute solutions of mineral acids and absorbs atmospheric carbon dioxide. Use Atomic Absorption Spectroscopy grade.

Lathosterol *(5α-Cholest-7-en-3β-ol),* $C_{27}H_{46}O$—**386.65** [80-99-9]—Use a suitable grade with a content of NLT 95%.

[NOTE—A suitable grade is available as catalog number 700069 from www.avantilipids.com or as catalog number C3652 from www.sigma-aldrich.com.]

Lauryl Dimethyl Amine Oxide *(N,N-Dimethyldodecyl amine-N-oxide),* $C_{14}H_{31}NO$—**229.41** [1643-20-5]—Use a suitable grade.

[NOTE—A suitable grade is available from Fluka, catalog number 40234, www.sigma-aldrich.com.]

Lead Acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$—**379.33** [6080-56-4]—Use ACS reagent grade.

Lead Acetate Paper: Immerse filter paper weighing about 80 g per square meter in a mixture of diluted acetic acid and lead acetate TS (1:10). Remove, dry, and cut into 15-mm × 40-mm strips.

Lead Monoxide *(Litharge),* PbO—**223.20** [1317-36-8]—Heavy, yellowish or reddish-yellow powder. Insoluble in water and in alcohol; soluble in acetic acid, in diluted nitric acid, and in warm solutions of the fixed alkali hydroxides.

Assay: Accurately weigh about 300 mg, freshly ignited in a muffle furnace at 600 ± 50°, and dissolve it by warming with 10 mL of water and 1 mL of glacial acetic acid. Dilute with 75 mL of water, heat to boiling, add 50.0 mL of 0.1 N potassium dichromate VS, and boil for 2 to 3 minutes. Cool, transfer to a 200-mL volumetric flask with the aid of water, dilute with water to volume, mix, and allow to settle. Withdraw 100.0 mL of the clear liquid, and transfer to a glass-stoppered flask. Add 10 mL of diluted sulfuric acid and 1 g of potassium iodide, insert the stopper, mix gently, and allow to stand for 10 minutes. Then titrate the liberated iodine, representing the excess of dichromate, with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached: each mL of 0.1 N potassium dichromate is equivalent to 7.440 mg of PbO. Not less than 98% is found.

Insoluble in Acetic Acid: Dissolve 2 g in 30 mL of dilute glacial acetic acid (1 in 2), boil gently for 5 minutes, filter, wash the residue with diluted acetic acid, and dry at 105° for 2 hours: the residue weighs not more than 10 mg (0.5%).

Substances Not Precipitated By Hydrogen Sulfide: Completely precipitate the lead from the filtrate obtained in the test for *Insoluble in Acetic Acid* by passing hydrogen sulfide into it, filter, and wash the precipitate with 20 mL of water. To one-half of the mixed filtrate and washings add 5 drops of sulfuric acid, evaporate to dryness, and ignite at 800 ± 25° for 15 minutes: the residue weighs not more than 5 mg (0.5%).

Volatile Substances: Accurately weigh about 5 g, and heat strongly in a covered porcelain crucible: it loses not more than 2.0% of its weight.

Lead Nitrate, $Pb(NO_3)_2$—**331.21** [10099-74-8]—Use ACS reagent grade.

Lead Perchlorate, $Pb(ClO_4)_2 \cdot 3H_2O$—**460.15**—Use ACS reagent grade.

Lead Tetraacetate, $C_8H_{12}O_8Pb$—**443.38** [546-67-8]—Colorless to pinkish crystals. Soluble in hot glacial acetic acid, in chloroform, in nitrobenzene, and in tetrachloroethane. Hydrolyzed by water, producing brown lead dioxide and acetic acid. Unstable in air.

Melting Range ⟨741⟩: between 175° and 180°.

Add the following:

▲**Lecithin** *(L-α-Lecithin; L-α-Phosphatidylcholine, Hydrogenated),* [8002-43-5]—Use a suitable grade with a content of NLT 98%. ▲ (USP 1-May-2020)

(*R*)-(+)-Limonene *((+)-p-Mentha-1,8-diene, (+)-Carvene, (R)-4-Isopropenyl-1-methyl-1-cyclohexene),* $C_{10}H_{16}$—**136.23** [5989-27-5]—Use a suitable grade with a GC chromatographic purity of NLT 99% (sum of enantiomers).

Linalool *(3,7-Dimethyl-1,6-octadien-3-ol),* $C_{10}H_{18}O$—**154.25** [78-70-6]—Use a suitable grade.

Linoleic Acid, $C_{18}H_{32}O_2$—**280.4** [60-33-3]—Clear, colorless liquid. Use a suitable grade.

Linolenic Acid *((Z,Z,Z)-9,12,15-Octadecatrienoic Acid),* $C_{18}H_{30}O_2$—**278.43** [463-40-1]—Use a suitable grade with a content of NLT 99%.

α-Lipoic Acid, $C_8H_{14}O_2S_2$—**206.3** [1077-28-7]—Yellow powder. Use a suitable grade.

Liquid Petrolatum—Use *Mineral Oil* (USP monograph).

Lithium, Li—**6.94** [7439-93-2]—Use a suitable grade (ribbon, granular, or wire) with a content of NLT 99.9%.

Lithium Chloride, LiCl—**42.39** [7447-41-8]—Use ACS reagent grade.

Lithium Hydroxide, $LiOH \cdot H_2O$—**41.96** [1310-65-2]—Use ACS reagent grade.

Lithium Metaborate, $LiBO_2$—**49.75** [13453-69-5]—Use ACS reagent grade.

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Lithium Nitrate, $LiNO_3$**—68.95** [7790-69-4]—Colorless crystals. Use a suitable grade labeled to contain not less than 97.0%.

Lithium Perchlorate, $LiClO_4$**—106.39** [7791-03-9]—Use ACS reagent grade.

Lithium Sulfate, $Li_2SO_4 \cdot H_2O$**—127.96** [10102-25-7]—Use ACS reagent grade.

Lithocholic Acid, $C_{24}H_{40}O_3$**—376.57** [434-13-9]—White powder. Use a suitable grade with a content of NLT 97% by titration.

Litmus [1393-92-6]—A blue pigment prepared from various species of *Rocella* DeCandolle, *Lecanora* Acharius, or other lichens (Fam. Parmeliaceae).

Description: Cubes, masses, fragments, or granules, of an indigo blue or deep violet color. Has the combined odor of indigo and violets, and tinges the saliva a deep blue. The indicator substances it contains are soluble in water and less soluble or insoluble in alcohol.

Ash: It yields not more than 60.0% of ash.

(*R*)-(–)-Littorine Hydrochloride *(R(–)-3α-(2-Hydroxy-3-phenylpropionyloxy)-tropane Hydrochloride),* $C_{17}H_{23}NO_3 \cdot HCl$**—325.83** [21956-47-8](free base)—Use a suitable grade. Store at 4°.

[NOTE—A suitable grade is available as catalog number 489377 from www.scbt.com.]

Locust Bean Gum—A gum obtained from the ground endosperms of *Ceratonia siliqua* Linné Taub. (Fam. Leguminosae). Use *Locust Bean Gum* (FCC monograph).

L-Lysine *(2,6-Diaminohexanoic Acid),* $C_6H_{14}N_2O_2$**—146.19** [56-87-1]—Crystalline needles or hexagonal plates. Soluble in water; very slightly soluble in alcohol; insoluble in ether.

- **SPECIFIC ROTATION** ⟨781⟩: between +25.5° and +26.0°.
  Test Solution: 20 mg per mL, in dilute hydrochloric acid (1 in 2).
- **NITROGEN DETERMINATION** ⟨461⟩, *Method I*: between 18.88% and 19.44% of N is found, corresponding to not less than 98.5% of $C_6H_{14}N_2O_2$, the test specimen previously having been dried at 105° for 2 hours.

Add the following:

▲**Lysolecithin** *(L-α-Lysolecithin; L-α-Lysophosphatidylcholine),* [9008-30-4]—Use a suitable grade from egg yolk with a content of NLT 99%.▲ (USP 1-May-2020)

Magnesium, Mg**—24.305** [7439-95-4]—Silvery metal in ribbon form. Reacts slowly with water at room temperature. Dissolves readily in dilute acids with the liberation of hydrogen.

Assay: Transfer 1 g, accurately weighed, to a 250-mL volumetric flask, and dissolve in a mixture of 15 mL of hydrochloric acid and 85 mL of water. When solution is complete, dilute with water to volume, and mix. Pipet 25 mL of the dilution into a 400-mL beaker, dilute with water to 250 mL, add 20 mL of ammonia–ammonium chloride TS and a few mg of eriochrome black T trituration, and titrate with 0.1 M edetate disodium VS to a blue endpoint. Each mL of 0.1 M edetate disodium VS is equivalent to 2.430 mg of Mg. Not less than 99% is found.

Magnesium Acetate, $Mg(C_2H_3O_2)_2 \cdot 4H_2O$**—214.45** [142-72-3]—Use ACS reagent grade.

Add the following:

▲**Magnesium Carbonate**—Use *Magnesium Carbonate (USP* monograph).▲ 2S (USP41)

Magnesium Chloride, $MgCl_2 \cdot 6H_2O$**—203.30** [7791-18-6]—Use ACS reagent grade.

Magnesium Nitrate, $Mg(NO_3)_2 \cdot 6H_2O$**—256.41** [13446-18-9]—Use ACS reagent grade.

Magnesium Oxide, MgO**—40.30** [1309-48-4]—Use ACS reagent grade.

Magnesium Oxide, Chromatographic—Use a suitable grade.

Magnesium Perchlorate, Anhydrous, $Mg(ClO_4)_2$**—223.21** [10034-81-8]—Use ACS reagent grade.

Magnesium Silicate, Activated—Use a suitable grade.

Magnesium Silicate, Chromatographic—Extremely white, hard, powdered (60- to 100-mesh) magnesia–silica gel. Suitable for use as an adsorbant in column chromatography.

Magnesium Sulfate, $MgSO_4 \cdot 7H_2O$**—246.48** [10034-99-8]—Use ACS reagent grade.

Magnesium Sulfate, Anhydrous, $MgSO_4$**—120.37** [7487-88-9]—Anhydrous Magnesium Sulfate may be prepared as follows. Place a suitable quantity of magnesium sulfate (see above), preferably powdered, in a shallow vessel, and expose to a temperature of about 80° for several hours with occasional stirring. Then heat at 275° to 300° until the weight is practically constant. Transfer the product while still warm to tight containers, as the anhydrous salt is very hygroscopic.

Malachite Green G—See *Brilliant Green.*

Maleic Acid, $C_4H_4O_4$**—116.07** [110-16-7]—White, crystalline powder. Soluble in 1.5 parts of water, in 2 parts of alcohol, and in 12 parts of ether.

Assay: Dissolve about 2 g, accurately weighed, in 100 mL of water and titrate with 1 N sodium hydroxide VS, using phenolphthalein TS as the indicator. Each mL of 1 N sodium hydroxide is equivalent to 58.04 mg of $C_4H_4O_4$: not less than 99% of $C_4H_4O_4$, calculated on the dried basis, is found.

Loss on Drying: Dry it in vacuum over phosphorus pentoxide for 2 hours: it loses not more than 1.5% of its weight.

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

Malonic Acid *(Propanedioic Acid),* $C_3H_4O_4$**—104.06** [141-82-2]—Use a suitable grade with a content of NLT 98%.

Maltotriose, $C_{18}H_{32}O_{16}$**—504.44** [1109-28-0]—Use a suitable grade with a content of not less than 95%.

Manganese, Mn**—54.94** [7439-96-5]—Powder, use a suitable grade with a content of NLT 99.99%.

Add the following:

▲**Manganese Sulfate** *(Manganese Sulfate Monohydrate; Manganese (II) Sulfate Monohydrate),* $MnSO_4 \cdot H_2O$**—169.01** [10034-96-5]—Use ACS reagent-grade manganese sulfate monohydrate.▲ (USP 1-May-2020)

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Add the following:

▲**Manganese Chloride Tetrahydrate** *[Manganese (II) Chloride Tetrahydrate; Manganous Chloride Tetrahydrate],* $MnCl_2 \cdot 4H_2O$—**197.90** [13446-34-9]—Use ACS reagent grade. ▲ (USP 1-May-2020)

Manganese Dioxide: see *Manganese Dioxide, Activated.*

Manganese Dioxide, Activated *(Manganese (IV) Oxide, Activated),* MnO_2—**86.94** [1313-13-9]—Use a suitable grade.

D-Mannitol—Use Mannitol USP.

Marfey's Reagent *[N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide, FDAA],* $C_9H_9FN_4O_5$—**272.19** [95713-52-3]—Use a suitable grade with a content of NLT 99.0%.

Medium-Chain Triglycerides *(Caprylic/Capric Triglycerides, Glyceryl Tricaprylate/Caprate)* [438544-49-1]—Use a suitable grade containing NLT 95% of saturated fatty acids with 8 and 10 carbon atoms.

[NOTE—A suitable grade is available as Captex 300 low C6 from www.abiteccorp.com.]

Melamine *(2,4,6-Triamino-1,3,5-triazine),* $C_3H_6N_6$—**126.1** [108-78-1]—Use a suitable grade.

2-Mercaptoethanol *(β-Mercaptoethanol),* C_2H_6OS—**78.13** [60-24-2]—Use a suitable grade.

3-Mercaptopropionic Acid $C_3H_6O_2S$—**106.14** [107-96-0]—Use a suitable grade with a content of NLT 99%.

Mercuric Acetate, $Hg(C_2H_3O_2)_2$—**318.68** [1600-27-7]—Use ACS reagent grade.

Mercuric Bromide, $HgBr_2$—**360.40** [7789-47-1]—Use ACS reagent grade.

Mercuric Chloride, $HgCl_2$—**271.50** [7487-94-7]—Use ACS reagent grade.

Mercuric Iodide, Red, HgI_2—**454.40** [7774-29-0]—Use ACS reagent grade.

Mercuric Nitrate, $Hg(NO_3)_2 \cdot xH_2O$—**342.62** [10045-94-0]—Use ACS reagent grade. This reagent is available as either the mono- or dihydrate.

Mercuric Oxide, Yellow, HgO—**216.59** [21908-53-2]—Use ACS reagent grade.

Mercuric Sulfate, $HgSO_4$—**296.65** [7783-35-9]—Use ACS reagent grade.

Mercuric Thiocyanate, $Hg(SCN)_2$—**316.76** [592-85-8]—White, crystalline powder. Very slightly soluble in water; soluble in solutions of sodium chloride; slightly soluble in alcohol and in ether.

Mercurous Nitrate Dihydrate *[Mercury (I) Nitrate Dihydrate],* $Hg_2(NO_3)_2 \cdot 2H_2O$—**561.22**—Use a suitable grade with a content of NLT 97%.

Mercury, Hg—**At. Wt. 200.59** [7439-97-6]—Use ACS reagent grade.

Mesityl Oxide, $C_6H_{10}O$—**98.14** [141-79-7]—Colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 150°; the detector temperature is maintained at 300°; the column temperature is maintained at 50° and programmed to rise 10° per minute to 200°. The area of the $C_6H_{10}O$ peak is not less than 98% of the total peak area.

Refractive Index ⟨831⟩: between 1.443 and 1.447 at 20°.

Metanil Yellow ***(Acid Yellow 36; 3-(4-Anilinophenylazo) benzenesulfonic Acid Sodium Salt),*** $C_{18}H_{14}N_3NaO_3S$—**375.38** [587-98-4]—Use a suitable grade with a dye content of NLT 70%.

Metaphenylenediamine Hydrochloride *(Metaphenylenediamine Dihydrochloride),* $C_6H_4(NH_2)_2 \cdot 2HCl$—**181.06**—White or slightly reddish-white, crystalline powder. Easily soluble in water. On exposure to light it acquires a reddish color. Store it protected from light.

Solubility: A solution of 1 g in 200 mL of water is colorless.

Residue on Ignition ⟨Reagent test⟩: Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

[NOTE—Metaphenylenediamine hydrochloride solution can be decolorized by treatment with a small quantity of activated charcoal.]

Metaphosphoric Acid *(Vitreous Sodium Acid Metaphosphate),* HPO_3—**79.98** [37267-86-0]—Use ACS reagent grade.

Methacrylic Acid [79-41-4]—Use a suitable grade.

Methanesulfonic Acid, CH_4O_3S—**96.11** [75-75-2]—Use a suitable grade.

Methanol *(Methyl Alcohol),* CH_3OH—**32.04** [67-56-1]—Use ACS reagent grade.

Methanol, Aldehyde-Free, CH_3OH—**32.04**—Dissolve 25 g of iodine in 1 L of methanol and pour the solution, with constant stirring, into 400 mL of 1 N sodium hydroxide. Add 150 mL of water, and allow to stand for 16 hours. Filter, and boil under a reflux condenser until the odor of iodoform disappears. Distill the solution by fractional distillation. It contains not more than 0.001% of aldehydes and ketones.

Methanol, Anhydrous—Use Methanol.

Methanol, Spectrophotometric—Use ACS reagent grade Methanol Suitable for Use in UV Spectrophotometry.

Methenamine *(Hexamethylenetetramine; Urotropine; Uritone; Hexamine),* $C_6H_{12}N_4$—**140.19** [100-97-0]—Use ACS reagent grade Hexamethylenetetramine.

L-Methionine Sulfoxide *(L-2-Amino-4-(methylsulfinyl)butanoic acid),* $C_5H_{11}NO_3S$—**165.21** [3226-65-1]—Use a suitable grade.

***p*-Methoxyacetophenone** *(4′-Methoxyacetophenone; 4-Acetylanisole),* $C_9H_{10}O_2$—**150.17** [100-06-1]—Use a suitable grade with a content of NLT 99%.

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7-Methoxycoumarin *(Herniarin; Methyl Umbelliferyl Ether)*, $C_{10}H_8O_3$—**176.17** [531-59-9]—Use a suitable grade with a content of not less than 98%.

Methoxyethanol *(Ethylene Glycol Monomethyl Ether; 2-Methoxyethanol)*, $CH_3OCH_2CH_2OH$—**76.09** [109-86-4]—Use ACS reagent grade.

2-Methoxyethanol *(Ethylene Glycol Monomethyl Ether; Methoxyethanol)*, $CH_3OCH_2CH_2OH$—**76.09** [109-86-4]—See *Methoxyethanol*.

5-Methoxy-1*H*-benzimidazole-2-thiol *(5-Methoxy-2-benzimidazolethiol)*, $C_8H_8N_2OS$—**180.23** [37052-78-1]—Use a suitable grade with a content of NLT 99.0%.

5-Methoxy-2-methyl-3-indoleacetic Acid, $C_{12}H_{13}NO_3$—**219.24** [2882-15-7]—Off-white powder.
Assay: Transfer about 110 mg, accurately weighed, to a 100-mL beaker. Add 30 mL of methanol and dissolve by stirring. Add 40 mL of water, and mix. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 21.92 mg of $C_{12}H_{13}NO_3$. Not less than 98% is found.
Melting Range ⟨741⟩: between 161° and 168°, but the range between beginning and end of melting does not exceed 3°.

Methoxyphenylacetic Acid *(α-Methoxyphenylacetic Acid)*, $C_9H_{10}O_3$—**166.2** [7021-09-2]—Use a suitable grade.

Methyl Acetate, $C_3H_6O_2$—**74.08** [74-20-9]—Colorless liquid. Soluble in water. Miscible with alcohol and with ether.
Specific Gravity ⟨841⟩: about 0.933.
Refractive Index ⟨831⟩: between 1.3615 and 1.3625 at 20°.
Boiling Range (Reagent test): Not less than 95% distills between 57° and 58°.

Methyl 4-Aminobenzoate, $C_8H_9NO_2$—**151.16** [619-45-4]—Off-white powder.
Assay: Dissolve about 38 mg, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.12 mg of $C_8H_9NO_2$. Not less than 99.0% is found.
Melting Range ⟨741⟩: between 108° and 110°.

Methyl Arachidate *(Eicosanoic acid, methyl ester)*, $C_{21}H_{42}O_2$—**326.56** [1120-28-1]—Off-white flakes.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal-conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2.0-mm × 1.8-m glass column packed with 5% G2 phase on support S1A; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 230° and programmed to rise 3° per minute to 280°. The area of the $C_2H_{42}O_2$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 46° and 51°.

Methyl Behenate, $C_{23}H_{46}O_2$—**354.61** [929-77-1]—White powder.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2.0-mm × 1.8-m glass column packed with 5% G3 phase on support S1A; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the initial temperature of the oven is 220°, which is held for 2 minutes, and then programmed to rise 3° per minute to attain a final temperature of 270°, which is held for 10 minutes. The area of the $C_{23}H_{46}O_2$ peak is not less than 98% of the total peak area.
Melting Range ⟨741⟩: between 54° and 56°.

Methyl Benzenesulfonate, $C_7H_8O_3S$—**172.20** [80-18-2]—Use a suitable grade.

Methyl Caprate, $C_{11}H_{22}O_2$—**186.29** [110-42-9]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the $C_{11}H_{22}O_2$ peak is not less than 98.5% of the total peak area.

Methyl Caprylate, $C_9H_{18}O_2$—**158.24** [111-11-5]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 230°; the detector temperature is maintained at 300°; the column temperature is maintained at 130° and programmed to rise 10° per minute to 280°. The area of the $C_9H_{18}O_2$ peak is not less than 98.5% of the total peak area.

Methyl Carbamate, $C_2H_5NO_2$—**75.07** [598-55-0]—White crystals. Freely soluble in water.
Melting Range ⟨741⟩: between 54° and 56°.

Methyl Chloroform *(Methylchloroform; 1,1,1-Trichloroethane)*, CH_3CCl_3—**133.40** [71-55-6]—Use ACS reagent grade.

Methyl *cis*-11-Eicosenoate *(cis-11-Eicosenoic acid methyl ester)*, $C_{21}H_{40}O_2$—**324.54** [2390-09-2]—Use a suitable grade with a content of NLT 98%.

Methyl Erucate, $C_{23}H_{44}O_2$—**352.59** [1120-34-9]—Colorless liquid.

Methyl Ethyl Ketone, $CH_3COC_2H_5$—**72.11** [78-93-3]—Use ACS reagent grade 2-butanone.

Methyl Green *(Methyl Green Zinc Chloride Double Salt; Ethyl Green Zinc Chloride Double Salt; C.I. 42590)*, $C_{27}H_{35}Cl_2N_3 \cdot ZnCl_2$—**608.78** [7114-03-6]—Use a suitable grade for microscopy.

Methyl Heptadecanoate, $C_{18}H_{36}O_2$—**284.48** [1731-92-6]—White, crystalline flakes.

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Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G8; the injection port temperature is maintained at 220°; the detector temperature is maintained at 220°; the column temperature is maintained at 180° and programmed to rise 4° per minute to 220°. The area of the $C_{18}H_{36}O_2$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 31° and 32°.

Methyl Hexanoate *(Caproic Acid Methyl Ester; Methyl Caproate)*, $C_7H_{14}O_2$—**130.18** [106-70-7]—Use a suitable grade with a content of NLT 99%.
[NOTE—A suitable grade is available as catalog 259942 from www.sigma-aldrich.com.]

Methyl Iodide *(Iodomethane)*, CH_3I—**141.94** [74-88-4]—Colorless, heavy, transparent liquid. Slightly soluble in water. Miscible with alcohol, with ether, and with solvent hexane. Turns brown on exposure to light as a result of liberation of iodine. Use a suitable grade with a content of not less than 99%.

Methyl Isobutyl Ketone—See 4-Methyl-2-pentanone.

Methyl Isobutyrate, $C_5H_{10}O_2$—**102.13** [547-63-7]— Use a suitable grade with a content of NLT 99%.

Methyl 12-Ketostearate *(Methyl 12-oxooctadecanoate)*, $C_{19}H_{36}O_3$—**312.49** [2380-27-0]—Use a suitable grade with a content of NLT 97%. [NOTE—A suitable grade is available as catalog number sc-235651 from www.scbt.com or as catalog number M5306 from www.usbio.net.]

Methyl Laurate, $C_{13}H_{26}O_2$—**214.34** [110-82-0]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{13}H_{26}O_2$ peak is not less than 99.45% of the total peak area.

Methyl Lignocerate, $C_{25}H_{50}O_2$—**382.66** [2442-49-1]—White crystals.

Methyl Linoleate, $C_{19}H_{34}O_2$—**294.47** [112-63-0]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 200° and programmed to rise 10° per minute to 300°. The area of the $C_{19}H_{34}O_2$ peak is not less than 99% of the total peak area.

Methyl Linolenate, $C_{19}H_{32}O_2$—**292.46** [301-00-8]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase 14% cyanopropylphenyl-86% dimethylpolysiloxane; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{19}H_{32}O_2$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.469 and 1.473 at 20°.

Methyl Methacrylate [80-62-6]—Use a suitable grade.

Methyl Myristate, $C_{15}H_{30}O_2$—**242.40** [124-10-7]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 200° and programmed to rise 10° per minute to 300°. The area of the $C_{15}H_{30}O_2$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.434 and 1.438 at 20°.

Methyl Oleate, $C_{19}H_{36}O_2$—**296.49** [112-62-9]—Colorless liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 230° and programmed to rise 10° per minute to 280°. The area of the $C_{19}H_{36}O_2$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: 1.452 at 20°.

Methyl Palmitate, $C_{17}H_{34}O_2$—**270.45** [112-39-0]—White solid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 200° and programmed to rise 10° per minute to 300°. The area of the $C_{17}H_{34}O_2$ peak is not less than 96.5% of the total peak area.

Methyl Palmitoleate *(Methyl cis-9-Hexadecenoate; Palmitoleic Acid Methyl Ester)*, $C_{17}H_{32}O_2$—**268.43** [1120-25-8]—Use a suitable grade with a content of NLT 99%.
[NOTE—A suitable grade is available as catalog number P9667 or 76176 from www.sigma-aldrich.com.]

Delete the following:

▲**Methyl Red** *(2-[4-Dimethylaminophenylazo]benzoic Acid; C. I. Acid Red 2)*, $C_{15}H_{15}N_3O_2$, free acid—**269.30** $C_{15}H_{14}N_3O_2Na$, sodium salt—**291.28** [493-52-7][845-10-3]—Use ACS reagent grade. The free acid is recommended for nonaqueous

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titrations, particularly when an aprotic solvent is used. The sodium salt is recommended for titrations in aqueous media and also for nonaqueous titrations where the medium is an amphiprotic solvent. The hydrochloride salt is recommended for titrations in aqueous media and amphiprotic solvents. ▲ (USP 1-May-2020)

Methyl Stearate, $C_{19}H_{38}O_2$**—298.50** [112-61-8]—Off-white, crystalline solid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; the column temperature is maintained at 200° and programmed to rise 10° per minute to 300°. The area of the $C_{19}H_{38}O_2$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 40° and 42°.

Methyl Sulfoxide [67-68-5]—See Dimethyl Sulfoxide.

Methyl *p*-Toluenesulfonate *(Methyl Toluene-4-sulfonate),* $C_8H_{10}SO_3$**—186.23** [80-48-8]—Use a suitable grade with a content of NLT 98%.

Methyl Yellow *(p-Dimethylaminoazobenzene),* $C_{14}H_{15}N_3$**—225.3** [60-11-7]—Use a suitable grade.

Methylamine, 40 Percent in Water, CH_5N**—31.06** [74-89-5]—Colorless liquid.
Assay: Using a syringe, transfer about 0.5 mL of a well-shaken specimen to 100 mL of water at a point below the surface of the water. Determine the weight of the specimen by weighing the syringe before and after the transfer. Mix, and titrate with 0.5 N hydrochloric acid VS, determining the endpoint potentiometrically, using a silver-silver chloride pH electrode and a calomel reference electrode. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N hydrochloric acid is equivalent to 15.53 mg of CH_5N: between 39.0% and 41.0% is found.
Refractive Index ⟨831⟩: between 1.3680 and 1.3710, at 20°.

Methylamine Hydrochloride, $CH_5N \cdot HCl$**—67.52** [593-51-1]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number M0505 from www.sigma-aldrich.com.]

***p*-Methylaminophenol Sulfate,** $(p\text{-}CH_3NHC_6H_4OH)_2 \cdot H_2SO_4$**—344.38** [55-55-0]—Use ACS reagent grade.

4-Methylbenzophenone, $C_{14}H_{12}O$**—196.25** [134-84-9]—Use a suitable grade.
Melting Range ⟨741⟩: between 56.5° and 57°.

Methylbenzothiazolone Hydrazone Hydrochloride, $C_8H_{10}ClN_3S \cdot H_2O$**—233.7** [38894-11-0] (monohydrate form)—**215.70** (anhydrous form); [149022-15-1] (hydrate form)—An almost white or yellowish, crystalline powder.
Suitability For Determination of Aldehydes: To 2 mL of aldehyde-free methanol add 60 µL of a 1 g per L solution of propionaldehyde in aldehyde-free methanol and 5 mL of a 4 g per L solution of methylbenzothiazolone hydrazone hydrochloride. Mix, and allow to stand for 30 minutes. Prepare a blank, omitting the propionaldehyde solution. Add 25.0 mL of a 2 g per L solution of ferric chloride to the test solution and to the blank, dilute with acetone to 100.0 mL, and mix. The absorbance of the test solution, measured at 660 nm using the blank as compensation liquid, is not less than 0.62. Use a suitable grade with a content of not less than 97%.

(*R*)-(+)-alpha-Methylbenzyl Isocyanate *((R)-(+)-1-Phenylethyl Isocyanate),* C_9H_9NO**—147.17** [33375-06-3]—Use a suitable grade with a content of NLT 99.0%.

(*S*)-(−)-α-Methylbenzyl Isocyanate, C_9H_9NO**—147.18** [14649-03-7]—Use a suitable grade.

Methylene Blue, $C_{16}H_{18}ClN_3S \cdot 3H_2O$**—373.90** [7220-79-3]—Dark green crystals or a crystalline powder, having a bronzelike luster. One g dissolves in about 25 mL of water and in about 65 mL of alcohol. Soluble in chloroform. Use a suitable grade with a dye content of not less than 85%.

Methylene Chloride *(Dichloromethane),* CH_2Cl_2**—84.93** [75-09-2]—Use ACS reagent grade Dichloromethane.

5,5′-Methylenedisalicylic Acid *(3,3′-Methylene-bis[6-hydroxybenzoic Acid]),* $C_{15}H_{12}O_6$**—288.25** [122-25-8]—Use a suitable grade.

3-*O*-Methylestrone, $C_{19}H_{24}O_2$**—284.39**—Use a suitable grade.
[NOTE—Commercially available as catalog number 1883-5 from Research Plus, Inc., P.O. Box 324, Bayonne, NJ 07002, fax number 908-754-2901, Web site: www.researchplus.com.]

1-Methylimidazole *(1-Methyl-1H-imidazole),* $C_4H_6N_2$**—82.10** [616-47-7]—Use a suitable grade with a content of NLT 99%.

2-Methylimidazole *(2-Methyl-1H-imidazole),* $C_4H_6N_2$**—82.10** [693-98-1]—Use a suitable grade with a content of NLT 99%.

2-Methyl-5-nitroimidazole, $C_4H_4N_3O_2$**—127.10** [88052-22-2]—Use a suitable grade.
[NOTE—Available as Catalog No. 13,625-5 from Sigma-Aldrich, www.sigma-aldrich.com.]

***N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide** *(p-Tolylsulfonylmethylnitrosamide),* $C_8H_{10}N_2O_3S$**—214.24**—Light yellow crystals or powder. Insoluble in water; soluble in benzene, in carbon tetrachloride, and in chloroform.
• **MELTING RANGE** ⟨741⟩: between 59° and 63°, but the range between beginning and end of melting does not exceed 2°.

4-Methylpentan-2-ol, $C_6H_{14}O$**—102.2**—Use a suitable grade.

2-Methylpentane *(2-Methyl-pentane; 1,1-Dimethylbutane; Isohexane),* C_6H_{14}**—86.18** [107-83-5]—Use a suitable grade with a content of not less than 99.0%.

3-Methyl-2-pentanone $C_6H_{12}O$**—100.16** [565-61-7]—Use a suitable grade with a content of NLT 99%.

4-Methyl-2-pentanone *(Methyl Isobutyl Ketone),* $(CH_3)_2CHCH_2COCH_3$**—100.16** [108-10-1]—Use ACS reagent grade.

2-Methyl-2-propyl-1,3-propanediol, $C_7H_{16}O_2$**—132.20** [78-26-2]—White crystals, melting at about 58°.

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***N*-Methylpyrrolidine** *(1-Methylpyrrolidine)*, $C_4H_8NCH_3$—**85.15** [120-94-5]—Use a suitable grade.

Mineral Acid—Use *Hydrochloric Acid* or *Sulfuric Acid* (NF monographs).

5,800, 23,700, and 100,000 Molecular Weight (MW) Pullulan Standards (a commercial Pullulan Standard set contains standards having several molecular weights: 5,800; 12,000; 24,000; 48,000; 100,000; 186,000; 380,000; and 750,000) [9057-02-7]—Use a suitable grade. Each individual Pullulan Standard with a different molecular weight, such as 5,800, 24,000, or 100,000, is equivalently used.
[NOTE—The standard set is available from Sigma-Aldrich (www.sigma-aldrich.com) and Waters (www.waters.com).]

Molybdenum, Mo—**95.94** [7439-98-7]—Wire. Use a suitable grade with a content of NLT 99.9%.

Molybdic Acid *(85 Percent Molybdic Acid)* [7782-91-4]—Use ACS reagent grade.

Monobasic Potassium Phosphate—See *Potassium Phosphate, Monobasic.*

Monobasic Sodium Phosphate—See *Sodium Phosphate, Monobasic.*

Monochloroacetic Acid ***(Chloroacetic Acid, Chloroethanoic Acid)***, $CH_2ClCOOH$—**94.50** [79-11-8]—Use ACS reagent grade.

1-Monodecanoyl-rac-glycerol *(Glycerol 1-decanoate; Capric acid α-monoglyceride; (2RS)-2,3-Dihydroxypropyl decanoate; α-Monocaprin)*, $C_{13}H_{26}O_4$—**246.34** [2277-23-8]—Use a suitable grade with a content of NLT 99%.

Monoethanolamine ***(2-Aminoethanol)***, C_2H_7NO—**61.08**—Use ACS reagent grade.

1-Monooctanoyl-rac-glycerol *(Glycerol 1-octanoate; 1-Capryloyl-rac-glycerol; (2RS)-2,3-Dihydroxypropyl octonoate; α-Monocaprylin)*, $C_{11}H_{22}O_4$—**218.29** [502-54-5]—Use a suitable grade with a content of NLT 99%.

Monosodium Glutamate *(L-Glutamic Acid Monosodium Salt Hydrate, (S)-2-Aminopentanedioic Acid)*, $C_5H_8NNaO_4 \cdot xH_2O$—**169.11 (anhydrous basis)** [142-47-2]—Use a suitable grade with a content of NLT 99%.

Montelukast Sodium Hydrate *(1-[[[(1R)-1-[3-[(1E)-2-(7-Chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic Acid Sodium Salt Hydrate)*, $C_{35}H_{35}ClNO_3S \cdot Na \cdot xH_2O$—**608.17 (anhydrous)** [151767-02-1]
(anhydrous)—Use a suitable grade with a content of NLT 98%.
[NOTE—A suitable grade is available as catalog number SML0101 from www.sigmaaldrich.com.]

Morin *(Morin Hydrate; 2′,3,4′,5,7-Pentahydroxyflavone Monohydrate)*, $C_{15}H_{10}O_7 \cdot H_2O$—**320.25** [480-16-0]—Use a suitable grade.

Morpholine *(Tetrahydro-1,4-oxazine)*, C_4H_9NO—**87.12** [110-91-8]—Use ACS reagent grade.

4-Morpholine Propane Sulfonic Acid *(MOPS; 3-(N-Morpholino) Propanesulfonic Acid; 4-Morpholinepropanesulfonic Acid)*, $C_7H_{15}NO_4S$—**209.26** [1132-61-2]—Use a suitable grade with a content of NLT 99%.

Myristyltrimethylammonium Bromide *(MTA, Tetradecyltrimethylammonium Bromide; Trimethyl(tetradecyl) ammonium Bromide)*, $C_{17}H_{38}BrN$—**336.39** [1119-97-7]—Use a suitable grade with a content of NLT 99%.

Naphthalene, $C_{10}H_8$—**128.17** [91-20-3]—Monoclinic prismatic plates, or white scales or powder. Use a suitable grade with a content of not less than 98%.

1,3-Naphthalenediol *(Naphthoresorcinol)*, $C_{10}H_6(OH)_2$—**160.17** [132-86-5]—Grayish-white to tan crystals or powder. Freely soluble in methanol; sparingly soluble in water, in alcohol, and in ether.
Melting Range ⟨741⟩: between 122° and 127°.
Solubility in Methanol: Dissolve 500 mg in 50 mL of methanol: the solution is clear and complete.

2,7-Naphthalenediol *(2,7-Dihydroxynaphthalene)*, $C_{10}H_8O_2$—**160.17** [582-17-2]—Off-white to yellow, crystalline solid or powder. Dissolves in acetone.
Melting Range ⟨741⟩: between 187° and 191°.

2-Naphthalenesulfonic Acid, $C_{10}H_8O_3S \cdot H_2O$—**226.25** [120-18-3]—Off-white to light gray crystals. Soluble in water.
Assay: Dissolve about 1 g, accurately weighed, in 100 mL of water, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 22.63 mg of $C_{10}H_8O_3S \cdot H_2O$. Not less than 98.0% is found.
Melting Range ⟨741⟩: between 122° and 126°, but the range between beginning and end of melting does not exceed 2°.

1-Naphthol *(Alphanaphthol)*, $C_{10}H_7OH$—**144.17** [90-15-3]—Use a suitable grade with a content of NLT 99%.

2-Naphthol *(Betanaphthol)*, $C_{10}H_7OH$—**144.17** [135-19-3]—White leaflets or crystalline powder. Discolors on exposure to light. Very slightly soluble in water; soluble in alcohol, in ether, in chloroform, and in solutions of alkali hydroxides.
Melting Range ⟨741⟩: between 121° and 123°.
Solubility in Alcohol: A solution of 1 g in 10 mL of alcohol is complete and colorless or practically so.
Residue on Ignition ⟨Reagent test⟩: not more than 0.05%.
Acidity: Shake 1 g with 50 mL of water occasionally during 15 minutes, and filter: the filtrate is neutral to litmus.
1-Naphthol: Boil 100 mg with 10 mL of water until dissolved, cool, and filter. Add to the filtrate 0.3 mL of 1 N sodium hydroxide and 0.3 mL of 0.1 N iodine: no violet color is produced.
Insoluble in Ammonia (naphthalene, etc.): Shake 500 mg with 30 mL of ammonia TS: the 2-naphthol dissolves completely and the solution is not darker than pale yellow.

Naphthol Dipotassium Disulfonate *(2-Naphthol-6,8-dipotassium Disulfonate)*, $C_{10}H_6K_2O_7S_2$—**380.48** [842-18-2]—Use a suitable grade.
[NOTE—A suitable grade is available as "2-naphthyl-6,8-disulfonic acid dipotassium salt" from Pfaltz and Bauer, Inc., www.pfaltzandbauer.com.]

Naphthol Disodium Disulfonate *(2-Naphthol-3,6-disodium Disulfonate)*, $C_{10}H_6Na_2O_7S_2$—**348.26**—Use a suitable grade.

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***p*-Naphtholbenzein,** $C_{27}H_{18}O_2$**—374.43** [145-50-6]—Red-brown powder. Use a suitable grade.

β-Naphthoquinone-4-sodium Sulfonate, $C_{10}H_5NaO_5S$**—260.20**—Yellow to orange-yellow crystals or crystalline powder. Soluble in about 10 parts of water; insoluble in alcohol.
Loss on Drying ⟨731⟩: Dry it in vacuum at about 50°: it loses not more than 2.0% of its weight.
Residue on Ignition (Reagent test): Ignite 1 g of dried sample with 3 mL of sulfuric acid: the residue weighs between 265 and 280 mg (between 26.5% and 28.0%).

Naphthoresorcinol *(1,3-Dihydroresorcinol),* $C_{10}H_8O_2$**—160.17** [132-86-5]—Use a suitable grade.

2-Naphthyl Chloroformate *(Chloroformic Acid 2-Naphthyl Ester),* $ClCOOC_{10}H_7$**—206.62** [7693-50-7]—Use a suitable grade.
[NOTE—A suitable grade is available from TCI America, www.tciamerica.com.]

1-Naphthylacetic Acid *(1-Naphthaleneacetic Acid, α-Naphthaleneacetic Acid Free Acid),* $C_{12}H_{10}O_2$**—186.21** [86-87-3]—Use a suitable grade with a content of NLT 95%.

1-Naphthylamine, $C_{10}H_9N$**—143.19** [134-32-7]—Use a suitable grade.

1-Naphthylamine Hydrochloride, $C_{10}H_7NH_2 \cdot HCl$**—179.65** [552-46-5]—White, crystalline powder that turns bluish upon exposure to light and air. Soluble in water, in alcohol, and in ether.
A 1 in 100 solution, make slightly acid with acetic acid, gives a violet color with 5 drops of ferric chloride TS. A 1 in 40 solution in diluted acetic acid is colorless and not more than slightly opalescent.
Residue on Ignition (Reagent test): Ignite 200 mg with a few drops of sulfuric acid: the weight of the residue is negligible.

***N*- (1-Naphthyl)ethylenediamine Dihydrochloride,** $C_{10}H_7NH(CH_2)_2NH_2 \cdot 2HCl$**—259.17** [1465-25-4]—Use ACS reagent grade.

Neutralized Alcohol—See *Alcohol, Neutralized.*

Nickel, Ni**—58.6934** [7440-02-0]—Use a suitable grade.

Nickel-Aluminum Catalyst—Use a suitable grade.
[NOTE—A suitable grade is "Raney Nickel, Active Catalyst," available as "aluminum–nickel alloy," catalog number 72240, available from Fluka Chemical Corp., fax 1-800-962-9591, Web site: www.sigma-aldrich.com.]

Nickel Nitrate Hexahydrate *[Nickel(III) Nitrate Hexahydrate, Nickelous Nitrate Hexahydrate],* $Ni(NO_3)_2 \cdot 6H_2O$**—290.81** [13478-00-7]—Use ACS reagent grade.

Nickel Sulfate, $NiSO_4 \cdot 6H_2O$**—262.85** [10101-97-0]—Use ACS reagent grade.

Nickel (II) Sulfate Heptahydrate, $NiSO_4 \cdot 7H_2O$**—280.9** [10101-98-1]—Use a suitable grade.

β-Nicotinamide Adenine Dinucleotide, $C_{21}H_{27}N_7O_{14}P_2$**—663.4** [53-84-9]—White, very hygroscopic powder. Freely soluble in water. Use a suitable grade with a content of NLT 98%.

Nicotinamide Adenine Dinucleotide Phosphate-adenosine-5′-triphosphate Mixture—Use a suitable grade.
Suitability: When used in the assay of lactulose, determine that a suitable absorbance-versus-concentration slope is obtained, using USP Lactulose RS, the reagent blank absorbance being not more than 0.020. The commercially available reagent contains 64 mg of nicotinamide adenine dinucleotide phosphate and 160 mg of adenosine-5′-triphosphate per vial. The mixture is buffered and stabilized. For use in the Assay of lactulose it is diluted with water to 100 mL.

Nicotinic Acid—Use *Niacin* (USP monograph).

Ninhydrin, $C_9H_4O_3 \cdot H_2O$**—178.14** [485-47-2]—Use ACS reagent grade.

Nitric Acid, HNO_3**—63.01** [7697-37-2]—Use ACS reagent grade.

Nitric Acid, Diluted, HNO_3 [7697-37-2]—Dilute 143 mL of nitric acid with water to 1000 mL.

Nitric Acid, Fuming *(90 Percent Nitric Acid),* HNO_3**—63.01** [7697-37-2]—Use ACS reagent grade Nitric Acid, 90 Percent.

Nitric Acid, Lead-Free: Use ACS reagent grade.
Lead: To 100 g add 0.1 g of anhydrous sodium carbonate and evaporate to dryness. Dissolve the residue in water, heating slightly, and dilute with the same solvent to 50.0 mL. Determine the lead content by atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩) measuring the absorbance at 283.3 nm or 217.0 nm using a lead hollow-cathode lamp and an air–acetylene flame. It contains not more than 0.1 ppm of lead (Pb).

Nitric Acid, Ultratrace, HNO_3 **—63.01** [7697-37-2]—Use ACS reagent grade.

Nitric Oxide–Nitrogen Dioxide Detector Tube: A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for an oxidizing layer and the indicator diphenyl benzidine.
• **MEASURING RANGE:** 0.5 to 10 ppm.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Nitrilotriacetic Acid, $N(CH_2COOH)_3$**—191.14** [139-13-9]—Use ACS reagent grade.

5-Nitro-1,10-phenanthroline, $C_{12}H_7N_3O_2$**—225.20** [4199-88-6]—White powder. Soluble in water.
Melting Range ⟨741⟩: between 198° and 200°.
Suitability as Redox Indicator: Dissolve 25 mg in a minimum volume of diluted sulfuric acid, add 10 mg of ferrous sulfate, and dilute with water to 100 mL: the solution is deep red in color and exhibits an absorption maximum at 510 nm. To 1.0 mL of the solution add 1.0 mL of 0.01 M ceric sulfate: the red color is discharged.

4′-Nitroacetophenone *(p′-Nitroacetophenone),* $C_8H_7NO_3$**—165.15** [100-19-6]—Yellow crystals.
Assay: Inject an appropriate ether solution of the specimen (about 0.5 µL) into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal

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conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: a 4-mm × 1.8-m stainless steel column containing 10% phase G1 on support S1A; the injection port and detector temperatures are maintained at 200° and 300°, respectively; the column temperature is maintained at 170° and programmed to rise 3° per minute to 220°. The area of the 4′-nitroacetophenone peak is not less than 97% of the total peak area.
Melting Range ⟨741⟩: between 78° and 80°.

***o*-Nitroaniline,** $NO_2C_6H_4NH_2$**—138.12** [88-74-4]—Orange-yellow crystals. Slightly soluble in cold water; soluble in hot water; freely soluble in alcohol and in chloroform. It forms water-soluble salts with mineral acids.
• **Melting Range** ⟨741⟩: between 71° and 72°.

Change to read:

***p*-Nitroaniline** ▲*(4-Nitroaniline),*▲ 2S (USP41) $NO_2C_6H_4NH_2$—**138.12** [100-01-6]—▲Use a suitable grade with a content of NLT 99%.▲ 2S (USP41)

Nitrobenzene, $C_6H_5NO_2$**—123.11** [98-95-3]—Use ACS reagent grade.

***p*-Nitrobenzenediazonium Tetrafluoroborate,** $NO_2C_6H_4N_2BF_4$**—236.92** [456-27-9]—Yellow-gold crystals. Soluble in acetonitrile. [**Caution**—Shock-sensitive; keep refrigerated.]
• **Assay:** Transfer about 30 mg, accurately weighed, to a low-actinic, 100-mL volumetric flask. Dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix. Using low-actinic glassware, dilute 2.0 mL of the resulting solution with spectrophotometric grade methanol to 50.0 mL. Measure the absorbance of this solution in a 1-cm cell at about 255 nm, using methanol as the blank. Calculate the absorptivity of the solution by dividing the measured absorbance by the concentration in g per mL. Calculate the assay value by the formula:

$$100a/59.4$$

in which a is the absorptivity of the solution: not less than 95.0% is found.

4-Nitrobenzoic Acid *(p-Nitrobenzoic Acid),* $C_7H_5NO_4$—**167.12** [62-23-7]—Use a suitable grade with a content of NLT 98%.

***p*-Nitrobenzyl Bromide,** $NO_2C_6H_4CH_2Br$**—216.03**—Almost white to pale yellow crystals, darkening on exposure to light. Practically insoluble in water; freely soluble in alcohol, in ether, and in glacial acetic acid. Store in tight, light-resistant containers.
• **Melting Range** ⟨741⟩: between 98° and 100°.
• **Solubility:** Separate 200-mg portions yield clear solutions in 5 mL of alcohol and in 5 mL of glacial acetic acid.
• **Residue on Ignition** (Reagent test): negligible, from 200 mg.

4-(*p*-Nitrobenzyl)pyridine, $C_{12}H_{10}N_2O_2$**—214.22** [1083-48-3]—Yellow crystals. Soluble in acetone. Use a suitable grade with a content of not less than 98%.

Nitrogen Certified Standard—A suitable NLT 99.99% nitrogen certified standard is available from most suppliers of specialty gases.

Nitromethane, CH_3NO_2**—61.04** [75-52-5]—Use ACS reagent grade.

Nitroso R Salt *(1-Nitroso-2-naphthol-3,6-disodium Disulfonate),* $NOC_{10}H_4OH(SO_3Na)_2$**—377.26** [525-05-3]—Yellow crystals or crystalline powder. One g dissolves in about 40 mL of water; insoluble in alcohol.
Sensitiveness: Dissolve 500 mg of sodium acetate in a solution of 0.4 mg of cobaltous chloride (0.1 mg of cobalt) in 5 mL of water. Add 1 mL of diluted acetic acid, and follow with 1 mL of a solution of the nitroso R salt (1 in 500): a red color, which is produced at once, persists when the solution is boiled with 1 mL of hydrochloric acid for 1 minute.

1-Nitroso-2-naphthol, $C_{10}H_7NO_2$**—173.17** [131-91-9]—Brown to yellowish-brown powder. Insoluble in water; soluble in alcohol, in benzene, in ether, in carbon tetrachloride, and in acetic acid.
Assay: Transfer about 250 mg, previously dried over silica gel to constant weight and accurately weighed, to a glass-stoppered flask, and dissolve in 10 mL of sodium hydroxide solution (1 in 10). Cool the solution in an ice bath, add dilute sulfuric acid (1 in 6) until a slight, permanent precipitate is formed and the solution is slightly acid, then add 3 g of potassium iodide, shake to dissolve, add 20 mL of dilute sulfuric acid (1 in 6), immediately insert the stopper in the flask, and allow to stand in the dark for 2 hours. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a complete blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 8.66 mg of $C_{10}H_7NO_2$: not less than 95.0% is found.
Melting Range ⟨741⟩: between 109° and 111°.
Residue on Ignition (Reagent test): not more than 0.2%.

Nitrous Oxide Certified Standard [10024-97-2]—A container of 99.9% nitrous oxide. It is available from most suppliers of specialty gases.

Nonadecane, $C_{19}H_{40}$**—268.52** [629-92-5]—White solid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: 3-mm × 1.8-m stainless steel column containing 5% phase G2 on support S1AB; the injection port temperature is maintained at 330°; the detector temperature is maintained at 300°; and the oven temperature is held initially at 190° and allowed to rise gradually to 250°. The area of the nonadecane peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 31.5° and 33.5°.

Nonanoic Acid, $C_9H_{18}O_2$**—158.24** [112-05-0]—Clear, colorless to faint yellow liquid. Miscible with water and with methanol.
Assay: Accurately weigh about 500 mg, transfer to a suitable container, add 30 mL of water, and mix. Add 40 mL of water, and mix. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 15.82 mg of $C_9H_{18}O_2$: not less than 96.0% of $C_9H_{18}O_2$ is found.
Refractive Index ⟨831⟩: about 1.432 at 20°.

Nonionic Wetting Agent: Use a suitable amphoteric surfactant.
[Note—A suitable grade is commercially available as Triton X-100 or Octoxynol 9.]

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Nonoxynol-9 (*Igepal CO-630, Semicid, Staycept, Tergitol TP-9, Sterox*), $(C_2H_4O)_nC_{15}H_{24}O$—[26027-38-3]—Use a suitable grade.

1-Nonyl Alcohol *(1-Nonanol),* $CH_3(CH_2)_8OH$—**144.25** [143-08-8]—Colorless liquid.
Assay: Not less than 97% of $C_9H_{20}O$ is found, a suitable gas chromatograph equipped with a flame-ionization detector and helium being used as the carrier gas at a flow rate of about 40 mL per minute. The following conditions have been found suitable: a 3.2-mm × 1.83-m stainless steel column packed with 20% phase G16 on support S1A; the injection port, column, and detector temperatures are maintained at about 250°, 160°, and 310°, respectively.
Refractive Index ⟨831⟩: between 1.432 and 1.434 at 20°.

***n*-Nonylamine** *(1-Aminononane),* $C_9H_{21}N$—**143.27** [112-20-9]—Use a suitable grade.

Nonylphenol Polyoxyethylene Ether, $(CH_3)_3CCH_2C(CH_3)_2CH_2C_6H_4O(CH_2CH_2O)_xH$, where *x* is approximately 40 to 1900–2100 [9016-45-9]—White solid; melts at approximately 44°. Use a suitable grade.

Nonylphenoxypoly(ethyleneoxy)ethanol—Clear, viscous, pale yellow liquid. May exhibit slight solidification on cooling; warming with agitation will restore to original condition. Density: about 1.06. Soluble in alcohol, in xylene, and in water. Suitable for use in gas–liquid chromatography.
[NOTE—A suitable grade is "Igepal CO 710," available from General Aniline and Film Corp., 140 West 51st St., New York, NY 10020.]

Normal Butyl Alcohol—See *Butyl Alcohol.*

Normal Butylamine—See *n-Butylamine.*

***n*-Octadecane,** $CH_3(CH_2)_{16}CH_3$—**254.49** [593-45-3]—Use a gas chromatographic standard with a content of not less than 99.5%.

Octadecyl Silane [18623-11-5]—This reagent is formed *in situ* by reaction of the column support with a suitable silylating agent such as octadecyl trichlorosilane.

***n*-Octane,** $CH_3(CH_2)_6CH_3$—**114.23** [111-65-9]—Use a suitable grade with a content of NLT 99.0%.
[NOTE—A suitable grade is available as catalog number 129370020 from Acros Organics, www.acros.com.]

Octanesulfonic Acid Sodium Salt *(Sodium 1-octanesulfonate),* $C_8H_{17}NaO_3S$, $C_8H_{17}NaO_3S \cdot H_2O$—**216.27, anhydrous; 234.27, hydrate** [5324-84-5, anhydrous; 207596-29-0, hydrate]—Use a suitable grade with a content of NLT 99.0%.

Octanesulfonic Acid Sodium Salt, Monohydrate *(1-Octanesulfonic Acid Sodium Salt, Monohydrate),* $C_8H_{17}NaO_3S \cdot H_2O$—**234.29** [207596-29-0]—Use a suitable grade with a content of NLT 99.0%.

1-Octanol *(Alcohol C8; Capryl Alcohol; Octyl Alcohol),* $C_8H_{18}O$—**130.23** [111-87-5]—Use ACS reagent grade.

Octanophenone, $C_{14}H_{20}O$—**204.31** [1674-37-9]—Colorless liquid.
Assay
Mobile phase: Prepare a filtered and degassed mixture of acetonitrile and water (7:3).
Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 254-nm detector and a 4.6-mm × 15.0-cm column that contains packing L1. The flow rate is about 2 mL per minute. The area of the $C_{14}H_{20}O$ peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: 1.5043 at 20°.

Octoxynol 9: see *Nonionic Wetting Agent.*

Add the following:

▲**Octylamine** *(1-Aminooctane; n-Octylamine; Caprylamine),* $C_8H_{19}N$—**129.24** [111-86-4]—Use a suitable grade with a content of NLT 99%.▲ (USP 1-Aug-2019)

Change to read:

(*p-tert*-Octylphenoxy) nonaethoxyethanol, $C_{34}H_{62}O_{11}$—**646.85** [▲127087-87-0▲ (USP 1-Aug-2019)]—Use a suitable grade. [NOTE—A suitable grade is available commercially as ▲Tergitol Type NP-9▲ (USP 1-Aug-2019) from reagent suppliers.]

(*p-tert*-Octylphenoxy)polyethoxyethanol—Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Triton 100" from reagent suppliers.]

Octyl Sulfate, Sodium Salt, $C_8H_{17}O_4SNa$—**232.27**—White powder.
Solubility: A 2-g portion dissolves in 100 mL of water.
Melting Range ⟨741⟩: between 195° and 197°, with decomposition.

Olefin Detector Tube: A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator in a stabilized form of permanganate.
• **MEASURING RANGE:** 0.06 to 3.2 Vol.–% Propylene, 0.04 to 2.4 Vol.–% Butylene.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com.]

Oligo-deoxythymidine—Polymeric length: 18. Use a suitable grade.
[NOTE—A suitable grade is available from BD Biosciences, www.bdbiosciences.com.]

Orange G *(the sodium salt of azobenzene-betanaphthol disulfonic acid),* $C_6H_5N{:}NC_{10}H_4(OH)(SO_3Na)_2$-2,6,8—**452.37** [1936-15-8]—Orange to brick-red powder or dark red crystals. Readily soluble in water, yielding an orange-yellow solution; slightly soluble in alcohol; insoluble in ether and in chloroform. The addition of tannic acid TS to its 1 in 500 solution causes no precipitation *(acid color).* The addition of hydrochloric acid to a mixture of 500 mg of zinc dust and 10 mL of its 1 in 500 solution produces decolorization. When filtered, the colorless filtrate, on standing exposed to air, does not regain its original color *(presence of azo-group).* When heated, orange G does not deflagrate (distinction from *nitro colors*). The addition of barium or calcium chloride TS to a concentrated solution of orange G produces a colored, crystalline precipitate. The addition of hydrochloric acid to its 1 in 500 solution produces no change; the addition of sodium hydroxide TS to a similar solution produces a yellowish red to a Bordeaux color but no precipitation. Orange G dissolves in sulfuric acid with an orange to yellowish-red color. No change in color results upon diluting the solution cautiously with water.

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Orcinol *(5-Methylresorcinol)*, $C_7H_8O_2 \cdot H_2O$—**142.15** [6153-39-5]—White to light tan crystals.
Assay: Transfer about 60 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask and dilute with methanol to volume, and mix. Using a suitable spectrophotometer, 1-cm cells, and methanol as the blank, record the absorbance of the solution at the wavelength of maximum absorbance at about 273 nm. From the observed absorbance, calculate the absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩): the absorptivity is not less than 13.2, corresponding to not less than 98% of $C_7H_8O_2 \cdot H_2O$.
Melting Range ⟨741⟩: between 58° and 61°.

Orthophenanthroline—See *1,10-Phenanthroline*.

Osmium Tetroxide *(Osmic Acid; Perosmic Anhydride)*, OsO_4—**254.23** [20816-12-0]—Use ACS reagent grade.

Oxalic Acid, $H_2C_2O_4 \cdot 2H_2O$—**126.07** [6153-56-6]—Use ACS reagent grade.

3,3′-Oxydipropionitrile, $O(CH_2CH_2CN)_2$—**124.14** [1656-48-0]—Clear, colorless to slightly yellow liquid. Refractive index: about 1.446 at 20°.
Boiling range: between 174° and 176° at 10 mm of mercury.

Oxygen Certified Standard—A suitable NLT 99.99% oxygen certified standard is available from most suppliers of specialty gases.

21% Oxygen Certified Standard—A suitable certified standard mixture of 21.0 ± 0.3% absolute oxygen in nitrogen is available from most suppliers of specialty gases.

93% Oxygen Certified Standard—A suitable certified standard mixture of 93.0 ± 0.1% absolute oxygen in nitrogen is available from most suppliers of specialty gases.

Oxygen-Helium Certified Standard—A mixture of 1.0% oxygen in industrial grade helium. It is available from most suppliers of specialty gases.

Delete the following:

▲Packings for High-Pressure Liquid Chromatography—See packings for high-pressure liquid chromatography in *Chromatography* ⟨621⟩, *Chromatographic Reagents*.▲ 2S(USP41)

Paired Ion Chromatography Reagent—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number WAT084283 from www.waters.com.]

Palladium Catalyst—Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Palladium Catalyst, Type I (5% Palladium on Calcium Carbonate)," from Engelhard Industries, Inc., fax number (864) 885-1375.]

Palladium Chloride, $PdCl_2$—**177.33** [7647-10-1]—Brown, crystalline powder. Soluble in water, in alcohol, in acetone, and in diluted hydrochloric acid.
Assay: Dissolve 80 mg, accurately weighed, in 10 mL of diluted hydrochloric acid, dilute with water to 50 mL, and add 25 mL of a 1 in 100 solution of dimethylglyoxime in alcohol. Allow to stand for 1 hour, and filter. Check for complete precipitation with the dimethylglyoxime solution. Ignite the precipitate in a tared platinum crucible at 850° for 2 hours, cool, and weigh the palladium. The weight of the residue is not less than 59.0% of the weight of the test specimen.

Palladous Chloride—See *Palladium Chloride*.

Pancreatic Digest of Casein (a bacteriological peptone; *Tryptone*)—A grayish-yellow powder, having a characteristic, but not putrescent, odor. Freely soluble in water; insoluble in alcohol and in ether.
Loss on Drying ⟨731⟩
Analysis: Dry at 100° to constant weight.
Acceptance criteria: NMT 7.0%
Residue on Ignition ⟨281⟩
Analysis: Ignite 500 mg with 1 mL of sulfuric acid.
Acceptance criteria: NMT 75 mg (15%)
Microbial Content: NMT 10^4 cfu/g
Nitrogen Content (Reagent test)
Analysis: Determine by the Kjeldahl method.
Acceptance criteria: 9.0%–14.0% is found
Amino Nitrogen: 3.5%–7.0%

Pancreatin [8049-47-6]—Use a grade of pancreatin which meets the USP requirements for amylase, lipase, and protease activities specified for the official substance.

Papaic Digest of Soybean Meal—A soluble nutrient material prepared by the action of the enzyme papain on soybean meal followed by suitable purification and concentration. It contains fermentable carbohydrates.
Loss on Drying ⟨731⟩
Analysis: Dry it at 100° to constant weight.
Acceptance criteria: NMT 7.0%
Residue on Ignition (Reagent test)
Analysis: Ignite 500 mg with 1 mL of sulfuric acid.
Acceptance criteria: NMT 75 mg (15.0%)
Microbial Content: NMT 10^4 cfu/g
Nitrogen Content (Reagent test)
Analysis: Determine by the Kjeldahl method, using a test specimen previously dried at 105° to constant weight.
Acceptance criteria: NLT 8.5% is found.
Amino Nitrogen: 1.8%–3.2%

***Para*- aminobenzoic Acid** *(p-Aminobenzoic Acid)*, $H_2NC_6H_4COOH$—**137.14** [150-13-0]—White or slightly yellow crystals or crystalline powder, becoming discolored on exposure to air or light. One g dissolves in 170 mL of water, in 9 mL of boiling water, in 8 mL of alcohol, and in 50 mL of ether. Freely soluble in solutions of alkali hydroxides and carbonates; soluble in warm glycerin; sparingly soluble in diluted hydrochloric acid; slightly soluble in chloroform. Store in tight, light-resistant containers.
Assay: Accurately weigh about 300 mg, previously dried at 105° for 2 hours, and transfer to a beaker or casserole. Add 5 mL of hydrochloric acid and 50 mL of water, and stir until dissolved. Cool to about 15°, add about 25 g of crushed ice, and slowly titrate with 0.1 M sodium nitrite VS until a glass rod dipped into the titrated solution produces an immediate blue ring when touched to starch iodide paper. When the titration is complete, the endpoint is reproducible after the mixture has been allowed to stand for 1 minute. Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of $C_7H_7NO_2$. Not less than 98.5% is found.
Melting Range ⟨741⟩: between 186° and 189°.
Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 0.2% of its weight.
Residue on Ignition (Reagent test): not more than 0.1%.

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Paraformaldehyde, $(CH_2O)_n$ [30525-89-4]—Fine, white powder.

Assay: Transfer about 1 g, accurately weighed, to a 250-mL conical flask containing 50.0 mL of 1 N sodium hydroxide VS, and mix by swirling. Immediately, and slowly, add 50 mL of hydrogen peroxide TS, previously neutralized to bromothymol blue, through a small funnel placed in the neck of the flask. After the reaction moderates, rinse the funnel and inner wall of the flask with water, allow the solution to stand for 30 minutes, add bromothymol blue TS, and titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 30.03 mg of HCHO: not less than 95% is found.

Residue on Ignition: not more than 0.1%.

Solubility in Ammonia: Dissolve 5 g in 50 mL of ammonia TS: a practically clear, colorless solution results.

Reaction: Shake 1 g with 20 mL of water for about 1 minute, and filter: the filtrate is neutral to litmus.

Pectate Lyase [9015-75-2]—An enzyme obtained from *Aspergillus sp.* Light brown, viscous liquid. Specific gravity is about 1.5. It is readily soluble in water. It is supplied at approximately 14 units per mL at pH 8.0 in Tris-HCl buffer [50 mM of Tris(hydroxymethyl)aminomethane containing 1 mM of $CaCl_2$, pH 8.0] in a solution of 50% glycerol and 0.02% sodium azide. One unit is defined as the enzyme activity that produces 1 µmol of unsaturated product per minute.

Activity

Pectin solution: Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a 100-mL volumetric flask.

[NOTE—Pectin has a molecular weight of 103,000 Da; its degree of esterification (percentage of galacturonic acid groups substituted with methyl) is 12.]

Moisten with 0.1 mL of 2-propanol. Add 50 mL of water to the flask, and mix the solution with a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the solution to a pH of 12. Stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 minutes. Adjust the solution with 0.5 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Tris buffer solution: Transfer 6.055 g of Tris(hydroxymethyl)aminomethane and 0.147 g of calcium chloride to a 1000-mL volumetric flask containing 950 mL of water, and mix. Adjust the solution with 1 N hydrochloric acid to a pH of 8.0. Dilute with water to volume.

Diluted pectate lyase: Transfer 0.5 mL of Pectate Lyase to a 50-mL volumetric flask, dilute with *Tris buffer solution* to volume, and mix.

Procedure: Add the solutions set forth in the table below to quartz cuvettes.

Label	Tris Buffer Solution (mL)	Pectin Solution (mL)	Diluted Pectate Lyase (mL)	Water (mL)
Enzyme blank	0.5	1.0	0	1.0
Test blank	0.5	0	0.5	1.5
Test solution	0.5	1.0	0.5	0.5

Perform the test on the solutions so obtained, using a suitable UV-Vis spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) and using water as the blank. Mix the solutions well at time 0, and immediately measure the absorbances at 235 nm. Record the value for the *Enzyme blank*, A_{0-EB}; for the *Test blank*, A_{0-TB}; and for the *Test solution*, A_{0-TS}. After incubation at room temperature for 30 minutes, determine the absorbance again at 235 nm for the *Enzyme blank*, A_{30-EB}; for the *Test blank*, A_{30-TB}; and for the *Test solution*, A_{30-TS}. One unit is defined as the enzymatic activity that produces 1 µmol of unsaturated product from pectin per minute. Calculate the Pectate Lyase activity, in units per mL, using the following formula:

$$50(10^3)\,[(A_{30-TS} - A_{30-EB} - A_{30-TB}) - (A_{0-TS} - A_{0-EB} - A_{0-TB})]/30E_{235}L$$

in which 50 is the volume, in mL, of *Diluted pectate lyase*; 10^3 is the unit conversion factor; 30 is the time, in minutes, of the reaction; E_{235} is the molar extinction coefficient, in $M^{-1}\,cm^{-1}$, of the reaction product (4600 $M^{-1}\,cm^{-1}$); and L is the path length, in cm, of the reaction cuvette (1 cm). Alternatively, these solutions, after being mixed in the cuvettes, can be immediately measured at 235 nm continuously in a recording UV-Vis spectrophotometer set up for kinetic assays. The result is obtained by correcting the blank determination, using the *Enzyme blank* and the *Test blank*.

Penicillinase—See *Beta-lactamase.*

Pentadecane, $C_{15}H_{32}$**—212.41** [629-62-9]—Colorless liquid.

Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; and the column temperature is maintained at 180° and programmed to rise 10° per minute to 280°. The area of the $C_{15}H_{32}$ peak is not less than 99% of the total peak area.

Refractive Index ⟨831⟩: between 1.430 and 1.434 at 20°.

1-Pentadecanol *(Pentadecyl Alcohol, n-Pentadecanol),* $C_{15}H_{32}O$**—228.41** [629-76-5]—Use a suitable grade with a content of NLT 98%.

Pentafluoropropanoic Acid *(Pentafluoropropionic Acid),* $C_3HF_5O_2$**—164.03** [422-64-0]—Use a suitable grade with a content of NLT 97%.

Pentane *(n-Pentane),* C_5H_{12}**—72.15** [109-66-0]—Clear, colorless, *flammable* liquid. Very slightly soluble in water. Miscible with alcohol, with ether, and with many organic solvents. Specific gravity: about 0.62.

Boiling Range (Reagent test): Not less than 95% distills between 34° and 36°.

1-Pentanesulfonic Acid Sodium Salt—See *Sodium 1-Pentanesulfonate.*

2-Pentanone, $C_5H_{10}O$**—86.13** [107-87-9]—Use a suitable grade.

Pepsin [9001-75-6]—E.C. 3.4.23.1

• **ACTIVITY DETERMINATION**

The activity determination of pepsin can be done by using a *Standard curve* or a *Single standard solution.* When the activity of pepsin is not known or is not expressed in USP Units/mg of weight, the activity is first determined by a *Standard curve.* If the correlation coefficient (r^2) of the

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standard curve is NLT 0.99, the next activity determinations can be done by using the *Single standard solution* procedure.

Standard curve

Diluted hydrochloric acid solution: Dilute 30 mL of 1 N hydrochloric acid with water to 1000 mL. Adjust with 1 N hydrochloric acid to a pH of 1.6 ± 0.1, if necessary.

Trichloroacetic acid (TCA) solution: 4.0% (w/v) trichloroacetic acid in water

Substrate solution: Transfer 4.0 g of USP Hemoglobin Protease Substrate RS to a 200-mL volumetric flask, and dissolve in 75 mL of *Diluted hydrochloric acid solution*. Once the hemoglobin is fully dissolved, adjust with 1 N hydrochloric acid to a pH of 1.6 ± 0.1, if necessary. Dilute with *Diluted hydrochloric acid solution* to volume.

Standard stock solution: Quantitatively prepare a solution of USP Pepsin for Assay RS with about 1.25 USP Pepsin Units/mL in *Diluted hydrochloric acid solution*.

Standard solutions: Prepare four test tubes capable of holding a minimum of 6 mL, and label the tubes S1, S2, S3, and S4. Add 5 mL of the *Standard stock solution* to tube S4. Add 4 mL of the *Standard stock solution* and then 1 mL of *Diluted hydrochloric acid solution* to tube S3. Add 3 mL of the *Standard stock solution*, then 2 mL of *Diluted hydrochloric acid solution* to tube S2. Add 2 mL of the *Standard stock solution*, then 3 mL of *Diluted hydrochloric acid solution* to tube S1.

[NOTE—Prepare these solutions immediately before use.]

Sample solution: Quantitatively prepare a solution of pepsin in *Diluted hydrochloric acid solution* containing approximately 0.90–1.00 USP Pepsin Units/mL.

[NOTE—Prepare this solution immediately before use.]

Procedure: Prepare test tubes as follows (use tubes that can accommodate and allow mixing on a vortex mixer of a volume of NLT 16 mL). Separately transfer 1.0-mL aliquots of each of the *Standard solutions* into four test tubes (two tubes for the analysis of each *Standard solution* and two blanks for each *Standard solution*).

Prepare a substrate blank by transferring 1.0 mL of *Diluted hydrochloric acid solution* into a single test tube.

Separately transfer 1.0-mL aliquots of the *Sample solution* into four test tubes (two tubes for the analysis of the *Sample solution* and two blanks for each *Sample solution*).

To each of the blank tubes add 10.0 mL of *TCA solution*, and mix on a vortex mixer.

Place all test tubes in a water bath maintained at 25 ± 1°, and allow equilibration for 5 min. Add 5.0 mL of the *Substrate solution* (previously equilibrated at 25 ± 1°) to each of the blank tubes, mix on a vortex mixer, and return the tubes to the water bath to incubate for 10 min.

At time equals zero, rapidly pipet 5.0 mL of the *Substrate solution* (previously equilibrated at 25 ± 1°) successively and at accurately timed intervals of at least 30 s into each of the tubes containing the *Sample solution* and *Standard solutions*. Mix each tube immediately after adding the *Substrate solution* by brief vortex mixing, and return the tube to the water bath. After 10.0 min, at the corresponding time intervals, stop the reaction by rapidly pipetting 10.0 mL of *TCA solution* into each tube. Mix each tube on a vortex mixer. Do not return the tubes to the water bath.

Allow all tubes, including blanks, to sit at room temperature for 25 min. After 25 min, mix the contents of each tube on a vortex mixer, and pass the contents through a fluted paper filter into clean tubes, discarding the first 5 mL of the filtrate. Refilter each of the filtrates through the same paper.

[NOTE—A suitable filter is the Whatman No. 41 ashless filter.]

Determine the absorbance of each filtrate using the following conditions.

Wavelength: 280 nm

Cell: 1 cm

Blank: Substrate blank

Calculate the average absorbance for each filtrate. Correct the absorbance of each *Standard solution*:

$$A_{SC} = A_S - A_{SB}$$

A_S = average absorbance of the *Standard solution*

A_{SB} = average absorbance of the *Standard solution* blank

Correct the absorbance of each *Sample solution*:

$$A_{UC} = A_U - A_{UB}$$

A_U = average absorbance of the *Sample solution*

A_{UB} = average absorbance of the *Sample solution* blank

Using linear regression, determine the correlation coefficient (r^2), slope (m) and y-intercept of the curve-corrected absorbance of the *Standard solutions* versus concentration (mg/mL).

Calculate the activity of pepsin in the sample:

$$\text{Activity (Units/mg)} = [(A_{UC} - b) \times P]/(m \times C_U)$$

A_{UC} = absorbance of each *Sample solution*

b = y-intercept of the standard curve

P = activity of the USP Pepsin for Assay RS (Units/mg)

m = slope of the standard curve

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

Single standard solution

Diluted hydrochloric acid solution: Dilute 30 mL of 1 N hydrochloric acid with water to 1000 mL. Adjust with 1 N hydrochloric acid to a pH of 1.6 ± 0.1, if necessary.

TCA solution: 4.0% (w/v) trichloroacetic acid in water

Substrate solution: Transfer 4.0 g of USP Hemoglobin Protease Substrate RS to a 200-mL volumetric flask, and dissolve in 75 mL of *Diluted hydrochloric acid solution*. Once the hemoglobin is fully dissolved, adjust with 1 N hydrochloric acid to a pH of 1.6 ± 0.1, if necessary. Dilute with *Diluted hydrochloric acid solution* to volume.

Standard solution: Using the USP Pepsin for Assay RS, prepare a solution containing 1.0 USP Pepsin Unit/mL in *Diluted hydrochloric acid solution*.

[NOTE—Prepare this solution immediately before use.]

Sample solution: Quantitatively prepare a solution of pepsin in *Diluted hydrochloric acid solution* containing approximately 0.90–1.00 USP Pepsin Units/mL.

[NOTE—Prepare this solution immediately before use.]

Procedure: Prepare test tubes as follows (use tubes that can accommodate and allow mixing on a vortex mixer of a volume of NLT 16 mL). Separately transfer 1.0-mL aliquots of the *Standard solution* into four test tubes (two tubes for the analysis of the *Standard solution* and two blanks for the *Standard solution*).

Prepare a substrate blank by transferring 1.0 mL of *Diluted hydrochloric acid solution* into a single test tube.

Separately transfer 1.0-mL aliquots of the *Sample solution* into four test tubes (two tubes for the analysis of

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the *Sample solution* and two blanks for each *Sample solution*).

To each of the blank tubes add 10.0 mL of *TCA solution*, and mix on a vortex mixer.

Place all test tubes in a water bath maintained at 25 ± 1°, and allow equilibration for 5 min. Add 5.0 mL of the *Substrate solution* (previously equilibrated to 25 ± 1°) to each of the blank tubes, mix on a vortex mixer, and return the tubes to the water bath to incubate for 10 min.

At time equals zero, rapidly pipet 5.0 mL of the *Substrate solution* (previously equilibrated to 25 ± 1°) successively and at accurately timed intervals of at least 30 s into each of the tubes containing the *Sample solution* and the *Standard solution*. Mix each tube immediately after adding the *Substrate solution* by brief vortex mixing, and return the tube to the water bath. After 10.0 min, at the corresponding time intervals, stop the reaction by rapidly pipetting 10.0 mL of *TCA solution* into each tube. Do not return the tubes to the water bath.

Allow all tubes, including blanks, to sit at room temperature for 25 min. After 25 min, mix the contents of each tube on a vortex mixer, and pass the contents through a fluted paper filter into clean tubes, discarding the first 5 mL of the filtrate.

[NOTE—A suitable filter is the Whatman No. 41 ashless filter.]

Determine the absorbance of each filtrate using the following conditions.

Wavelength: 280 nm
Cell: 1 cm
Blank: Substrate blank

Calculate the average absorbance for each filtrate. Correct the absorbance of each *Standard solution*:

$$A_{SC} = A_S - A_{SB}$$

A_S = average absorbance of the *Standard solution*
A_{SB} = average absorbance of the *Standard solution* blank

Correct the absorbance of each *Sample solution*:

$$A_{UC} = A_U - A_{UB}$$

A_U = average absorbance of the *Sample solution*
A_{UB} = average absorbance of the *Sample solution* blank

Calculate the activity of pepsin in the sample:

$$\text{Activity (Units/mg)} = (A_{UC}/A_{SC}) \times (C_S/C_U) \times P$$

A_{UC} = absorbance of each *Sample solution*
A_{SC} = absorbance of each *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = activity of the USP Pepsin for Assay RS (Units/mg)

One Pepsin Unit is defined as the quantity of enzyme that produces the equivalent of 1 µmol of tyrosine per min under the conditions of the activity determination procedure.

Pepsin, Purified—A white or yellowish-white powder, spongy mass, or translucent scales or granules. Freely soluble in water, producing more or less opalescence; practically insoluble in alcohol, in chloroform, and in ether. Purified Pepsin used in the second tier of the *Dissolution* test has an activity that is determined by the following method.

Activity

Pepsin solution: Transfer about 2.5 mg of Purified Pepsin, accurately measured, to a 100-mL volumetric flask, dilute with 10 mM hydrochloric acid to volume, and mix.

[NOTE—Prepare immediately before use.]

2.0% Hemoglobin solution: Dissolve and dilute 2.5 g of bovine hemoglobin with water to 100 mL. Dilute 80 mL of this solution with 0.3 M hydrochloric acid to a volume of 100 mL.

Trichloroacetic acid solution: Dilute 5 g of trichloroacetic acid with water to 100 mL.

Test Solution: Transfer 5.0 mL of *2.0% Hemoglobin solution* to a suitable container equilibrated at 37°. Add 1.0 mL of *Pepsin solution*, mix by swirling, and incubate at 37° for 10 minutes. Immediately add 10.0 mL of *Trichloroacetic acid solution*, mix by swirling, and incubate at 37° for 5 minutes. Pass through a filter having a 0.8-µm or finer porosity.

Control solution: Transfer 5.0 mL of *2.0% Hemoglobin solution* to a suitable container equilibrated at 37°. Mix by swirling, and incubate at 37° for 10 minutes. Immediately add 10.0 mL of *Trichloroacetic acid solution* and 1.0 mL of *Pepsin solution*, mix by swirling, and incubate at 37° for 5 minutes. Pass through a filter having a 0.8-µm or finer porosity.

Procedure: Determine the absorbances of the *Test solution* and *Control solution*, in 1-cm cells, at a wavelength of about 280 nm, using water as the reference. Calculate the activity of the portion of Purified Pepsin taken by the formula:

$$10{,}000(A_U - A_C)$$

in which A_U and A_C are the absorbances of the *Test solution* and the *Control solution*, respectively.

Peptic Digest of Animal Tissue (a bacteriological peptone)—Tan powder, having a characteristic, but not putrescent, odor. Soluble in water; insoluble in alcohol and in ether. An autoclaved solution (2 in 100) is clear and is neutral or nearly so in its reaction.

Loss on Drying ⟨731⟩
Analysis: Dry at 100° to constant weight.
Acceptance criteria: NMT 7.0%
Residue on Ignition ⟨281⟩
Analysis: Ignite 500 mg with 1 mL of sulfuric acid.
Acceptance criteria: NMT 75 mg (15%)
Microbial Content: NMT 10^4 cfu/g
Nitrogen Content
Analysis: Determine by the Kjeldahl method, using a test specimen previously dried at 105° to constant weight.
Acceptance criteria: Between 9.0% and 14.0%
Amino Nitrogen: 2.7%–3.7%

Peptone, Dried *(Meat Peptone)*—Reddish-yellow to brown powder, having a characteristic, but not putrescent, odor. Soluble in water, forming a yellowish-brown solution having a slight acid reaction; insoluble in alcohol and in ether.

Nitrogen Compounds (Reagent test): Determine by the Kjeldahl method, using a test specimen previously dried at 105° to constant weight: between 12% and 18% of nitrogen is found.

Residue on Ignition (Reagent test): Ignite 500 mg with 1 mL of sulfuric acid: the residue weighs NMT 75 mg (15.0%).

Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses NMT 7.0% of its weight.

Coagulable Protein: Heat a filtered solution (1 in 20) to boiling: no precipitate forms.

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Microbial Content: NMT 10^4 cfu/g

Perchloric Acid *(70 Percent Perchloric Acid),* $HClO_4$—**100.46** [7601-90-3]—Use ACS reagent grade (containing between 69.0% and 72.0% of $HClO_4$).

Periodic Acid, H_5IO_6—**227.94** [10450-60-9]—White to pale yellow crystals. Very soluble in water. Undergoes slow decomposition to iodic acid. Use ACS reagent grade.

Add the following:

▲**Peroxidase** *(Horseradish Peroxidase),* [9003-99-0]—Use a suitable grade Type I from horseradish.▲ (USP 1-May-2019)

Petroleum Benzin—See *Hexane, Solvent.*

Petroleum Ether *(Ligroin; Light Petroleum),*[8032-32-4]—Use ACS reagent grade.

Delete the following:

▲**Phases for Gas Chromatography**—See phases for gas chromatography in *Chromatography* ⟨621⟩, *Chromatographic Columns.*▲ 2S(USP41)

(*R*)-(–)-α-Phellandrene *((–)-p-Mentha-1,5-diene, (R)-5-Isopropyl-2-methyl-cyclohexa-1,3-diene, (R)-5-Isopropyl-2-methyl-1,3-cyclohexadiene),* $C_{10}H_{16}$—**136.23** [4221-98-1]—Use a suitable grade with a GC chromatographic purity of NLT 95% (sum of enantiomers).

Phenacetin[62-44-2]—Use a suitable grade.

1,10-Phenanthroline *(Orthophenanthroline),* $C_{12}H_8N_2 \cdot H_2O$—**198.22** [5144-89-8]—Use ACS reagent grade.

***o*-Phenanthroline Monohydrochloride Monohydrate,** $C_{12}H_8N_2 \cdot HCl \cdot H_2O$—**234.69** [3829-86-5]—Use a suitable grade.

Phenol [108-95-2]—Use ACS reagent grade.

Phenol Red, Sodium, $C_{19}H_{13}O_5SNa$—**376.4** [34487-61-1]—Red to brown powder. Use ACS reagent grade.

Phenolsulfonphthalein—Use *Phenol Red* (see *Indicators* under *Indicators and Indicator Test Papers*).

Phenoxybenzamine Hydrochloride *[N-(2-Chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine Hydrochloride],* $C_{18}H_{22}ClNO \cdot HCl$—**340.29** [63-92-3]—White, crystalline powder.

Melting Range ⟨741⟩: between 137° and 140°.

Absorptivity: Its absorptivity, 1%, 1 cm, in the range of 272 nm to 290 nm, in chloroform solution is about 178.

3-Phenoxybenzoic Acid, $C_{13}H_{10}O_3$—**214.22** [3739-38-6]—Use a suitable grade.

Melting Range ⟨741⟩: between 149° and 150°.

2-Phenoxyethanol, $C_6H_5OCH_2CH_2OH$—**138.16** [122-99-6]—Colorless, slightly viscous liquid. Soluble in water. Miscible with alcohol, with acetone, and with glycerin. Density: about 1.107.

Assay: To 2 g, accurately weighed, add 10 mL of a freshly prepared solution made by dissolving 25 g of acetic anhydride in 100 g of anhydrous pyridine. Swirl to mix the liquids, heat on a steam bath for 45 minutes, add 10 mL of water, heat for 2 additional minutes, and cool. Add 10 mL of normal butyl alcohol, shake vigorously, add phenolphthalein TS, and titrate with 1 N sodium hydroxide VS. Perform a blank test using the same quantities of the same reagents, and in the same manner, and make any necessary correction. Each mL of 1 N sodium hydroxide is equivalent to 138.2 mg of $C_8H_{10}O_2$. Not less than 99% is found.

Phenol: Add 0.2 mL of it to 20 mL of water, mix, and to 5 mL of the mixture add 0.2 mL of Millon's reagent. Warm the solution at 60° for 90 seconds, and allow to stand: no pink or red color is produced within 1 minute.

Phenyl Ether—See *Diphenyl Ether.*

Phenyl Isocyanate, C_6H_5NCO—**119.12** [103-71-9]—Clear, colorless to straw-yellow liquid of medium volatility. [**CAUTION**—Phenyl Isocyanate is a violent lacrimator, and the vapor is highly toxic. Handle with care.]

Assay: Transfer 250 mg, accurately weighed, to a glass-stoppered, 250-mL flask. Exercise care to avoid loss by volatilization, and avoid breathing the vapor. Add 20 mL of butylamine solution (25 g of butylamine diluted to 1000 mL with dioxane previously dried over potassium hydroxide pellets), insert the stopper in the flask, and allow to stand for 15 minutes. Add a few drops of methyl red TS and 25 mL of water, and titrate the excess amine with 0.1 N sulfuric acid VS. Perform a blank titration on 20 mL of the butylamine solution (see *Residual Titrations* ⟨541⟩). Subtract the volume of 0.1 N sulfuric acid consumed in the test specimen titration from that consumed in the blank titration. Each mL of 0.1 N sulfuric acid, representing this difference, is equivalent to 11.91 mg of C_6H_5NCO: not less than 97.0% of C_6H_5NCO is found.

2-Phenylacetamide *(α-Phenylacetamide),* C_8H_9NO—**135.16** [103-81-1]—Bimorphous plates or leaflets. Slightly soluble in water. Use a suitable grade.

Melting Range ⟨741⟩: between 156° and 158°.

Phenylacetic Acid *(α-Toluic Acid; α-Tolylic Acid; Benzeneacetic Acid),* $C_8H_8O_2$—**136.15** [103-82-2]—Use a suitable grade with a content of NLT 99%.

***dl*-Phenylalanine,** $C_9H_{11}NO_2$—**165.19** [150-30-1]—Use a suitable grade.

L-Phenylalanine *((S)-2-Amino-3-phenylpropionic acid),* $C_9H_{11}NO_2$—**165.19** [63-91-2]—Use a suitable grade with a content of NLT 98%.

***o*-Phenylenediamine Dihydrochloride,** $C_6H_8N_2 \cdot 2HCl$—**181.1**—White powder.

• **ASSAY:** When tested by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of butyl alcohol, water, and acetic acid (12:5:3), and examined under short-wavelength UV light, a single spot is exhibited, with trace impurities.

***p*-Phenylenediamine Dihydrochloride**—See *p-Phenylenediamine Hydrochloride.*

***p*-Phenylenediamine Hydrochloride** *(1,4-Diaminobenzene Dihydrochloride),* $C_6H_8N_2 \cdot 2HCl$—**181.06**—White to pale tan crystals or crystalline powder, turning red on exposure to air. Freely soluble in water; slightly soluble in alcohol and in ether. Preserve in well-closed containers, protected from light.

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- **INSOLUBLE MATTER:** Dissolve 1 g in 10 mL of water: the solution is clear and complete.
- **MOLAR ABSORPTIVITY** (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩): Dissolve 60 mg in 100.0 mL of water, and mix. Pipet 2 mL of this solution into a 50-mL volumetric flask, dilute with pH 7 buffer solution to volume, and mix. The molar absorptivity of this solution, at 239 nm, is not less than 9000.

Phenylglycine (*D*(−)-*2-Phenylglycine*), $(C_6H_5CH(NH_2)COOH)$ **—151.17** [875-74-1]—Use a suitable grade.

Phenylhydrazine, $C_6H_5NHNH_2$**—108.14** [100-63-0]—A colorless, or slightly yellowish, highly refractive liquid.

[NOTE—Protect from light, and distill under reduced pressure shortly prior to use.]

Congealing Temperature ⟨651⟩: not below 16°.

Insoluble Matter: Shake 1 mL with 20 mL of diluted acetic acid: the resulting solution is clear or practically so.

Residue on Ignition (Reagent test): Ignite 1 mL with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

Phenylhydrazine Hydrochloride, $C_6H_5NHNH_2 \cdot HCl$**—144.60** [59-88-1]—White or yellowish crystals or powder. Soluble in water and in alcohol. Store in tight containers, protected from light. Use a suitable grade with a content of not less than 99%.

Phenylmethylsulfonyl Fluoride, $C_7H_7FO_2S$**—174.2** [329-98-6]—White to faint yellow powder. Use a suitable grade.

[NOTE—A suitable grade is available from Sigma-Aldrich, www.sigma-aldrich.com.]

3-Phenylphenol (*m-Phenylphenol*), $C_6H_5C_6H_4OH$**—170.21** [580-51-8]—White to off-white, crystalline powder.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G1; the injection port temperature is maintained at 250°; the column temperature is maintained at 150° and programmed to rise 15° per minute to 250°; and the detector temperature is maintained at 310°. The area of the 3-phenylphenol peak is not less than 98% of the total peak area.

Melting Range ⟨741⟩: between 76° and 79°.

Phloroglucinol, $C_6H_3(OH)_3 \cdot 2H_2O$**—162.14** [6099-90-7]—White or yellowish-white crystals or a crystalline powder. Soluble in alcohol and in ether; slightly soluble in water.

Insoluble in Alcohol: Dissolve 1 g in 20 mL of alcohol: a clear and complete solution results.

Melting Range, *Class Ia* ⟨741⟩: between 215° and 219°.

Residue on Ignition (Reagent test): Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 1 mg (0.1%).

Diresorcinol: Heat to boiling a solution of 100 mg in 10 mL of acetic anhydride, cool the solution, and superimpose it upon 10 mL of sulfuric acid: no violet color appears at the zone of contact of the liquids.

Phloxine B (*Acid red 92; Eosin 10B; 2′,4′,5′,7′-tetrabromo-4,5,6,7-tetrachlorofluorescein disodium salt*) $C_{20}H_2Br_4Cl_4Na_2O_5$ **—829.63** [18472-87-2]—Use a suitable grade with a dye content of not less than 80%, certified by the Biological Stain Commission.

Phosphatase Enzyme, Alkaline—Use a suitable grade from intestinal origin.

[NOTE—A suitable grade is available from Worthington Biochemical Corp., www.worthington-biochem.com.]

Phosphatic Enzyme—An enzyme preparation of microbial origin, high in both phosphatase and amylase activity, the former being the property that renders it suitable for use in the liberation of thiamine from its orthophosphate and pyrophosphate esters. Light cream-colored or slightly gray powder. Freely soluble in water. It hydrolyzes 300 times its weight of starch in 30 minutes.

Amylase Activity: Place in a test tube 5 mL of a 1 in 50 solution of soluble starch in 0.2 M, pH 5 sodium acetate buffer (containing 1.6 g of anhydrous sodium acetate in each L and sufficient glacial acetic acid to adjust to a pH of 5), and add 4 mL of water. Mix, and place in a water bath at 40°. Add 1 mL of a solution containing 0.3 mg of the phosphatic enzyme, mix, and note the exact time. After 30 minutes remove 1.0 mL of the mixture, and add it to 5.0 mL of 0.0005 N iodine in a 20- × 150-mm test tube: a clear, red color results.

Phosphomolybdic Acid, approximately $20MoO_3 \cdot P_2O_5 \cdot 51H_2O$**—3939.49** [51429-74-4]—Use ACS reagent grade.

Phosphoric Acid, H_3PO_4**—98.00** [7664-38-2]—Use ACS reagent grade.

Phosphorous Acid (*Phosphonic Acid*), H_3O_3P**—82.00** [13598-36-2]—Use a suitable grade with a content of not less than 99%.

Phosphorus, Red, P**—At. Wt. 30.97376**—A dark red powder. Insoluble in water and in dilute acids; soluble in dehydrated alcohol.

Yellow Phosphorus: Shake 20 g with 75 mL of carbon disulfide in a glass-stoppered vessel, and allow to stand in the dark overnight. Filter, and wash the residue with carbon disulfide until the filtrate, collected in a graduated cylinder, measures 100 mL. Evaporate the solvent to 10 mL by immersing the cylinder in hot water. Dip a strip of cupric sulfate test paper in the remaining solvent: no more color is produced than in a similar strip dipped into 10 mL of solution in carbon disulfide containing 3 mg of yellow phosphorus (0.015% as P).

Soluble Substances: Digest 2 g with 30 mL of acetic acid on a steam bath for 15 minutes. Cool, dilute with water to 40 mL, and filter. Evaporate 20 mL of the filtrate on a steam bath, and dry at 105° for 2 hours: the residue weighs not more than 6 mg (0.6%).

Phosphorus Pentoxide (*Phosphoric Anhydride*), P_2O_5**—141.94** [1314-56-3]—Use ACS reagent grade.

Phosphotungstic Acid, approximately $24WO_3 \cdot P_2O_5 \cdot 51H_2O$ **—6624.84**—White or yellowish-green crystals or a crystalline powder. Soluble in water, in alcohol, and in ether.

- **INSOLUBLE MATTER** (Reagent test): not more than 1 mg, from 5 g (0.02%).
- **CHLORIDE** (Reagent test): One g shows not more than 0.3 mg of Cl (0.03%).
- **NITRATE:** Dissolve 500 mg in 10 mL of water, and add about 10 mg of sodium chloride, 0.1 mL of indigo carmine TS, and 10 mL of sulfuric acid: the blue color does not disappear within 1 minute (about 0.01%).
- **SULFATE** (Reagent test, *Method I*): A 500-mg portion shows not more than 0.1 mg of SO_4 (0.02%).

o- Phthalaldehyde (*Phthalic Dicarboxaldehyde*), $C_6H_4(CHO)_2$ **—134.13** [643-79-8]—Use a suitable grade.

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Phthalazine, $C_8H_6N_2$**—130.15** [253-52-1]—Yellow to tan crystals.
Melting Range ⟨741⟩: between 89° and 92°.

Phthalic Acid, $C_8H_6O_4$**—166.13** [88-99-3]—Use ACS reagent grade.

Phthalic Anhydride, $C_8H_4O_3$**—148.12** [85-44-9]—Use ACS reagent grade.

Phthalimide, $C_8H_5NO_2$**—147.13** [85-41-6]—White powder.
Assay
Mobile phase: Prepare a mixture of isooctane and methyl-*tert*-butyl ether (88:12).
Procedure: Inject about 20 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 230-nm detector and a 4.6-mm × 15-cm column that contains packing L3. The flow rate is about 2 mL per minute. The area of the $C_8H_5NO_2$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 233° and 235°, with decomposition.

2-Picoline, C_6H_7N**—93.13** [109-06-8]—Colorless to yellowish liquid.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2-mm × 2-m glass column packed with 20% liquid phase G16 on 80- to 100-mesh support S1C; the injection port temperature is maintained at 140°; the detector temperature is maintained at 300°; the column temperature is maintained at 90° and programmed to rise 3° per minute to 140°. The area of the C_6H_7N peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: 1.500 ± 0.002 at 20°.

Picric Acid *(2,4,6-Trinitrophenol; Trinitrophenol),* $C_6H_2(OH)(NO_2)_3$-1,2,4,6**—229.10** [88-89-1]—Use ACS reagent grade.

Picrolonic Acid *(3-Methyl-4-nitro-1- (p-nitrophenyl) -5-pyrazolone),* $C_{10}H_8N_4O_5$**—264.19** [550-74-3]—Yellow to brownish-yellow, crystalline powder. Slightly soluble in water; soluble in alcohol, in chloroform, in ether, in benzene, and in solutions of alkali hydroxides.
Melting Range ⟨741⟩: between 115° and 117°.
Residue on Ignition (Reagent test): negligible, from 200 mg.
Sensitiveness: Dissolve 25 mg in 10 mL of warm water containing 0.1 mL of glacial acetic acid, and filter the solution, if necessary. Dissolve 100 mg of calcium chloride in 250 mL of water, and mix. Heat 1 mL of the calcium chloride solution in a test tube to about 60°, then add to it 1 mL of the picrolonic acid solution: a bulky precipitate forms in 5 minutes or less.

(+)-α-Pinene *((1R,5R)-2-Pinene, (1R,5R)-2,6,6,-Trimethylbicyclo[3.1.1]hept-2-ene),* $C_{10}H_{16}$**—136.23** [7785-70-8]—Use a suitable grade with a GC chromatographic purity of NLT 99%.

β-Pinene *((±)-2(10)-Pinene, 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane),* $C_{10}H_{16}$**—136.23** [127-91-3]—Use a suitable grade with a GC chromatographic purity of NLT 95%.

[NOTE—A suitable grade is available as catalog number 89335 from www.phytolab.com.]

Pipemidic Acid *(8-Ethyl-3,8-dihydro-5-oxo-2-(1-piperazinyl) pyrido[2,3-d]-pyrimidine-6-carboxylic acid),* $C_{14}H_{17}N_5O_3$**—303.3** [51940-44-4]—Use a suitable grade.

Piperazine *(Diethylenediamine),* $C_4H_{10}N_2 \cdot 6H_2O$**—194.23**—Use a suitable grade.

Piperidine, $C_5H_{11}N$**—85.15** [110-89-4]—Colorless liquid. Miscible with water and with alcohol. Specific gravity: about 0.860.
Congealing Range ⟨651⟩: between 12° and 15°.
Boiling Range (Reagent test): Not less than 95% distills between 104° and 106°.
Refractive Index: about 1.454.

Platinic Chloride *(Chloroplatinic Acid),* $H_2PtCl_6 \cdot 6H_2O$**—517.90** [18497-13-7]—Use ACS reagent grade Chloroplatinic Acid.

Add the following:

▲Poly(dimethylsiloxane-co-methylphenylsiloxane), $[Si(CH_3)_2O]_x[Si(CH_3)(C_6H_5)O]_y$[63148-52-7]—Use a suitable grade with a viscosity of 125 centistokes (cSt).▲ (USP 1-Aug-2019)

Polydimethylsiloxane, viscosity 0.65 centistokes *(Hexamethyldisiloxane),* $(CH_3)_3SiOSi(CH_3)_3$**—162.38** [107-46-0]—Liquid. Freezes at about 0°.
Refractive Index ⟨831⟩: about 1.3770.
Specific Gravity ⟨841⟩: about 0.760.

Polyethylene Glycol 200, $H(OCH_2CH_2)_nOH$ in which the average value of *n* is 4**—average molecular weight 200** [25322-68-3]—Clear, colorless or almost colorless, viscous, hygroscopic liquid. Very soluble in acetone and in alcohol; practically insoluble in ether and in fatty oils. Use a suitable grade.
- **REFRACTIVE INDEX** ⟨831⟩: 1.4590 at 20°.
- **DENSITY:** 1.127 at 25°.
- **VISCOSITY:** 4.3 centistokes at 98.9°.

Add the following:

▲Polyethylene Glycol 400—Molecular weight range: 380–420 [25322-68-3]—Use *Polyethylene Glycol (NF monograph).*▲ (USP 1-May-2019)

Polyethylene Glycol 600 [25322-68-3]—A clear, practically colorless, viscous liquid condensation polymer represented by $H(OCH_2CH_2)_nOH$, in which *n* varies from 12 to 14. Its average molecular weight is about 600.
It meets the requirements of all of the tests under *Polyethylene Glycol* (NF monograph) except *Limit of Ethylene Glycol and Diethylene Glycol.*

Add the following:

▲Polyethylene Glycol 4000—Molecular weight range: 3600–4400 [25322-68-3]—Use *Polyethylene Glycol (NF monograph).*▲ (USP 1-May-2019)

Polyethylene Glycol 20,000 [25322-68-3]—Molecular weight range: 15,000–20,000. Hard, white, waxy solid, usually supplied in flake form. Soluble in water with subsequent gel formation.

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Viscosity—Capillary Methods ⟨911⟩, *Viscosity of 25% solution*: Add 50.0 g of test specimen to a 250-mL wide-mouth, screw-cap jar containing 150.0 g of water. Attach the cap securely to the jar, and roll on a mechanical roller until the test specimen is completely dissolved, in 2 to 4 hours. Allow the solution to stand until all air bubbles have disappeared. Another 2 to 4 hours may be required. Adjust the temperature of the solution to 37.8 ± 0.1°, and determine the kinematic viscosity on a suitable viscometer of the Ubbelohde type. The viscosity is not less than 100 centistokes.
pH ⟨791⟩: between 6.5 and 8.0 in a solution (1 in 20).
[NOTE—A five-fold dilution of the test solution prepared for the *Viscosity of 25% Solution* test may be used.]
Residue on Ignition ⟨281⟩: not more than 0.7%, the use of sulfuric acid being omitted.

Polyethylene Glycol Standards with Molecular Weights of 1000, 2000, 3000, 4000, and 6000 Daltons (g/mol) *[Poly(oxy-1,2-ethanediyl), α-hydroxy-ω-hydroxy-; 1,2-Ethanediol, homopolymer],* $H(OCH_2CH_2)_nOH$, in which the average values of *n* are 22, 45, 69, 92, and 149—**average molecular weight (M_w), 1020, 2010, 3060, 4080, and 6550** [25332-68-3]—Use molecular weight standard grades. [NOTE—Suitable grades are available from www.polymer.de.]

Polyoxyethylene 10 Lauryl Ether *(Decaethylene Glycol Monododecyl Ether)* $C_{32}H_{66}O_{11}$—**626.86** [9002-92-0]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number P9769 at http://www.sigma-aldrich.com.]

Polyoxyethylene (23) Lauryl Ether *(Brij-35)*: Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Brij-35."]

Polyoxyethylene (20) Sorbitan Monolaurate[9005-64-5]—Viscous, light yellow to yellow-green liquid. Use a suitable grade.

Add the following:

▲**Polypeptone**—Pancreatic digest of casein and peptic digest of animal tissue combined in equal parts. Use a suitable grade. ▲ (USP 1-May-2020)

Polysaccharide Molecular Weight Standards—Polymaltotriose polymers of different weight-average molecular weight, M_w, values ranging from 5,000 to 400,000 Da.
[NOTE—A suitable set is available from Shodex (www.shodex.com) as Kit P-82.]

Add the following:

▲**Polysorbate 20**—Use *Polysorbate 20 (NF* monograph). ▲ (USP 1-May-2020)

Polysorbate 80 *(Polyoxyethylenesorbitan Monooleate; Tween 80; Polyethylene Glycol Sorbitan Monooleate)*—**micellar average molecular weight 79,000** [9005-65-6]—Use a suitable grade.

Polystyrene Cation-Exchange Resin—See *Cation-Exchange Resin, Polystyrene.*

Polytef—See Poly(tetrafluoroethylene).

Poly(tetrafluoroethylene) *(Polytef; Polytetrafluoroethylene Resin; Teflon),* $(C_2F_4)_n$, where *n* is at least 20,000 [9002-84-0]—Use a suitable grade.

Polyvinyl Alcohol, $(C_2H_4O)_n$ [9002-89-5]—White powder. Soluble in water; insoluble in organic solvents.
- **pH** ⟨791⟩: 5.0–8.0, in a solution (1 in 25)
- **Loss on Drying:** Dry at 110° to constant weight. It loses NMT 5% of its weight.
- **Residue on Ignition:** NMT 0.75%
[NOTE—Suitable grades are available as catalog number U232 from www.avantormaterials.com, catalog number 151940 from www.mpbio.com, and catalog number 114266 from www.emdmillipore.com.]

Potassium Acetate, $KC_2H_3O_2$—**98.14** [127-08-2]—Use ACS reagent grade.

Potassium Alum—Use *Potassium Alum* (USP monograph).

Potassium Arsenate Monobasic, KH_2AsO_4—**180.03** [7784-41-0]—Use a suitable grade with a content of NLT 98%.

Potassium Bicarbonate, $KHCO_3$—**100.12** [298-14-6]—Use ACS reagent grade.

Potassium Biphosphate—See *Potassium Phosphate, Monobasic.*

Potassium Biphthalate *(Acid Potassium Phthalate; Phthalic Acid Monopotassium Salt; Potassium Hydrogen Phthalate Acidimetric Standard),* $KHC_6H_4(COO)_2$—**204.22** [877-24-7]—Use ACS reagent grade Potassium Hydrogen Phthalate, Acidimetric Standard.

Potassium Bisulfate, $KHSO_4$—**136.17** [7646-93-7]—Fused, white, deliquescent masses or granules. Very soluble in water. When ignited, it evolves SO_3 and H_2O, changing first to potassium pyrosulfate, then to sulfate.
Acidity: Dissolve 4 g, accurately weighed, in 50 mL of water, add phenolphthalein TS, and titrate with 1 N alkali: it contains between 34% and 36%, calculated as H_2SO_4.
Insoluble Matter and **Ammonium Hydroxide Precipitate:** Dissolve 10 g in 100 mL of water, add methyl red TS, render slightly alkaline with ammonia TS, boil for 1 minute, and digest on a steam bath for 1 hour. Pass through a tared filtering crucible, wash thoroughly, and dry at 105° for 2 hours: the precipitate weighs not more than 1 mg (0.01%).
For the following tests, prepare a *Test solution* as follows. Dissolve 6 g in 45 mL of water, add 2 mL of hydrochloric acid, boil gently for 10 minutes, cool, and dilute with water to 60 mL.
Heavy Metals (Reagent test): To 30 mL of *Test solution* add phenolphthalein TS, and neutralize with ammonia TS. Add 0.5 mL of glacial acetic acid, dilute with water to 40 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 10 mL of *Test solution* and 0.02 mg of added Pb (0.001%).
Iron ⟨241⟩: To 5 mL of *Test solution* add 2 mL of hydrochloric acid, and dilute with water to 47 mL: the solution shows not more than 0.01 mg of Fe (0.002%).

Potassium Bromate, $KBrO_3$—**167.00** [7758-01-2]—Use ACS reagent grade.

Potassium Bromide, KBr—**119.00** [7758-02-3]—Use ACS reagent grade.

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Potassium Carbonate—See Potassium Carbonate, Anhydrous.

Potassium Carbonate, Anhydrous, K_2CO_3**—138.21** [584-08-7]—Use ACS reagent grade.

Potassium Chlorate, $KClO_3$**—122.55** [3811-04-9]—Use ACS reagent grade.

Potassium Chloride, KCl**—74.55** [7447-40-7]—Use ACS reagent grade.

Potassium Chloroplatinate, K_2PtCl_6**—485.99**—Heavy, yellow powder. Soluble in hydrochloric acid and in nitric acid.

Assay: Accurately weigh about 300 mg, transfer to a 600-mL beaker, add 20 mL of hydrochloric acid, and heat gently if necessary to achieve complete solution. Add zinc granules, slowly, until no more dissolves, then add 2 mL of hydrochloric acid, and digest for 1 hour on a steam bath to coagulate the reduced platinum. Add more acid, if necessary, to ensure that all of the zinc has dissolved. Filter through paper, rinsing the beaker with diluted hydrochloric acid until all of the precipitate is transferred to the filter, then wash with several small portions of water. Ignite the filter in a tared crucible at 800 ± 25° to constant weight. Each mg of residue is equivalent to 1.0 mg of platinum. Not less than 40% is found.

Potassium Chromate, K_2CrO_4**—194.19** [7789-00-6]—Use ACS reagent grade.

Potassium Cyanide, KCN**—65.12** [151-50-8]—Use ACS reagent grade.

Potassium Dichromate, $K_2Cr_2O_7$**—294.18** [7778-50-9]—Use ACS reagent grade.

[NOTE—Potassium dichromate of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 136.]

Potassium Ferricyanide, $K_3Fe(CN)_6$**—329.24** [13746-66-2]—Use ACS reagent grade.

Potassium Ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$**—422.39** [14459-95-1]—Use ACS reagent grade.

Potassium Hyaluronate—White to cream-colored powder. Freely soluble in water. Store in a tight container, in a refrigerator.

Inhibitor Content: Prepare as directed in the *Assay* under *Hyaluronidase for Injection* (USP monograph) a quantity of *Standard solution* containing 1 USP Hyaluronidase Unit in each mL, and a similar quantity of acetate-buffered *Standard solution* using as the solvent 0.1 M, pH 6 sodium acetate buffer (prepared by diluting the 0.2 M buffer prepared as directed below with an equal volume of water). Prepare from the potassium hyaluronate under test 10 mL of *Potassium hyaluronate stock solution*, and dilute 2 mL of it with the specified *Phosphate buffer solution* to make a *Hyaluronate solution*. In the same way, and concurrently, dilute a second 2-mL portion of the stock solution with 0.2 M, pH 6 sodium acetate buffer (containing 16.4 g of anhydrous sodium acetate and 0.45 mL of glacial acetic acid in each 1000 mL).

Place 0.50-mL portions of the *Hyaluronate solution* in each of four 16- × 100-mm test tubes, and place 0.50-mL portions of the acetate-buffered *Hyaluronate solution* in two similar tubes. To two of the four tubes containing *Hyaluronate solution* add 0.50 mL of *Diluent for hyaluronidase solutions*, prepared as directed in the *Assay* under *Hyaluronidase for Injection* (USP monograph). To the remaining two tubes, on a rigid schedule, at 30-second intervals, add 0.50 mL of *Standard solution*. Similarly, to the two tubes containing acetate-buffered *Hyaluronate solution* add at 30-second intervals 0.50-mL portions of acetate-buffered *Standard solution*. Then proceed as directed in the second paragraph for *Procedure*, beginning with "Mix the contents," as far as "Plot the average." The reduction in absorbance of acetate-buffered *Hyaluronate solution* is not less than 25% of that observed in the *Hyaluronate solution*.

Turbidity production: The average absorbance of the solutions in the two tubes containing *Hyaluronate solution* and *Diluent for hyaluronidase solutions* prepared in the test for *Inhibitor Content* is not less than 0.26 at a wavelength of 640 nm in a suitable spectrophotometer using a 1-cm cell.

Potassium Hydrogen Sulfate, $KHSO_4$**—136.17**—White crystals. Soluble in water.

Melting Point ⟨741⟩: about 197°.

Potassium Hydroxide, KOH**—56.11** [1310-58-3]—Use ACS reagent grade.

Potassium Iodate, KIO_3**—214.00** [7758-05-6]—Use ACS reagent grade.

Potassium Iodide, KI**—166.00** [7681-11-0]—Use ACS reagent grade.

Potassium Metabisulfite *(Potassium Disulfite; Potassium Pyrosulfite),* $K_2S_2O_5$**—222.32** [16731-55-8]—Use a suitable grade with a content of not less than 98%.

Potassium Nitrate, KNO_3**—101.10** [7757-79-1]—Use ACS reagent grade.

Potassium Nitrite, KNO_2**—85.10** [7758-09-0]—Use ACS reagent grade.

Potassium Perchlorate, $KClO_4$**—138.55** [7778-74-7]—Use ACS reagent grade.

Potassium Periodate *(Potassium meta-Periodate),* KIO_4**—230.00** [7790-21-8]—Use ACS reagent grade.

Potassium Permanganate, $KMnO_4$**—158.03** [7722-64-7]—Use ACS reagent grade.

Potassium Persulfate, $K_2S_2O_8$**—270.32** [7727-21-1]—Use ACS reagent grade Potassium Peroxydisulfate.

Potassium Phosphate, Dibasic *(Dipotassium Hydrogen Phosphate; Dipotassium Phosphate),* K_2HPO_4**—174.18** [7758-11-4]—Use ACS reagent grade.

Potassium Phosphate, Dibasic, Trihydrate *(Dipotassium Hydrogen Phosphate Trihydrate; Dipotassium Phosphate),* $K_2HPO_4 \cdot 3H_2O$**—228.22** [16788-57-1]—Use a suitable grade with a content of NLT 99.0%.

Potassium Phosphate, Monobasic *(Potassium Biphosphate; Potassium Dihydrogen Phosphate),* KH_2PO_4**—136.09** [7778-77-0]—Use ACS reagent grade.

[NOTE—Certified Potassium Dihydrogen Phosphate is available from the National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 186.]

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Potassium Phosphate, Tribasic, K_3PO_4**—212.27** [7778-53-2]—Deliquescent, orthorhombic crystals. Use ACS reagent grade.

Change to read:

Potassium Pyroantimonate *(Potassium Hexahydroxyantimonate),* $KSb(OH)_6$ ▲or $KSb(OH)_6 \cdot xH_2O$▲ 2S (USP41)**—262.90** [*12208-13-8*]—Use a suitable grade.

Potassium Pyrophosphate, $K_4P_2O_7$**—330.34** [7320-34-5]—Colorless, deliquescent granules. Freely soluble in water; insoluble in alcohol.

Potassium Pyrosulfate [7790-62-7]—Usually available as a mixture of potassium pyrosulfate ($K_2S_2O_7$) and potassium bisulfate ($KHSO_4$). Use ACS reagent grade.

Potassium Sodium Tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$**—282.22** [6381-59-5]—Use ACS reagent grade.

Potassium Sulfate, K_2SO_4**—174.26** [7778-80-5]—Use ACS reagent grade.

Potassium Tellurite *(Potassium Tellurate IV),* K_2TeO_3**—253.79** [7790-58-1]—White, granular powder. Soluble in water. Its solution is alkaline.

Assay: Weigh accurately about 120 mg, transfer to a beaker, and dissolve in a mixture of 10 mL of nitric acid, 10 mL of sulfuric acid, and 25 mL of water. Heat to boiling, and boil until copious fumes of sulfur trioxide are evolved. Cool, cautiously add 100 mL of water, heat to boiling, add 6 g of sodium fluoride, and titrate the hot solution with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate is equivalent to 12.69 mg of K_2TeO_3. Not less than 98% is found.

Chloride ⟨Reagent test⟩: One g shows not more than 0.1 mg of Cl (0.01%).

Potassium Thiocyanate, KSCN**—97.18** [333-20-0]—Use ACS reagent grade.

Potato Starch—See *Starch, Potato.*

Pregnenolone Acetate *(3β-Acetoxy-5-pregnen-20-one, 5-Pregnen-3β-ol-20-one Acetate),* $C_{23}H_{34}O_3$**—358.51** [1778-02-5]—Use a suitable grade with a content of NLT 99%.

Pregnenolone Isobutyrate *(5-Pregnen-3-ol-20-one Isobutyrate),* $C_{25}H_{38}O_3$**—386.57**—Use a suitable grade with a content of NLT 99%.

[NOTE—A suitable grade is available as catalog number EPP2920000 from www.lgcstandards.com or catalog number P2920000 from http://crs.edqm.eu.]

Propionaldehyde, C_3H_6O**—58.08** [123-38-6]—Use a suitable grade.

Propionic Acid *(Propanoic Acid),* $C_3H_6O_2$**—74.08** [79-09-4]—Use ACS reagent grade.

Propionic Anhydride, $C_6H_{10}O_3$**—130.14** [123-62-6]—Colorless liquid. Is decomposed by water. Soluble in methanol, in alcohol, in ether, and in chloroform.

Assay: Accurately weigh about 350 mg into a tared, glass-stoppered flask containing 50 mL of dimethylformamide previously neutralized to the thymol blue endpoint with 0.1 N sodium methoxide in methanol VS. Titrate with 0.1 N sodium methoxide in methanol VS to the thymol blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 13.014 mg of $C_6H_{10}O_3$. Not less than 97.0% is found.

Refractive Index ⟨831⟩: between 1.4035 and 1.4045 at 20°.

Propiophenone, $C_9H_{10}O$**—134.18** [93-55-0]—Use a suitable grade.

***iso*- Propyl Alcohol**—See *Isopropyl Alcohol.*

***n*-Propyl Alcohol** *(1-Propanol),* $CH_3CH_2CH_2OH$**—60.10** [71-23-8]—Use ACS reagent grade.

Propylamine Hydrochloride *(1-Propanamine hydrochloride; n-Propylamine hydrochloride),* $C_3H_9N \cdot HCl$**—95.57** [556-53-6]—Use a suitable grade with a content of not less than 99%.

Add the following:

▲**Propylene Glycol** *(1,2-Propanediol),* $C_3H_8O_2$**—76.10** [57-55-6]—Use ACS reagent grade 1,2-Propanediol.▲ (USP 1-May-2019)

Propylparaben—Use *Propylparaben* ⟨NF monograph⟩.

Protein Molecular Weight Standard: Also known as protein molecular weight markers (for SDS-PAGE) and consists of a mixture of several proteins of well-defined molecular weights. The products are generally available in a suitable buffer containing a suitable reducing agent (generally, 100 mM DTT), a preservative (for example, sodium azide), and 50% glycerol to prevent freezing. Use a suitable grade. Store at –20°.

Protein Standard Solution (8 g/dL)—A solution containing 5 g of Albumin Human and 3 g of human gamma globulin per dL.

[NOTE—A suitable grade is available as Protein Standard Solution, catalog number 540-10, from Sigma-Aldrich, www.sigma-aldrich.com.]

Protocatechuic Acid ***(3,4-Dihydroxybenzoic acid)***, $C_7H_6O_4$**—154.12** [99-50-3]—Use a suitable grade.

Pullulanase *(Amylopectin-6-gluconohydrolase)* [9075-68-7]—An enzyme obtained from *Kleibsiella pneumoniae.* It contains not less than 30 units per mg of protein. One unit defined for enzymatic activity will liberate 1.0 µmol of maltotriose (measured as glucose) from pullulan per minute at pH 5.0 at 30°. It can be suspended in 3.2 M ammonium sulfate solution, pH 6.2.

• **MEASUREMENT OF RELATIVE PULLULANASE ACTIVITY**

Determination of pullulanase activity

Substrate: Dissolve pullulan[1] in water to make a 1.25% (w/v) solution.

[NOTE—Add pullulan to the water. Clumping will occur if water is added to pullulan.]

Buffer solution A, pH 5.0: Add 0.1 M disodium phosphate (27 g of dibasic sodium phosphate in each L of the solution) to 0.1 M citric acid (21 g of citric acid in each L of the solution) to adjust pH to 5.0.

Buffer solution B, pH 6.0: Add diluted acetic acid to 1 M sodium acetate (136 g of sodium acetate in each L of solution) to adjust the pH to 6.0. Dilute with water to

[1] A suitable supplier for pullulan is www.intl.hayashibara.co.jp.

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prepare the final buffer solution as 0.01 M acetic acid buffer, pH 6.0.

Somogyi reagent: Add 54 g of disodium phosphate heptahydrate or 28 g of anhydrous disodium hydrogen phosphate and 40 g of potassium sodium tartrate to about 650 mL of water or about 700 mL for anhydrous disodium hydrogen phosphate. Add 100 mL of 1 N sodium hydroxide to this solution and mix. Add 80 mL of 10% cupric sulfate to the solution, and mix. Heat until any solid is completely dissolved. Add 180 g of anhydrous sodium sulfate to the solution and adjust the volume to 1 L. Allow the solution to stand at room temperature for 1 or 2 days to let insoluble matter precipitate. Filter the solution with standard filter paper, and keep the solution in a brown bottle with a ground-glass stopper.

Nelson reagent: Dissolve 50 g of ammonium molybdate in 900 mL of water. Add 42 g of sulfuric acid, and mix. Dissolve 6 g of sodium arsenate or 3.6 g of monobasic potassium arsenate in 50 g of water. Allow the solution to stand in a brown bottle with a ground-glass stopper at 37° for 1 or 2 days.

Glucose standard solution: Dry anhydrous glucose crystals under less than 50-mm Hg at 60° for 5 hours, and calculate the water content. Transfer 10.00 g of dried glucose to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL to a 1-L volumetric flask and dilute to volume with water. Each mL contains 100 µg of glucose.

Pullulanase diluent: Dilute pullulanase with *Buffer solution B, pH 6.0* to prepare a solution having the enzyme activity of about 0.2 units per mL.

[NOTE—The measurement range is between 0.1 and 0.4 units per mL.]

Record the dilution factor (F_{PD}). This diluent is used as a diluted enzyme solution.

Procedure: Transfer 4 mL of *Substrate* to a test tube and add 0.5 mL of *Buffer solution A, pH 5.0,* mix, and incubate at 30°. Add 0.5 mL of *Pullulanase diluent,* and mix thoroughly. After 30 seconds, transfer 1 mL of this solution to a test tube labeled as "Pullulan test solution 1", and add 2 mL of *Somogyi reagent,* and mix. After 30 minutes and 30 seconds, transfer 1 mL of the mixture of *Substrate* and *Pullulanase diluent* to a second test tube labeled as "Pullulan test solution 2", add 2 mL of *Somogyi reagent,* and mix. In a third test tube labeled as "Standard blank", mix 2 mL of *Somogyi reagent* and 1 mL of water. In a fourth test tube labeled as "Glucose standard solution", mix 2 mL of *Somogyi reagent* and 1 mL of *Glucose standard solution,* and add 1 mL of water. Incubate the fourth test tube in a boiling water bath for exactly 10 minutes. Remove the tube and allow it to cool in cold running water. Add 2 mL of *Nelson reagent,* mix well, and allow the solution to stand for at least 15 minutes. Add 5 mL of water to each of the four test tubes, and mix thoroughly. Determine the absorbance at 520 nm of the Standard blank, A_{blank}, of the *Glucose standard solution,* A_{Std}, of the Pullulan test solution 1, A_0, and of the Pullulan test solution 2, A_{30}, using water as the blank. One unit is defined as the enzymatic activity that produces 1 µmol of maltotriose (measured as glucose) from pullulan per minute. Calculate the pullulanase activity, PA, in units per mL, using the formula:

$$PA = [(A_{30} - A_0)/(A_{Std} - A_{blank})] \times 0.185 \times F_{PD}$$

• **MEASUREMENT OF PROTEIN AMOUNT (MEASURED AS ALBUMINOID AMOUNT) FOR THE CALCULATION OF SPECIFIC ACTIVITY**

Reagent A: Prepare a solution having known concentrations of about 0.1 N sodium hydroxide and about 0.2 M sodium carbonate.

Reagent B: Transfer 0.5 g of cupric sulfate and 1.0 g of sodium citrate dihydrate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Lowry solution: Mix *Reagent A* and *Reagent B* at the proportion of 50:1.

Diluted Folin-Ciocalteu's phenol reagent (for albuminoid quantification): Prepare a two-fold dilution of 2 N Folin-Ciocalteu's phenol reagent commercially available or prepare a solution by making an appropriate dilution from Folin-Ciocalteu Phenol TS (see *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩, *Method 2*).

Bovine serum albumin standard stock solution: Transfer 0.05 g of bovine serum albumin to a 500-mL volumetric flask, dissolve in and dilute with water to volume, and mix. It contains 100 µg of bovine serum albumin per mL.

Standard solutions: Using appropriate dilutions of *Bovine serum albumin standard stock solution* in water, prepare five *Standard solutions* having concentration equally spaced between 5 and 100 µg of bovine serum albumin per mL.

Test solution: Dilute pullulanase with *Buffer solution B, pH 6.0* in order to obtain a solution having a concentration between 60 and 70 µg of albuminoid per mL.

[NOTE—Water can be used as diluent.]

Record the dilution factor, F_{TS}.

Blank solution: Use water.

Procedure: To 0.3 mL in separate tubes of the *Standard solutions,* the *Test solution,* and the *Blank solution,* add 3 mL of *Lowry solution,* and mix. Allow to incubate at room temperature for 10 minutes. Add 0.3 mL of *Diluted Folin-Ciocalteu's phenol reagent* to each tube, mix immediately, and allow to stand at room temperature for 60 minutes. Determine the absorbances of the *Standard solutions* and the *Test solution* at the wavelength of maximum absorbance at about 750 nm, using the *Blank solution* as the blank.

Calculation: [NOTE—The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using linear regression method, plot the absorbances of the *Standard solutions* versus the protein (bovine serum albumin) concentrations, in µg per mL, and determine the best fit curve. Using the plot, determine the concentration, $C_{albuminoid}$, in µg per mL, of protein (albuminoid amount) in the *Test solution.* Calculate the albuminoid concentration, in mg per mL, in the pullulanase taken by the formula:

$$C_{protein} = (C_{albuminoid} \times F_{TS})/1000$$

Calculate the specific activity, SA, in units per mg, of pullulanase using the formula:

$$SA = PA/C_{protein}$$

Pumice—A substance of volcanic origin consisting chiefly of complex silicates of aluminum and alkali metals. Occurs as very light, hard, rough, porous, gray masses, or as a gray-colored powder. Is insoluble in water and is not attacked by diluted acids.

Acid- and Water-Soluble Substances: Boil 2.0 g of powdered pumice with 50 mL of diluted hydrochloric acid under a reflux condenser for 30 minutes. Cool, and filter. To half of the filtrate add 5 drops of sulfuric acid, evaporate to

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dryness, ignite, and weigh: the residue weighs not more than 60 mg (6.0%).

Purine, $C_5H_4N_4$**—120.11** [120-73-0]—White to off-white powder.
Melting Range ⟨741⟩: between 214° and 217°.
A single spot is exhibited when it is examined by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of butyl alcohol, water, and glacial acetic acid (60:25:15).

Putrescine Dihydrochloride, $C_4H_{12}N_2 \cdot 2HCl$**—161.07** [333-93-7]—White, crystalline powder. Use a suitable grade.

Pyrazole, $C_3H_4N_2$ [288-13-1]—White to pale yellow crystals or crystalline powder. Soluble in water, in alcohol, and in ether.
Melting Range ⟨741⟩: between 67° and 71°.

Pyrene, $C_{16}H_{10}$**—202.25** [129-00-0]—White to light yellow crystals.
Assay: Transfer about 9 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Using a suitable spectrophotometer, 1-cm cells, and methanol as the blank, record the absorbance of the solution at the wavelength of maximum absorbance at about 238 nm. From the observed absorbance, calculate the absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩): the absorptivity is not less than 432.9, corresponding to not less than 98% of $C_{16}H_{10}$.
Melting Range ⟨741⟩: between 149° and 153° over a 2° range.

Pyridine, C_5H_5N**—79.10** [110-86-1]—Use ACS reagent grade.

Add the following:

▲Pyridine, Anhydrous, C_5H_5N**—79.1** [110-86-1]—Use a suitable grade with a content of NLT 99% and a water content of NMT 0.003% (see *Water Determination* ⟨921⟩, *Method I*).▲ (USP 1-May-2019)

Pyridine, Dried [110-86-1]—Use ACS reagent grade.

Pyridoxal Hydrochloride, $C_8H_9NO_3 \cdot HCl$**—203.62** [65-22-5]—White to slightly yellow crystals or crystalline powder. Gradually darkens on exposure to air or sunlight. One g dissolves in about 2 mL of water and in about 25 mL of alcohol. Insoluble in acetone, in chloroform, and in ether. Its solutions are acid (pH about 3).
Melting Range ⟨741⟩: between 171° and 175° with some decomposition.
Residue on Ignition (Reagent test): not more than 0.1%.
Loss on drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Nitrogen Content (Reagent test): Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 6.7% and 7.1% of N is found.
Chloride Content: Accurately weigh about 500 mg, previously dried at 105° for 2 hours, and dissolve in 50 mL of water. Add 3 mL of nitric acid and 50.0 mL of 0.1 N silver nitrate VS, then add 5 mL of nitrobenzene, shake for about 2 minutes, add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS: each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 17.2% and 17.7% is found.

Pyridoxal 5-Phosphate, $4\text{-}CHOC_5HN\text{-}2\text{-}CH_3$, 3-OH, $5\text{-}CH_2PO_4H_2 \cdot H_2O$**—265.16** [41468-25-1]—Light yellow powder. Use a suitable grade.

Pyridoxamine Dihydrochloride, $C_8H_{12}N_2O_2 \cdot 2HCl$**—241.11** [524-36-7]—White to slightly yellow crystals or crystalline powder. Gradually darkens on exposure to air or sunlight. One g dissolves in about 1 mL of water and in about 60 mL of alcohol. Insoluble in chloroform and in ether. Its solutions are acid.
Melting Range ⟨741⟩: between 225° and 230°, with some decomposition.
Residue on Ignition (Reagent test): not more than 0.15%.
Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Nitrogen Content (Reagent test): Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 11.3% and 11.8% of N is found.
Chloride Content: Determine as directed in the test for *Chloride Content* under Pyridoxal Hydrochloride: between 29.1% and 29.6% of Cl is found.

1-(2-Pyridylazo)-2-naphthol, $C_{15}H_{11}N_3O$**—249.27** [85-85-8]—Stable, orange-red crystals. Soluble in alcohol and in hot solutions of dilute alkalies; slightly soluble in water.
Melting Range ⟨741⟩: between 140° and 142°.
Sensitiveness: Add 0.1 mL of a 1 in 1000 solution of it in alcohol to a mixture of 10 mL of water and 1 mL of a buffer solution prepared by mixing 80 mL of 0.2 M acetic acid and 20 mL of sodium acetate solution (8.2 in 100), and mix. To this solution add 1 mL of a mixture of 1 mL of cupric sulfate TS and 2 mL of water, and mix: the color changes from yellow to red.

4-(2-Pyridylazo)resorcinol (*PAR*), $C_{11}H_9N_3O_2$, free acid; $C_{11}H_8N_3NaO_2$, monosodium salt free acid monosodium salt**—215.21, —237.21,** [1141-59-9, free acid; 16593-81-0, monosodium salt]—Use ACS reagent grade.

3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic Acid, Disodium Salt *(3-(2-Pyridyl)-5,6-bis(5-sulfo-2-furyl)-1,2,4-triazine, Disodium Salt Hydrate),* $C_{16}H_8N_4Na_2O_8S_2$**—494.37** [79551-14-7]—Use a suitable grade.
[NOTE—A suitable grade is available as product number P4272 from Sigma-Aldrich, 1-800-558-9160; www.sigma-aldrich.com.]

Pyrogallol, $C_6H_3(OH)_3$**—126.11** [87-66-1]—Use ACS reagent grade.

Pyrrole, C_4H_5N**—67.09** [109-97-7]—Clear liquid, colorless when freshly distilled, becoming yellow in a few days. Specific gravity: about 0.94. Insoluble in water; soluble in alcohol, in benzene, and in ether.
Boiling Range (Reagent test): Not less than 90% distills between 128° and 132°.

2-Pyrrolidone, C_4H_7NO**—85.11** [616-45-5]—Use a suitable grade with a content of NLT 98.0% (by GC).
[NOTE—A suitable grade is available as catalog number TCI-P0575 from www.spectrumchemical.com.]

Pyruvic Acid, $CH_3COCOOH$**—88.06** [127-17-3]—Colorless to light yellow liquid. Miscible with water, with alcohol, and with ether.
Refractive Index ⟨831⟩: about 1.43 at 20°.
Assay: Accurately weigh about 1 g, transfer to a suitable container, and add 100 mL of water. Mix, add

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phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS. Each mL of 0.5 N sodium hydroxide is equivalent to 44.03 mg of $CH_3COCOOH$: not less than 98% of $CH_3COCOOH$ is found.

Quinhydrone, $C_6H_4(OH)_2 \cdot C_6H_4O_2$**—218.21** [106-34-3]—Green crystals having a metallic luster. Slightly soluble in cold water; soluble in hot water, in alcohol, and in ether.

Assay: Transfer about 450 mg, accurately weighed, to a glass-stoppered flask, add 50 mL of 1 N sulfuric acid and 3 g of potassium iodide, insert the stopper in the flask, and shake until dissolved. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.405 mg of quinone ($C_6H_4O_2$). Between 49.0% and 51.0% is found.

Alcohol-Insoluble Matter: Dissolve 10 g in 100 mL of hot alcohol, filter through a suitable tared crucible of fine porosity, and wash with hot alcohol until the last washing is colorless. Dry at 105°, cool in a desiccator, and weigh: the residue weighs not more than 1.0 mg (0.010%).

Residue on Ignition ⟨Reagent test⟩: not more than 0.050%, a 2.0-g test specimen being used. Save the residue.

Sulfate: Transfer 1 g to a platinum crucible, add 10 mL of hot water and 0.5 g of sodium carbonate, evaporate to dryness, and ignite, protected from the sulfur in the flame, until the residue is nearly white. Cool, add 20 mL of water and 1 mL of 30 percent hydrogen peroxide, boil gently for a few minutes, add 2 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Cool, dissolve the residue in 20 mL of water, filter, and to the filtrate add 1 mL of 1 N hydrochloric acid and 3 mL of barium chloride TS: any turbidity produced within 10 minutes does not exceed that in a control containing 0.2 mg of added SO_4 and 0.5 mg of sodium carbonate, 1 mL of 30 percent hydrogen peroxide, and 2 mL of hydrochloric acid previously evaporated on a steam bath to dryness (0.02%).

Heavy Metals: To the residue retained from the test for *Residue on Ignition* add 2 mL of hydrochloric acid and 0.5 mL of nitric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 30 mL of hot water containing 1 mL of 1 N hydrochloric acid, cool, dilute with water to 40 mL, and mix. Dilute 20 mL of this solution (retain the rest of the solution) with water to 25 mL, adjust to a pH between 3.0 and 4.0 by the addition of 1 N acetic acid or 6 N ammonium hydroxide as necessary, dilute with water to 40 mL, and add 10 mL of freshly prepared hydrogen sulfide TS: any brown color produced does not exceed that in a control containing 0.02 mg of added Pb (0.002%).

Iron ⟨241⟩: To 10 mL of the solution retained from the test for *Heavy Metals* add 2 mL of hydrochloric acid, and dilute with water to 47 mL: the solution shows not more than 0.01 mg of Fe (0.002%).

Quinone—See *p-Benzoquinone*.

Rayon—Use *Purified Rayon* (USP monograph).

Direct Red 80, $C_{45}H_{26}N_{10}Na_6O_{21}S_6$**—1373.07** [2610-10-8]—Red powder. Soluble in water; poorly soluble in alcohol. Use a suitable grade.

Red-Cell Lysing Agent: The reagent is available as a solution containing 0.33% potassium cyanide and 0.11% sodium nitroprusside and a quaternary ammonium salt as the surface active agent (5.5%).

[NOTE—The reagent is manufactured by Coulter Electronics Diagnostics, Hialeah, FL and is available from many suppliers under the name of Zapoglobin® (or Zap-oglobin®).]

Red Phosphorus—See *Phosphorus, Red*.

Resazurin (Sodium), $C_{12}H_6NNaO_4$**—251.17** [62758-13-8]—A brownish-purple, crystalline powder. One g dissolves in 100 mL of water, forming a deep-violet-colored solution.

Hydrogen sulfide and other compounds containing the thiol group decolorize solutions of resazurin sodium, forming dihydroresorufin. When the decolorized solution is shaken in the presence of air, a rose color develops as a result of the formation of resorufin.

Resorcinol *(1,3-Benzenediol),* $C_6H_4(OH)_2$**—110.11** [108-46-3]—Use ACS reagent grade 1,3-Benzenediol.

9*Z*-Retinoic Acid *(9-cis-Tretinoin; 9-cis Retinoic Acid; Alitretinoin),* $C_{20}H_{28}O_2$**—300.44** [5300-03-8]—Use a suitable grade with a content of NLT 98%. Store at −20°. [NOTE—A suitable grade is available as catalog number R4643 from www.sigma-aldrich.com.]

Retinyl Palmitate, $C_{36}H_{60}O_2$**—524.9**—Yellow liquid.

Assay

Mobile phase: Prepare a mixture of acetonitrile and tetrahydrofuran (55:15).

Procedure: Inject about 10 µL into a suitable liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 320-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. The area of the $C_{36}H_{60}O_2$ peak is not less than 93% of the total peak area.

Reverse Transcriptase—Use a suitable grade.

[NOTE—A suitable grade is available from BD Biosciences, www.bdbiosciences.com.]

Rhodamine B *(Tetraethylrhodamine),* $C_{28}H_{31}ClN_2O_3$**—479.01** [81-88-9]—Green crystals or a reddish-violet powder. Very soluble in water, yielding a bluish-red solution that is strongly fluorescent when dilute. Very soluble in alcohol; slightly soluble in dilute acids and in alkali solutions. In strong acid solution, it forms a pink complex with antimony that is soluble in isopropyl ether.

Clarity of Solution: Its solution (1 in 200) is complete and clear.

Residue on Ignition ⟨Reagent test⟩: Ignite 1 g with 1 mL of sulfuric acid: the residue weighs not more than 2 mg (0.2%).

Rhodamine 6G, $C_{28}H_{31}C_1N_2O_3$**—479.02** [989-38-8]—Use a suitable grade.

Ribonuclease Inhibitor—Use a suitable grade.

[NOTE—A suitable grade is available from BD Biosciences, www.bdbiosciences.com.]

Rose Bengal Sodium *(Disodium Salt of 4,5,6,7-Tetrachloro-2′,4′,5′,7′-tetraiodofluorescein),* $C_{20}H_2Cl_4I_4Na_2O_5$**—1017.64** [632-69-9]—Fine, rose-colored crystals or crystalline powder. Soluble in water.

[NOTE—Render commercially available material suitably pure by the following treatment. Dissolve 8 g in 200 mL of water, and adjust to a pH between 10 and 11, using short-range pH indicator paper. Add 200 mL of acetone, while stirring gently, then add dilute hydrochloric acid (1 in 10), while continuing to stir, until the pH of the solution reaches 4.0. Add 400 mL more of water, with stirring, and continue the stirring for 5 minutes. Filter the crystals on a filtering funnel, and return the crystals to the beaker used for crystallization. Recrystallize three more times in the same manner, and dry the crystals at 110° for 12 hours.

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Store in an amber bottle in a refrigerator at a temperature between 2° and 8°. Prepare this reagent fresh monthly.]

Chromatographic Purity: Dissolve 100 mg of rose bengal sodium, prepared as described above, in 100 mL of water, and apply 10 µL of the solution on suitable chromatographic paper. Develop the chromatogram by ascending chromatography, using a mixture of 1 part of dilute alcohol (1 in 4) and 1 part of dilute stronger ammonia water (1 in 12). Examine the chromatogram in daylight and under UV light (360 nm): no colored or fluorescent spot is visible other than the rose bengal sodium spot.

[NOTE—A suitable grade is available commercially as "Silica Gel H."]

Ruthenium Red *(Ruthenium Oxychloride, Ammoniated)*, $Ru_2(OH)_2Cl_4 \cdot 7NH_3 \cdot 3H_2O$—**551.23** [11103-72-3]—A brownish-red to dark purple powder. Soluble in water.

S Designations—See supports for gas chromatography under *Reagents, Chromatographic Columns*.

Sabinene *(Thuj-4(10)-ene, 4-Methylene-1-isopropylbicyclo[3.1.0]hexane, 1-Isopropyl-4-methylenebicyclo[3.1.0]hexane)*, $C_{10}H_{16}$—**136.23** [*3387-41-5*]—Use a suitable grade with a GC chromatographic purity of NLT 95%.

[NOTE—A suitable grade is available as catalog number 82342 from www.phytolab.com.]

Saccharose—Use *Sucrose* (NF monograph).

Safranin O [477-73-6]—Dark red powder consisting of a mixture of 3,7-diamino-2,8-dimethyl-5-phenylphenazinium chloride, $C_{20}H_{19}ClN_4$—350.85, and 3,7-diamino-2,8-dimethyl-5-*o*-tolylphenazinium chloride, $C_{21}H_{21}ClN_4$—364.88—Sparingly soluble in 70 percent alcohol yielding a clear red solution with a yellowish-red fluorescence.

IDENTIFICATION

- **A.** To 10 mL of a 0.5% w/v solution add 5 mL of hydrochloric acid: a bluish violet solution is produced.
- **B.** To 10 mL of a 0.5% w/v solution add 5 mL of sodium hydroxide solution (1 in 5): a brownish-red precipitate is produced.
- **C.** To 100 mg add 5 mL of sulfuric acid: a green solution is produced, which, on dilution, changes to blue and finally to red.

Absorption Characteristics: Dissolve 50 mg in 250 mL of 50 percent alcohol. Dilute 3 mL of this solution with 50 percent alcohol to 200 mL. Determine the absorbance, in a 1-cm cell, with a suitable spectrophotometer. The absorbance maximum is in the range of 530 to 533 nm; the ratio $(P - 15)/(P + 15)$ is between 1.10 and 1.32, in which P is the wavelength of maximum absorbance.

[NOTE—A suitable grade is available as catalog number 10,214-8 from Sigma-Aldrich, www.sigma-aldrich.com.]

Salicylaldazine, $C_{14}H_{12}N_2O_2$—**240.26** [959-36-4]—Use a suitable grade or prepare as follows. Dissolve 300 mg of hydrazine sulfate in 5 mL of water, add 1 mL of glacial acetic acid and 2 mL of a freshly prepared 1-in-5 solution of salicylaldehyde in isopropyl alcohol, mix, and allow to stand until a yellow precipitate is formed. Extract the mixture with two 15-mL portions of methylene chloride. Combine the methylene chloride extracts, and dry over anhydrous sodium sulfate. Decant the methylene chloride solution, and evaporate it to dryness. Recrystallize the residue of salicylaldazine from a mixture of warm toluene and methanol (60:40) with cooling. Filter, and dry the crystals under vacuum.

[NOTE—A suitable grade is available as catalog number 218090 from MP Biochemicals (www.mpbio.com).]

Salicylaldehyde, *(2-Hydroxybenzaldehyde)*, $2\text{-}HOC_6H_4CHO$—**122.12** [90-02-8]—Use a suitable grade with a content of NLT 98.0%.

Salicylic Acid—Use *Salicylic Acid* (USP monograph).

Sand, Standard 20- to 30-Mesh—Silica sand, composed almost entirely of naturally rounded grains of nearly pure quartz. Predominantly graded to pass an 850-µm (No. 20) sieve (85 to 100 percentage passing) and be retained on a 600-µm (No. 30) sieve (0 to 5 percentage passing).

[NOTE—A suitable grade is available as Ottawa Standard Sand from Thomas Scientific, 99 High Hill Road at I-295, P.O. Box 99, Swedesboro, NJ 08085-0099.]

Sand, Washed—It may be prepared as follows. Digest clean, hard sand at room temperature with a mixture of 1 part of hydrochloric acid and 2 parts of water (about 13% of HCl) for several days, or at an elevated temperature for several hours. Collect the sand on a filter, wash with water until the washings are neutral and show only a slight reaction for chloride, and finally dry. Washed sand meets the following tests.

Substances Soluble in Hydrochloric Acid: Digest 10 g with a mixture of 10 mL of hydrochloric acid and 40 mL of water on a steam bath for 4 hours, replacing from time to time the water lost by evaporation. Filter, and to 25 mL of the filtrate add 5 drops of sulfuric acid, evaporate, and ignite to constant weight: the residue weighs not more than 8 mg (0.16%).

Chloride (Reagent test): Shake 1 g with 20 mL of water for 5 minutes, filter, and add to the filtrate 1 mL of nitric acid and 1 mL of silver nitrate TS: any turbidity produced corresponds to not more than 0.03 mg of Cl (0.003%).

Sawdust, Purified—It may be prepared as follows. Extract sawdust in a percolator, first with sodium hydroxide solution (1 in 100), and then with dilute hydrochloric acid (1 in 100) until the acid percolate gives no test for alkaloid with mercuric-potassium iodide TS or with iodine TS. Then wash with water until free from acid and soluble salts, and dry. Purified sawdust meets the following test.

Alkaloids: To 5 g of purified sawdust contained in a flask add 50 mL of a mixture of 2 volumes of ether and 1 volume of chloroform and 10 mL of ammonia TS, and shake frequently for 2 hours. Decant 20 mL of the clear, ether-chloroform liquid, and evaporate to dryness. Dissolve the residue in 2 mL of dilute hydrochloric acid (1 in 12), and divide into two portions. To 1 portion add mercuric-potassium iodide TS, and to the other add iodine TS: no turbidity is produced in either portion.

Scandium Oxide, Sc_2O_3—**137.91** [12060-01-1]—Fine, white powder.

Secondary Butyl Alcohol—See *Butyl Alcohol, Secondary*.

Selenious Acid *(Selenous Acid)*, H_2SeO_3—**128.97** [7783-00-8]—Colorless or white crystals, efflorescent in dry air and hygroscopic in moist air. Soluble in water and in alcohol.

Assay: Accurately weigh about 100 mg, transfer to a glass-stoppered flask, and dissolve in 50 mL of water. Add 10 mL of potassium iodide solution (3 in 10) and 5 mL of hydrochloric acid, mix, insert the stopper in the flask, and allow to stand for 10 minutes. Dilute with 50 mL of water, add 3 mL of starch TS, and titrate with 0.1 N sodium thiosulfate VS until the color is no longer diminished, then

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titrate with 0.1 N iodine VS to a blue color. Subtract the volume of 0.1 N iodine solution from the volume of 0.1 N sodium thiosulfate to give the volume of 0.1 N thiosulfate equivalent to selenious acid. Each mL of 0.1 N sodium thiosulfate is equivalent to 3.225 mg of H_2SeO_3: not less than 93% is found.
Insoluble Matter: Dissolve 1 g in 5 mL of water: the solution is clear and complete.
Residue on Ignition ⟨Reagent test⟩: not more than 1.0 mg (0.01%), from 10 g.
Selenate and **Sulfate:** Dissolve 500 mg in 10 mL of water, and add 0.1 mL of hydrochloric acid and 1 mL of barium chloride TS: no turbidity or precipitate is formed within 10 minutes.

Selenium, Se—At. Wt. 78.96 [7782-49-2]—Dark-red amorphous, or bluish-black, crystalline powder. Soluble in solutions of sodium and potassium hydroxides or sulfides; insoluble in water. Use a suitable grade with a content of not less than 99.99%.

Selenomethionine, $C_5H_{11}NO_2Se$—196.11 [1464-42-2]—[CAUTION—Handle with care, as this reagent is highly toxic.]
Assay: Weigh accurately about 750 mg, dissolve in 100 mL of methanol, add crystal violet TS, and titrate with 0.1 N perchloric acid to a blue-green endpoint. Each mL of 0.1 N perchloric acid is equivalent to 19.61 mg of $C_5H_{11}NO_2Se$: between 97.0% and 103.0%, calculated on the as-is basis, is found.
Melting Range ⟨741⟩: about 260°, with decomposition.
Nitrogen Determination ⟨461⟩: Determine by the Kjeldahl method: between 6.8% and 7.4%, calculated on the as-is basis, is found.

Silica, Calcined Diatomaceous—See *Diatomaceous Silica, Calcined*.

Silica, Chromatographic, Silanized, Flux-Calcined, Acid-washed—Use a suitable grade.
[NOTE—Suitable grades are available commercially as "Aeropak 30," "Diatoport S," and "Gas-Chrom Z."]

Silica Gel—An amorphous, partly hydrated SiO_2 occurring in glassy granules of varying size. When used as a desiccant, it frequently is coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated (i.e., may regain their capacity to absorb water) by being heated at 110° until the gel assumes the original color.
For use as a desiccant, use ACS Silica Gel Desiccant grade.

Silica Gel, Binder-Free—Silica gel for chromatographic use formulated without a binder, since only activated forms of the silica gel are used as the binding agent.
[NOTE—A suitable grade is available commercially as "Silica Gel H."]

Silica Gel, Chromatographic—Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Silica Gel G."]

Silica Gel, Octadecylsilanized Chromatographic—Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Analtech Reversed Phase Uniplates" from www.spectrumchemical.com.]

Add the following:

▲**Silica Gel, Precoated Plates, with Fluorescence Indicator F_{254}**—Plates coated with a uniform 200-µm layer of porous (60 Å pore size) silica gel having irregular particles of 2–10 µm and an average particle size of 5 µm, a polymeric binder, and a fluorescence indicator (F_{254}).▲ (USP 1-Aug-2019)

Silica Gel, Porous—Use a grade suitable for high-pressure liquid chromatography.
[NOTE—A suitable grade for reverse phase high-pressure liquid chromatography is available as "LiChrosorb SI60, Reverse Phase."]

Silica Gel–Impregnated Glass Microfiber Sheet—Use a suitable grade.
[NOTE—A suitable grade is available commercially as "Seprachrom" Chamber with Type SG ITLC, Product No. 51923, from Gelman Instrument Co., Ann Arbor, MI 48106.]

Silica Gel Mixture, Chromatographic—A mixture of silica gel with a suitable fluorescing substance.
[NOTE—A suitable grade is available commercially as "Silica Gel GF 254."]

Silica Gel Mixture, Chromatographic, with Chemically Bound Amino Groups—Use a suitable grade.

Silica Gel Mixture, Dimethylsilanized, Chromatographic—Use a suitable grade.
[NOTE—A suitable grade is available as "Silica Gel 60 silanized RP-2 F_{254}," from EMD Chemicals, www.emdchemicals.com.]

Silica Gel Mixture, Octadecylsilanized Chromatographic—Use a suitable grade.
[NOTE—A suitable grade is available commercially as KC-18F from Whatman Chemical Separation, Inc., 9 Bridewell Place, Clifton, NJ 07014.]

Silica Gel Mixture, Octylsilanized, Chromatographic—Use a mixture of RP-8 chromatographic silica gel with a suitable fluorescing substance agent.

Silica Microspheres—Use a suitable grade.
[NOTE—A suitable grade, in a controlled-diameter, spherical, porous form, is available commercially as "Zorbax Sil," from Agilent, www.agilent.com.]

Siliceous Earth, Chromatographic—For gas chromatography, use a specially prepared grade meeting the following general description: Purified siliceous earth of suitable mesh size that has been acid- and/or base-washed. It may or may not be silanized.
For column partition chromatography, it is essential that the material be free from interfering substances. If such interferences are known or thought to be present, purify the material as follows: Place a pledget of glass wool in the base of a chromatographic column having a diameter of 100 mm or larger, and add *Purified Siliceous Earth* (NF monograph) to a height equal to 5 times the diameter of the column. Add a volume of hydrochloric acid equivalent to one-third the volume of siliceous earth, and allow the acid to percolate into the column. Wash the column with methanol, using small volumes at first to rinse the walls of the column, and continue washing with methanol until the last washing is neutral to moistened litmus paper. Extrude the washed column into shallow dishes, heat on a steam bath to remove the excess methanol, and dry at 105° until the material is powdery and

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free from traces of methanol. Store the dried material in well-closed containers.

[NOTE—A suitable grade is "Chromosorb W-AW."]

[NOTE—Suitable silanized grades for gas chromatography are "Gas Chrom Q," and "Chromosorb W (AW-DMCS-treated).]

[NOTE—A suitable grade for column chromatography is acid-washed "Celite 545," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Siliceous Earth, Chromatographic, Silanized—Place about 450 g of purified siliceous earth in a large, open, glass crystallizing dish in a vacuum desiccator containing 30 mL of a suitable silane, e.g., a mixture of 1 volume of dimethyldichlorosilane and 1 volume of trimethylchlorosilane, or a mixture of 1 volume of methyltrichlorosilane and 2 volumes of dimethyldichlorosilane. Apply vacuum intermittently for several hours, until no liquid silane remains. Float the treated purified siliceous earth on water, and gently agitate to allow any uncoated particles to sink. Skim the silanized material off the surface, wash it on a sintered-glass funnel with warm methanol until the filtrate no longer is acidic, and dry at 110°.

Silicic Acid, $SiO_2 \cdot xH_2O$**—(anhydrous) 60.08** [1343-98-2]—White, amorphous powder. Insoluble in water and in acids; soluble in hot solutions of strong alkalies.

- **RESIDUE ON IGNITION** (Reagent test): not less than 80.0%.
- **NONVOLATILE WITH HYDROFLUORIC ACID:** Heat 500 mg with 1 mL of sulfuric acid and 10 mL of hydrofluoric acid in a platinum crucible to dryness, and ignite to constant weight: the weight of the residue does not exceed 1.0 mg (0.2%).
- **CHLORIDE** (Reagent test): One g shows not more than 0.05 mg of Cl (0.005%).
- **SULFATE** (Reagent test): Boil 2 g with 20 mL of dilute hydrochloric acid (1 in 40), filter, neutralize the filtrate with ammonia TS, and dilute with water to 20.0 mL. A 10-mL aliquot of the solution shows not more than 0.1 mg of SO_4 (0.01%).
- **HEAVY METALS** (Reagent test): Boil 2.5 g with 50 mL of dilute hydrochloric acid (1 in 10) for 5 minutes, filter while hot, and evaporate the filtrate on a steam bath to dryness. Take up the residue in 20 mL of dilute hydrochloric acid (1 in 500), digest for 5 minutes, cool, add water to make 100 mL, and filter. To 40 mL of the filtrate add 10 mL of hydrogen sulfide TS: any color produced is not darker than that produced by adding 10 mL of hydrogen sulfide TS to a control containing 0.03 mg of Pb (0.003%).
- **IRON** ⟨241⟩: To 20 mL of the filtrate obtained in the test for *Heavy Metals* add 1 mL of hydrochloric acid, and dilute with water to 47 mL: the solution shows not more than 0.015 mg of Fe (0.003%).

Silicic Acid—Impregnated Glass Microfilament Sheets with Fluorescent Indicator—Use a suitable grade.

[NOTE—One example of a suitable grade is "ITLC Type SAF" sheets, available from Gelman Instrument Co., 600 South Wagner Rd., Ann Arbor, MI 48106.]

Silicon Carbide, SiC**—40.10** [409-21-2]—In small clean chips, suitable for use in promoting ebullition.

Silicone (75 Percent Phenyl, Methyl)—Use a suitable grade.

[NOTE—A suitable grade is available as "OV-25."]

Silicotungstic Acid, *n*-Hydrate *(Tungstosilicic Acid),* $H_4Si(W_3O_{10})_4 \cdot nH_2O$**—2878.17 (anhydrous)** [12027-38-2]

- **ASSAY:** Dissolve about 1 g, accurately weighed, in 25 mL of dilute hydrochloric acid (1 in 5). Add 50 mL of a solution of 5 g of cinchonine in dilute hydrochloric acid (1 in 2). Warm on a steam bath for about 30 min. Cool, filter through a tared crucible, and ignite at 800° to constant weight. The weight of the residue multiplied by 1.047 is equal to the weight of silicotungstic acid dihydrate in the sample taken. NLT 98% is found.

Silver Diethyldithiocarbamate, $(C_2H_5)_2NCS_2Ag$**—256.14** [1470-61-7]—Use ACS reagent grade.

Silver Nitrate, $AgNO_3$**—169.87** [7761-88-8]—Use ACS reagent grade.

Silver Oxide, Ag_2O**—231.74** [20667-12-3]—Brownish-black, heavy powder. Slowly decomposes on exposure to light. Absorbs carbon dioxide when moist. Practically insoluble in water; freely soluble in dilute nitric acid and in ammonia; insoluble in alcohol. Store in well-closed containers; do not expose to ammonia fumes or easily oxidizable substances.

Assay: Dissolve about 500 mg, previously dried at 120° for 3 hours and accurately weighed, in a mixture of 20 mL of water and 5 mL of nitric acid. Dilute with 100 mL of water, add 2 mL of ferric ammonium sulfate TS, and titrate with 0.1 N ammonium thiocyanate VS to a permanent reddish-brown color. Each mL of 0.1 N ammonium thiocyanate is equivalent to 11.59 mg of Ag_2O: not less than 99.7% of Ag_2O is found.

Loss on Drying: Dry it at 120° for 3 hours: it loses not more than 0.25% of its weight.

Nitrate: To 500 mg add 30 mg of sodium carbonate and 2 mL of phenoldisulfonic acid TS, mix, and heat on a steam bath for 15 minutes. Cool, *cautiously* add 20 mL of water, render alkaline with ammonia TS, and dilute with water to 30 mL: any color produced by the test solution is not darker than that produced in a control containing 0.01 mg of NO_3 (0.002%).

Substances Insoluble in Nitric Acid: Dissolve 5 g in a mixture of 5 mL of nitric acid and 10 mL of water, dilute with water to about 65 mL, and filter any undissolved residue on a tared filtering crucible (retain the filtrate for the test for *Substances Not Precipitated By Hydrochloric Acid*). Wash the crucible with water until the last washing shows no opalescence with 1 drop of hydrochloric acid, and dry at 105° to constant weight: the residue weighs not more than 1 mg (0.02%).

Substances Not Precipitated By Hydrochloric Acid: Dilute the filtrate obtained in the test for *Substances Insoluble in Nitric Acid* with water to 250 mL, heat to boiling, and add, dropwise, sufficient hydrochloric acid to precipitate all of the silver (about 5 mL), avoiding any great excess. Cool, dilute with water to 300 mL, and allow to stand overnight. Filter, evaporate 200 mL of the filtrate in a suitable tared porcelain dish to dryness, and ignite: the residue weighs not more than 1.7 mg (0.05%).

Alkalinity: Heat 2 g with 40 mL of water on a steam bath for 15 minutes, cool, and dilute with water to 50 mL. Filter, discarding the first 10 mL of the filtrate. To 25 mL of the subsequent filtrate add 2 drops of phenolphthalein TS, and titrate with 0.02 N hydrochloric acid VS to the disappearance of any pink color: not more than 0.20 mL is required (0.016% as NaOH).

Silver Sulfate, Ag_2SO_4**—311.80** [10294-26-5]—Use ACS reagent grade.

β-Sitosterol, *(22:23 Dihydrostigmasterol),* $C_{29}H_{50}O$**—414.7** [83-46-5]—White powder. Soluble in chloroform. Store in a freezer.

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Specific Rotation ⟨781S⟩: between –33° and –39°, determined in a solution containing 0.5 g of test specimen per mL of chloroform.
Water Determination, *Method I* ⟨921⟩: not more than 6%.

Soda Lime—Use *Soda Lime* (NF monograph).

Sodium, Na—**At. Wt. 22.98977** [7440-23-5]—Use ACS reagent grade.

Sodium Acetate, $NaC_2H_3O_2 \cdot 3H_2O$—**136.08** [6131-90-4]—Use ACS reagent grade Sodium Acetate Trihydrate.

Sodium Acetate, Anhydrous, $NaC_2H_3O_2$—**82.03** [127-09-3]—Use ACS reagent grade.

Sodium Alizarinsulfonate *(Alizarin Red S; Alizarin Sodium Monosulfonate),* $C_{14}H_7NaO_7S \cdot H_2O$—**360.27**—Yellow-brown or orange-yellow powder. Freely soluble in water, with production of a yellow color; sparingly soluble in alcohol.
Sensitiveness: Add 3 drops of a solution of it (1 in 100) to 100 mL of water, and add 0.25 mL of 0.02 N sodium hydroxide: a red color is produced. Add 0.25 mL of 0.02 N hydrochloric acid: the original yellow color returns.

Sodium Ammonium Phosphate—See *Sodium Ammonium Phosphate Tetrahydrate.*

Sodium Ammonium Phosphate Tetrahydrate *(Microcosmic Salt),* $NaNH_4HPO_4 \cdot 4H_2O$—**209.07**—Colorless crystals or white granules. Freely soluble in water; insoluble in alcohol. Effloresces in air and loses ammonia.
• **Insoluble Matter and Ammonium Hydroxide Precipitate**
Analysis: Dissolve 10 g in 100 mL of water, add 10 mL of ammonia TS, and heat on a steam bath for 1 hr. If any precipitate is formed, filter, wash well with water, and ignite.
Acceptance criteria: NMT 1 mg (0.01%)
Chloride (Reagent test): One g shows NMT 0.02 mg of Cl (0.002%).
• **Heavy Metals**
Analysis: Dissolve 3 g in 25 mL of water, add 15 mL of 1 N sulfuric acid, then add 10 mL of hydrogen sulfide TS.
Acceptance criteria: Any brown color developed in 1 min is not darker than that of a control containing 3 mL of Standard lead solution TS and 0.5 mL of 1 N sulfuric acid (0.001%).
• **Nitrate**
Analysis: Dissolve 1 g in 10 mL of water, add 0.1 mL of indigo carmine TS, then add, with stirring, 10 mL of sulfuric acid.
Acceptance criteria: Blue color persists for 10 min (about 0.005%).
• **Sulfate** (Reagent test, *Method II*)
Analysis: Dissolve 10 g in 100 mL of water, add 5 mL of hydrochloric acid, and filter if necessary.
Acceptance criteria: NMT 5 mg (0.02%)

Sodium Arsenate *(Arsenic Acid; Sodium Salt),* $Na_2HAsO_4 \cdot 7H_2O$—**312.01** [10048-95-0]—Use ACS reagent grade.

Sodium Arsenite, $NaAsO_2$—**129.91** [7784-46-5]—White, crystalline powder. Soluble in water; slightly soluble in alcohol.
• **Assay:** Transfer about 5.5 g, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Pipet 25 mL of this solution into a suitable container, add 50 mL of water and 5 g of dibasic sodium phosphate, swirl to dissolve, and titrate with 0.1 N iodine VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N iodine is equivalent to 3.746 mg of As. Between 57.0% and 60.5% is found (equivalent to 98.8% to 104.9% of $NaAsO_2$).
• **Chloride** (Reagent test): One g shows not more than 0.10 mg of Cl (0.01%).
• **Heavy Metals:** Dissolve 200 mg in 8 mL of dilute hydrochloric acid (3 in 8), and evaporate on a steam bath to dryness. Dissolve the residue in 5 mL of dilute hydrochloric acid (2 in 5), and again evaporate to dryness. Dissolve the residue in 10 mL of water, and add 2 mL of diluted acetic acid and 10 mL of hydrogen sulfide TS. Any brown color produced is not darker than that of a control containing 0.01 mg of added Pb (0.005%).
• **Iron:** Dissolve 1 g in 20 mL of dilute hydrochloric acid (1 in 5), and add, dropwise, a slight excess of bromine TS. Boil the solution to remove the excess bromine, cool, dilute with water to 40 mL, and add 10 mL of ammonium thiocyanate solution (3 in 10). Any red color produced is not darker than that of a control containing 0.02 mg of added Fe (0.02%).
• **Sulfide:** Dissolve 1 g in 20 mL of water, and add 5 drops of lead acetate TS: no brown color is produced (about 0.0005%).
• **Sulfate** (Reagent test, *Method II*): Dissolve 5 g in 100 mL of water, add methyl orange TS, neutralize with 1 N hydrochloric acid, add 3 mL of the acid in excess, and filter: the filtrate yields not more than 3 mg of residue (0.02%).

Sodium Azide, NaN_3—**65.01** [26628-22-8]—White powder.
Assay
[**Caution**—Sodium azide is a potent poison. Its conjugate acid HN_3 is more toxic than hydrogen cyanide and is readily liberated from neutral aqueous solutions. Contact of NaH_3 or hydrazoic acid (HN_3) with certain metals may produce explosive salts. Work in a well-ventilated hood, and handle the sample with care.]
Dissolve about 100 mg, accurately weighed, in 50 mL of water, and add 3 drops of phenolphthalein. Adjust the pH, if necessary, to 7.0, and add 35.0 mL of 0.1 N perchloric acid. Pipet, while stirring, 2.5 mL of 1.0 M sodium nitrite into the solution, and stir for 15 seconds. Titrate rapidly to the phenolphthalein endpoint with 0.1 N sodium hydroxide. The endpoint should be reached in less than 4 minutes after addition of perchloric acid because HN_3 is readily volatile. Calculate the percentage of azide by the formula:

$$[(N_P)(V_P) - (N_S)(V_S)](65.01)(100)/2C$$

where N_P is the normality of perchloric acid solution; V_P is the volume of perchloric acid, in mL, taken; N_S is the normality of sodium hydroxide solution; V_S is the volume, in mL, of sodium hydroxide taken; 65.01 is the molecular weight of sodium azide; and C is the weight, in mg, of sodium azide. Not less than 98.5% of NaN_3 is found.

Sodium Bicarbonate, $NaHCO_3$—**84.01** [144-55-8]—Use ACS reagent grade.

Sodium Biphenyl, $C_{12}H_9Na$—**176.19**—Available as a solution in 2-ethoxyethyl ether, or in 1,2-dimethoxyethane (diethylene glycol diether).
• **Activity:** Place 20 mL of dry toluene in a titration flask equipped with a magnetic stirring bar and a stopper having a hole through which the delivery tip of a weight buret may be inserted. Add a quantity of sodium biphenyl sufficient to produce a blue color in the mixture, and titrate with amyl alcohol, contained in a weight buret, to the disappearance of the blue color. (Disregard the amounts of sodium

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biphenyl and amyl alcohol used in this adjustment.) Weigh accurately the weight buret containing the amyl alcohol. Transfer the contents of a vial of well-mixed test specimen to the titration flask, and titrate quickly with the amyl alcohol to the disappearance of the blue color. Weigh the buret to determine the weight of amyl alcohol consumed, and calculate the activity, in mEq/vial, by the formula:

$$\text{Result} = 11.25W$$

in which W is the weight of amyl alcohol consumed. NLT 10% activity is found.

- **IODINE CONTENT:** Add 10 mL to 5 mL of toluene contained in a 125-mL separator fitted with a suitable inert plastic stopcock, and shake vigorously for 2 min. Extract gently with three 10-mL portions of dilute phosphoric acid (1 in 3), combining the lower phases in a 125-mL iodine flask. Add sodium hypochlorite TS, dropwise, to the combined extracts until the solution turns brown, then add 0.5 mL in excess. Shake intermittently for 3 min, add 5 mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 min, accurately timed. Add 1 g of potassium iodide, shake for 30 s, add 3 mL of starch TS, and titrate with 0.1 N sodium thiosulfate VS: NMT 0.1 mL of 0.1 N sodium thiosulfate is consumed.

  [NOTE—A suitable grade is available as catalog number 277134 from www.sigma-aldrich.com or as catalog number 54101 from www.gfschemicals.com.]

Sodium Biphosphate, $NaH_2PO_4 \cdot H_2O$**—137.99**—Use ACS reagent grade Sodium Phosphate, Monobasic.

Sodium Bisulfite [7631-90-5]—This reagent is usually a mixture of sodium bisulfite and sodium metabisulfite [7681-57-4]. Use ACS reagent grade Sodium Bisulfite.

Sodium Bitartrate, $NaHC_4H_4O_6 \cdot H_2O$**—190.08** [6131-98-2]—White crystals or a crystalline powder. Soluble in cold water.

- **ASSAY:** Dissolve about 500 mg, accurately weighed, in 30 mL of water, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS: each mL of 0.1 N sodium hydroxide is equivalent to 19.01 mg of $NaHC_4H_4O_6 \cdot H_2O$. Between 99% and 100.5% is found.
- **INSOLUBLE MATTER** (Reagent test): not more than 1 mg, from 10 g (0.01%).
- **CHLORIDE** (Reagent test): One g shows not more than 0.2 mg of Cl (0.02%).
- **HEAVY METALS** (Reagent test): Dissolve 4 g in 25 mL of water, add 2 drops of phenolphthalein TS, and then add ammonia TS, dropwise, until the solution is slightly pink. Add 4 mL of 1 N hydrochloric acid, dilute with water to 40 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 0.04 mg of added Pb (0.001%).
- **SULFATE** (Reagent test, *Method I*): One g shows not more than 0.2 mg of SO_4 (0.02%).

Sodium Borate *(Borax; Sodium Tetraborate),* $Na_2B_4O_7 \cdot 10H_2O$**—381.37** [1303-96-4]—Use ACS reagent grade.

[NOTE—Certified Borax is available from the National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 187.]

Sodium Borohydride, $NaBH_4$**—37.83** [16940-66-2]—White, crystalline solid. Freely soluble in water; soluble (with reaction) in methanol. Its solutions are rapidly decomposed by boiling.

Assay

Potassium iodate solution: Dissolve 8.917 g, previously dried at 110° to constant weight and accurately weighed, in water to make 1000.0 mL.

Procedure: Dissolve about 500 mg, accurately weighed, in 125 mL of sodium hydroxide solution (1 in 25) in a 250-mL volumetric flask, dilute with the sodium hydroxide solution to volume, and mix. Pipet 10 mL of the solution into a 250-mL iodine flask, add 35.0 mL of *Potassium iodate solution,* and mix. Add 2 g of potassium iodide, mix, add 10 mL of dilute sulfuric acid (1 in 10), insert the stopper in the flask, and allow to stand in the dark for 3 minutes. Titrate the solution with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Calculate the amount, in mg, of $NaBH_4$ in the specimen titrated by the formula:

$$([(35.0)(0.25)] - 0.1V)4.729$$

in which V is the volume, in mL, of 0.1 N sodium thiosulfate used in the titration. Not less than 98% is found.

Sodium Bromide, NaBr**—102.89** [7647-15-6]—Use ACS reagent grade.

Add the following:

▲**Sodium Butyrate** *(Butyric Acid Sodium Salt),* $C_4H_7O_2Na$**—110.09** [156-54-7]—Use a suitable grade with a content of NLT 98.5%.▲ (USP 1-May-2020)

Sodium Carbonate—Use *Sodium Carbonate, Anhydrous.*

Sodium Carbonate, Anhydrous, Na_2CO_3**—105.99** [497-19-8]—Use ACS reagent grade.

Sodium Carbonate, Monohydrate, $Na_2CO_3 \cdot H_2O$**—124.00** [5968-11-6]—Use ACS reagent grade.

Sodium Chloride, NaCl**—58.44** [7647-14-5]—Use ACS reagent grade.

Sodium Chloride Solution, Isotonic—Use *Saline TS.*

Sodium Cholate Hydrate *(3α,7α,12α-trihydroxy-5β-cholan-24-oic acid sodium salt; cholalic acid sodium salt),* $C_{24}H_{39}NaO_5 \cdot xH_2O$**—430.55 (anhydrous)** [206986-87-0]—It can be from ox or sheep bile. Use a suitable grade with a content of not less than 99%.

Sodium Chromate, $Na_2CrO_4 \cdot 4H_2O$**—234.03** [7775-11-3]—Lemon-yellow crystals. Soluble in water.

Assay: Accurately weigh about 300 mg, and dissolve in 10 mL of water contained in a 500-mL flask. Add 3 g of potassium iodide and 10 mL of diluted sulfuric acid, and dilute with 350 mL of oxygen-free and carbon dioxide-free water. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate consumed is equivalent to 7.802 mg of $Na_2CrO_4 \cdot 4H_2O$. Not less than 99% is found.

Insoluble Matter (Reagent test): not more than 1 mg, from 20 g dissolved in 150 mL of water (0.005%).

Aluminum: Dissolve 20 g in 140 mL of water, filter, and add 5 mL of glacial acetic acid to the filtrate. Add stronger ammonia water until alkaline, and digest for 2 hours on a steam bath. Pass through hardened filter paper, wash thoroughly, ignite, and weigh: the residue weighs not more than 0.8 mg (0.002%).

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Calcium: Determine as directed in the test for calcium for ACS reagent grade Potassium Chromate (0.005%).
Chloride: Determine as directed in the test for chloride for ACS reagent grade Potassium Chromate (about 0.005%).
Sulfate: Determine as directed in the test for sulfate for ACS reagent grade Potassium Dichromate, but add 4.5 mL of hydrochloric acid to the water used to dissolve the test specimen: the residue weighs not more than 2.4 mg (0.01%).

Sodium Chromotropate—See *Chromotropic Acid.*

Sodium Citrate Dihydrate *(2-Hydroxy-1,2,3-propanetricarboxylic Acid, Trisodium Salt, Dihydrate),* $Na_3C_6H_5O_7 \cdot 2H_2O$—**294.10** [6132-04-3]—Use ACS reagent grade.

Sodium Cobaltinitrite, $Na_3Co(NO_2)_6$—**403.94** [13600-98-1]—Use ACS reagent grade.

Sodium Cyanide, NaCN—**49.01** [1433-33-9]—Use ACS reagent grade.

Sodium 1-Decanesulfonate *(1-Decanesulfonic Acid Sodium Salt),* $C_{10}H_{21}NaO_3S$—**244.33** [13419-61-9]—Use a suitable grade for ion pair chromatography with a content of not less than 99.0%.

Sodium Desoxycholate—Use *Bile Salts.*

Sodium Dichromate, $Na_2Cr_2O_7 \cdot 2H_2O$ (for chromic acid cleaning mixture)—**298.00** [7789-12-0]—Use ACS reagent grade.

Sodium Diethyldithiocarbamate, $(C_2H_5)_2NCS_2Na \cdot 3H_2O$—**225.31** [20624-25-3]—Use ACS reagent grade.

Sodium 2,2-dimethyl-2-silapentane-5-sulfonate—See *Sodium 3-(trimethylsilyl)-1-propane sulfonate.*

Sodium Dithionite—Use *Sodium Hydrosulfite.*

Sodium 1-Dodecanesulfonate *(1-Dodecanesulfonic Acid Sodium Salt),* $C_{12}H_{25}NaSO_3$—**272.38** [2386-53-0]—Use a suitable grade with a content of NLT 99%.

Sodium Dodecyl Sulfate *(Sodium Lauryl Sulfate),* $C_{12}H_{25}SO_4Na$—**288.38** [151-21-3]—Light yellow, crystalline powder.

Sodium Ferrocyanide, $Na_4Fe(CN)_6 \cdot 10H_2O$—**484.06** [13601-19-9]—Yellow crystals or granules. Freely soluble in water.
Assay: Dissolve 2 g, accurately weighed, in 400 mL of water, add 10 mL of sulfuric acid, and titrate with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate is equivalent to 48.41 mg of $Na_4Fe(CN)_6 \cdot 10H_2O$. Not less than 98% is found.
Insoluble Matter (Reagent test): not more than 1 mg, from 10 g (0.01%).
Chloride (Reagent test): Dissolve 1 g in 75 mL of water, add a solution prepared by dissolving 1.2 g of cupric sulfate in 25 mL of water, mix, and allow to stand for 15 minutes. To 20 mL of the decanted, clear liquid add 2 mL of nitric acid and 1 mL of silver nitrate TS: any turbidity produced does not exceed that of a control containing 0.02 mg of Cl, 2 mL of nitric acid, 1 mL of silver nitrate TS, and sufficient cupric sulfate to match the color of the Test solution.
Sulfate: Dissolve 5 g in 100 mL of water without heating, filter, and to the filtrate add 0.25 mL of glacial acetic acid and 5 mL of barium chloride TS: no turbidity is produced in 10 minutes (about 0.01% as SO_4).

Sodium Fluorescein, $C_{20}H_{10}Na_2O_5$—**376.28**—Orange-red, hygroscopic powder. Freely soluble in water; slightly soluble in alcohol. Its water solution is yellowish red in color and exhibits a strong yellowish green fluorescence that disappears when the solution is acidified and reappears when the solution is neutralized or made basic.
Loss on Drying ⟨731⟩: Dry it at 120° to constant weight: it loses not more than 7.0% of its weight.

Sodium Fluoride, NaF—**41.99** [7681-49-4]—Use ACS reagent grade.

Sodium Glycocholate, $C_{26}H_{42}NNaO_6$—**487.60** [863-57-0]—White to tan powder. Is hygroscopic. Freely soluble in water and in alcohol.
- **SPECIFIC ROTATION** ⟨781⟩: between +28° and +31°, calculated on the dried basis (it is rendered anhydrous by drying at 100° for 2 hours), determined at 20° in a solution containing 10 mg per mL.
- **NITROGEN DETERMINATION** ⟨461⟩, *Method I*: between 2.6% and 3.2% of N is found, calculated on the dried basis.

Sodium 1-Heptanesulfonate *(1-Heptanesulfonic Acid Sodium Salt),* $C_7H_{15}NaO_3S$—**202.25** [22767-50-6]—Use a suitable grade.

Change to read:

Sodium 1-Heptanesulfonate Monohydrate, $C_7H_{15}NaO_3S \cdot H_2O$—**220.26** [▲207300-90-1▲ (USP 1-Aug-2019)]—Use a suitable grade.

Sodium 1-Hexanesulfonate *(1-Hexanesulfonic Acid Sodium Salt),* $C_6H_{13}NaO_3S$—**188.22** [2832-45-3]—Use a suitable grade.

Sodium 1-Hexanesulfonate Monohydrate, $C_6H_{13}NaO_3S \cdot H_2O$—**206.23** [207300-91-2]—Use a suitable grade.

Sodium Hydrogen Sulfate *(Sodium Bisulfate),* $NaHSO_4$—**120.06** [7681-38-1]—Freely soluble in water; very soluble in boiling water. It decomposes in alcohol into sodium sulfate and free sulfuric acid. Use a suitable reagent grade.
Melting Point ⟨741⟩: about 315°.

Sodium Hydrosulfite *(Sodium Dithionite),* $Na_2S_2O_4$—**174.11** [7775-14-6]—White or grayish-white crystalline powder. Soluble in water; slightly soluble in alcohol. Gradually oxidizes in air, more readily when in solution, to bisulfite, acquiring an acid reaction. Is affected by light.

Assay: Accurately weigh about 1 g, dissolve it in a mixture of 10 mL of formaldehyde TS and 10 mL of water contained in a small glass-stoppered flask, and allow to stand for 30 minutes with frequent agitation. Transfer the solution to a 250-mL volumetric flask, add 150 mL of water and 3 drops of methyl orange TS, and then add, dropwise, 1 N sulfuric acid to a slightly acid reaction. Dilute with water to 250 mL, and mix. To 50.0 mL of the dilution add 2 drops of phenolphthalein TS and just sufficient 0.1 N sodium hydroxide to produce a slight, pink color, then titrate with 0.1 N iodine, adding 3 mL of starch TS as the indicator. Then discharge the blue color of the solution with 1 drop of 0.1 N sodium thiosulfate, and titrate with 0.1 N sodium hydroxide VS to a pink color: each mL of 0.1 N sodium hydroxide is equivalent to 3.482 mg of $Na_2S_2O_4$. Not less than 88% is found.

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Sulfide: Add sodium hydroxide solution (1 in 10) to lead acetate TS until the precipitate dissolves. Add 5 drops of this solution to a solution of 1 g of the sodium hydrosulfite in 10 mL of water: no immediate darkening is observed.
Heavy Metals: Dissolve 1 g in 10 mL of water, add 10 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water and 0.5 mL of diluted hydrochloric acid, filter, and add to the filtrate 10 mL of hydrogen sulfide TS: no darkening is produced. Render the solution alkaline with ammonia TS: a slight, greenish color may be produced, but not a dark or white precipitate.
Suitability for Riboflavin Assay: To each of 2 or more tubes add 10 mL of water and 1.0 mL of a standard riboflavin solution containing 20 µg of riboflavin in each mL, and mix. To each tube add 1.0 mL of glacial acetic acid, mix, add with mixing, 0.5 mL of potassium permanganate solution (1 in 25), and allow to stand for 2 minutes. Then to each tube add, with mixing, 0.5 mL of hydrogen peroxide TS: the permanganate color is destroyed within 10 seconds. Shake the tubes vigorously until excess oxygen is expelled. If gas bubbles remain on the sides of tubes after foaming has ceased, remove the bubbles by tipping the tubes so that the solution flows slowly from end to end. In a suitable fluorometer, measure the fluorescence of the solution. Then add, with mixing, 8.0 mg of sodium hydrosulfite: the riboflavin is completely reduced in not more than 5 seconds.

Sodium Hydroxide, NaOH—**40.00** [1310-73-2]—Use ACS reagent grade.

Sodium Hypochlorite Solution[7681-52-9]—A solution of sodium hypochlorite (NaClO) in water. Usually yellow to yellowish-green in color. Has an odor of chlorine. Is affected by light and gradually deteriorates. Store it in light-resistant containers, preferably below 25°. [**CAUTION**—This solution is corrosive and may evolve gases that are corrosive and toxic. It is a powerful oxidant that can react violently with reducing agents. It is irritating and corrosive to skin and mucous membranes.]
Assay: Transfer about 3 mL to a tared, glass-stoppered iodine flask, and weigh accurately. Add 50 mL of water, 2 g of potassium iodide, and 10 mL of acetic acid. Insert the stopper in the flask, and allow to stand in the dark for 10 min. Remove the stopper, rinse the walls of the flask with a few mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is neared. Each mL of 0.1 N sodium thiosulfate consumed is equivalent to 3.722 mg of NaClO: NLT 5.25% is found. If it is desired to calculate the percentage of available chlorine, note that each mL of 0.1 N sodium thiosulfate consumed is equivalent to 3.545 mg of available chlorine.
Calcium: Transfer 10.0 g to a 150-mL beaker, dissolve in 10 mL of water, and add 5 mL of hydrochloric acid and 2 g of potassium iodide. Heat the mixture for 5 min, cool, and add 2 mL of 30% hydrogen peroxide. Evaporate to dryness, cool, and add 2 mL of hydrochloric acid and 2 mL of 30% hydrogen peroxide. Rinse the inner walls of the beaker with a few mL of water, and evaporate to dryness. Take up the residue in 20 mL of water, and filter if necessary. To the filtrate add ammonium hydroxide until the solution is just alkaline, then add 4 drops of ammonium hydroxide and 5 mL of ammonium oxalate TS: any turbidity produced within 15 min does not exceed that in a blank containing 0.1 mg of added calcium carried through the entire procedure (0.001%).
Phosphate (Reagent test): Transfer 2 g to a beaker, and add 5 mL of hydrochloric acid and 2 g of potassium iodide. Heat the solution for 5 min, and cool. Add 2 mL of 30% hydrogen peroxide, and evaporate the solution to dryness. Rinse the walls of the beaker with a few mL of water, and add 2 mL of hydrochloric acid and 2 mL of 30% hydrogen peroxide. Evaporate again to dryness: the residue shows NMT 0.01 mg of PO_4 (5 ppm).

Sodium Iodate, $NaIO_3$—**197.9** [7681-55-2]—White to yellowish-white powder. Use a suitable reagent grade.

Sodium Lauryl Sulfate: See Sodium Dodecyl Sulfate.

Sodium Metabisulfite, $Na_2S_2O_5$—**190.11** [7681-57-4]—Use ACS reagent grade.

Sodium Metaperiodate, $NaIO_4$—**213.89** [7790-28-5]—Use ACS reagent grade Sodium Periodate.

Sodium Methoxide, CH_3ONa—**54.02** [124-41-4]—Fine, white powder. Reacts violently with water with evolution of heat. Soluble in alcohol and in methanol.
Assay: Transfer about 220 mg to a tared, glass-stoppered flask, and weigh accurately. Dissolve the test specimen in about 10 mL of methanol, then add 100 mL of water slowly, with stirring. Add phenolphthalein TS, and titrate with 0.1 N hydrochloric acid VS to a colorless endpoint: each mL of 0.1 N hydrochloric acid VS is equivalent to 5.402 mg of CH_3ONa. Not less than 98.0% is found.

Sodium Molybdate, $Na_2MoO_4 \cdot 2H_2O$—**241.95** [7631-95-0]—Use ACS reagent grade.

Sodium Nitrate, $NaNO_3$—**84.99** [7631-99-4]—Use ACS reagent grade.

Sodium Nitrite, $NaNO_2$—**69.00** [7632-00-0]—Use ACS reagent grade.

Sodium Nitroferricyanide *(Sodium Nitroprusside),* $Na_2Fe(NO)(CN)_5 \cdot 2H_2O$—**297.95** [13755-38-9]—Use ACS reagent grade.

Sodium 1-Octanesulfonate—See Octanesulfonic Acid Sodium Salt.

Add the following:

▲**Sodium Oleate** *(cis-9-Octadecenoic Acid Sodium Salt; Oleic Acid Sodium Salt),* $C_{18}H_{33}NaO_2$—**304.44** [143-19-1]—Use a suitable grade with a content of NLT 99%.▲ (USP 1-May-2020)

Sodium Oxalate, $Na_2C_2O_4$—**134.00** [62-76-0]—Use ACS reagent grade.
[NOTE—Sodium Oxalate of a quality suitable as a primary standard is available from the Office of Standard Reference Materials, National Institute of Standards and Technology, Washington, DC, www.nist.gov, as standard sample No. 40.]

Sodium Pentacyanoammineferrate(II) *[Trisodium Amminepentacyanoferrate (3-)],* $C_5H_3FeN_6Na_3$ —**271.93** [14099-05-9]—Yellow to tan powder. Soluble in water.
SOLUBILITY: Dissolve 500 mg in 50 mL of water, and allow to stand for 1 h. The solution is clear and free from foreign matter.
SENSITIVITY
1,1-Dimethylhydrazine standard solution: Place 500 mL of water in a 1-L volumetric flask, and add from a buret 1.27 mL of anhydrous 1,1-dimethylhydrazine. Dilute with water to volume, and mix. Pipet 10 mL of this solution

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into a 100-mL volumetric flask, and dilute with water to volume. Each milliliter of this solution contains the equivalent of 100 µg of 1,1-dimethylhydrazine.

Buffer solution: Transfer 4.8 g of citric acid monohydrate to a 1-L volumetric flask, dissolve in water, add 14.6 g of sodium phosphate, swirl to dissolve, and dilute with water to volume.

Test preparation: Dissolve 100 mg of sodium pentacyanoammineferrate(II) in 100 mL of water.

Procedure: Into each of five 25-mL volumetric flasks pipet 0, 0.25, 0.50, 1.0, and 1.5 mL, respectively, of *1,1-Dimethylhydrazine standard solution*; to each add 15 mL of *Buffer solution*, and swirl to mix. To each flask, add by pipet 2 mL of *Test preparation*, mix, dilute with *Buffer solution* to volume, and allow to stand for 1 h. Using a suitable spectrophotometer, 1-cm cells, and the solution containing no *1,1-Dimethylhydrazine standard solution* as the blank, determine the absorbances of the remaining solutions at 500 nm. Plot the observed absorbance as the ordinate versus the concentration of standard as the abscissa on coordinate paper, and draw the curve of best fit. The plot is linear and the absorbance of the 150-µg solution is NLT 0.65.

[NOTE—A suitable grade is available as catalog number 14099-05-9 from www.bocsci.com.]

Sodium (tri) Pentacyanoamino Ferrate—See *Sodium Pentacyanoammineferrate(II)*.

Sodium 1-Pentanesulfonate *(1-Pentanesulfonic Acid Sodium Salt)*, $C_5H_{11}NaO_3S \cdot H_2O$—**192.21** [207605-40-1]—Use a suitable grade with a content of NLT 98.0%.

Sodium 1-Pentanesulfonate, Anhydrous ***(1-Pentanesulfonic Acid Sodium Salt, Anhydrous)***, $C_5H_{11}SO_3Na$—**174.19** [22767-49-3]—Use a suitable grade with a content of NLT 98%.

Sodium Perchlorate, $NaClO_4 \cdot H_2O$—**140.46** [7791-07-3]—Use ACS reagent grade.

Sodium Peroxide, Na_2O_2—**77.98** [1313-60-6]—Use ACS reagent grade.

Sodium Phosphate *(Sodium Orthophosphate; Trisodium Phosphate)*, Na_3PO_4—**163.94** [*7601-54-9*]—Use a suitable grade with a content NLT 95%.

Sodium Phosphate, Dibasic *(Disodium Phosphate; Disodium Hydrogen Phosphate; Sodium Phosphate, Dibasic, Heptahydrate)*, $Na_2HPO_4 \cdot 7H_2O$—**268.07** [7782-85-6]—Use ACS reagent grade Sodium Phosphate, Dibasic, Heptahydrate.

Sodium Phosphate, Dibasic, Anhydrous *(Anhydrous Disodium Hydrogen Phosphate) (for buffer solutions)*, Na_2HPO_4—**141.96** [7558-79-4]—Use ACS reagent grade Sodium Phosphate, Dibasic, Anhydrous.

Sodium Phosphate, Dibasic, Dihydrate *(Sodium Monohydrogen Phosphate; Disodium Hydrogen Phosphate)*, $Na_2HPO_4 \cdot 2H_2O$—**177.99** [10028-24-7]—Use a suitable grade with a content of not less than 99.5%.

[NOTE—A suitable grade is available from www.emdchemicals.com, catalog number SX0713.]

Sodium Phosphate Dibasic, Dodecahydrate *(Disodium Hydrogen Phosphate, Dodecahydrate)*, $Na_2HPO_4 \cdot 12H_2O$—**358.14** [10039-32-4]—Use a suitable grade with a content of between 98.0% and 102.0% of $Na_2HPO_4 \cdot 12H_2O$.

Sodium Phosphate, Dibasic, Heptahydrate *(Disodium Hydrogen Phosphate Heptahydrate; Disodium Phosphate)*, $Na_2HPO_4 \cdot 7H_2O$—**268.07** [7782-85-6]—Use ACS reagent grade.

Sodium Phosphate, Monobasic *(Sodium Biphosphate; Sodium Dihydrogen Phosphate; Acid Sodium Phosphate; Monosodium Orthophosphate)*, $NaH_2PO_4 \cdot H_2O$—**137.99** [10049-21-5]—Use ACS reagent grade.

Sodium Phosphate, Monobasic, Anhydrous *(Sodium Biphosphate; Sodium Dihydrogen Phosphate; Acid Sodium Phosphate; Monosodium Orthophosphate)*, NaH_2PO_4—**119.98** [7558-80-7]—Use a suitable grade with a content of not less than 99.0%.

Sodium Phosphate, Monobasic, Dihydrate *(Sodium Dihydrogen Phosphate, Dihydrate)*, $NaH_2PO_4 \cdot 2H_2O$—**156.01** [13472-35-0]—Use a suitable grade with a content of not less than 99.0%.

Sodium Phosphate, Tribasic, $Na_3PO_4 \cdot 12H_2O$—**380.12** [10101-89-0]—Use ACS reagent grade.

Add the following:

▲**Sodium Phosphate, Tribasic, Anhydrous**, Na_3PO_4—**163.94** [7601-54-9]—Use a suitable grade with a content of NLT 96.0%.▲ (USP 1-May-2019)

Sodium Phosphite Pentahydrate *(Disodium Hydrogen Phosphite)*, $Na_2HPO_3 \cdot 5H_2O$—**216.04** [13517-23-2]—Use a suitable grade with a content of not less than 98%.

[NOTE—A suitable grade is available from Fluka, www.sigma-aldrich.com, catalog number 04283.]

Add the following:

▲**Sodium Propionate** *(Propionic Acid Sodium Salt)*, $C_3H_5O_2Na$—**96.06** [137-40-6]—Use a suitable grade with a content of NLT 99%.▲ (USP 1-May-2020)

Sodium Pyrophosphate, $Na_4P_2O_7 \cdot 10H_2O$—**446.06** [13472-36-1]—Use ACS reagent grade.

Sodium Pyruvate, CH_3COCO_2Na—**110.04** [113-24-6]—White to practically white powder or crystalline solid. Soluble in water.

Assay: Transfer about 300 mg, accurately weighed, to a high-form titration beaker, add 150 mL of glacial acetic acid, and stir until dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode modified to use 0.1 N tetramethylammonium chloride in methanol as the electrolyte. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 11.00 mg of CH_3COCO_2Na: not less than 98.0% is found.

Solubility: Dissolve 1.5 g in 25 mL of water: the solution is clear and complete.

Free Acid: Dissolve 10 g in 150 mL of water, and titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically: not more than 2.8 mL of 0.5 N sodium hydroxide is consumed (about 1% as $C_3H_4O_3$).

Sodium Salicylate [54-21-7]—It complies with the specifications under *Sodium Salicylate* (USP monograph), and in addition meets the requirements of the following test.

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Nitrate: Dissolve 100 mg in 5 mL of water, and superimpose the solution upon 5 mL of sulfuric acid: no brownish-red color appears at the junction of the two liquids.

Sodium Selenite, Na_2SeO_3—**172.94** [10102-18-8]—White, odorless, crystalline powder, usually partially hydrated. Freely soluble in water; insoluble in alcohol.

Assay: Accurately weigh about 180 mg, previously dried at 120° to constant weight, and dissolve it in 50 mL of water in a glass-stoppered flask. Add, successively, 3 g of potassium iodide and then 5 mL of hydrochloric acid, insert the stopper, and allow to stand for 10 minutes. Add 50 mL of water, 50.0 mL of 0.1 N sodium thiosulfate VS, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to a blue color. Perform a blank determination. The difference in volumes of 0.1 N iodine is equivalent to 4.323 mg of Na_2SeO_3. Between 98% and 101% is found.

Solubility: One g in 10 mL of water shows not more than a faint haze.

Carbonate: To 500 mg add 1 mL of water and 2 mL of diluted hydrochloric acid: no effervescence is produced.

Chloride (Reagent test): A 500-mg portion shows not more than 0.05 mg of Cl (0.01%).

Nitrate (Reagent test): A 200-mg portion dissolved in 3 mL of water shows not more than 0.02 mg of NO_3 (0.01%).

Selenate and **Sulfate** (***as*** SO_4): To 500 mg in a small evaporating dish add 20 mg of sodium carbonate and 10 mL of hydrochloric acid. Slowly evaporate the solution on a steam bath under a hood to dryness. Wash the sides of the dish with 5 mL of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in a mixture of 15 mL of hot water and 1 mL of hydrochloric acid. Proceed as directed under *Sulfate in Reagents* (Reagent test, *Method I*), beginning with "Filter the solution." The test specimen shows no more turbidity than that produced by 0.15 mg of SO_4 (0.03%).

Sodium Stearyl Fumarate *(Fumaric Acid Octadecyl Ester Sodium Salt, Fumaric Acid Stearyl Ester Sodium Salt, Sodium Octadecyl Fumarate),* $C_{22}H_{39}NaO_4$— **390.54** [4070-80-8]—Use a suitable grade with a content of NLT 98.0%.

[NOTE—A suitable grade is available as catalog number S0879 from www.tcichemicals.com.]

Sodium Sulfate *(Glauber's Salt),* $Na_2SO_4 \cdot 10H_2O$—**322.20** [7727-73-3]—Use ACS reagent grade.

Sodium Sulfate, Anhydrous, Na_2SO_4—**142.04** [7757-82-6]—Use ACS reagent grade.

For use in assaying alkaloids by gas–liquid chromatography, it conforms to the following additional test.

Suitability for Alkaloid Assays: Transfer about 10 mg of atropine, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with alcohol to volume. Pipet 3 mL of the solution into each of two 60-mL separators, and add to each 10 mL of water, 1 mL of 1 N sodium hydroxide, and 10 mL of chloroform. Shake thoroughly, and allow the layers to separate. Filter the organic phase from one separator through phase-separating paper, previously washed with 5 mL of chloroform, supported in a funnel, and collect the filtrate in a suitable container. Add 10 mL of chloroform to the separator, shake thoroughly, and filter the organic layer through the same phase-separating paper, collecting and combining the filtrates in the same container. Designate the combined filtrates as *Solution A*. Filter the organic phase from the second separator through 30 g of the Anhydrous Sodium Sulfate, supported on a pledget of glass wool in a small funnel, and previously washed with chloroform, and collect the filtrate in a suitable container. Add 10 mL of chloroform to the separator, shake thoroughly, and filter the organic layer through the same portion of anhydrous sodium sulfate, collecting and combining the filtrates in the same container. Designate the combined filtrates as *Solution B*. Evaporate the two solutions in vacuum to a volume of about 1 mL. Inject an accurately measured volume of *Solution A* into a suitable gas chromatograph, and record the peak height. Repeat the determination with a second accurately measured volume of *Solution A*, record the peak height, and obtain the average of the two results. In a similar manner, determine the peak height of two portions of *Solution B*, and obtain the average of the results. The average value obtained for *Solution B* is within 5.0% of the value obtained for *Solution A*.

Under typical conditions, the gas chromatograph contains a 4-mm × 1.2-m glass column packed with 3% phase G3 on packing S1A. After curing and conditioning, the column temperature is maintained at 210°, the injector port temperature at 225°, and the detector block temperature at 240° during the determinations. The carrier gas is helium, flowing at a rate of 60 mL per minute.

Sodium Sulfate Decahydrate—Use *Sodium Sulfate*.

Sodium Sulfide, $Na_2S \cdot 9H_2O$—**240.18** [1313-84-4]—Use ACS reagent grade.

Sodium Sulfite—Use *Sodium Sulfite, Anhydrous*.

Sodium Sulfite, Anhydrous *(Exsiccated Sodium Sulfite),* Na_2SO_3—**126.04** [7757-83-7]—Use ACS reagent grade.

Sodium *p*-Sulfophenylazochromotropate *[Trisodium Salt of 4,5-Dihydroxy-3-(p-sulfophenylazo)-2,7-naphthalenedisulfonic Acid],* $C_{16}H_9N_2Na_3O_{11}S_3 \cdot 3H_2O$—**624.47**—Bright red powder. Very soluble in water; insoluble in alcohol. Combines with zirconium oxychloride to form a soluble pink zirconium lake.

[NOTE—The reagent is available as Catalog No. 7309 from Distillation Products Industries, Eastman Organic Chemicals Dept., Rochester, NY 14650. A procedure for its preparation is described in *Z. Anal. Chem.*, 146, 417 (1955).]

Sodium Tartrate, $Na_2C_4H_4O_6 \cdot 2H_2O$—**230.08** [6106-24-7]—Use ACS reagent grade.

Add the following:

▲**Sodium Taurocholate** *(Taurocholic Acid Sodium Salt),* $C_{26}H_{44}NNaO_7S \cdot xH_2O$—**537.68 (anhydrous basis)** [345909-26-4]—Use a suitable grade with a content of NLT 97%.▲ (USP 1-May-2020)

Add the following:

▲**Sodium Taurodeoxycholate** *(2-([3α,12α-Dihydroxy-24-oxo-5β-cholan-24-yl]amino)ethanesulfonic Acid; Taurodeoxycholic Acid Sodium Salt Hydrate),* $C_{26}H_{44}NO_6SNa$—**521.69 (anhydrous basis)** [207737-97-1]—Use a suitable grade with a content of NLT 95%.▲ (USP 1-May-2020)

Sodium Tetraphenylborate, $NaB(C_6H_5)_4$—**342.22** [143-66-8]—Use ACS reagent grade.

Sodium Tetraphenylboron—See *Sodium Tetraphenylborate*.

Sodium Thioglycolate *(Sodium Thioglycollate),* $HSCH_2COONa$—**114.10** [367-51-1]—A white, crystalline powder. Very soluble in water; slightly soluble in alcohol. Is hygroscopic, and oxidizes in air. Store in tight, light-resistant

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containers. It should not be used if it is pale yellow or darker in color.

Assay: Accurately weigh about 250 mg, and dissolve in 50 mL of oxygen-free water. Add 5 mL of diluted hydrochloric acid, boil for 2 minutes, cool, and titrate the solution with 0.1 N iodine VS, adding 3 mL of starch TS toward the end: each mL of 0.1 N iodine is equivalent to 11.41 mg of $HSCH_2COONa$. Not less than 75% is found.

Insoluble Matter: A solution of 1 g in 10 mL of water is clear, and practically complete.

Sulfide: Dissolve 500 mg in 10 mL of water in a small flask, add 2 mL of hydrochloric acid, then place a strip of filter paper, moistened with lead acetate TS, over the mouth of the flask, and bring the solution to a boil: the lead acetate paper is not darkened.

Sodium Thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$—**248.19** [10102-17-7]—Use ACS reagent grade.

Sodium L-Thyroxine—Use *Levothyroxine Sodium* (USP monograph).

Sodium 3-(trimethylsilyl)-1-propane sulfonate *(Sodium 2,2-dimethyl-2-silapentane-5-sulfonate),* $C_6H_{15}SiNaO_3S$—**218.32**—Use a suitable grade.

Sodium Tungstate, $Na_2WO_4 \cdot 2H_2O$—**329.85** [10213-10-2]—Use ACS reagent grade.

Solvent Hexane—See *Hexane, Solvent.*

Sorbitol—Use *Sorbitol* (NF monograph).

Stachyose Hydrate, $C_{24}H_{42}O_{21} \cdot xH_2O$—**666.58** [10094-58-3]—Use a suitable grade.

Standard Sand, 20- to 30-Mesh—See *Sand, Standard 20- to 30-mesh.*

Stannous Chloride, $SnCl_2 \cdot 2H_2O$—**225.65** [10025-69-1]—Use ACS reagent grade.

Starch, Potato—The starch separated from the tubers of *Solanum tuberosum* Linné (Fam. Solanaceae). A more or less finely granular powder, consisting of starch grains of characteristic shape and appearance when examined microscopically.

Starch, Soluble *(for iodimetry)* [9005-84-9]—Use ACS reagent grade.

Soluble Starch—See *Starch, Soluble.*

Starch, Soluble, Purified: White, amorphous powder; under microscopic examination it shows the characteristic form of potato starch. Soluble in hot water; very slightly soluble in alcohol.

Test Solution for Determination of pH and Sensitivity: Stir 2.0 g in 10 mL of water, add boiling water to make 100 mL, and boil for 2 minutes. The hot solution is almost clear. On cooling, the solution may become opalescent or turbid, but does not gel. Use it as the *Test solution.*

pH ⟨791⟩: The pH of the *Test solution* is between 6.0 and 7.5.

Sensitivity: Mix 2.5 mL of *Test solution,* 97.5 mL of water, and 0.50 mL of 0.010 N iodine: a distinct blue color results, and it disappears upon the addition of 0.50 mL of 0.010 N sodium thiosulfate.

Absorbance: Prepare a pH 5.3 buffer solution by dissolving 43.5 g of sodium acetate (trihydrate) and 4.5 mL of glacial acetic acid in water, transferring the resultant solution to a 250-mL volumetric flask, adding water to volume, and mixing.

Dissolve 1.00 g of Soluble Purified Starch in 2.5 mL of the buffer solution by warming, transfer to a 100-mL volumetric flask, add water to volume, and mix. Add 0.50 mL of this solution to a 100-mL volumetric flask containing about 75 mL of water, 1 mL of 1 N hydrochloric acid, and 1.5 mL of 0.020 N iodine, swirling the flask during the addition. Add water to volume, mix, and allow to stand in the dark for 1 hour. The absorbance of this solution, measured at 575 nm in a 1-cm cell against a blank, is between 0.5 and 0.6.

Reducing Substances: Shake 10.0 g with 100 mL of water for 15 minutes, and allow to settle for about 12 hours. Filter a portion of the supernatant through fine sintered glass. To 50 mL of the filtrate add 50 mL of alkaline cupric tartrate TS, and boil for 1 to 2 minutes. Filter the resulting cuprous oxide, wash it with hot water and then with alcohol, and dry it at 105° for 2 hours: not more than 47 mg is found, corresponding to 0.7% of reducing sugars as maltose.

Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 10% of its weight.

Residue on Ignition ⟨281⟩: not more than 0.5%.

Stearic Acid, $C_{18}H_{36}O_2$—**284.48** [57-11-4]—Hard, white crystals or amorphous, white powder. Freely soluble in chloroform and in ether; soluble in alcohol and in solvent hexane.

Congealing Temperature ⟨651⟩: between 67° and 69°.

Acid Value ⟨401⟩: between 196 and 199.

Iodine Value ⟨401⟩: not more than 1.

Saponification Value ⟨401⟩: between 197 and 200.

Palmitic Acid: Determine as directed in the *Assay* under *Stearic Acid* (NF monograph): not more than 5.0% is found.

Stearyl Alcohol *(1-Octadecanol),* $C_{18}H_{38}O$—**270.49** [112-92-5]—White flakes, granules, or crystals. Soluble in alcohol, in ether, in acetone, and in benzene; insoluble in water.

Melting Range ⟨741⟩: between 56° and 58°.

Other Requirements: It conforms to the tests for *Acid Value, Iodine Value,* and *Hydroxyl Value* under *Stearyl Alcohol* (NF monograph).

Stronger Ammonia Water—See Ammonia Water, Stronger.

Strontium Acetate, $Sr(CH_3COO)_2 \cdot ½H_2O$—**214.72** [543-94-2]—White, crystalline powder. Soluble in 3 parts of water; slightly soluble in alcohol.

Assay: Ignite about 3 g, accurately weighed, in a platinum crucible, protecting from sulfur in the flame. Cool, transfer the crucible with the residue to a beaker, and add 50 mL of water and 40.0 mL of 1 N hydrochloric acid VS. Boil gently for 30 minutes or longer, if necessary; filter; wash with hot water until the washings are neutral; add methyl red TS; and titrate the excess acid with 1 N sodium hydroxide VS. Each mL of 1 N hydrochloric acid is equivalent to 107.4 mg of $Sr(CH_3COO)_2 \cdot ½H_2O$: not less than 99% is found.

Insoluble Matter (Reagent test): not more than 2 mg, from 10 g (0.02%).

Free Alkali or Free Acid: Dissolve 3 g in 30 mL of water, and add 3 drops of phenolphthalein TS: no pink color is produced. Titrate with 0.1 N sodium hydroxide VS to a pink color: not more than 0.30 mL of the 0.1 N sodium hydroxide is required.

Barium: Dissolve 1 g in 10 mL of water, and add 1 drop of glacial acetic acid and 5 drops of potassium dichromate

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solution (1 in 10): no turbidity is produced within 2 minutes (about 0.02%).
Calcium: Ignite 1 g until completely carbonized. Warm the residue with a mixture of 3 mL of nitric acid and 10 mL of water, filter, wash with 5 mL of water, and evaporate the filtrate on a steam bath to dryness. Powder the residue, and dry it at 120° for 3 hours. Reflux the dried powder with 15 mL of dehydrated alcohol for 10 minutes, cool in ice, and filter. Repeat the extraction with 10 mL of dehydrated alcohol. Evaporate the combined filtrates to dryness, add 0.5 mL of sulfuric acid, and ignite: the weight of the residue is not more than 10 mg (0.3% of Ca).
Chloride (Reagent test): One g shows not more than 0.1 mg of Cl (0.01%).
Heavy Metals (Reagent test): 0.001%.
Iron ⟨241⟩: Dissolve 1.0 g in 45 mL of water, and add 2 mL of hydrochloric acid: the solution shows not more than 0.01 mg of Fe (0.001%).
Alkali Salts: Dissolve 2 g in 80 mL of water, heat to boiling, add an excess of ammonium carbonate TS, boil for 5 minutes, dilute with water to 100 mL, and filter. Evaporate 50 mL of the filtrate, and ignite: the residue, after correcting for the ignition residue from half the volume of the clear ammonium carbonate TS used above, is not more than 3 mg (0.3%).
Nitrate: Dissolve 1 g in 10 mL of water, add 0.10 mL of indigo carmine TS, and then add 10 mL of sulfuric acid: the blue color persists for 5 minutes (about 0.01% of NO_3).

Strontium Hydroxide *(Strontium Hydroxide Octahydrate)*, $Sr(OH)_2 \cdot 8H_2O$—**265.76** [18480-07-4]—White, crystalline, free-flowing powder. Sparingly soluble in water. May absorb carbon dioxide from the air. Keep tightly closed.
Assay and **Carbonate:** Accurately weigh about 5 g, dissolve in 200 mL of warm carbon dioxide-free water in a glass-stoppered, 500-mL flask, add phenolphthalein TS, and titrate with 1 N hydrochloric acid VS to determine the hydroxide alkalinity. Then add methyl orange TS, and titrate with 1 N hydrochloric acid VS. Each mL of 1 N hydrochloric acid required to reach the phenolphthalein endpoint is equivalent to 132.9 mg of $Sr(OH)_2 \cdot 8H_2O$, and each additional mL of 1 N hydrochloric acid VS required to reach the methyl orange endpoint is equivalent to 73.8 mg of $SrCO_3$. Not less than 95.0% of $Sr(OH)_2 \cdot 8H_2O$ and not more than 3.0% of $SrCO_3$ are found.
Chloride (Reagent test): Dissolve 1.0 g in 100 mL of water, and filter if necessary: 1.0 mL of the solution shows not more than 0.01 mg of Cl (0.1%).
Calcium (Reagent test)
Test solution: Dissolve 5.0 g in water, and dilute with water to 100 mL.
Sample solution: Dilute 10.0 mL of the *Test solution* with water to 100 mL.
Control solution: To 10.0 mL of the *Test solution* add 0.50 mg of calcium ion (Ca), and dilute with water to 100 mL.
Procedure: Determine the background emission at 416.7 nm: the limit is 0.1%.
Iron: Dissolve 1 g in warm water, and dilute with water to 100 mL. To 20 mL of this solution add 2 mL of hydrochloric acid and 0.1 mL of 0.1 N potassium permanganate, allow to stand for 5 minutes, and add 3 mL of ammonium thiocyanate solution (3 in 10). Any red color produced is not darker than that of a control containing 0.03 mg of added Fe (0.015%).
Heavy Metals: Dissolve 2.0 g in 14 mL of dilute hydrochloric acid (1 in 6), and evaporate on a steam bath to dryness. Take up the residue in 25 mL of water, filter, and dilute with water to 100 mL (*Test solution*). To 5.0 mL of the *Test solution* add 0.02 mg of lead (Pb), and dilute with water to 30 mL, to provide the standard. For the test specimen, use 30 mL of the *Test solution*. Adjust each solution with diluted acetic acid or ammonia TS to a pH between 3.0 and 4.0 (using short-range pH paper), dilute with water to 40 mL, and add 10 mL of freshly prepared hydrogen sulfide TS: any brown color developed in the sample solution is not darker than that in the control solution (0.004%).

Strychnine Sulfate, $(C_{21}H_{22}N_2O_2)_2 \cdot H_2SO_4 \cdot 5H_2O$—**856.98** [60-41-3]—Colorless or white crystals, or a white, crystalline powder. Its solutions are levorotatory. One g dissolves in about 35 parts of water, in 85 mL of alcohol, and in about 220 mL of chloroform. Insoluble in ether.
Solubility: A solution of 500 mg in 25 mL of water is complete, clear, and colorless.
Residue on Ignition (Reagent test): not more than 0.1%.
Brucine: To 100 mg add 1 mL of dilute nitric acid (1 in 2): a yellow color may be observed, but not a red or reddish-brown color.

Styrene-Divinylbenzene Anion-exchange Resin, 50- to 100-Mesh—See *Anion-exchange Resin, 50- to 100-Mesh, Styrene-Divinylbenzene.*

Styrene-Divinylbenzene Cation-exchange Resin, Strongly Acidic—See *Cation-exchange Resin, Styrene-Divinylbenzene, Strongly Acidic.*

Styrene-Divinylbenzene Copolymer Beads—Neutral, porous, cross-linked beads, 200–400 mesh, molecular weight operating range up to 2,000 (based on beads fully swollen in benzene). Suitable for use in the gel permeation separation of lipophilic polymers and other solutes requiring organic eluant.
[NOTE—A suitable grade is available commercially as "BioBeads S-X" from Bio-Rad, www.bio-rad.com.]

Succinic Acid, $C_4H_6O_4$—**118.09** [110-15-6]—Use ACS reagent grade.

Sudan III, $C_{22}H_{16}N_4O$—**352.39** [85-86-9]—Red to red-brown powder. Use a suitable grade.
Assay: When tested by thin-layer chromatography (see *Chromatography* ⟨621⟩) with the use of plates coated with chromatographic silica gel mixture and a developing system consisting of a mixture of hexane and ethyl acetate (80:20), and examined under short-wavelength UV light, a single spot is exhibited, with trace impurities.

Sudan IV, $C_{24}H_{20}N_4O$—**380.44** [85-83-6]—Brown to reddish-brown powder.
Assay: Transfer about 25 mg, accurately weighed, to a 100-mL volumetric flask. Dissolve in chloroform, dilute with chloroform to volume, and mix. Dilute 2.0 mL of the resulting solution with chloroform to 50.0 mL. Determine the absorbance of this solution in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the percentage of Sudan IV in the test specimen taken by the formula:

$$(100A)/(85C)$$

in which A is the absorbance at 520 nm and C is the concentration of the test specimen in g per L. Not less than 90% is found.
Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 10% of its weight.

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Sulfaguanidine *(4-Amino-N-(aminoiminomethyl) benzenesulfonamide, 4-Amino-N-guanylbenzenesulfonamide),* $C_7H_{10}N_4O_2S$—**214.24** [57-67-0]—Use a suitable grade.

Sulfamerazine ***(4-Amino-N-(4-methyl-2-pyrimidinyl) benzenesulfonamide),*** $C_{11}H_{12}N_4O_2S$—**264.30** [127-79-7]—Use a suitable grade with a content of NLT 99.0%.

Sulfamic Acid, HSO_3NH_2—**97.09** [5329-14-6]—Use ACS reagent grade.

Sulfan Blue *(Patent Blue VF; Acid Blue V),* $C_{27}H_{31}N_2NaO_6S_2$ — **566.66** [129-17-9]—Use a suitable grade with a dye content of NLT 50%.

Sulfanilamide, $C_6H_8N_2O_2S$—**172.20** [63-74-1]—Use USP Sulfanilamide Melting Point RS.

Sulfanilic Acid, p-$NH_2C_6H_4SO_3H \cdot H_2O$—**191.21** [121-57-3] —Use ACS reagent grade.

Sulfatase Enzyme Preparation—Use a suitable grade.
[NOTE—A suitable grade is available commercially under catalog number S-9626 from Sigma-Aldrich, Web site: www.sigma-aldrich.com.]

Sulfathiazole Sodium *(4-Amino-N-2-thiazolylbenzenesulfonamide Sodium Salt),* $C_9H_8N_3NaO_2S_2$— **277.29** [144-74-1]—Use a suitable grade.

Add the following:

▲**Sulfiram** *(Diethylthiocarbamic Thioanhydride),* $C_{10}H_{20}N_2S_3$— **264.47** [*95-05-6*]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number MM1945.00 from www.lgcstandards.com.]
▲ 2S (USP41)

Sulfonic Acid Cation-exchange Resin—See *Cation-exchange Resin, Sulfonic Acid.*

Sulfosalicylic Acid, $C_6H_3(COOH)(OH)(SO_3H)$-1,2,5 · $2H_2O$— **254.22** [97-05-2]—Use ACS reagent grade.

Sulfur—Use *Precipitated Sulfur* (USP monograph).

Sulfur Dioxide Detector Tube: A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for an iodine-starch indicator.
Measuring Range: 1 to 25 ppm.
[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Sulfuric Acid, H_2SO_4—**98.08** [7664-93-9]—Use ACS reagent grade.

Sulfuric Acid, Diluted—Transfer 57 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Sulfuric Acid, Fluorometric—Use ACS reagent grade Sulfuric Acid that conforms to the following additional test:
Fluorescence: Using a suitable fluorometer having a sharp cut-off 360-nm excitation filter and a sharp cut-off 415-nm excitation filter, determine the fluorescence of the sulfuric acid in a cuvette previously rinsed with water followed by several portions of the acid under examination: the fluorescence does not exceed that of quinine sulfate solution (1 in 1,600,000,000), similarly measured.

Sulfuric Acid, Fuming, H_2SO_4 plus free SO_3[8014-95-7]— having a nominal content of 15%, 20%, or 30% of free SO_3— Use ACS reagent grade (containing between 15.0% and 18.0%, between 20.0% and 23.0%, or between 30.0% and 33.0% of free SO_3).

Sulfuric Acid, Nitrogen Free, H_2SO_4—**98.08** [7664-93-9]— Use a suitable grade.
[NOTE—A suitable grade is available as Sulfuric Acid, Ultrex II, catalog number 6902-05 from www.avantormaterials.com.]

Sulfurous Acid, H_2SO_3—**82.08** [7782-99-2]—A water solution of sulfur dioxide. Use ACS reagent grade.

Sunflower Oil[8001-21-6]—Use a suitable grade.

Delete the following:

▲**Supports for Gas Chromatography**—See supports for gas chromatography in *Chromatography* ⟨621⟩, *Chromatographic Reagents.*▲ 2S(USP41)

Tannic Acid *(Tannin)* [1401-55-4]—Use ACS reagent grade.

Tartaric Acid, $H_2C_4H_4O_6$—**150.09**—Use ACS reagent grade.

***tert*-Butyl Hydroperoxide Solution** *(T-HYDRO, TBHP),* $C_4H_{10}O_2$—**90.12** [75-91-2]—70%, by weight, in water.
[NOTE—A suitable grade is available as catalog number 458139 from www.sigma-aldrich.com.]

Butyl Alcohol, Tertiary, $(CH_3)_3COH$—**74.12** [75-65-0]— Use ACS reagent grade *tert*-Butyl Alcohol.

Testosterone Benzoate *(4-Androsten-17β-ol-3-one benzoate),* $C_{26}H_{32}O_3$—**392.54**—Use a suitable grade.

2′,4′,5′,7′-Tetrabromofluorescein *(Eosin Y, Eosin Yellowish, Eosin Bromo ES, Solvent Red 43, Acid Red 87),* $C_{20}H_8Br_4O_5$— **167.8** [630-20-6]—Dark red to brown powder. Solubility 0.1% in water (clear orange solution). Use a suitable grade.

Tetrabromophenolphthalein Ethyl Ester, $C_{22}H_{14}Br_4O_4$— **661.96**—Use ACS reagent grade.

Tetrabutylammonium Bromide, $(C_4H_9)_4NBr$—**322.37** [1643-19-2]—Use ACS reagent grade.

Tetrabutylammonium Hydrogen Sulfate, $C_{16}H_{37}NO_4S$— **339.54** [32503-27-8]—White, crystalline powder. Soluble in alcohol yielding a slightly hazy, colorless solution.
Assay: Dissolve about 170 mg, accurately weighed, in 40 mL of water. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 33.95 mg of $C_{16}H_{37}NO_4S$. Not less than 97.0% is found.
Melting Range ⟨741⟩: between 169° and 173°.

Tetrabutylammonium Hydrogen Sulfate Ion Pairing Reagent—A mixture of tetrabutylammonium hydrogen sulfate and phosphate buffer. Use a suitable grade.

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[NOTE—A suitable grade is available as catalog number WAT084189 from the Waters Corporation (www.waters.com).]

0.4 M Aqueous Tetrabutylammonium Hydroxide, $C_{16}H_{37}NO$**—259.47** [2052-49-5]—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number 420120025 from www.fishersci.com.]

Tetrabutylammonium Hydroxide, 1.0 M in Methanol [2052-49-5]—Use a suitable grade.

Tetrabutylammonium Hydroxide 30-Hydrate, $C_{16}H_{37}NO \cdot 30H_2O$**—799.93** [147741-30-8]—Use a suitable grade with a content of NLT 98.0%.

Tetrabutylammonium Hydroxide, 40 Percent in Water, $[CH_3(CH_2)_3]_4NOH$**—259.47** [2052-49-5]—Use a suitable grade.

Tetrabutylammonium Iodide, $(C_4H_9)_4NI$**—369.37** [311-28-4]—White, shiny, crystalline flakes. Soluble in alcohol and in ether; slightly soluble in water.
Assay: Dissolve 370 mg, accurately weighed, in 60 mL of acetone with vigorous stirring. Stir the solution by mechanical means, add 10 mL of 16% sulfuric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a glass–silver electrode system, and adding the titrant in 0.1-mL increments as the endpoint is approached. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N silver nitrate is equivalent to 36.94 mg of $(C_4H_9)_4NI$: not less than 99.0% is found.

Tetrabutylammonium Phosphate, $(C_4H_9)_4NH_2PO_4$**—339.46** [5574-97-0]—White to off-white powder. Soluble in water.
Assay: Dissolve about 1.5 g, accurately weighed, in 100 mL of water. Without delay, titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 169.7 mg of $(C_4H_9)_4NH_2PO_4$. Not less than 97.0% is found.

Tetradecylammonium Bromide *(Tetrakis(decyl)ammonium Bromide),* $C_{40}H_{84}BrN$**—659.01** [14937-42-9]—Use a suitable grade with a content of NLT 99%.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled, $^{13}C_{12}H_4Cl_4O_2$**—333.84**—Clear, colorless liquid.
• **ASSAY:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with an electron-capture detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; and the column temperature is maintained at 70° and programmed to rise 15° per minute to 300°. The area of the $^{13}C_{12}H_4Cl_4O_2$ peak is not less than 99.0% of the total peak area.
[NOTE—A suitable grade is available from Cambridge Isotopes Laboratories (www.isotope.com).]

2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled, $^{13}C_{12}H_4Cl_4O$**—317.84**—Clear, colorless liquid.
• **ASSAY:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with an electron-capture detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.32-mm × 60-m capillary column coated with a 1-µm layer of phase G27; the injection port temperature is maintained at 300°; the detector temperature is maintained at 350°; and the column temperature is maintained at 70° and programmed to rise 15° per minute to 275°. The area of the $^{13}C_{12}H_4Cl_4O$ peak is not less than 99.0% of the total peak area.
[NOTE—A suitable grade is available from Cambridge Isotopes Laboratories (www.isotope.com).]

1,1,2,2-Tetrachloroethane, $C_2H_2Cl_4$**—167.8** [630-20-6]—Colorless clear liquid. Specific gravity: 1.553. Refractive index at 20° is 1.481. Use a suitable grade.

Tetracosane, $C_{24}H_{50}$**—338.66** [646-31-1]—White powder.
Melting Range ⟨741⟩: between 51° and 53°.

Tetradecane, $C_{14}H_{30}$**—198.39** [629-59-4]—Clear, colorless liquid.
Assay: When examined by gas-liquid chromatography, it shows a purity of not less than 98%. The following conditions have been found suitable for assaying the reagent: a 3-mm × 2.4-m stainless steel column packed with phase G16 on support S1; the carrier gas is helium, flowing at a rate of 27.5 mL per minute; the column temperature is maintained at 250°, the injection port is maintained at 200°, and the detector is maintained at 280°. A flame-ionization detector is employed.
Melting Range, *Class II* ⟨741⟩: between 4° and 8°, within a 2° range.
Refractive Index ⟨831⟩: between 1.4280 and 1.4300 at 20°.

Tetraethylammonium Chloride *(TEA Chloride),* $C_8H_{20}NCl$**—165.7** [56-34-8]—Use a suitable grade with a content of NLT 98%.

Tetraethylammonium Perchlorate, $(C_2H_5)_4NClO_4$**—229.70**—White crystals. Soluble in water. Use a suitable grade.

Tetraethylene Glycol, $C_8H_{18}O_5$**—194.23** [112-60-7]—Nearly colorless liquid. Refractive index: about 1.46.
Assay: When examined by gas–liquid chromatography, using suitable gas chromatographic apparatus and conditions, it shows a purity of not less than 90%.
Boiling Range (Reagent test): between 177° and 187°, at a pressure of 9 mm of Hg.

Tetraethylenepentamine, $C_8H_{23}N_5$**—189.31** [112-57-2]—Colorless liquid.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 280°. The area of the $C_8H_{23}N_5$ peak is not less than 30% of the total peak area.
Refractive Index ⟨831⟩: between 1.503 and 1.507 at 20°.

Tetraheptylammonium Bromide, $(C_7H_{15})_4NBr$**—490.70** [4368-51-8]—White, flaky powder.
Melting Range ⟨741⟩: between 89° and 91°.

Tetrahexylammonium Hydrogen Sulfate, $C_{24}H_{53}NO_4S$**—451.75** [32503-34-7]—Use a suitable grade with a content of not less than 98.0%.

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Delta-8-tetrahydrocannabinol *(Δ-8-Tetrahydrocannabinol)*, $C_{21}H_{30}O_2$—**314.47** [5957-75-5]—Use a suitable grade which may be a solid material or a solution in methanol. [NOTE—A suitable grade of a methanolic solution (1 mg/mL) is available from www.cerilliant.com, catalog number T-032.]

Tetrahydrofuran, C_4H_8O—**72.11** [109-99-9]—Use ACS reagent grade.

For Chromatographic Application: Use a suitable grade, inhibitor-free, with a content of NLT 99.9%.

Tetrahydrofuran, Peroxide-Free, C_4H_8O— **72.11** —Use ACS reagent grade.

Peroxide: Transfer 8 mL of potassium iodide and starch TS to a 12-mL ground glass-stoppered cylinder about 15 mm in diameter. Fill completely with the substance under test, mix, and allow to stand protected from light for 5 minutes. No color develops.

Tetrahydrofuran, Stabilizer-Free: Use a suitable grade.

Tetrahydro-2-furancarboxylic Acid *(±Tetrahydro-2-furoic Acid)*, $C_5H_8O_3$—**116.12** [16874-33-2]—Use a suitable grade with a content of not less than 97%. [NOTE—A suitable grade is available from www.sigma-aldrich.com, catalog number 341517.]

Change to read:

***N*-(2-Tetrahydrofuroyl)piperazine** *(1-[(Tetrahydro-2-furanyl)carbonyl]piperazine)*, $C_9H_{16}N_2O_2$—**184.23**—Use a suitable grade.

[NOTE—A suitable grade can be obtained from ▲Oakwood Chemical, www.oakwoodchemical.com.▲ (ERR 1-Aug-2018)]

1,2,3,4-Tetrahydronaphthalene, $C_{10}H_{12}$—**132.21** [119-64-2]—Colorless liquid.

Refractive Index ⟨831⟩: 1.5401 at 20°.

Tetramethylammonium Bromide, $(CH_3)_4NBr$—**154.05** [64-20-0]—Use ACS reagent grade.

Tetramethylammonium Chloride, $(CH_3)_4NCl$—**109.60** [75-57-0]—Colorless crystals. Soluble in water and in alcohol; insoluble in chloroform.

Assay: Transfer about 200 mg, accurately weighed, to a beaker, add 50 mL of water and 10 mL of diluted nitric acid, swirl to dissolve the test specimen, add 50.0 mL of 0.1 N silver nitrate VS, and mix. Add 2 mL of ferric ammonium sulfate TS and 5 mL of nitrobenzene, shake, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS: each mL of 0.1 N silver nitrate is equivalent to 10.96 mg of $(CH_3)_4NCl$. Not less than 98% is found.

Tetramethylammonium Hydroxide, $(CH_3)_4NOH$—**91.15** [75-59-2]—Available as an approximately 10% or approximately 25% aqueous solution, or as the crystalline pentahydrate. Is clear and colorless. Tetramethylammonium hydroxide is a stronger base than ammonia and rapidly absorbs carbon dioxide from the air. Store in tight containers.

Assay: Accurately weigh a glass-stoppered flask containing about 15 mL of water. Add a quantity of a solution of tetramethylammonium hydroxide, equivalent to about 200 mg of $(CH_3)_4NOH$, and again weigh. Add methyl red TS, and titrate the solution with 0.1 N hydrochloric acid VS: each mL of 0.1 N hydrochloric acid is equivalent to 9.115 mg of $(CH_3)_4NOH$.

Residue on Evaporation: Evaporate 5 mL of solution on a steam bath, and dry at 105° for 1 hour: the weight of the residue is equivalent to not more than 0.02% of the weight of the test specimen.

Ammonia and Other Amines: Accurately weigh a quantity of solution, corresponding to about 300 mg of $(CH_3)_4NOH$, in a low-form weighing bottle tared with 5 mL of water. Add a slight excess of 1 N hydrochloric acid (about 4 mL), evaporate on a steam bath to dryness, and dry at 105° for 2 hours: the weight of the tetramethylammonium chloride so obtained, multiplied by 0.8317, represents the quantity, in mg, of $(CH_3)_4NOH$ in the portion of test specimen taken and corresponds to within 0.2% above or below that found in the *Assay*.

Tetramethylammonium Hydroxide, Pentahydrate, $(CH_3)_4NOH \cdot 5H_2O$—**181.23** [10424-65-4]—White to off-white crystals. Is hygroscopic. Strong base. Keep well-closed. Soluble in water and in methanol.

Assay: Accurately weigh about 800 mg, dissolve in 100 mL of water, and titrate with 0.1 N hydrochloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N hydrochloric acid is equivalent to 18.22 mg of $(CH_3)_4NOH \cdot 5H_2O$: not less than 98% is found.

Tetramethylammonium Hydroxide Solution in Methanol [75-59-2]—A solution in methanol of tetramethylammonium hydroxide [$(CH_3)_4NOH$—91.15]. Is generally available in concentrations of 10% and 25%. The following specifications apply specifically to the 25% concentration; for other concentrations, appropriate adjustments in the procedures may be necessary.

Assay: Accurately weigh about 1 g of the solution, and dilute with water to about 50 mL. Add phenolphthalein TS, and titrate with 0.1 N hydrochloric acid VS to the disappearance of the pink color: each mL of 0.1 N hydrochloric acid VS is equivalent to 91.15 mg of $(CH_3)_4NOH$. Between 23% and 25% is found.

Clarity: A portion of it in a test tube is clear, or only slightly turbid, when viewed transversely.

Tetramethylammonium Nitrate, $(CH_3)_4NNO_3$—**136.15** [1941-24-8]—White crystals. Freely soluble in water.

Tetramethylbenzidine *(4-(4-Amino-3,5-dimethylphenyl)-2,6-dimethylaniline; 3,3′,5,5′ Tetramethylbenzidine; 3,3′5,5′-Tetramethyl-[1,1′-biphenyl] 4,4′-diamine)*, $C_{16}H_{20}N_2$—**240.34** [54827-17-7]—Use a suitable grade.

1,1,3,3-Tetramethylbutylamine *(tert-Octylamine)*, $C_8H_{19}N$—**129.24** [107-45-9]—Use a suitable grade with a content of NLT 95%.

4,4′-Tetramethyldiaminodiphenylmethane *[(4,4′-Methylenebis(N,N-dimethylaniline)]*, $[(CH_3)_2NC_6H_4]_2CH_2$—**254.38** [101-61-1]—Off-white crystals.

Melting Range ⟨741⟩: between 87° and 90°.

Tetramethylethylenediamine *(N,N,N′,N′-Tetramethylethylenediamine; 1,2-Bis(dimethylamino)ethane; TEMED; TMEDA)*, $C_6H_{16}N_2$ —**116.20** [110-18-9]—Use a suitable grade with a content of NLT 99%.

Tetramethylsilane, $(CH_3)_4Si$—**88.23** [75-76-3]—Use ACS reagent grade.

Tetrapropylammonium Chloride, $C_{12}H_{28}ClN$—**221.82** [5810-42-4]—Use a suitable grade with a content of not less than 98.0%.

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Tetrasodium Ethylenediaminetetraacetate *((Ethylenedinitrilo)tetraacetic Acid Tetrasodium Salt),* $C_{10}H_{12}N_2Na_4O_8$—**380.17**—Fine, white, crystalline powder. Soluble in water.

Loss on Drying ⟨731⟩: Dry it at 105° for 4 hours: it loses not more than 8% of its weight.

Thallous Chloride, TlCl—**239.84**—Fine, white, crystalline powder. Soluble in about 260 parts of cold water and in about 70 parts of boiling water; insoluble in alcohol. *Poisonous; use with adequate ventilation.*

Assay: Dissolve about 500 mg, accurately weighed, in a mixture of 80 mL of water and 0.5 mL of sulfuric acid. When dissolution is complete, add 20 mL of hydrochloric acid. Heat to 60° and maintain this temperature while titrating with 0.1 N ceric sulfate VS, determining the endpoint potentiometrically, using silver-silver chloride and platinum electrodes. Each mL of 0.1 N ceric sulfate is equivalent to 11.99 mg of TlCl. Not less than 99% is found.

Theobromine, $C_7H_8N_4O_2$—**180.17** [83-67-0]—White, crystalline solid. Very slightly soluble in water and in alcohol; almost insoluble in benzene, in ether, and in chloroform.

Assay: Dissolve about 34 mg, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.02 mg of $C_7H_8N_4O_2$. Not less than 95% is found.

Thiazole Yellow *(CI Direct Yellow 9; Clayton Yellow; Titan Yellow),* $C_{28}H_{19}N_5Na_2O_6S_4$—**695.74** [1829-00-1]—Yellowish-brown powder. Soluble in water and in alcohol to yield in each instance a yellow solution; soluble in dilute alkali to yield a brownish-red solution. Protect from light.

Solubility: A 200-mg portion mixed with 50 mL of water shows not more than a faint haze.

Residue on Ignition: Accurately weigh about 1.5 g, previously dried at 105° for 2 hours, and ignite until thoroughly charred. Cool, add 2 mL of nitric acid and 2 mL of sulfuric acid, ignite gently to expel excess acids, then at 600° to 800° to constant weight: the residue of sodium sulfate (Na_2SO_4) is between 19.8% and 21.5% of the weight of the test specimen (theory is 20.4%).

Sensitiveness to Magnesium: Add 0.2 mL of a solution (1 in 10,000) and 2 mL of 1 N sodium hydroxide to a mixture of 9.5 mL of water and 0.5 mL of a solution prepared by dissolving 1.014 g of clear crystals of magnesium sulfate in water, diluting with water to 100 mL, then diluting 10 mL of the resulting solution with water to 1 L: a distinct pink color is produced within 10 minutes.

Thioacetamide, C_2H_5NS—**75.13** [62-55-5]—Use ACS reagent grade.

2-Thiobarbituric Acid, $C_4H_4N_2O_2S$—**144.15** [504-17-6]—White leaflets. Slightly soluble in water.

Melting Temperature ⟨741⟩: 236°, with decomposition.

2,2′-Thiodiethanol, $(HOCH_2CH_2)_2S$—**122.19** [111-48-8]—Pale yellow to colorless liquid.

Assay: Not less than 98% of $C_4H_{10}O_2S$ is found, a suitable gas chromatograph equipped with a flame-ionization detector being used. The following conditions have been found suitable: a 4.0-mm × 1.83-m glass column is packed with 10% phase G25 on support S1A; the column, injection port, and detector temperatures are maintained at 200°, 250°, and 310°, respectively.

Refractive Index ⟨831⟩: between 1.4250 and 1.4270, at 20°.

Thioglycolic Acid, $HSCH_2COOH$—**92.12** [68-11-1]—A colorless or nearly colorless liquid. Miscible with water. Soluble in alcohol.

Sensitiveness: Mix 1 mL with 2 mL of stronger ammonia water, and dilute with water to 20 mL. Add 1 mL of this solution to a mixture of 20 mL of water and 0.1 mL of dilute ferric chloride TS (1 in 100), then add 5 mL of ammonia TS: a distinct pink color is produced.

Thionine Acetate, $C_{12}H_9N_3S \cdot C_2H_4O_2$—**287.34** [78338-22-4]—Use a suitable grade.

Thiourea, $(NH_2)_2CS$—**76.12** [62-56-6]—Use ACS reagent grade.

Thorium Nitrate, $Th(NO_3)_4 \cdot 4H_2O$—**552.12** [13470-07-0]—Use ACS reagent grade.

Thrombin Human *(Factor II_a)*—~**33,600** [9002-04-4]—A preparation of a serine protease (enzyme) that converts human fibrinogen into fibrin. It is obtained from human plasma and may be prepared by precipitation with suitable salts and organic solvents under controlled conditions of pH, ionic strength, and temperature. A yellowish-white powder, freely soluble in a 9 g per L solution of sodium chloride, which forms a cloudy, pale yellow solution. Store in a sealed, sterile container under nitrogen, protected from light, at a temperature below 0°. One unit corresponds to the amount of enzyme that hydrolyzes 1 µmol of Tos-Gly-Pro-Arg-4-nitroaniline acetate per minute at a pH of 8.4 and a temperature of 37°.

Thromboplastin [9035-58-9]—Buff-colored powder, or opalescent or turbid suspension. It exhibits thrombokinase activity derived from the acetone-extracted brain and/or lung tissue of freshly killed rabbits. It may contain sodium chloride and calcium chloride in suitable proportions, and it may contain a suitable antimicrobial agent. It is used in suspension form for the determination of the prothrombin time and activity of blood. Its thrombokinase activity is such that it gives a clotting time of 11 to 16 seconds with normal human plasma and the proper concentration of calcium ions. Store in tight containers, preferably at a temperature below 5°.

Loss on Drying ⟨731⟩:

[NOTE—This test is applicable only to the dry form.]

Dry it in vacuum at 60° for 6 hours: it loses not more than 5.0% of its weight.

Thymidine, $C_{10}H_{14}N_2O_5$—**242.2** [50-89-5]—White powder. Use a suitable grade.

Thymol, $C_6H_3[CH_3][OH][CH(CH_3)_2]1,3,4$—**150.22** [89-83-8]—Colorless, often large, crystals, or a white, crystalline powder. Is affected by light. Has greater density than water, but when liquefied by fusion is less dense than water. Its alcohol solutions are neutral to litmus. One g dissolves in about 1000 mL of water, in 1 mL of alcohol, in 1 mL of chloroform, in 1.5 mL of ether, and in about 2 mL of olive oil. Soluble in glacial acetic acid and in fixed or volatile oils. Store in tight, light-resistant containers.

Melting Range ⟨741⟩: between 48° and 51°, but when melted it remains liquid at a considerably lower temperature.

Nonvolatile Matter: Volatilize 2 g on a steam bath, and dry at 105° to constant weight: the residue weighs not more than 1 mg (0.05%).

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Thyroglobulin, [9010-34-8]—A protein having a molecular weight of 670 kDa. Available as a slightly beige, freeze-dried powder made from bovine or porcine thyroid gland. Use a suitable grade.

Tin, Sn—**At. Wt. 118.71** [7440-31-5]—Use ACS reagent grade.

Titanium Tetrachloride, $TiCl_4$—**189.68** [7550-45-0]—Clear, colorless liquid. Fumes in air. [**CAUTION**—It reacts violently with water.]

Assay: Accurately weigh 0.75 g into 100 mL of 2 N sulfuric acid contained in a Smith weighing buret. Pour the solution through a zinc–mercury reduction column into 50 mL of 0.1 N ferric ammonium sulfate VS. Elute with 100 mL of 2 N sulfuric acid and 100 mL of water. Add 10 mL of phosphoric acid, and titrate with 0.1 N potassium permanganate VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N potassium permanganate is equivalent to 18.97 mg of $TiCl_4$. Not less than 99.5% is found.

Boiling Range (Reagent test): between 135° and 140°.

Titanium Trichloride *(Titanous Chloride),* $TiCl_3$—**154.23** [7705-07-9]—Black, hygroscopic powder, unstable in air. Soluble in water, the solution depositing titanic acid on exposure to air. Is available usually as 15% to 20%, dark violet-blue, aqueous solutions. Store the solution in tightly closed, glass-stoppered bottles, protected from light.

***o*-Tolidine** *(4,4′-Diamino-3,3′-dimethylbiphenyl),* $(NH_2)(CH_3)C_6H_3 \cdot C_6H_3(CH_3)(NH_2)4,3,3′,4′$—**212.29** [119-93-7]—White to reddish crystals or crystalline powder. Slightly soluble in water; soluble in alcohol, in ether, and in dilute acids. Preserve in well-closed containers, protected from light. [**CAUTION**—Avoid contact with *o*-tolidine and mixtures containing *o*-tolidine, and conduct all tests in a well-ventilated fume hood.]

Use a suitable grade with a content of NLT 98.0%.

Tolualdehyde *(o-Tolualdehyde),* C_8H_8O—**120.15** [529-20-4]—Use a suitable grade.

***p*-Tolualdehyde,** C_8H_8O—**120.15** [104-87-0]—Colorless to yellow, clear liquid.

- **ASSAY:** When examined by gas-liquid chromatography, it shows a purity of not less than 98%. The following conditions have been found suitable for assaying the article: A 3-mm × 1.8-m stainless steel column packed with a 5% phase G4 on support S1. Nitrogen, having a flow rate of about 12 mL per minute, is the carrier gas, the detector and column temperature are about 125°, and the injection port temperature is about 205°. A flame-ionization detector is employed and the specimen is a 5% solution in carbon disulfide.
- **REFRACTIVE INDEX** ⟨831⟩: between 1.544 and 1.546, at 20°.

Toluene *(Toluol),* $C_6H_5CH_3$—**92.14** [108-88-3]—Use ACS reagent grade.

***p*-Toluenesulfonic Acid,** $CH_3C_6H_4SO_3H \cdot H_2O$—**190.22** [6192-52-5]—Use ACS reagent grade.

***p*-Toluenesulfonyl-L-arginine Methyl Ester Hydrochloride** *($N_α$-p-Tosyl-L-arginine methyl ester hydrochloride; TAME),* $C_{14}H_{22}N_4O_4S \cdot HCl$—**378.88** [1784-03-8]—Determine its suitability as directed in the test for *Trypsin* under *Chymotrypsin* (USP monograph).

***p*-Toluic Acid,** $CH_3C_6H_4COOH$—**136.15** [99-94-5]—White, crystalline powder. Sparingly soluble in hot water; very soluble in alcohol, in methanol, and in ether.

- **ASSAY:** Transfer about 650 mg, accurately weighed, to a suitable container, dissolve in 125 mL of alcohol, add 25 mL of water, and mix. Titrate with 0.5 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sodium hydroxide is equivalent to 68.07 mg of $C_8H_8O_2$: not less than 98% is found.
- **MELTING RANGE** ⟨741⟩: over a range of 2° that includes 181°.

***o*-Toluidine** *(2-Aminotoluene; 2-Methylaniline),* $C_6H_4(CH_3)(NH_2)1,2$—**107.15** [95-53-4]—Light yellow liquid becoming reddish brown on exposure to air and light. Soluble in alcohol, in ether, and in dilute acids; slightly soluble in water. Preserve in well-closed containers, protected from light.

Specific Gravity ⟨841⟩: 1.008 at 20°.

Boiling Range (Reagent test): between 200° and 202°.

***p*-Toluidine,** C_7H_9N—**107.15** [106-49-0]—White to beige crystals or flakes.

Freely soluble in alcohol, in acetone, in methanol, and in dilute acids; slightly soluble in water.

- **ASSAY:** Dissolve 400 mg, accurately weighed, in 100 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 10.72 mg of $CH_3C_6H_4NH_2$. Not less than 98%, calculated on the dried basis, is found.
- **LOSS ON DRYING:** Weigh accurately about 1 g, and dry at 30° to constant weight: it loses not more than 2% of its weight.

Toluidine Blue, $(C_{15}H_{16}ClN_3S)_2 \cdot ZnCl_2$—**747.95** [6586-04-5]—Use a suitable grade.

Toluidine Blue O, $C_{15}H_{16}N_3SCl$—**305.8** [92-31-9]—Use a suitable grade.

Tosylchloramide Sodium *(Chloramine-T Trihydrate; N-Chloro-4-toluenesulfonamide, Sodium Salt, Trihydrate),* $H_3CC_6H_4SO_2NClNa \cdot 3H_2O$—**281.69** [127-65-1]—Use Chloramine-T Trihydrate, ACS reagent grade.

Add the following:

▲Triacetin, $C_9H_{14}O_6$—**218.2** [102-76-1]—Use a suitable grade with a content of NLT 99%.▲ (USP 1-May-2019)

***n*-Triacontane,** $C_{30}H_{62}$—**422.81** [638-68-6]—Use a suitable grade.

2,4,6-Triamino-5-nitrosopyrimidine, $C_4H_6N_6O$—**154.13**—Pink powder.

Assay: Dissolve about 34 mg, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 15.41 mg of $C_4H_6N_6O$. Not less than 97% is found.

Triazolam, $C_{17}H_{12}Cl_2N_4$—**343.21** [28911-01-5]—Use *Triazolam* (USP monograph).

Tributyl Phosphate *(Tri-n-butyl Phosphate),* $(C_4H_9)_3PO_4$—**266.31** [126-73-8]—Clear, almost colorless liquid. Slightly

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soluble in water. Miscible with common organic solvents. Specific gravity: about 0.976.
Refractive Index ⟨831⟩: between 1.4205 and 1.4225.

Tributylethylammonium Hydroxide, $C_{14}H_{33}NO$**—231.42**—Use a suitable grade.

Tributyrin *(Glyceryl Tributyrate),* $C_{15}H_{26}O_6$**—302.36** [60-01-5]—Colorless, oily liquid. Insoluble in water; very soluble in alcohol and in ether.
Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, nitrogen being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 1.8-m stainless steel column containing phase G4 on support S1A; the injection port temperature is maintained at 270°; and the detector temperature is maintained at 300°. The area of the tributyrin peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: between 1.4345 and 1.4365 at 20°.
Acid Content: Transfer 1.0 g, accurately weighed, to a beaker, add 75 mL of methanol, and dissolve by stirring. When dissolution is complete, add 25 mL of water, and titrate with 0.05 N potassium hydroxide VS, using phenolphthalein TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N potassium hydroxide is equivalent to 88.1 mg of butyric acid: not more than 0.5% is found.

Trichloroacetic Acid, CCl_3COOH**—163.39** [76-03-9]—Use ACS reagent grade.

Trichloroethane—See *Methyl Chloroform.*

2,2,2-Trichloroethanol *(Trichloroethyl Alcohol),* $C_2H_3OCl_3$**—149.40** [115-20-8]—Use a suitable grade with a content of NLT 99%.

Trichlorofluoromethane, CCl_3F**—137.37** [75-69-4]—Colorless liquid.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal conductivity detector, helium being used as the carrier gas. The following conditions have been found suitable: a 2.0-mm × 1.8-m glass column packed with 10% G1 phase on support S1A; the injection port temperature is maintained at 50°; the detector temperature is maintained at 300°; and the column temperature is maintained at 0° and programmed to rise 3° per minute to 50°. The area of the CCl_3F peak is not less than 99% of the total peak area.
Refractive Index ⟨831⟩: between 1.380 and 1.384 at 20°.

Trichlorotrifluoroethane—Use a suitable grade.
[NOTE—A suitable preparation, listed as "Freon-TF aerosol," is available from E. I. du Pont de Nemours and Co., Wilmington, DE 19898.]

Triethanolamine—Use *Trolamine* (NF monograph).

Triethylamine, $(C_2H_5)_3N$**—101.19** [121-44-8]—Colorless liquid. Slightly soluble in water. Miscible with alcohol, with ether, and with cold water. Store in well-closed containers. Use a suitable grade with a content of not less than 99.5%.

Triethylamine Hydrochloride, $C_6H_{15}N \cdot HCl$**—137.65** [554-68-7]—White to off-white powder.
Assay: Transfer about 35 mg, accurately weighed, to a suitable beaker, add 50 mL of glacial acetic acid, and dissolve by stirring. Add 5 mL of mercuric acetate TS, with stirring. When solution is complete, titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank titration, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.77 mg of $C_6H_{15}N \cdot HCl$. Not less than 97.5% of $C_6H_{15}N \cdot HCl$ is found.
Melting Point ⟨741⟩: between 256° and 259°, with decomposition.

Triethylamine Phosphate *(Triethylammonium Phosphate),* $C_6H_{15}N \cdot H_3O_4P$**—199.19**—Use a suitable grade.
[NOTE—A suitable grade is available from www.tciamerica.com, catalog number T1300.]

Triethylammonium Acetate, 1 M, $C_8H_{19}NO_2$**—161.2** [5204-74-0]—Use a suitable grade. [NOTE—A suitable grade is available as catalog number 625718 from www.calbiochem.com.]

Triethylene Glycol, $C_6H_{14}O_4$**—150.17** [112-27-6]—Colorless to pale yellow liquid. Is hygroscopic. Miscible with water, with alcohol, and with toluene.
Assay: Inject an appropriate test specimen into a suitable gas chromatograph equipped with a flame-ionization detector (see *Chromatography* ⟨621⟩), helium being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 1.85-m stainless steel column packed with support S2; the injection port, column, and detector temperatures are maintained at 250°, 230°, and 310°, respectively. The area of the $C_6H_{14}O_4$ peak is not less than 97% of the total peak area.
Refractive Index ⟨831⟩: between 1.4550 and 1.4570, at 20°.

Triethylenediamine *(1,4-Diazobicyclo[2.2.2]octane),* $C_6H_{12}N_2$**—112.17** [280-57-9]—Use a suitable grade with a content of not less than 98%.

Trifluoroacetic Acid, $C_2HF_3O_2$**—114.02** [76-05-1]—Colorless liquid. Miscible with ether, with acetone, with ethanol, with benzene, with carbon tetrachloride, and with hexane.
Assay: Dissolve about 300 mg, accurately weighed, in 25 mL of water and 25 mL of alcohol. Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N sodium hydroxide is equivalent to 11.40 mg of $C_2HF_3O_2$. Not less than 99% is found.

Add the following:

▲**Trifluoroacetic Acid Ethyl Ester** *(Ethyl Trifluoroacetate),* $C_4H_5F_3O_2$**—142.08** [383-63-1]—Use a suitable grade with a content of NLT 99%. ▲ (USP 1-May-2019)

Trifluoroacetic Anhydride, $(F_3CCO)_2O$**—210.03** [407-25-0]—Colorless liquid. Boils between 40° and 42°. Extremely volatile. Avoid exposure to air or water.
Assay: Transfer about 0.8 g, accurately weighed, to a glass-stoppered flask containing 50 mL of methanol. Add 500 mg of phenolphthalein, and titrate with 0.1 N sodium methoxide VS to a pink endpoint. Calculate A by the formula:

$$V/W$$

in which V is the volume, in mL, of 0.1 N sodium methoxide and W is the weight, in mg, of test specimen. To a second glass-stoppered flask containing 50 mL of a mixture of

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dimethylformamide and water (1:1) transfer 0.4 g, accurately weighed, of the specimen under test, add 500 mg of phenolphthalein, and titrate with 0.1 N sodium hydroxide VS to a pink endpoint. Calculate B by the formula:

$$V^1/W^1$$

in which V^1 is the volume, in mL, of 0.1 N sodium hydroxide and W^1 is the weight, in mg, of test specimen. Calculate the percentage of $(F_3CCO)_2O$ by the formula:

$$2100.3(B - A)$$

Not less than 97% is found. If 2A is greater than B, calculate the percentage of F_3CCOOH by the formula:

$$1140.3(2A - B)$$

α,α,α-Trifluoro-*p*-cresol *(4-hydroxybenzotrifluoride, 4-trifluoromethylphenol)*, $C_7H_5F_3O$—**162.11** [402-45-9]
Melting Range ⟨741⟩: between 48° and 52°.

2,2,2-Trifluoroethanol, CF_3CH_2OH—**100.04** [75-89-8]—Colorless liquid.
Assay: Inject an appropriate specimen into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 100°; the detector temperature is maintained at 150°; and the column temperature is maintained at 0° and programmed to rise 10° per minute to 150°. The area of the CF_3CH_2OH peak is not less than 99% of the total peak area.
Boiling Range: between 77° and 80°.

2,2,2-Trifluoroethyldifluoromethyl Ether *(Difluoromethyl-2,2,2-trifluoroethyl ether)*, $C_3H_3F_5O$—**150.05**—Clear liquid. Use a suitable grade.
Boiling Range: between 28° and 30°.
[NOTE—A suitable grade is available from PCR Incorporated, P.O. Box 1466, Gainesville, FL 32602. Tel: 904-376-8246. The catalogue number is 17151-2.]

(*m*-Trifluoromethylphenyl) Trimethylammonium Hydroxide in Methanol—Use a suitable grade.
[NOTE—A suitable grade is available as "Meth-Prep II" from Alltech, www.fishersci.com.]

5-(Trifluoromethyl)uracil, $C_5H_3F_3N_2O_2$—**180.08** [54-20-6]—White to off-white powder.
Assay: When tested by thin-layer chromatography, with the use of plates coated with chromatographic silica gel mixture, a developing system consisting of chloroform, methanol, and acetic acid (17:2:1), and examined visually and under long-wavelength UV light, a single spot is exhibited.

Trifluorovinyl Chloride Polymer ***(Fluorolube; 1-Chloro-1,2,2-trifluoro-ethene Homopolymer)***, $(C_2ClF_3)_x$—Use a suitable grade.
[NOTE—A suitable grade is available as catalog number Z123552 from www.sigma-aldrich.com.]

Trimethylacethydrazide Ammonium Chloride *(Betaine Hydrazide Chloride; Girard Reagent T)*, $[(CH_3)_3N^+CH_2CONHNH_2]Cl^-$—**167.64** [123-46-6]—Colorless or white crystals. Freely soluble in water. One g dissolves in about 25 mL of alcohol. Insoluble in chloroform and in ether. Hygroscopic.
Melting Range ⟨741⟩: between 185° and 192°, determined after recrystallization from hot alcohol, if necessary.
Residue on Ignition (Reagent test): Ignite 1 g with 0.5 mL of sulfuric acid: the residue weighs not more than 10 mg (1%).

Add the following:

▲**Trimethylamine Hydrochloride** *(Trimethylammonium Chloride)*, $C_3H_9N \cdot HCl$—**95.57** [*593-81-7*]—Use a suitable grade with a content of NLT 98.5%. ▲ 2S (USP41)

Trimethylchlorosilane—See *Chlorotrimethylsilane.*

2,2,4-Trimethylpentane *(Isooctane)*, C_8H_{18}—**114.23** [540-84-1]—Use ACS reagent grade.

2,4,6-Trimethylpyridine *(5-Collidine)*, $C_8H_{11}N$—**121.18** [108-75-8]—Clear, colorless liquid. Soluble in cold water and less soluble in hot water; soluble in alcohol, in chloroform, and in methanol. Miscible with ether.
Assay: Inject an appropriate test specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩), helium being used as a carrier gas. The following conditions have been found suitable: a 3-mm × 1.85-m stainless steel column containing phase G16 on support S1A; the injection port, column, and detector temperatures are maintained at 180°, 165°, and 270°, respectively; and a flame-ionization detector is used. The area of the $C_8H_{11}N$ peak is not less than 98% of the total peak area.
Refractive Index ⟨831⟩: between 1.4970 and 1.4990, at 20°.

***N*-(Trimethylsilyl)-imidazole,** $C_6H_{12}N_2Si$—**140.26** [18156-74-6]—A clear, colorless, to light yellow liquid.
• **REFRACTIVE INDEX** ⟨831⟩: between 1.4744 and 1.4764 at 20°.

Trimethyltin Bromide, C_3H_9BrSn—**243.72** [1066-44-0]—Use a suitable grade.

2,4,6-Trinitrobenzenesulfonic Acid, $C_6H_2(NO_2)_3SO_3H \cdot 3H_2O$—**347.21** [2508-19-2]—Pale yellow to tan crystals. Use a suitable grade. Also available as a 5% (w/v) or a 1 M aqueous solution.

Trinitrophenol—See *Picric Acid.*

Trioctylphosphine Oxide, $C_{24}H_{51}PO$—**386.63** [78-50-2]—White, crystalline powder. Insoluble in water; soluble in organic solvents.
Melting Range ⟨741⟩: between 54° and 56°.

1,3,5-Triphenylbenzene, $(C_6H_5)_3C_6H_3$—**306.41** [612-71-5]—White to off-white powder.
Melting Range ⟨741⟩: between 172° and 175°.

Triphenylene *(9,10-Benzophenanthrene)*, $C_{18}H_{12}$—**228.29** [217-59-4]—Use a suitable grade with a content of NLT 98%.

Triphenylmethane, $C_{19}H_{16}$—**244.34** [519-73-3]—Light brown powder.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a

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1-µm layer of phase G2; the injection port temperature is maintained at 300°; the detector temperature is maintained at 300°; and the column temperature is maintained at 200° and programmed to rise 10° per minute to 300°. The area of the $C_{19}H_{16}$ peak is not less than 99% of the total peak area.
Melting Range ⟨741⟩: between 92° and 94°.

Triphenylmethanol, $C_{19}H_{16}O$**—260.34** [76-84-6]—White to off-white powder.
Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 280°; the detector temperature is maintained at 300°; and the column temperature is maintained at 180°. The area of the $C_{19}H_{16}O$ peak is not less than 96.5% of the total peak area.

Add the following:

▲Triphenylphosphine Oxide *(Triphenyl Phosphorus Oxide; Triphenylphosphine Monoxide),* $(C_6H_5)_3PO$**—278.28** [791-28-6]—Use a suitable grade with a content of NLT 98%.▲ (USP 1-Dec-2019)

Triphenyltetrazolium Chloride, $C_{19}H_{15}ClN_4$**—334.80** [298-96-4]—White to yellowish, crystalline powder. Soluble in about 10 parts of water and of alcohol; slightly soluble in acetone; insoluble in ether. Usually contains solvent of crystallization, and when dried at 105° it melts at about 240°, with decomposition.
Solubility: Separate 100-mg portions dissolve completely in 10 mL of water and in 10 mL of alcohol, respectively, to yield solutions that are clear, or practically so.
Loss on Drying ⟨731⟩: Dry it at 105° to constant weight: it loses not more than 5.0% of its weight.
Residue on Ignition (Reagent test): negligible, from 100 mg.
Sensitiveness: Dissolve 10 mg in 10 mL of dehydrated alcohol (*A*). Then dissolve 10 mg of dextrose in 20 mL of dehydrated alcohol (*B*). To 0.2 mL of *B* add 1 mL of dehydrated alcohol and 0.5 mL of dilute tetramethylammonium hydroxide TS (1 volume diluted with 9 volumes of dehydrated alcohol), then add 0.2 mL of *A:* a pronounced red color develops within about 10 minutes.

Tris(2-aminoethyl)amine, $C_6H_{18}N_4$**—146.23** [4097-89-6]—Yellow liquid. Soluble in methanol.
Assay: Dissolve about 80 mg in 30 mL of methanol. Add 40 mL of water, and titrate with 1 N hydrochloric acid, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 1 N hydrochloric acid is equivalent to 48.75 mg of $C_6H_{18}N_4$. Not less than 98.0% is found.
Refractive Index ⟨831⟩: between 1.4956 and 1.4986 at 20°.

Tris(hydroxymethyl)aminomethane [77-86-1]—Use ACS reagent grade—See also Tromethamine.

Tris(hydroxymethyl)aminomethane Acetate, $C_4H_{11}O_3 \cdot NCH_3$**—181.19** [6850-28-8]—White powder with lumps. Use a suitable grade.

Tris(hydroxymethyl)aminomethane Hydrochloride, $C_4H_{11}NO_3 \cdot HCl$**—157.60** [1185-53-1]—Colorless crystals. Use a suitable grade.

***N*-Tris(hydroxymethyl)methylglycine,** $C_6H_{13}NO_5$**—179.2** [5704-04-1]—White, crystalline powder. Use a suitable grade.

***Tritirachium Album* Proteinase K:** Use a suitable grade.
[NOTE—A suitable grade can be obtained from Qiagen, Inc., www.qiagen.com.]

Tromethamine *[Tris(hydroxymethyl)aminomethane; THAM; 2-Amino-2-(hydroxymethyl)-1,3-propanediol],* $C_4H_{11}NO_3$**—121.14**—Use ACS reagent grade Tris(hydroxymethyl)amino methane.

Tropaeolin OO *(Acid Orange 5),* $C_{18}H_{14}N_3NaO_3S$**—375.38** [554-73-4]—Orange-yellow scales, or yellow powder. Soluble in water.
pH range: from 1.4 (red) to 2.6 (yellow).

Tropic Acid, $C_9H_{10}O_3$**—166.18** [529-64-6]—Use a suitable grade.

Tropine, $C_8H_{15}NO$**—141.2** [120-29-6]—Use a suitable grade.

Trypan Blue *(Direct Blue 14),* $C_{34}H_{24}N_6Na_4O_{14}S_4$**—960.8** [72-57-1]—Use a suitable grade.

Add the following:

▲Tryptamine Hydrochloride *(3-(2-Aminoethyl)indole hydrochloride),* $C_{10}H_{13}ClN_2$**—196.68** [343-94-2]—Use a suitable grade with a content of NLT 98%. [NOTE—A suitable grade is available as catalog #15798 from www.acros.com.]▲ (USP 1-Aug-2019)

Tryptone—Use *Pancreatic Digest of Casein.*

L-Tryptophane, $C_{11}H_{12}N_2O_2$**—204.23** [73-22-3]—White or not more than slightly yellow leaflets or powder. One g dissolves in about 100 mL of water; soluble in dilute acids and in solutions of the alkali hydroxides; slightly soluble in alcohol.
- **ASSAY:** Accurately weigh about 300 mg, dissolve in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Each mL of 0.1 N perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$. Between 98.0% and 102.0%, calculated on the dried basis, is found.
- **SPECIFIC ROTATION** ⟨781⟩: between −30.0° and −33.0°, determined in a solution containing 1.0 g of test specimen, previously dried at 105° for 3 hours, in 100 mL.
- **LOSS ON DRYING** ⟨731⟩: Dry it at 105° for 3 hours: it loses not more than 0.3% of its weight.
- **RESIDUE ON IGNITION** (Reagent test): not more than 0.1%.
- **TYROSINE:** Dissolve 100 mg in 3 mL of diluted sulfuric acid, add 10 mL of mercuric sulfate TS, and heat on a steam bath for 10 minutes. Filter, wash with 5 mL of mercuric sulfate TS, and add to the combined filtrate 0.5 mL of sodium nitrite solution (1 in 20): no red color is produced within 15 minutes.

Tuberculin, Purified Protein Derivative ***(Tuberculin PPD):*** Derived from the human strain of *Mycobacterium tuberculosis,* and available either as a solution or as a lyophilized powder. For lyophilized powder, reconstitute according to the manufacturer's instructions using the diluent provided by the manufacturer. Solutions may contain a stabilizer and a preservative. One Tuberculin Unit (TU) is equivalent to 0.02 µg of Tuberculin PPD.

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Tubocurarine Chloride ***(7′,12′-Dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethyltubocuraranium Chloride),*** $C_{37}H_{42}Cl_2N_2O_6$—**681.65** [6989-98-6]—Use a suitable grade with an assay result between 98.0% and 102.0%.

[NOTE—A suitable grade is available from Acros Organics, catalog number 24349 at www.acros.com.]

Tungstic Acid, H_2WO_4—**249.85** [7783-03-1]—Use a suitable grade with a content of not less than 99%.

L-Tyrosine Disodium, $C_9H_9NO_3Na_2$—**225.2** [69849-45-6]—Off-white to tan powder. Use a suitable grade.

Tyrosol *(2-(4-Hydroxyphenyl)ethanol),* $C_8H_{10}O_2$—**138.16** [501-94-0]—Use a suitable grade with a content of NLT 99%.

Uracil, $C_4H_4N_2O_2$—**112.09** [66-22-8]—White to cream-colored, crystalline powder. Melts above 300°. One g dissolves in about 500 mL of water; less soluble in alcohol; soluble in ammonia TS and in sodium hydroxide TS. Its solutions yield no precipitate with the usual alkaloidal precipitants.

Residue on Ignition ⟨Reagent test⟩: negligible, from 100 mg.

Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 2% of its weight.

Uranyl Acetate *(Uranium Acetate),* $UO_2(C_2H_3O_2)_2 \cdot 2H_2O$—**424.15** [541-09-3]—Use ACS reagent grade.

Urea, NH_2CONH_2—**60.06** [57-13-6]—Use ACS reagent grade.

Urethane *(Ethyl carbamate),* $C_3H_7NO_2$—**89.09** [51-79-6]—White powder with chunks. Freely soluble in water.

Melting Range ⟨741⟩: between 48° and 50°.

Uridine, $C_9H_{12}N_2O_6$—**244.20** [58-96-8]—White powder.

Assay

Mobile phase: Prepare a mixture of methanol and 0.2 M ammonium acetate (10:90), and adjust with phosphoric acid to a pH of 7.0.

Test solution: 0.5 mg per mL in water.

Procedure: Inject about 20 µL of the *Test solution* into a liquid chromatograph (see *Chromatography* ⟨621⟩), equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. The area of the $C_9H_{12}N_2O_6$ peak is not less than 99% of the total peak area.

Melting Range ⟨741⟩: between 166° and 171°.

Valeric Acid, $C_5H_{10}O_2$—**102.13** [109-52-4]—Clear, colorless liquid.

Assay: Accurately weigh about 500 mg, transfer to a suitable container, add 30 mL of water, and mix. Add 40 mL of water, and mix. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 10.21 mg of $C_5H_{10}O_2$: not less than 99.0% of $C_5H_{10}O_2$ is found.

Valerophenone, $C_{11}H_{14}O$—**162.23** [1009-14-9]—Colorless liquid.

Assay: Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a capillary column coated with a 1-µm layer of phase G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 150° and programmed to rise 10° per minute to 300°. The area of the $C_{11}H_{14}O$ peak is not less than 98% of the total peak area.

Refractive Index ⟨831⟩: 1.5149 at 20°.

Boiling Range: between 105° and 107°, at a pressure of 5 mm of mercury.

Vanadium Pentoxide, V_2O_5—**181.88** [1314-62-1]—Fine, yellow to orange-yellow powder. Slightly soluble in water; soluble in concentrated acids and in alkalies; insoluble in alcohol.

Assay: Transfer about 400 mg, accurately weighed, to a 500-mL conical flask, and add 150 mL of water and 30 mL of dilute sulfuric acid (1 in 2). Boil the solution on a hot plate for 5 minutes, add 50 mL of water, and continue boiling until a yellow solution is obtained. Transfer the hot plate and the flask to a well-ventilated hood, and bubble sulfur dioxide gas through the solution for 10 minutes, or until the solution is a clear, brilliant blue color. Rinse the gas delivery tube into the flask with a few mL of water, then bubble carbon dioxide gas through the solution for 30 minutes while continuing to boil the solution gently. Cool the solution to about 80°, and titrate with 0.1 N potassium permanganate VS to a yellow-orange endpoint. Perform a complete blank determination, and make any necessary correction. Each mL of 0.1 N potassium permanganate is equivalent to 9.095 mg of V_2O_5. Not less than 99.5% is found.

Vanadyl Sulfate, $VOSO_4 \cdot xH_2O$ (anhydrous)—**163.00** [27774-13-6]—Blue, hygroscopic crystals. Slowly and usually incompletely soluble in water.

Assay: Accurately weigh about 400 mg of the dried test specimen obtained in the test for *Water,* and transfer with 15 to 20 mL of water into a beaker. Add 3 mL of sulfuric acid, cover the beaker with a watch glass, and heat on a steam bath until all dissolves. Cool, dilute with 125 mL of water, and titrate with 0.1 N potassium permanganate VS to the production of a pinkish color that persists for 1 minute: each mL of 0.1 N potassium permanganate is equivalent to 16.30 mg of $VOSO_4$. Not less than 97% is found.

Water: Dry about 1 g, accurately weighed, at 220° to constant weight: it loses not more than 50.0% of its weight.

Pentavalent Vanadium: Heat 1 g, accurately weighed, with 50 mL of water and 5 mL of hydrochloric acid in a flask until dissolved. Cool, add 2 g of potassium iodide, insert the stopper, and allow to stand for 30 minutes. Add 50 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Correct for the volume of thiosulfate consumed by a blank. Each mL of 0.1 N thiosulfate is equivalent to 5.095 mg of vanadium (V). Not more than 0.5% is found, calculated on the dried basis.

Substances Not Precipitated By Ammonia: Dissolve 1.0 g by heating with 20 mL of water and 2 mL of hydrochloric acid. Dilute with water to about 75 mL, and neutralize to litmus paper with ammonia TS. Transfer the solution to a cylinder, slowly add 5 mL of ammonia TS and sufficient water to make 100 mL, and allow to stand overnight. Decant 50 mL of the supernatant through a filter, add 5 drops of sulfuric acid, evaporate to dryness, and ignite: the residue weighs not more than 10 mg (2.0%).

Vinyl Acetate, $CH_3COOCH{=}CH_2$—**86.09** [108-05-4]—Liquid.

Assay: Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found

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suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of G2; the injection port temperature is maintained at 100°; the detector temperature is maintained at 300°; and the column temperature is maintained at 100° and programmed to rise 10° per minute to 150°. The area of the $CH_3COOCH=CH_2$ peak is not less than 99% of the total peak area.

2-Vinylpyridine, C_7H_7N**—105.14** [100-69-6]
Boiling Range: between 79° and 82°, at 29 mm of mercury.
Density: 0.975 at 25°.
Refractive Index ⟨831⟩: about 1.5490 at 20°.

Vinylpyrrolidinone *(1-Vinyl-2-pyrrolidinone; 1-Vinyl-2-pyrrolidone; N-Vinylpyrrolidinone; N-Vinylpyrrolidone),* C_6H_9NO**—111.14** [88-12-0]—Colorless liquid.
- **Assay:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-µm layer of G2; the injection port temperature is maintained at 250°; the detector temperature is maintained at 300°; and the column temperature is maintained at 100° and programmed to rise 10° per min to 250°. The area of the C_6H_9NO peak is not less than 99.0% of the total peak area.
- **Water Determination,** *Method I* ⟨921⟩: not more than 0.1%, determined on 2.5 g, using a mixture of 50 mL of methanol and 10 mL of butyrolactone as the solvent.
[Note—A suitable grade is available from Merck KGaA/EMD chemicals, catalogue number 8.08518.0250, www.emdchemicals.com.]

Washed Sand—See *Sand, Washed.*

Water—Use USP *Purified Water.*

Change to read:

Water, Ammonia-Free,$_2$—18.02—Use ▲*High-Purity Water* as defined in *Water for Pharmaceutical Purposes* ⟨1231⟩, *3. Waters Used for Pharmaceutical Manufacturing and Testing Purposes, 3.3 Nonmonographed Waters, 3.3.10 High-Purity Water.*▲ (USP 1-Dec-2019)

Water, Carbon Dioxide-Free—It is *Purified Water* that has been boiled vigorously for 5 min or more and allowed to cool while protected from absorption of carbon dioxide (CO_2) from the atmosphere, or *Purified Water* that has a resistivity of NLT 18 Mohm-cm.

Water, Deaerated—For purposes other than dissolution and drug release testing, it is *Purified Water* that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 min and cooling or by application of ultrasonic vibration.

Water, Organic-Free—It is *Purified Water* that produces no significantly interfering peaks when chromatographed as indicated in *Residual Solvents* ⟨467⟩, *Identification, Control, and Quantification of Residual Solvents.*

Water, Particle-Free—It is *Purified Water* that has been passed through a suitable filter of 0.22-µm pore size.

Water Vapor Detector Tube: A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator, which consists of a selenium sol in suspension in sulfuric acid.
- **Measuring Range:** 5 to 250 mg per cubic meter.
[Note—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nextteq.com.]

Wright's Stain [68988-92-1]—A mixture of methylene blue, methylene azure, and the eosinates of both, available as a solid and as a solution in methanol. Use a suitable grade.
[Note—If a solid is used, dissolve 6.0 g of Wright's stain powder (CAS# *68988-92-1,* dark green powder) and 0.6 g of Giemsa stain powder (CAS# *51811-82-6,* dark green to black powder or crystals) in 1000 mL of methanol. Stir overnight, and filter before use.]

Xanthine, $C_5H_4N_4O_2$**—152.11** [69-89-6]—White, crystalline powder. Decomposes on heating. Slightly soluble in water and in alcohol; soluble in sodium hydroxide TS; sparingly soluble in diluted hydrochloric acid. When subjected to the murexide reaction, a purple color is produced with the ammonia, but on the subsequent addition of fixed alkali hydroxides, the color is not discharged but is changed to violet.
Residue on Ignition (Reagent test): negligible, from 100 mg.
Loss on Drying ⟨731⟩: Dry it at 105° for 2 hours: it loses not more than 1% of its weight.

Xanthydrol, $C_{13}H_{10}O_2$**—198.22** [90-46-0]—Pale yellow, crystalline powder. Insoluble in water; soluble in alcohol, in chloroform, and in ether. Soluble in glacial acetic acid, forming a practically colorless solution; but when the powder is treated with diluted hydrochloric acid, a lemon-yellow color is produced.
Melting Range ⟨741⟩: between 121° and 123°.
Residue on Ignition: Ignite 500 mg with 0.5 mL of sulfuric acid: the residue weighs not more than 10 mg (2.0%).

Xylene, C_8H_{10}**—106.17** [1330-20-7]—Use ACS reagent grade.

***m*-Xylene,** $C_6H_4(CH_3)_2$**—106.17** [108-38-3]—Clear, colorless, flammable liquid. Insoluble in water; miscible with alcohol and with ether. Use a suitable grade.

***o*-Xylene,** C_8H_{10}**—106.17** [95-47-6]—Clear, colorless, mobile, flammable liquid. Insoluble in water; miscible with alcohol and with ether.
- **Assay:** When examined by gas-liquid chromatography, it shows a purity of not less than 95%. The following conditions have been found suitable for assaying the substance: A 3-mm × 1.8-m stainless steel column packed with 1.75% hydrated aluminum silicate plus 5.0% diisodecyl phthalate on support S1. Helium, having a flow rate of about 27.5 mL per minute is the carrier gas, the detector temperature is about 280°, the injection port temperature is about 180°, and the column temperature is 80°. A flame-ionization detector is employed.
- **Refractive Index** ⟨831⟩: between 1.5040 and 1.5060, at 20°.

***p*-Xylene,** C_8H_{10}**—106.17** [106-42-3]—Colorless liquid.
- **Assay:** Inject an appropriate volume into a gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a

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1-µm layer of phase G14; the column temperature is maintained at 50° and programmed to rise 10° per minute to 100°; the injection port is maintained at 130°; and the detector is maintained at 300°. The area of the C_8H_{10} peak is not less than 99% of the total peak area.

• **Refractive Index** ⟨831⟩: between 1.493 and 1.497 at 20°.

Xylene Cyanole FF, $C_{25}H_{27}N_2NaO_6S_2$**—538.61** [2650-17-1]—Gray-blue to dark blue powder. Soluble in water.

Assay: Transfer about 50 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 2.0 mL of the solution to a 50-mL volumetric flask, dilute with pH 7.0 phosphate buffer to volume (See *Solutions*, in this section), and mix. Using a suitable spectrophotometer, 1-cm cells, and water as the blank, record the absorbance of the solution at the wavelength of maximum absorbance at about 614 nm. From the observed absorbance, calculate the absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩): the absorptivity is not less than 55.9, corresponding to about 83% of $C_{25}H_{27}N_2NaO_6S_2$.

Loss on Drying ⟨731⟩: Dry it at 110° to constant weight: it loses not more than 6.0% of its weight.

Xylometazoline Hydrochloride—Use *Xylometazoline Hydrochloride* (USP monograph).

Xylose, $C_5H_{10}O_5$**—150.13** [58-86-6]—Use a suitable grade.

Yeast Extract—A water-soluble, peptone-like derivative of yeast cells (*Saccharomyces*) prepared under optimum conditions, clarified, and dried to a reddish-yellow to brown powder, having a characteristic but not putrescent odor. Soluble in water, forming a yellow to brown solution, having a slightly acid reaction. Contains no added carbohydrate.

Loss on Drying ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 6%
Residue on Ignition (Reagent test)
Analysis: Ignite 500 mg with 1 mL of sulfuric acid
Acceptance criteria: NMT 75 mg (15.0%)
Chloride (Reagent test): NMT 5% of chloride (Cl), calculated as sodium chloride
Nitrogen Content (Reagent test)
Analysis: Determine by the Kjeldahl method, previously dried at 105° to constant weight.
Acceptance criteria: 7.2%–13.0% of N is found.
Microbial Content: NMT 10^4 cfu/g
Amino Nitrogen: 6.0%–6.9%

Yellow Mercuric Oxide—See *Mercuric Oxide, Yellow*.

Zinc, Zn**—At. Wt. 65.39** [7440-66-6]—Use ACS reagent grade.

Zinc, Activated—Place zinc pellets in a conical flask, and add a sufficient quantity of a 50-ppm solution of chloroplatinic acid to cover all pellets. Allow the metal to remain in contact with the solution for 10 min, drain, wash, and dry immediately.

Assay: To 5 g of activated zinc add 15 mL of hydrochloric acid, 25 mL of water, 0.1 mL of stannous chloride solution (235 mg/mL in hydrochloric acid), and 5 mL of potassium iodide solution (166 mg/mL in water). No stain is produced when in contact with mercuric bromide paper. Repeat the test for arsenic, using the same reagents and adding a solution containing 1 µg of arsenic. An appreciable stain appears when in contact with mercuric bromide paper.

Zinc Acetate, $Zn(CH_3COO)_2 \cdot 2H_2O$**—219.51** [557-34-6]—Use ACS reagent grade.

Zinc Amalgam—Add 54 g of mossy or granular zinc to 100 mL of mercury in a beaker. Heat, with stirring, on a hot plate *under a hood* [**Caution**—Mercury vapor is extremely toxic] until solution of the zinc is complete or practically so. Allow to cool to room temperature, and if necessary add sufficient mercury to prevent solidification of the amalgam. Transfer the amalgam to a glass-stoppered bottle, and shake a few times with dilute hydrochloric acid (1 in 2), to remove any zinc oxide formed.

Change to read:

Zinc Chloride, Anhydrous, Powdered—Use *Zinc Chloride* (USP monograph) that has been ▲powdered and dried at 105° until constant weight.▲ (USP 1-May-2019)

Zinc Sulfate Heptahydrate, $ZnSO_4 \cdot 7H_2O$**—287.54** [7446-20-0]—Odorless crystals, granules, or powder.

Melting Point ⟨741⟩: 100°.
Density: 1.97.

Zirconyl Nitrate, $ZrO(NO_3)_2$**—231.23** [13826-66-9]—Use a suitable grade.

Indicators and Indicator Test Papers

INDICATORS

1. SCOPE

Indicators are required in Pharmacopeial tests and assays either to indicate the completion of a chemical reaction in volumetric analysis or to indicate the hydrogen-ion concentration (pH) of solutions. The necessary solutions of indicators are listed among the *Test Solutions*, abbreviated TS.

2. PREPARATION OF SOME INDICATORS

Solution of indicators of the basic type and of the phthaleins are prepared by dissolving in alcohol.

With indicators containing an acidic group, the acid must first be neutralized with sodium hydroxide (NaOH) as follows. Triturate 100 mg of the indicator in a smooth-surfaced mortar with the volume of 0.05 N sodium hydroxide specified in the directions for preparing its test solution, or with the equivalent of 0.02 N sodium hydroxide. After solubilization of the indicator, dilute the solution with carbon dioxide-free water to 200 mL (final concentration 0.05%). Store the solution in suitably resistant containers, protected from light.

3. pH RANGE AND COLOR CHANGE FOR SOME USEFUL INDICATORS

Indicator	pH Range	Color Change
Malachite green oxalate	0.0–2.0	yellow–green
Thymol blue	1.2–2.8	red–yellow
Quinaldine red	1.4–3.2	colorless–red
Methyl yellow	2.9–4.0	red–yellow
Bromophenol blue	3.0–4.6	yellow–blue

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Indicator	pH Range	Color Change
Methyl orange	3.2–4.4	pink–yellow
Bromocresol green	4.0–5.4	yellow–blue
Methyl red	4.2–6.2	red–yellow
Bromocresol purple sodium salt	5.0–6.8	greenish yellow–purple-violet
Bromocresol purple	5.2–6.8	yellow–purple
Bromothymol blue	6.0–7.6	yellow–blue
Phenol red	6.8–8.2	yellow–red
Neutral red	6.8–8.0	red–orange
Cresol red	7.2–8.8	yellow–red
Thymol blue	8.0–9.2	yellow–blue
Thymolphthalein	8.6–10.0	colorless–blue
Phenolphthalein	8.0–10.0	colorless–red
p-Naphtholbenzein	8.8–10.0	orange–green
Nile blue hydrochloride	9.0–13.0	blue–pink

Alphazurine 2G—Use a suitable grade.

Azo Violet [*4-(p-Nitrophenylazo)resorcinol*], $C_{12}H_9N_3O_4$—**259.22**—Red powder. It melts at about 193°, with decomposition.

Bismuth Sulfite—Use a suitable grade.

Brilliant Green—See *Brilliant Green* in the section *Reagents*.

Brilliant Yellow (*C.I. 24890*), $C_{26}H_{18}N_4Na_2O_8S$—**592.49**—Orange to rust-colored powder. Soluble in water.
• **Loss on Drying** ⟨731⟩: Dry it in vacuum at 60° for 1 hour: it loses not more than 5% of its weight.

Bromocresol Blue—Use *Bromocresol Green*.

Bromocresol Green (*Bromocresol Blue; Tetrabromo-m-cresol-sulfonphthalein*), $C_{21}H_{14}Br_4O_5S$—**698.01**—White or pale buff-colored powder. Slightly soluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 4.0 to 5.4. Color change: from yellow to blue.

Bromocresol Green Sodium Salt—Use a suitable grade.

Bromocresol Purple (*Dibromo-o-cresolsulfonphthalein*), $C_{21}H_{16}Br_2O_5S$—**540.22**—White to pink, crystalline powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 5.2 to 6.8. Color change: from yellow to purple.

Bromocresol Purple Sodium Salt, $C_{21}H_{15}Br_2O_5SNa$—**562.20**—Black powder. Soluble in water. Transition interval: from pH 5.0 to 6.8. Color change: from greenish yellow to purple-violet.
Melting Range ⟨741⟩: between 261° and 264°.

Bromophenol Blue (*3′,3″,5′,5″-Tetrabromophenolsulfonphthalein*), $C_{19}H_{10}Br_4O_5S$—**669.96**—Pinkish crystals. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 3.0 to 4.6. Color change: from yellow to blue.

Bromophenol Blue Sodium—The sodium salt of 3′,3″,5′,5″ (*Tetrabromophenolsulfonphthalein*), $C_{19}H_9Br_4O_5SNa$—**646.36**—Pinkish crystals. Soluble in water and in alcohol. Transition interval: from pH 3.0 to 4.6. Color change: from yellow to blue.

Bromothymol Blue (*3′,3″-Dibromothymolsulfonphthalein*), $C_{27}H_{28}Br_2O_5S$—**624.38**—Cream-colored powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 6.0 to 7.6. Color change: from yellow to blue.

Congo Red—See *Congo Red* in the section *Reagents*.

Cresol Red (*o-Cresolsulfonphthalein*), $C_{21}H_{18}O_5S$—**382.43**—Red-brown powder. Slightly soluble in water; soluble in alcohol and in dilute solutions of alkali hydroxides. Transition interval: from pH 7.2 to 8.8. Color change: from yellow to red.

Crystal Violet (*Hexamethyl-p-rosaniline Chloride*), $C_{25}H_{30}ClN_3$—**407.98**—Dark-green crystals. Slightly soluble in water; sparingly soluble in alcohol and in glacial acetic acid. Its solutions are deep violet in color.
Sensitiveness: Dissolve 100 mg in 100 mL of glacial acetic acid, and mix. Pipet 1 mL of the solution into a 100-mL volumetric flask, and dilute with glacial acetic acid to volume: the solution is violet-blue in color and does not show a reddish tint. Pipet 20 mL of the diluted solution into a beaker, and titrate with 0.1 N perchloric acid VS, adding the perchloric acid slowly from a microburet: not more than 0.10 mL of 0.1 N perchloric acid is required to produce an emerald-green color.

4,5-Dihydroxy-3-(*p*-sulfophenylazo)-2,7-naphthalenedisulfonic Acid, Trisodium Salt—See *2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic Acid, Trisodium Salt*.

Eosin Y (*Indicator grade Eosin Y, Sodium tetrabromofluorescein*), $C_{20}H_6Br_4Na_2O_5$—**691.86** [17372-87-1]—Red to brownish-red pieces or powder. One g dissolves in about 2 mL of water and in 50 mL of alcohol. Dye content about 80%.

Change to read:

Eriochrome Black T [*Sodium 1-(1-hydroxy-2-naphthylazo) 5-nitro-2-naphthol-4-sulfonate*], $C_{20}H_{12}N_3NaO_7S$—**461.38** ▲[1787-61-7]▲ (USP 1-Dec-2019)—Brownish-black powder having a faint, metallic sheen. Soluble in alcohol, in methanol, and in hot water.
▲**Suitability as Complexometric Indicator**▲ (USP 1-Aug-2019)
▲**pH 10 ammoniacal buffer:** Transfer 90 g of ammonium chloride to a 500-mL volumetric flask containing 375 mL of ammonium hydroxide. Dilute with water to volume.
Magnesium standard solution: Transfer 1.014 g of magnesium sulfate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. Dilute 10 mL of this solution with water to 1 L.This solution contains 0.01 mg of magnesium per milliliter.
Sample solution: Dissolve 0.1 g of eriochrome black T in 100 mL of water, warming if necessary.
Procedure: In a suitable container, add 10 mL of *pH 10 ammoniacal buffer* to 100 mL of water. Add 0.5 mL of the *Sample solution* and 10.0 mL of *Magnesium standard solution*.
Acceptance criteria: The reagent meets the criteria for its use as a complexometric indicator if the color of the

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resulting solution is red-violet, and if turns into blue after the addition of 0.10 mL of 0.1 M edetate disodium VS.▲ (USP 1-Dec-2019)

Eriochrome Black T Trituration—Grind 200 mg of eriochrome black T to a fine powder with 20 g of potassium chloride.

Litmus—Blue powder, cubes, or pieces. Partly soluble in water and in alcohol. Transition interval: from approximately pH 4.5 to 8. Color change: from red to blue. Litmus is unsuitable for determining the pH of alkaloids, carbonates, and bicarbonates.

Malachite Green Oxalate, $[C_{23}H_{25}N_2^+]_2 \cdot [C_2HO_4^-]_2 \cdot C_2H_2O_4$—**927.00**—The oxalate salt, crystallized with oxalic acid, of a triphenylmethane dye. Dark-green powder, having a metallic luster. Sparingly soluble in water; soluble in glacial acetic acid. Transition interval: from pH 0.0 to 2.0. Color change: from yellow to green.

Methyl Orange (*Helianthin* or *Tropaeolin D*), $C_{14}H_{14}N_3NaO_3S$—**327.33**—The sodium salt of dimethylaminoazobenzene sulfonic acid or dimethylaminoazobenzene sodium sulfonate. An orange-yellow powder or crystalline scales. Slightly soluble in cold water; readily soluble in hot water; insoluble in alcohol. Transition interval: from pH 3.2 to 4.4. Color change: from pink to yellow.

Change to read:

Methyl Red (*2-[[4-(Dimethylamino)phenyl]azo]benzoic Acid; ▲C. I. Acid Red 2*), $C_{15}H_{15}N_3O_2$ (free acid)—**269.30** [493-52-7], $C_{15}H_{14}N_3O_2Na$ (sodium salt)—**291.29** [845-10-3], $C_{15}H_{15}N_3O_2 \cdot HCl$ (hydrochloride)—**305.76** [63451-28-5]—Use ACS reagent grade. The free acid is recommended for nonaqueous titrations, particularly when an aprotic solvent is used. The sodium salt is recommended for titrations in aqueous media and also for nonaqueous titrations where the medium is an amphiprotic solvent. The hydrochloride salt is recommended for titrations in aqueous media and amphiprotic solvents.▲ (USP 1-May-2020)

Methyl Red Sodium—The sodium salt of 2-[[4-(dimethylamino)phenyl]azo]benzoic acid. $2\text{-}[4\text{-}(CH_3)_2NC_6H_4N{:}N]C_6H_4COONa$—**291.28**—Orange-brown powder. Freely soluble in cold water and in alcohol. Transition interval: from pH 4.2 to 6.2. Color change: from red to yellow.

Methyl Yellow (*p-Dimethylaminoazobenzene*), $C_{14}H_{15}N_3$—**225.29**—Yellow crystals, melting between 114° and 117°. Insoluble in water; soluble in alcohol, in benzene, in chloroform, in ether, in dilute mineral acids, and in oils. Transition interval: from pH 2.9 to 4.0. Color change: from red to yellow.

***p*-Naphtholbenzein** (*4-[α-(4-Hydroxy-1-naphthyl) benzylidene]-1(4H)-naphthalenone*), $(4\text{-}HOC_{10}H_6)C(:C_{10}H_6\text{-}4:O)(C_6H_5)$—**374.43**—Reddish brown powder. Insoluble in water; soluble in alcohol, in benzene, in ether, and in glacial acetic acid. Transition interval: from pH 8.8 to 10.0. Color change: from orange to green.

Neutral Red (*3-Amino-7-dimethylamino-2-methylphenazine Monohydrochloride*), $C_{15}H_{16}N_4 \cdot HCl$—**288.78**—Reddish to olive-green, coarse powder. Sparingly soluble in water and in alcohol. Transition interval: from pH 6.8 to 8.0. Color change: from red to orange.

Nile Blue Hydrochloride (*Nile Blue A, as the hydrochloride; 5-Amino-9-(diethylamino)benzo[a]phenoxazin-7-ium chloride*), $C_{20}H_{20}ClN_3O$—**353.85**—Slightly soluble in alcohol and in glacial acetic acid. Transition interval: from pH 9.0 to 13.0. Color change: from blue to pink.

Phenol Red [*4,4′-(3H-2,1-Benzoxathiol-3-ylidene)diphenol, S,S-Dioxide*], $C_{19}H_{14}O_5S$—**354.38**—Crystalline powder, varying in color from bright to dark red. Very slightly soluble in water; freely soluble in solutions of alkali carbonates and hydroxides; slightly soluble in alcohol. Transition interval: from pH 6.8 to 8.2. Color change: from yellow to red.

Phenolphthalein [*3,3-Bis(p-hydroxyphenyl)phthalide*], $C_{20}H_{14}O_4$—**318.32** [77-09-8]—White or faintly yellowish-white, crystalline powder. Insoluble in water; soluble in alcohol. Transition interval: from pH 8.0 to 10.0. Color change: from colorless to red.

Quinaldine Red (*5-Dimethylamino-2-styrylethylquinolinium Iodide*), $C_{21}H_{23}IN_2$—**430.33**—Dark blue-black powder. Sparingly soluble in water; freely soluble in alcohol. Melts at about 260°, with decomposition. Transition interval: from pH 1.4 to 3.2. Color change: from colorless to red.

2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic Acid, Trisodium Salt (*4,5-Dihydroxy-3-(p-sulfophenylazo)-2,7-naphthalenedisulfonic Acid, Trisodium Salt*), $C_{16}H_9N_2O_{11}S_3Na_3$—**570.42**—Red powder. Soluble in water.

Thymol Blue (*Thymolsulfonphthalein*), $C_{27}H_{30}O_5S$—**466.59**—Dark-colored, crystalline powder. Slightly soluble in water; soluble in alcohol and in dilute alkali solutions. *Acid*—Transition interval: from pH 1.2 to 2.8. Color change: from red to yellow. *Alkaline*—Transition interval: from pH 8.0 to 9.2. Color change: from yellow to blue.

Thymolphthalein, $C_{28}H_{30}O_4$—**430.54**—White to slightly yellow, crystalline powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 9.3 to 10.5. Color change: from colorless to blue.

Change to read:

Xylenol Orange (*N,N′-[3H-2,1-Benzoxathiol-3-ylidenebis-[(6-hydroxy-5-methyl-3,1-phenylene)methylene]] bis[N-(carboxy-methyl)glycine] S,S-dioxide*), $C_{31}H_{28}N_2Na_4O_{13}S$ ▲(salt)—**760.59**, $C_{31}H_{30}N_2O_{13}S$ (free acid)—**672.66**▲ (USP 1-May-2020)—Orange powder. ▲The acid form is soluble in alcohol and the salt form is soluble in water.▲ (USP 1-May-2020) In acid solution, it is lemon-yellow in color, and its metal complexes are intensely red. It yields a distinct endpoint where a metal such as bismuth, cadmium, lanthanum, lead, mercury, scandium, thorium, or zinc is titrated with edetate disodium.

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Indicator and Test Papers

1. DEFINITION

Indicator and test papers are strips of paper of suitable dimension and grade impregnated with an indicator or a reagent to provide a convenient form of the impregnated substance.

Some test papers may be obtained from commercial sources of laboratory supplies. Alternatively, they may be prepared in the laboratory.

2. PREPARATION OF INDICATOR AND TEST PAPERS

Wet white filter paper completely with hydrochloric acid, and wash with water until the last washing does not produce a red color when treated with methyl red TS. Then treat with ammonia TS, and wash again with water until the last washing does not produce a red color when treated with phenolphthalein TS.

Carefully dry in still air, unless otherwise specified, by suspending it from rods of glass or other inert material in a space free from acid, alkali, and other fumes, saturate the paper with the proper strength or concentration of indicator solution, and carefully dry using the same drying procedure.

Cut the paper into strips of convenient size, and store the papers in well-closed containers, protected from light and moisture.

Cupric Sulfate Test Paper—Use *cupric sulfate TS*.

Eriochrome Black T–Sodium Chloride Indicator: Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate until the mixture becomes homogenous.

Lead Acetate Test Paper—Usually about 6 × 80 mm in size. Use lead acetate TS, and dry the paper at 100°, avoiding contact with metal.

Litmus Paper, Blue—Usually about 6 × 50 mm in size. It meets the requirements of the following tests.

Phosphate ***(Reagent test)*****:** Cut 5 strips into small pieces, mix with 500 mg of magnesium nitrate in a porcelain crucible, and ignite. To the residue add 5 mL of nitric acid, and evaporate to dryness: the residue shows not more than 0.02 mg of PO_4.

Residue on Ignition: Ignite carefully 10 strips of the paper to constant weight: the weight of the residue corresponds to not more than 0.4 mg per strip of about 3 square cm.

Rosin Acids: Immerse a strip of the blue paper in a solution of 100 mg of silver nitrate in 50 mL of water: the color of the paper does not change in 30 seconds.

Sensitiveness: Drop a 10- to 12-mm strip into 100 mL of 0.0005 N acid contained in a beaker, and stir continuously: the color of the paper is changed within 45 seconds. The 0.0005 N acid is prepared by diluting 1 mL of 0.1 N hydrochloric acid with freshly boiled and cooled purified water to 200 mL.

Add the following:

▲**Litmus Paper, Neutral**—Usually about 6 × 50 mm in size. Neutral litmus paper meets the requirements of the tests for *Phosphate*, *Residue on Ignition*, and *Sensitiveness* under Litmus Paper, Blue. It changes from its original color to red in acid conditions, and from its original color to blue in basic conditions.▲ (USP 1-Aug-2019)

Litmus Paper, Red—Usually about 6 × 50 mm in size. Red litmus paper meets the requirements of the tests for *Phosphate*, *Residue on Ignition*, and *Rosin Acids*, under Litmus Paper, Blue.

Sensitiveness: Drop a 10- to 12-mm strip into 100 mL of 0.0005 N sodium hydroxide contained in a beaker, and stir continuously: the color of the paper changes within 30 seconds. The 0.0005 N sodium hydroxide is prepared by diluting 1 mL of 0.1 N sodium hydroxide with freshly boiled and cooled purified water to 200 mL.

Mercuric Bromide Test Paper—Place a 50 mg/mL solution of mercuric bromide in dehydrated alcohol in a dish, and immerse in it pieces of white filter paper weighing 80 g/m² (speed of filtration = filtration time expressed in s for 100 mL of water at 20° with a filter surface of 10 cm² and a constant pressure of 6.7 kPa; 40–60 s), each measuring 1.5 cm by 20 cm and folded in the middle. Allow the excess of liquid to drain, and allow the paper to dry, protected from light, suspended over a nonmetallic thread. Discard 1 cm from each end of each strip, and cut the remainder into 1.5-cm squares or discs of 1.5-cm diameter. Store in a glass-stoppered container wrapped with black paper.

Methyl Green–Iodomercurate Paper—Immerse thin strips of suitable filter paper in a 40 g per L solution of methyl green, and allow to air-dry. Immerse the strips for 1 hour in a solution containing 140 g per L of potassium iodide and 200 g per L of mercuric iodide. Wash with water until the washings are practically colorless, and allow to air-dry. Store protected from light, and use within 48 hours.

Methyl Yellow Paper—Use a 1 in 2000 solution of methyl yellow in alcohol.

pH Indicator Paper, Short-Range—Use a suitable grade.

Phenolphthalein Paper—Use a 1 in 1000 solution of phenolphthalein in diluted alcohol.

Starch Iodate Paper—Use a mixture of equal volumes of starch TS and potassium iodate solution (1 in 20).

Starch Iodide Paper—Use a solution of 500 mg of potassium iodide in 100 mL of freshly prepared starch TS.

Thiazole Yellow Paper: Use a 1 in 2000 solution of thiazole yellow in water.

Turmeric Paper—Use a solution prepared as follows: Macerate 20 g of powdered turmeric, the dried root of *Curcuma longa* Linné (Fam. Zingiberaceae), with four 100-mL portions of cold water, decanting the clear liquid portion each time and discarding it. Dry the residue at a temperature not over 100°. Macerate with 100 mL of alcohol for several days, and filter.

Sensitiveness: Dip a strip of the paper, of about 1.5-cm length, in a solution of 1.0 mg of boric acid in 5 mL of water, previously mixed with 1 mL of hydrochloric acid. After 1 minute remove the paper from the liquid, and allow it to dry: the yellow color changes to brown. Then moisten the paper with ammonia TS: the color of the paper changes to greenish black.

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BUFFER SOLUTIONS

1. DEFINITION

A solution is said to be buffered if it resists changes in the activity of an ion on the addition of substances that are expected to change the activity of that ion. Buffers are substances or combinations of substances that impart this resistance to a solution. Buffered solutions are systems in which the ion is in equilibrium with substances capable of removing or releasing the ion.

2. BUFFER CAPACITY

It refers to the amount of material that may be added to a solution without causing a significant change in ion activity. It is defined as the ratio of acid or base added (in gram-equivalents/L) to the change in pH units. The capacity of a buffered solution is adjusted to the conditions of use, usually by adjustment of the concentrations of buffer substances.

3. USES

Buffers are used to establish and maintain an ion activity within narrow limits. The most common systems are used for the following:

1. To establish hydrogen-ion activity for the standardization of pH meters
2. In the preparation of dosage forms
3. In analytical procedures
4. To maintain stability of various dosage forms

Buffers used in physiological systems are carefully chosen so as not to interfere with pharmacological activity of the medicament or normal function of the organism.

4. STANDARD BUFFER SOLUTIONS

Standard solutions of definite pH are readily available in buffer solutions prepared from the appropriate reagents.

Buffer solutions, buffer tablets, and buffer solids may be obtained from commercial sources in convenient prepackaged form.

4.1 Preparation

Previously dry the crystalline reagents at 110°–120° for 1 h, except for boric acid and sodium acetate trihydrate.

Where water is specified for solution or dilution of test substances in pH determinations, use carbon dioxide-free water.

Store the prepared solutions in chemically resistant, tight containers such as Type 1 glass bottles. Use the solutions within 3 months.

Standard buffer solutions for various ranges between pH 1.2 and 10.0 may be prepared by appropriate combinations of the solutions described herein, used in the proportions shown in the table below.

The volumes shown in the table below are for 200 mL of buffer solution, except for *Acetate Buffer* where the volumes are for 1000 mL of buffer solution and for *Citrate Buffer* where the volumes are for 100 mL of buffer solution.

1. *Hydrochloric Acid, 0.2 M and Sodium Hydroxide, 0.2 M*: Prepare and standardize as directed in *Volumetric Solutions*.
2. *Potassium Biphthalate, 0.2 M*: 40.85 g/L of potassium biphthalate in water
3. *Potassium Phosphate, Monobasic 0.2 M*: 27.22 g/L of monobasic potassium phosphate in water
4. *Boric Acid and Potassium Chloride, 0.2 M*: 12.37 g/L of boric acid and 14.91 g/L of potassium chloride in water
5. *Potassium Chloride, 0.2 M*: 14.91 g/L of potassium chloride in water
6. *Acetic Acid, 2 N*: Prepare and standardize as directed in *Volumetric Solutions*.
7. *Citric Acid, 0.1 M*: 21.01 g/L of citric acid in water
8. *Sodium Citrate, 0.1 M*: 29.41 g/L of sodium citrate dihydrate in water

Composition of Standard Buffer Solutions

Hydrochloric Acid Buffer

Place 50 mL of the potassium chloride solution in a 200-mL volumetric flask, add the specified volume of the hydrochloric acid solution, then add water to volume.

pH	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2
0.2 M HCl, mL	85.0	67.2	53.2	41.4	32.4	26.0	20.4	16.2	13.0	10.2	7.8

Acid Phthalate Buffer

Place 50 mL of the potassium biphthalate solution in a 200-mL volumetric flask, add the specified volume of the hydrochloric acid solution, then add water to volume.

pH	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
0.2 M HCl, mL	49.5	42.2	35.4	28.9	22.3	15.7	10.4	6.3	2.9	0.1

Neutralized Phthalate Buffer

Place 50 mL of the potassium biphthalate solution in a 200-mL volumetric flask, add the specified volume of the sodium hydroxide solution, then add water to volume.

pH	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
0.2 M NaOH, mL	3.0	6.6	11.1	16.5	22.6	28.8	34.1	38.8	42.3

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Phosphate Buffer											
Place 50 mL of the monobasic potassium phosphate solution in a 200-mL volumetric flask, add the specified volume of the sodium hydroxide solution, then add water to volume.											
pH	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
0.2 M NaOH, mL	3.6	5.6	8.1	11.6	16.4	22.4	29.1	34.7	39.1	42.4	44.5	46.1

Alkaline Borate Buffer											
Place 50 mL of the boric acid and potassium chloride solution in a 200-mL volumetric flask, add the specified volume of the sodium hydroxide solution, then add water to volume.											
pH	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0
0.2 M NaOH, mL	3.9	6.0	8.6	11.8	15.8	20.8	26.4	32.1	36.9	40.6	43.7

Acetate Buffer										
Place the specified amount of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) in a 1000-mL volumetric flask, add the specified volume of the acetic acid solution, then add water to volume, and mix.										
pH	4.1	4.3	4.5	4.7	4.9	5.1	5.2	5.3	5.4	5.5
pH (measured)	4.10	4.29	4.51	4.70	4.90	5.11	5.18	5.30	5.40	5.48
$NaC_2H_3O_2 \cdot 3H_2O$, g	1.5	1.99	2.99	3.59	4.34	5.08	5.23	5.61	5.76	5.98
2 N CH_3COOH, mL	19.5	17.7	14.0	11.8	9.1	6.3	5.8	4.4	3.8	3.0

Citrate Buffer																	
Mix *0.1 M Citric Acid* with *0.1 M Sodium Citrate* in the proportions given below.																	
pH	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2
0.1 M Citric Acid, mL	82.0	77.5	73.0	68.5	63.5	59.0	54.0	49.5	44.5	40.0	35.0	30.5	25.5	21.0	16.0	11.5	8.0
0.1 M Sodium Citrate, mL	18.0	22.5	27.0	31.5	36.5	41.0	46.0	50.5	55.5	60.0	65.0	69.5	74.5	79.0	84.0	88.5	92.0

COLORIMETRIC SOLUTIONS (CS)

1. DEFINITION

Colorimetric solutions are used in the preparation of the colorimetric standards for certain drugs and for evaluating color in some monographs.

For the preparation of matching fluids, see *Color and Achromicity* ⟨631⟩.

2. STORAGE

Store colorimetric solutions in suitably resistant, tight containers.

Change to read:

3. COMPARISON OF COLORS

Comparison of colors as directed in the Pharmacopeial tests preferably is made in matched color-comparison tubes or in a suitable colorimeter under conditions that ensure that the colorimetric reference solution and that of the specimen under test are treated alike in all respects. The comparison of colors is best made in layers of equal depth, and viewed transversely against a white background (see also ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).

It is particularly important that the sample and standard be compared at the same temperature, preferably 25°.

Cobaltous Chloride CS—Dissolve about 65 g of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 5 mL of this solution into a 250-mL iodine flask, add 5 mL of hydrogen peroxide TS and 15 mL of sodium hydroxide solution (1 in 5), boil for 10 minutes, cool, and add 2 g of potassium iodide and 20 mL of dilute sulfuric acid (1 in 4). When the precipitate has dissolved, titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 23.79 mg of $CoCl_2 \cdot 6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 59.5 mg of $CoCl_2 \cdot 6H_2O$.

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Cupric Sulfate CS—Dissolve about 65 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask, add 40 mL of water, 4 mL of acetic acid, 3 g of potassium iodide, and 5 mL of hydrochloric acid, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 24.97 mg of $CuSO_4 \cdot 5H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 62.4 mg of $CuSO_4 \cdot 5H_2O$.

Ferric Chloride CS—Dissolve about 55 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask, add 15 mL of water, 3 g of potassium iodide, and 5 mL of hydrochloric acid, and allow the mixture to stand for 15 minutes. Dilute with 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 27.03 mg of $FeCl_3 \cdot 6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water so that each mL contains 45.0 mg of $FeCl_3 \cdot 6H_2O$.

INDICATORS SOLUTIONS

See *TEST SOLUTIONS*.

TEST SOLUTIONS (TS)

1. USE AS INDICATORS

Certain test solutions are intended for use as detectors of a shift in solution composition (e.g., hydrogen ion, complexometric precipitation, iodometric determination, etc.).

2. VOLUMETRIC SOLUTIONS USED AS TEST SOLUTIONS

Where it is directed that a volumetric solution be used as the test solution in a qualitative procedure, standardization of the solution used as the test solution is not required.

3. SOLUTIONS PREPARED FRESH

In general, the directive to prepare a solution "fresh" indicates that the solution is of limited stability and must be prepared on the day of use.

Acetaldehyde TS—Mix 4 mL of acetaldehyde, 3 mL of alcohol, and 1 mL of water. Prepare this solution fresh.

Acetate Buffer TS—Dissolve 320 g of ammonium acetate in 500 mL of water, add 5 mL of glacial acetic acid, dilute with water to 1000.0 mL, and mix. This solution has a pH between 5.9 and 6.0.

1 M Acetic Acid TS—Transfer 57.4 mL of glacial acetic acid to a 1000-mL volumetric flask. Dilute with water to volume.

2 M Acetic Acid TS—Transfer 114.8 mL of glacial acetic acid to a 1000-mL volumetric flask. Dilute with water to volume.

0.008 M Acetic Acid TS—Transfer 0.5 mL of glacial acetic acid to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute with water to volume.

0.3 N Acetic Acid TS—Transfer 17.2 mL of glacial acetic acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Acetic Acid, Glacial, TS—Determine the water content of a specimen of glacial acetic acid by the *Titrimetric Method* (see *Water Determination* ⟨921⟩). If the acid contains more than 0.4% of water, add a few mL of acetic anhydride, mix, allow to stand overnight, and again determine the water content. If the acid contains less than 0.02% of water, add sufficient water to make the final concentration between 0.02% and 0.4%, mix, allow to stand overnight, and again determine the water content. Repeat the adjustment with acetic anhydride or water, as necessary, until the resulting solution shows a water content of not more than 0.4%.

Acetic Acid, Strong, TS—Add 300.0 mL of glacial acetic acid, and dilute with water to 1000 mL. This solution contains about 30% (v/v) of CH_3COOH and has a concentration of about 5 N.

Acetic Acid–Ammonium Acetate Buffer TS—Dissolve 77.1 g of ammonium acetate in water, add 57 mL of glacial acetic acid, and dilute with water to 1000 mL.

Acetone, Buffered, TS—Dissolve 8.15 g of sodium acetate and 42 g of sodium chloride in about 100 mL of water, and add 68 mL of 0.1 N hydrochloric acid and 150 mL of acetone. Mix, and dilute with water to 500 mL.

Acid Ferric Chloride TS—Mix 60 mL of glacial acetic acid with 5 mL of sulfuric acid, add 1 mL of ferric chloride TS, mix, and cool.

Acid Ferrous Sulfate TS—See *Ferrous Sulfate, Acid, TS.*

Acid Stannous Chloride TS—See *Stannous Chloride, Acid, TS.*

Acid Stannous Chloride TS, Stronger—See *Stannous Chloride, Acid, Stronger, TS.*

Albumen TS—Carefully separate the white from the yolk of a strictly fresh hen's egg. Shake the white with 100 mL of water until mixed and all but the chalaza has undergone solution; then filter. Prepare the solution fresh.

Alcohol–Phenol TS—Dissolve 780 mg of phenol in alcohol to make 100 mL.

Alcoholic TS—It contains 95 parts of specially denaturated alcohol 3A with 5 parts of isopropyl alcohol. The final concentrations are approximately 90% alcohol, 5% methanol, and 5% isopropanol.

[NOTE—A suitable grade is available as Reagent alcohol, catalog number R8382, available at www.sigma-aldrich.com.]

Alcoholic Ammonia TS—See *Ammonia TS, Alcoholic.*

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Alcoholic Mercuric Bromide TS—See *Mercuric Bromide TS, Alcoholic.*

Alcoholic Potassium Hydroxide TS—Use *0.5 N Alcoholic Potassium Hydroxide VS.*

Alkaline Cupric Citrate TS—See *Cupric Citrate TS, Alkaline.*

Alkaline Cupric Citrate TS 2—See *Cupric Citrate TS 2, Alkaline.*

Alkaline Cupric Iodide TS—See *Cupric Iodide TS, Alkaline.*

Alkaline Cupric Tartrate TS *(Fehling's Solution)*—See *Cupric Tartrate TS, Alkaline.*

Alkaline Mercuric–Potassium Iodide TS—See *Mercuric–Potassium Iodide TS, Alkaline.*

Alkaline Picrate TS—See *Picrate TS, Alkaline.*

Alkaline Sodium Hydrosulfite TS—See *Sodium Hydrosulfite TS, Alkaline.*

Amaranth TS—Dissolve 20 mg of amaranth in 10 mL of water.

Aminonaphtholsulfonic Acid TS—Accurately weigh 5 g of sodium sulfite, 94.3 g of sodium bisulfite, and 700 mg of 1,2,4-aminonaphtholsulfonic acid, and mix. Prepare aminonaphtholsulfonic acid TS fresh on the day of use by dissolving 1.5 g of the dry mixture in 10 mL of water.

Ammonia TS—It contains between 9.5% and 10.5% of NH_3. Prepare by diluting 350 mL of *Ammonia Water, Stronger* (see in the section, *Reagents*) with water to make 1000 mL.

Ammonia TS 2—Prepare by diluting 13.5 mL of Ammonia Water, Stronger (see *Reagent Specifications* in the section *Reagents*) with water to make 100 mL.

Ammonia TS, Alcoholic—A solution of ammonia gas in alcohol. Clear, colorless liquid having a strong odor of ammonia. Specific gravity: about 0.80. It contains between 9% and 11% of NH_3. Store it in alkali-resistant containers, in a cold place.

Ammonia TS, Stronger—Use *Ammonia Water, Stronger* (see in the section *Reagents*).

Ammonia–Ammonium Chloride Buffer TS—Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonium hydroxide, and dilute with water to 1000 mL.

Ammonia–Cyanide TS—Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

Ammoniacal Potassium Ferricyanide TS—Dissolve 2 g of potassium ferricyanide in 75 mL of water, add 25 mL of ammonium hydroxide, and mix.

Ammoniated Cupric Oxide TS—See *Cupric Oxide, Ammoniated, TS.*

Ammonium Acetate TS—Dissolve 10 g of ammonium acetate in water to make 100 mL.

Ammonium Carbonate TS—Dissolve 20 g of ammonium carbonate and 20 mL of ammonia TS in water to make 100 mL.

Ammonium Carbonate TS 2—Prepare a 158-mg/mL solution of ammonium carbonate in water.

Ammonium Chloride TS—Dissolve 10.5 g of ammonium chloride in water to make 100 mL.

Ammonium Chloride–Ammonium Hydroxide TS—Mix equal volumes of water and ammonium hydroxide, and saturate with ammonium chloride.

1 M Ammonium Hydroxide TS—Transfer 67.6 mL of ammonium hydroxide to a 1000-mL volumetric flask. Dilute with water to volume.

2 M Ammonium Hydroxide TS—Transfer 135 mL of ammonium hydroxide to a 1000-mL volumetric flask. Dilute with water to volume.

Ammonium Molybdate TS—Dissolve 6.5 g of finely powdered molybdic acid in a mixture of 14 mL of water and 14.5 mL of ammonium hydroxide. Cool the solution, and add it slowly, with stirring, to a well-cooled mixture of 32 mL of nitric acid and 40 mL of water. Allow to stand for 48 hours, and filter through a fine-porosity, sintered-glass crucible. This solution deteriorates upon standing and is unsuitable for use if, upon the addition of 2 mL of dibasic sodium phosphate TS to 5 mL of the solution, an abundant yellow precipitate does not form at once or after slight warming. Store it in the dark. If a precipitate forms during storage, use only the clear supernatant.

Ammonium Oxalate TS—Dissolve 3.5 g of ammonium oxalate in water to make 100 mL.

Ammonium Phosphate, Dibasic, TS ***(Ammonium Phosphate TS)***—Dissolve 13 g of dibasic ammonium phosphate in water to make 100 mL.

Ammonium Polysulfide TS—Yellow liquid, made by saturating ammonium sulfide TS with sulfur.

Ammonium Pyrrolidinedithiocarbamate, Saturated, TS—Add about 10 g of ammonium pyrrolidinedithiocarbamate to a 1000-mL volumetric flask, and dilute with water to volume.

Ammonium Reineckate TS—Shake about 500 mg of ammonium reineckate with 20 mL of water frequently during 1 hour, and filter. Use within 2 days.

Ammonium Sulfide TS—Use ACS reagent-grade Ammonium Sulfide Solution.

Ammonium Thiocyanate TS—Dissolve 8 g of ammonium thiocyanate in water to make 100 mL.

Ammonium Vanadate TS—Dissolve 2.5 g of ammonium vanadate in 500 mL of boiling water, cool, and add 20 mL of nitric acid. Mix, cool, and add water to make 1 L. Store in polyethylene containers.

Anthrone TS—Within 12 hours of use, rapidly dissolve 35 mg of anthrone in a hot mixture of 35 mL of water and 65 mL of sulfuric acid. Immediately cool in an ice bath to room temperature, and filter through glass wool. Allow the solution to stand at room temperature for 30 minutes before use.

Antimony Trichloride TS—Dissolve 20 g of antimony trichloride in chloroform to make 100 mL. Filter if necessary.

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10% Ascorbic Acid TS—Transfer 10 g of ascorbic acid to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Barium Chloride TS—Dissolve 12 g of barium chloride in water to make 100 mL.

Barium Hydroxide TS—A saturated solution of barium hydroxide in recently boiled water. Prepare the solution fresh.

Barium Nitrate TS—Dissolve 6.5 g of barium nitrate in water to make 100 mL.

Betanaphthol TS—See *2-Naphthol TS*.

Biuret Reagent TS—Dissolve 1.5 g of cupric sulfate and 6.0 g of potassium sodium tartrate in 500 mL of water in a 1000-mL volumetric flask. Add 300 mL of carbonate-free sodium hydroxide solution (1 in 10), dilute with carbonate-free sodium hydroxide solution (1 in 10) to 1000 mL, and mix.

Blue Tetrazolium TS—Dissolve 500 mg of blue tet razolium in alcohol to make 100 mL.

Brilliant Blue G TS—Transfer 25 mg of brilliant blue G to a 100-mL volumetric flask, add 12.5 mL of alcohol and 25 mL of phosphoric acid, dilute with water to volume, and mix.

Bromine TS ***(Bromine Water)***—A saturated solution of bromine, prepared by agitating 2 to 3 mL of bromine with 100 mL of cold water in a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum. Store it in a cold place, protected from light.

Bromine–Sodium Acetate TS—Dissolve 100 g of sodium acetate in 1000 mL of glacial acetic acid, add 50 mL of bromine, and mix.

***p*-Bromoaniline TS**—Add 8 g of *p*-bromoaniline to a mixture of 380 mL of thiourea-saturated glacial acetic acid, 10 mL of sodium chloride solution (1 in 5), 5 mL of oxalic acid solution (1 in 20), and 5 mL of dibasic sodium phosphate solution (1 in 10) in a low-actinic glass bottle. Mix, and allow to stand overnight before using. Protect from light, and use within 7 days.

Bromocresol Blue TS—Use *Bromocresol Green TS*.

Bromocresol Green TS—Dissolve 50 mg of bromocresol green in 100 mL of alcohol, and filter if necessary.

Bromocresol Green–Methyl Red TS—Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in 180 mL of alcohol, and dilute with water to 200 mL.

Bromocresol Purple TS—Dissolve 250 mg of bromocresol purple in 20 mL of 0.05 N sodium hydroxide, and dilute with water to 250 mL.

Bromophenol Blue TS—Dissolve 100 mg of bromophenol blue in 100 mL of diluted alcohol, and filter if necessary.

Bromothymol Blue TS—Dissolve 100 mg of bromothymol blue in 100 mL of diluted alcohol, and filter if necessary.

Buffered Acetone TS—See *Acetone, Buffered, TS*.

Calcium Chloride TS—Dissolve 7.5 g of calcium chloride in water to make 100 mL.

Calcium Hydroxide TS—Use *Calcium Hydroxide Topical Solution* (USP monograph).

Calcium Sulfate TS—A saturated solution of calcium sulfate in water.

Ceric Ammonium Nitrate TS—Dissolve 6.25 g of ceric ammonium nitrate in 10 mL of 0.25 N nitric acid. Use within 3 days.

Chloral Hydrate TS—Dissolve 50 g of chloral hydrate in a mixture of 15 mL of water and 10 mL of glycerin.

Chlorine TS ***(Chlorine Water)***—A saturated solution of chlorine in water. Place the solution in small, completely filled, light-resistant containers. Chlorine TS, even when kept from light and air, is apt to deteriorate. Store it in a cold, dark place. For full strength, prepare this solution fresh.

Chromotropic Acid TS—Dissolve 50 mg of chromotropic acid or its disodium salt in 100 mL of 75% sulfuric acid, which may be made by cautiously adding 75 mL of sulfuric acid to 33.3 mL of water.

Add the following:

▲Citric Acid TS—100 g/L of anhydrous citric acid in water▲ (USP 1-May-2019)

Cobalt–Uranyl Acetate TS—Dissolve, with warming, 40 g of uranyl acetate in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Similarly, prepare a solution containing 200 g of cobaltous acetate in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Mix the two solutions while still warm, and cool to 20°. Maintain the temperature at 20° for about 2 hours to separate the excess salts from solution, and then pass through a dry filter.

Cobaltous Chloride TS—Dissolve 2 g of cobaltous chloride in 1 mL of hydrochloric acid and sufficient water to make 100 mL.

Congo Red TS—Dissolve 500 mg of congo red in a mixture of 10 mL of alcohol and 90 mL of water.

***m*-Cresol Purple TS**—Dissolve 0.10 g of metacresol purple in 13 mL of 0.01 N sodium hydroxide, dilute with water to 100 mL, and mix.

Cresol Red TS—Triturate 100 mg of cresol red in a mortar with 26.2 mL of 0.01 N sodium hydroxide until solution is complete, then dilute the solution with water to 250 mL.

Cresol Red–Thymol Blue TS—Add 15 mL of thymol blue TS to 5 mL of cresol red TS, and mix.

Crystal Violet TS—Dissolve 100 mg of crystal violet in 10 mL of glacial acetic acid.

Cupric Acetate TS—Dissolve 100 mg of cupric acetate in about 5 mL of water to which a few drops of acetic acid have been added. Dilute to 100 mL, and filter, if necessary.

Cupric Acetate TS, Stronger ***(Barfoed's Reagent)***—Dissolve 13.3 g of cupric acetate in a mixture of 195 mL of water and 5 mL of acetic acid.

Cupric-Ammonium Sulfate TS—To cupric sulfate TS add ammonia TS, dropwise, until the precipitate initially formed is nearly but not completely dissolved. Allow to settle, and decant the clear solution. Prepare this solution fresh.

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Cupric Citrate TS—Dissolve 25 g of cupric sulfate, 50 g of citric acid, and 144 g of anhydrous sodium carbonate in water, and dilute with water to 1000 mL.

Cupric Citrate TS, Alkaline—With the aid of heat, dissolve 173 g of dihydrated sodium citrate and 117 g of monohydrated sodium carbonate in about 700 mL of water, and filter through paper, if necessary, to obtain a clear solution. In a separate container dissolve 17.3 g of cupric sulfate in about 100 mL of water, and slowly add this solution, with constant stirring, to the first solution. Cool the mixture, add water to make 1000 mL, and mix.

Cupric Citrate TS 2, Alkaline—With the aid of heat, dissolve about 173 g of sodium citrate dihydrate and 117 g of sodium carbonate monohydrate in about 700 mL of water, and filter. In a second flask, dissolve about 27.06 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in about 100 mL of water. Slowly combine the two solutions while stirring, and dilute with water to 1000 mL.

Cupric Iodide TS, Alkaline—Dissolve 7.5 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in about 100 mL of water. In a separate container dissolve 25 g of anhydrous sodium carbonate, 20 g of sodium bicarbonate, and 25 g of potassium sodium tartrate in about 600 mL of water. With constant stirring, add the cupric sulfate solution to the bottom of the alkaline tartrate solution by means of a funnel that touches the bottom of the container. Add 1.5 g of potassium iodide, 200 g of anhydrous sodium sulfate, 50 to 150 mL of 0.02 M potassium iodate, and sufficient water to make 1000 mL.

Cupric Oxide, Ammoniated, TS ***(Schweitzer's Reagent)***—Dissolve 10 g of cupric sulfate in 100 mL of water, add sufficient sodium hydroxide solution (1 in 5) to precipitate the copper hydroxide, collect the latter on a filter, and wash free from sulfate with cold water. Dissolve the precipitate, which must be kept wet during the entire process, in the minimum quantity of ammonia TS necessary for complete solution.

Cupric Sulfate TS—Dissolve 12.5 g of cupric sulfate in water to make 100 mL.

Cupric Tartrate TS, Alkaline ***(Fehling's Solution)***
The Copper Solution (A): Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, showing no trace of efflorescence of adhering moisture, in water to make 500 mL. Store this solution in small, tight containers.
The Alkaline Tartrate Solution (B): Dissolve 173 g of crystallized potassium sodium tartrate and 50 g of sodium hydroxide in water to make 500 mL. Store this solution in small, alkali-resistant containers.
For use, mix exactly equal volumes of *Solutions A* and *B* at the time required.

Delafield's Hematoxylin TS—Prepare 400 mL of a saturated solution of ammonium alum *(Solution A)*. Dissolve 4 g of hematoxylin in 25 mL of alcohol, mix it with *Solution A*, and allow it to stand for 4 days in a flask closed with a pledget of purified cotton and exposed to light and air *(Solution B)*. Then filter *Solution B*, and add to it a *Solution C* consisting of a mixture of 100 mL of glycerin and 100 mL of methanol. Mix, and allow the mixture to stand in a warm place, exposed to light, for 6 weeks until it becomes dark-colored. Store in tightly stoppered bottles.
For use in staining endocrine tissue, dilute this test solution with an equal volume of water.

Denaturated Alcoholic TS—A specially denaturated alcohol containing either rubber hydrocarbon solvent of heptane or toluene. [NOTE—A suitable grade is available from www.lyondell.com or from www.sasol.com, as Ethanol SDA 2B HEP 200, or Ethanol SDA 2B TOL 200, or Ethanol SDA 2B TOL 190, or Alcohol SDA 2B-2.]

Denigès' Reagent—See *Mercuric Sulfate TS*.

Diazobenzenesulfonic Acid TS—Place in a beaker 1.57 g of sulfanilic acid, previously dried at 105° for 3 hours, add 80 mL of water and 10 mL of diluted hydrochloric acid, and warm on a steam bath until dissolved. Cool to 15° (some of the sulfanilic acid may separate but will dissolve later), and add slowly, with constant stirring, 6.5 mL of sodium nitrite solution (1 in 10). Then dilute with water to 100 mL.

Dichlorofluorescein TS—Dissolve 100 mg of dichlorofluorescein in 60 mL of alcohol, add 2.5 mL of 0.1 N sodium hydroxide, mix, and dilute with water to 100 mL.

2,7-Dihydroxynaphthalene TS—Dissolve 100 mg of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow the solution to stand until the yellow color disappears. If the solution is very dark, discard it and prepare a new solution from a different supply of sulfuric acid. This solution is stable for approximately 1 month if stored in a dark bottle.

Diiodofluorescein TS—Dissolve 500 mg of diiodofluorescein in a mixture of 75 mL of alcohol and 30 mL of water.

Diluted Lead Subacetate TS—See *Lead Subacetate TS, Diluted*.

***p*-Dimethylaminobenzaldehyde TS**—Dissolve 125 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 65 mL of sulfuric acid and 35 mL of water, and add 0.05 mL of ferric chloride TS. Use within 7 days.

Dinitrophenylhydrazine TS—Carefully mix 10 mL of water and 10 mL of sulfuric acid, and cool. To the mixture, contained in a glass-stoppered flask, add 2 g of 2,4-dinitrophenylhydrazine, and shake until dissolved. To the solution add 35 mL of water, mix, cool, and filter.

Diphenylamine TS—Dissolve 1.0 g of diphenylamine in 100 mL of sulfuric acid. The solution should be colorless.

Diphenylcarbazone TS—Dissolve 1 g of crystalline diphenylcarbazone in 75 mL of alcohol, then add alcohol to make 100 mL. Store in a brown bottle.

Dithizone TS—Dissolve 25.6 mg of dithizone in 100 mL of alcohol. Store in a cold place, and use within 2 months.

Dragendorff's TS—Mix 850 mg of bismuth subnitrate with 40 mL of water and 10 mL of glacial acetic acid *(Solution A)*. Dissolve 8 g of potassium iodide in 20 mL of water *(Solution B)*. Mix equal portions of *Solution A* and *Solution B* to obtain a stock solution, which can be stored for several months in a dark bottle. Mix 10 mL of the stock solution with 20 mL of glacial acetic acid, and dilute with water to make 100 mL.

Edetate Disodium TS—Dissolve 1 g of edetate disodium in 950 mL of water, add 50 mL of alcohol, and mix.

0.01 M Edetate Disodium TS—Transfer 100 mL of 0.1 M edetate disodium VS to a 1000-mL volumetric flask. Dilute with water to volume.

Eosin Y TS (adsorption indicator)—Dissolve 50 mg of eosin Y in 10 mL of water.

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Eriochrome Black TS—Dissolve 200 mg of eriochrome black T and 2 g of hydroxylamine hydrochloride in methanol to make 50 mL.

Eriochrome Cyanine TS—Dissolve 750 mg of eriochrome cyanine R in 200 mL of water, add 25 g of sodium chloride, 25 g of ammonium nitrate, and 2 mL of nitric acid, and dilute with water to 1000 mL.

Fehling's Solution—See *Cupric Tartrate TS, Alkaline.*

Ferric Ammonium Sulfate TS—Dissolve 8 g of ferric ammonium sulfate in water to make 100 mL.

Ferric Chloride TS—Dissolve 9 g of ferric chloride in water to make 100 mL.

Ferroin TS—Dissolve 0.7 g of ferrous sulfate and 1.76 g of *o*-phenanthroline monohydrochloride monohydrate in water, and dilute with water to 100 mL.

Ferrous Sulfate TS—Dissolve 8 g of clear crystals of ferrous sulfate in about 100 mL of recently boiled and thoroughly cooled water. Prepare this solution fresh.

Ferrous Sulfate, Acid, TS—Dissolve 7 g of ferrous sulfate crystals in 90 mL of recently boiled and thoroughly cooled water, and add sulfuric acid to make 100 mL. Prepare this solution immediately prior to use.

Folin-Ciocalteu Phenol TS—Into a 1500-mL flask introduce 100 g of sodium tungstate, 25 g of sodium molybdate, 700 mL of water, 50 mL of phosphoric acid, and 100 mL of hydrochloric acid. Gently reflux the mixture for about 10 hours, and add 150 g of lithium sulfate, 50 mL of water, and a few drops of bromine. Boil the mixture, without the condenser, for about 15 minutes, or until the excess bromine is expelled. Cool, dilute with water to 1 L, and filter: the filtrate has no greenish tint. Before use, dilute 1 part of the filtrate with 1 part of water. When used for protein determination (i.e., Lowry assay), this reagent must be further diluted (1:5) with water. See *Method 2* in *Total Protein Assay* under *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.

Formaldehyde TS—Use *Formaldehyde Solution* (see in the section *Reagents*).

Fuchsin–Pyrogallol TS—Dissolve 100 mg of basic fuchsin in 50 mL of water that previously has been boiled for 15 minutes and allowed to cool slightly. Cool, add 2 mL of a saturated solution of sodium bisulfite, mix, and allow to stand for not less than 3 hours. Add 0.9 mL of hydrochloric acid, mix, and allow to stand overnight. Add 100 mg of pyrogallol, shake until solution is effected, and dilute with water to 100 mL. Store in an amber-colored glass bottle in a refrigerator.

Fuchsin–Sulfurous Acid TS—Dissolve 200 mg of basic fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution of 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid. Dilute the solution with water to 200 mL, and allow to stand for at least 1 hour. Prepare this solution fresh.

Gastric Fluid, Simulated, TS—Dissolve 2.0 g of sodium chloride and 3.2 g of purified pepsin, that is derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protein, in 7.0 mL of hydrochloric acid and sufficient water to make 1000 mL. [NOTE—Pepsin activity is described in the *Food Chemicals Codex* specifications under *General Tests and Assays*.] This test solution has a pH of about 1.2.

Gelatin TS (for the assay of *Corticotropin Injection*)—Dissolve 340 g of acid-treated precursor gelatin (Type A) in water to make 1000 mL. Heat the solution in an autoclave at 115° for 30 minutes after the exhaust line temperature has reached 115°. Cool the solution, and add 10 g of phenol and 1000 mL of water. Store in tight containers in a refrigerator.

Glacial Acetic Acid TS—See *Acetic Acid, Glacial, TS.*

Glucose Oxidase–Chromogen TS—A solution containing, in each mL, 0.5 µmol of 4-aminoantipyrine, 22.0 µmol of sodium *p*-hydroxybenzoate, not less than 7.0 units of glucose oxidase, and not less than 0.5 units of peroxidase, and buffered to a pH of 7.0 ± 0.1.

Suitability: When used for determining glucose in Inulin, ascertain that no significant color results by reaction with fructose, and that a suitable absorbance-versus-concentration slope is obtained with glucose.

[NOTE—Glucose oxidase can be from *Aspergillus niger*.]

Glycerin Base TS—To 200 g of glycerin add water to bring the total weight to 235 g. Add 140 mL of 1 N sodium hydroxide and 50 mL of water.

Gold Chloride TS—Dissolve 1 g of gold chloride in 35 mL of water.

0.001 N Hydrochloric Acid TS—Transfer 1.0 mL of 1 N hydrochloric acid VS to a 1000-mL volumetric flask and dilute with water to volume.

0.01 N Hydrochloric Acid TS—Transfer 100 mL of 0.1 N hydrochloric acid VS to a 1000-mL volumetric flask. Dilute with water to volume.

0.025 N Hydrochloric Acid TS—Transfer 25 mL of 1 N hydrochloric acid VS to a 1000-mL volumetric flask. Dilute with water to volume.

0.05 N Hydrochloric Acid TS—Transfer 4.1 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.06 N Hydrochloric Acid TS—Transfer 20.0 mL of 3 N hydrochloric acid TS to a 1000-mL volumetric flask. Dilute with water to volume.

0.36 N Hydrochloric Acid TS—Transfer 120 mL of 3 N hydrochloric acid TS to a 1000-mL volumetric flask. Dilute with water to volume.

2 N Hydrochloric Acid TS—Transfer 17.0 mL of hydrochloric acid to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume.

3 N Hydrochloric Acid TS—Transfer 246 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

4 N Hydrochloric Acid TS—Transfer 331 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

5 N Hydrochloric Acid TS— Slowly transfer 413 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

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6 N Hydrochloric Acid TS—Slowly transfer 49.8 mL of hydrochloric acid to a 100-mL volumetric flask containing about 45 mL of water. Cool and dilute with water to volume.

0.08 N Hydrochloric Acid TS—Transfer 4.0 mL of 2 N hydrochloric acid TS to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume.

0.125 N Hydrochloric Acid TS—Transfer 10.3 mL of hydrochloric acid to a 1000-mL volumetric flask which contains about 500 mL of water. Cool and dilute with water to volume.

Hydrogen Peroxide TS—Use *Hydrogen Peroxide Topical Solution* (USP monograph).

Hydrogen Sulfide TS—A saturated solution of hydrogen sulfide, made by passing H_2S into cold water. Store it in small, dark amber-colored bottles, filled nearly to the top. It is unsuitable unless it possesses a strong odor of H_2S, and unless it produces at once a copious precipitate of sulfur when added to an equal volume of ferric chloride TS. Store in a cold, dark place.

Hydroxylamine Hydrochloride TS—Dissolve 3.5 g of hydroxylamine hydrochloride in 95 mL of 60% alcohol, and add 0.5 mL of bromophenol blue solution (1 in 1000 of alcohol) and 0.5 N alcoholic potassium hydroxide until a greenish tint develops in the solution. Then add 60% alcohol to make 100 mL.

8-Hydroxyquinoline TS—Dissolve 5 g of 8-hydroxy quinoline in alcohol to make 100 mL.

Indigo Carmine TS ***(Sodium Indigotindisulfonate TS)***—Dissolve a quantity of sodium indigotindisulfonate, equivalent to 180 mg of $C_{16}H_8N_2O_2(SO_3Na)_2$, in water to make 100 mL. Use within 60 days.

Indophenol–Acetate TS (for the assay of *Corticotropin Injection*)—To 60 mL of standard dichlorophenol-indophenol solution (see in the section *Volumetric Solutions*) add water to make 250 mL. Add to the resulting solution an equal volume of sodium acetate solution freshly prepared by dissolving 13.66 g of anhydrous sodium acetate in water to make 500 mL and adjusting with 0.5 N acetic acid to a pH of 7. Store in a refrigerator, and use within 2 weeks.

Intestinal Fluid, Simulated, TS—Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, and add 77 mL of 0.2 N sodium hydroxide and 500 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with either 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 6.8 ± 0.1. Dilute with water to 1000 mL.

Iodine TS—Use *0.1 N Iodine* (see in the section *Volumetric Solutions*).

Iodine, Diluted TS: Transfer 10.0 mL of 0.1 N iodine VS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Iodine Monochloride TS—Dissolve 10 g of potassium iodide and 6.44 g of potassium iodate in 75 mL of water in a glass-stoppered container. Add 75 mL of hydrochloric acid and 5 mL of chloroform, and adjust to a faint iodine color (in the chloroform) by adding dilute potassium iodide or potassium iodate solution. If much iodine is liberated, use a stronger solution of potassium iodate than 0.01 M at first, making the final adjustment with the 0.01 M potassium iodate. Store in a dark place, and readjust to a faint iodine color as necessary.

Iodine and Potassium Iodide TS 1—Dissolve 500 mg of iodine and 1.5 g of potassium iodide in 25 mL of water.

Iodine and Potassium Iodide TS 2—Dissolve 12.7 g of iodine and 20 g of potassium iodide in water, and dilute with water to 1000.0 mL. To 10.0 mL of this solution, add 0.6 g of potassium iodide, and dilute with water to 100.0 mL. Prepare immediately before use.

Iodine and Potassium Iodide TS 3—Dissolve 0.127 g of iodine and 0.20 g of potassium iodide in water, and dilute with water to 10.0 mL.

Iodobromide TS—Dissolve 20 g of iodine monobromide in glacial acetic acid to make 1000 mL. Store in glass containers, protected from light.

Iodochloride TS—Dissolve 16.5 g of iodine monochloride in 1000 mL of glacial acetic acid.

Iodoplatinate TS—Dissolve 300 mg of platinic chloride in 97 mL of water. Immediately prior to use, add 3.5 mL of potassium iodide TS, and mix.

Iron–Phenol TS ***(Iron-Kober Reagent)***—Dissolve 1.054 g of ferrous ammonium sulfate in 20 mL of water, and add 1 mL of sulfuric acid and 1 mL of 30 percent hydrogen peroxide. Mix, heat until effervescence ceases, and dilute with water to 50 mL. To 3 volumes of this solution contained in a volumetric flask add sulfuric acid, with cooling, to make 100 volumes. Purify phenol by distillation, discarding the first 10% and the last 5%, collecting the distillate, with exclusion of moisture, in a dry, tared glass-stoppered flask of about twice the volume of the phenol. Solidify the phenol in an ice bath, breaking the top crust with a glass rod to ensure complete crystallization. Weigh the flask and its contents, add to the phenol 1.13 times its weight of the iron–sulfuric acid solution prepared as directed, insert the stopper in the flask, and allow to stand, without cooling but with occasional mixing, until the phenol is liquefied. Shake the mixture vigorously until mixed, allow to stand in the dark for 16 to 24 hours, and again weigh the flask and its contents. To the mixture add 23.5% of its weight of a solution of 100 volumes of sulfuric acid in 110 volumes of water, mix, transfer to dry glass-stoppered bottles, and store in the dark, protected from atmospheric moisture. Use within 6 months. Dispense the reagent from a small-bore buret, arranged to exclude moisture, capable of delivering 1 mL in 30 seconds or less, and having no lubricant, other than reagent, on its stopcock. Wipe the buret tip with tissue before each addition.

Iron Salicylate TS—Dissolve 500 mg of ferric ammonium sulfate in 250 mL of water containing 10 mL of diluted sulfuric acid, and add water to make 500 mL. To 100 mL of the resulting solution add 50 mL of a 1.15% solution of sodium salicylate, 20 mL of diluted acetic acid, and 80 mL of a 13.6% solution of sodium acetate, then add water to make 500 mL. Store in a well-closed container. Protect from light. Use within 2 weeks.

Lanthanum Nitrate TS—Dissolve 5.0 g of lanthanum nitrate hexahydrate in 100 mL of water.

Lead Acetate TS—Dissolve 9.5 g of clear, transparent crystals of lead acetate in recently boiled water to make 100 mL. Store in well-stoppered bottles.

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Lead Acetate TS, Alcoholic—Dissolve 2 g of clear, transparent crystals of lead acetate in alcohol to make 100 mL. Store in tight containers.

Lead Nitrate Stock Solution TS—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which 1 mL of nitric acid has been added, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Lead Subacetate TS—Dissolve 40.0 g of lead acetate in 90 mL of carbon dioxide-free water. Adjust with 10 M sodium hydroxide to a pH of 7.5, centrifuge, and use the clear supernatant. It contains NLT 16.7% (w/w) and NMT 17.4% (w/w) of Pb in a form corresponding to the formula $C_8H_{14}O_{10}Pb_3$. The solution remains clear when stored in a well-closed container.

Lead Subacetate TS, Diluted—Dilute 3.25 mL of lead subacetate TS with water, recently boiled and cooled, to make 100 mL. Store in small, well-filled, tight containers.

Litmus TS—Digest 25 g of powdered litmus with three successive 100-mL portions of boiling alcohol, continuing each extraction for about 1 hour. Filter, wash with alcohol, and discard the alcohol filtrate. Macerate the residue with about 25 mL of cold water for 4 hours, filter, and discard the filtrate. Finally digest the residue with 125 mL of boiling water for 1 hour, cool, and filter.

Locke-Ringer's Solution—See *Locke-Ringer's TS*.

Locke-Ringer's TS *(Locke-Ringer's Solution)*

Sodium Chloride	9.0 g
Potassium Chloride	0.42 g
Calcium Chloride	0.24 g
Magnesium Chloride	0.2 g
Sodium Bicarbonate	0.5 g
Dextrose	0.5 g
Water, recently distilled from a hard-glass flask, a sufficient quantity to make	1000 mL

Prepare fresh each day. The constituents (except the dextrose and the sodium bicarbonate) may be made up in stock solutions and diluted as needed.

Magnesia Mixture TS—Dissolve 5.5 g of magnesium chloride and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, set the mixture aside for a few days in a well-stoppered bottle, and filter. If the solution is not perfectly clear, filter it before using.

Magnesium Sulfate TS—Dissolve 12 g of crystals of magnesium sulfate, selected for freedom from efflorescence, in water to make 100 mL.

Malachite Green TS—Dissolve 1 g of malachite green oxalate in 100 mL of glacial acetic acid.

Mallory's Stain—Dissolve 500 mg of water-soluble aniline blue, 2 g of orange G, and 2 g of oxalic acid in 100 mL of water.

Mayer's Reagent—See *Mercuric–Potassium Iodide TS*.

Mercuric Acetate TS—Dissolve 6.0 g of mercuric acetate in glacial acetic acid to make 100 mL. Store in tight containers, protected from direct sunlight.

Mercuric–Ammonium Thiocyanate TS—Dissolve 30 g of ammonium thiocyanate and 27 g of mercuric chloride in water to make 1000 mL.

Mercuric Bromide TS, Alcoholic—Dissolve 5 g of mercuric bromide in 100 mL of alcohol, employing gentle heat to facilitate solution. Store in glass containers, protected from light.

Mercuric Chloride TS—Dissolve 6.5 g of mercuric chloride in water to make 100 mL.

Mercuric Iodide TS *(Valser's Reagent)*—Slowly add potassium iodide solution (1 in 10) to red mercuric iodide until almost all of the latter is dissolved, and filter off the excess. A solution containing 10 g of potassium iodide in 100 mL dissolves approximately 14 g of HgI_2 at 20°.

Mercuric Nitrate TS—Dissolve 40 g of mercuric oxide (red or yellow) in a mixture of 32 mL of nitric acid and 15 mL of water. Store in glass containers, protected from light.

Mercuric–Potassium Iodide TS *(Mayer's Reagent)*—Dissolve 1.358 g of mercuric chloride in 60 mL of water. Dissolve 5 g of potassium iodide in 10 mL of water. Mix the two solutions, and dilute with water to 100 mL.

Mercuric–Potassium Iodide TS, Alkaline *(Nessler's Reagent)*—Dissolve 143 g of sodium hydroxide in 700 mL of water. Dissolve 50 g of red mercuric iodide and 40 g of potassium iodide in 200 mL of water. Pour the iodide solution into the hydroxide solution, and dilute with water to 1000 mL. Allow to settle, and use the clear supernatant.

Mercuric Sulfate TS *(Denigès' Reagent)*—Mix 5 g of yellow mercuric oxide with 40 mL of water, and while stirring slowly add 20 mL of sulfuric acid, then add another 40 mL of water, and stir until completely dissolved.

Mercurous Nitrate TS—Dissolve 15 g of mercurous nitrate in a mixture of 90 mL of water and 10 mL of diluted nitric acid. Store in dark, amber-colored bottles in which a small globule of mercury has been placed.

Metaphenylenediamine Hydrochloride TS—Dissolve 1 g of metaphenylenediamine hydrochloride in 200 mL of water. The solution must be colorless when used. If necessary, decolorize by heating with activated charcoal.

Metaphosphoric–Acetic Acids TS—Dissolve 15 g of metaphosphoric acid in 40 mL of glacial acetic acid and sufficient water to make 500 mL. Store in a cold place, and use within 2 days.

Methoxyphenylacetic TS—Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of *Tetramethylammonium Hydroxide TS*, and add 20 mL of dehydrated alcohol. Store in a polyethylene container.

Methyl Orange TS—Dissolve 100 mg of methyl orange in 100 mL of water, and filter if necessary.

Methyl Purple TS—Use *Methyl Red–Methylene Blue TS*.

Methyl Red TS—Dissolve 100 mg of methyl red in 100 mL of alcohol, and filter if necessary.

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Methyl Red TS 2—To 1.86 mL of 0.1 M sodium hydroxide and 50 mL of alcohol, add 50 mg of methyl red, and dilute with water to 100 mL.

Methyl Red TS, Methanolic—Dissolve 1 g of methyl red in 100 mL of methanol, and filter, if necessary. Store protected from light, and use within 21 days.

Methyl Red–Methylene Blue TS—Add 10 mL of methyl red TS to 10 mL of methylene blue TS, and mix.

Methyl Violet TS—Use *Crystal Violet TS*.

Methyl Yellow TS: Prepare a solution containing 0.10 mg per mL in alcohol.

Methyl Yellow–Methylene Blue TS—Dissolve 1 g of methyl yellow and 100 mg of methylene blue in 125 mL of methanol.

3-Methyl-2-benzothiazolinone Hydrazone Hydrochloride TS—Dissolve 0.1 g of 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate in 10 mL of water, dilute the resulting solution with methanol to 100 mL, and mix.

Methylene Blue TS—Dissolve 125 mg of methylene blue in 100 mL of alcohol, and dilute with alcohol to 250 mL.

Methylthionine Perchlorate TS—To 500 mL of potassium perchlorate solution (1 in 1000) add dropwise, with constant shaking, methylene blue solution (1 in 100) until a slight, permanent turbidity results. Allow the precipitate to settle, decant the supernatant through paper, and use only the clear solution.

Millon's Reagent—To 2 mL of mercury in a conical flask add 20 mL of nitric acid. Shake the flask under a hood to break up the mercury into small globules. After about 10 minutes, add 35 mL of water, and, if a precipitate or crystals appear, add sufficient dilute nitric acid (1 in 5, prepared from nitric acid from which the oxides have been removed by blowing air through it until it is colorless) to dissolve the separated solid. Add sodium hydroxide solution (1 in 10) dropwise, with thorough mixing, until the curdy precipitate that forms after the addition of each drop no longer redissolves but is dispersed to form a suspension. Add 5 mL more of the dilute nitric acid, and mix. Prepare this solution fresh.

Molybdo-phosphotungstate TS *(Folin-Denis Reagent)*—To about 350 mL of water contained in a round-bottom flask add 50 g of sodium tungstate, 12 g of phosphomolybdic acid, and 25 mL of phosphoric acid. Boil the mixture under a reflux condenser for 2 hours, then cool, dilute with water to 500 mL, and mix. Store in tight containers, protected from light, and in a cold place.

0.05 M Monobasic Sodium Phosphate TS—Transfer 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

0.1 M Monobasic Sodium Phosphate TS—Transfer 13.8 g of monobasic sodium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

1-Naphthol Reagent—Dissolve 1 g of 1-naphthol in 25 mL of methanol. Prepare this solution fresh.

1-Naphthol TS—Use *1-Naphthol Reagent*.

2-Naphthol TS *(Betanaphthol TS)*—Dissolve 1 g of 2-naphthol in 100 mL of sodium hydroxide solution (1 in 100).

***p*-Naphtholbenzein TS**—Dissolve 250 mg of *p*-naphtholbenzein in 100 mL of glacial acetic acid.

***N*-(1-Naphthyl)ethylenediamine Dihydrochloride TS**—Dissolve 100 mg of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 mL of a mixture of 7 parts of acetone and 3 parts of water.

Nessler's Reagent—See *Mercuric–Potassium Iodide TS, Alkaline*.

Neutral Red TS—Dissolve 100 mg of neutral red in 100 mL of 50% alcohol.

Nickel Standard Solution TS—Dissolve 4.78 g of nickel(II) sulfate heptahydrate in water, and dilute with water to 1000 mL. Immediately prior to use, dilute 10.0 mL of the solution so obtained with water to 1000 mL. Suitable nickel standard solutions are also available commercially.

Ninhydrin TS—Use *Triketohydrindene Hydrate TS*.

0.01 N Nitric Acid TS—Transfer 10.0 mL of 1 N nitric acid TS to a 1000-mL volumetric flask. Dilute with water to volume.

0.2 N Nitric Acid TS—Slowly transfer 12.7 mL of nitric acid to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute with water to volume.

1 N Nitric Acid TS—Transfer 63.7 mL of nitric acid to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute with water to volume.

2 N Nitric Acid TS—Transfer 12.74 mL of nitric acid slowly to a 100-mL volumetric flask containing about 40 mL of water. Cool and dilute with water to volume.

***p*-Nitroaniline TS**—To 350 mg of *p*-nitroaniline add 1.5 mL of hydrochloric acid, and mix. Dilute with water to 50 mL, mix, and allow to settle. Place 5 mL of the clear supernatant in a 100-mL volumetric flask, and immerse it in an ice bath. While it is in the ice bath, add 1 mL of hydrochloric acid, then add, in small portions, 2 mL of sodium nitrite solution (1 in 100), dilute with water to volume, and mix.

Nitrophenanthroline TS—Dissolve 150 mg of 5-nitro-1,10-phenanthroline in 15 mL of freshly prepared ferrous sulfate solution (1 in 140).

Orthophenanthroline TS—Dissolve 150 mg of orthophenanthroline in 10 mL of a solution of ferrous sulfate, prepared by dissolving 700 mg of ferrous sulfate in 100 mL of water. The ferrous sulfate solution must be prepared immediately before dissolving the orthophenanthroline. Store in well-closed containers.

Oxalic Acid TS—Dissolve 6.3 g of oxalic acid in water to make 100 mL.

Palladium Chloride TS, Buffered—Weigh 500 mg of palladium chloride into a 250-mL beaker, add 5 mL of concentrated hydrochloric acid, and warm the mixture on a steam bath. Add 200 mL of hot water in small increments with continued heating until solution is complete. Transfer the solution to a 250-mL volumetric flask, and dilute with water to volume. Transfer 50 mL to a 100-mL volumetric flask. Add

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10 mL of 1 M sodium acetate and 9.6 mL of 1 N hydrochloric acid. Dilute with water to volume.

Perchloric Acid TS—Dilute 8.5 mL of perchloric acid with water to 100 mL.

Phenol TS—Dissolve 1.2 g of phenol in alcohol to make 10 mL. Prepare weekly.

Phenol Red TS *(Phenolsulfonphthalein TS)*—Dissolve 100 mg of phenolsulfonphthalein in 100 mL of alcohol, and filter if necessary.

pH 4.7 Phenol Red TS—Dissolve 33 mg of phenolsulfonphthalein in 1.5 mL of 2 N sodium hydroxide solution, dilute with water to 100 mL, and mix *(Solution A)*. Dissolve 25 mg of ammonium sulfate in 235 mL of water, add 105 mL of 2 N sodium hydroxide solution and 135 mL of 2 N acetic acid, and mix *(Solution B)*. Add 25 mL of *Solution A* to *Solution B*, and mix. If necessary, adjust the pH of this solution to 4.7.

Phenoldisulfonic Acid TS—Dissolve 2.5 g of phenol in 15 mL of sulfuric acid in a flask of suitable capacity. Add 7.5 mL of fuming sulfuric acid, stir well, and heat at 100° for 2 hours. Transfer the product, while still fluid, to a glass-stoppered bottle, and, when desired for use, warm in a water bath until liquefied.

Phenolphthalein TS—Dissolve 1 g of phenolphthalein in 100 mL of alcohol.

Phenylhydrazine Acetate TS—Dissolve 10 mL of phenylhydrazine and 5 mL of glacial acetic acid in water to make 100 mL.

Phenylhydrazine–Sulfuric Acid TS—Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of a cooled mixture of equal volumes of sulfuric acid and water.

Phloroglucinol TS—Dissolve 500 mg of phloroglucinol in 25 mL of alcohol. Store in tight containers, protected from light.

Phosphatic Enzyme TS—Dissolve 5 g of phosphatic enzyme in water to make 50 mL. Prepare this solution fresh.

Phosphomolybdic Acid TS—Dissolve 20 g of phosphomolybdic acid in alcohol to make 100 mL. Filter the solution, and use only the clear filtrate.

0.01 M Phosphoric Acid TS—Transfer 0.7 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.05 M Phosphoric Acid TS—Transfer 3.4 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.06 M Phosphoric Acid TS—Transfer 4.2 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.75 M Phosphoric Acid TS—Transfer 51 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Add the following:

▲1 M Phosphoric Acid TS—Transfer 68 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool down to room temperature and dilute with water to volume.▲ (USP 1-Aug-2019)

1.5 M Phosphoric Acid TS—Transfer 10.5 mL of phosphoric acid to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume.

1 N Phosphoric Acid TS—To a 1000-mL volumetric flask containing about 800 mL of water, slowly add 22.8 mL of phosphoric acid. Cool and dilute with water to volume.

Add the following:

▲18 N Phosphoric Acid TS—Transfer 410.5 mL of phosphoric acid to a 1000-mL volumetric flask containing about 400 mL of water. Cool and dilute with water to volume.▲ (USP 1-May-2020)

0.01% Phosphoric Acid TS—Transfer 12 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Dilute with water to volume. Transfer 10 mL to a 1000-mL volumetric flask and dilute with water to volume.

0.02 M Phosphoric Acid TS—Transfer 1.4 mL of phosphoric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

10% Phosphoric Acid TS—Transfer 7 mL of phosphoric acid to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume.

20% Phosphoric Acid TS—Transfer 14 mL of phosphoric acid to a 100-mL volumetric flask containing about 50 mL of water. Cool and dilute with water to volume.

Phosphotungstic Acid TS—Dissolve 1 g of phosphotungstic acid in water to make 100 mL.

Picrate TS, Alkaline—Mix 20 mL of trinitrophenol solution (1 in 100) with 10 mL of sodium hydroxide solution (1 in 20), dilute with water to 100 mL, and mix. Use within 2 days.

Picric Acid TS—See *Trinitrophenol TS*.

Platinic Chloride TS—Dissolve 2.6 g of platinic chloride in water to make 20 mL.

Platinum–Cobalt TS—Dissolve 1.246 g of potassium chloroplatinate (K_2PtCl_6) and 1.000 g of cobalt chloride ($CoCl_2 \cdot 6H_2O$) in water, add 100 mL of hydrochloric acid, and dilute with water to 1 L.

Potassium Acetate TS—Dissolve 10 g of potassium acetate in water to make 100 mL.

Potassium–Bismuth Iodide TS—Dissolve 12.5 g of tartaric acid in 25 mL of water, then dissolve 1.06 g of bismuth subnitrate in this mixture *(Solution A)*. Dissolve 20 g of potassium iodide in 25 mL of water *(Solution B)*. Dissolve 100 g of tartaric acid in 450 mL of water *(Solution C)*. Add *Solutions A* and *B* to *Solution C*, and mix.

Potassium Carbonate TS—Dissolve 7 g of anhydrous potassium carbonate in water to make 100 mL.

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Potassium Chromate TS—Dissolve 10 g of potassium chromate in water to make 100 mL.

Potassium Dichromate TS—Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

Potassium Ferricyanide TS—Dissolve 1 g of potassium ferricyanide in 10 mL of water. Prepare this solution fresh.

Potassium Ferrocyanide TS—Dissolve 1 g of potassium ferrocyanide in 10 mL of water. Prepare this solution fresh.

Potassium Hydroxide TS—Dissolve 6.5 g of potassium hydroxide in water to make 100 mL.

10 M Potassium Hydroxide TS—Transfer 660.1 g of potassium hydroxide to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

1.8 N Potassium Hydroxide TS—Dissolve 101 g of potassium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL.

2 N Potassium Hydroxide TS—Dissolve 112.2 g of potassium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL.

45% Potassium Hydroxide TS—Transfer 53 g of potassium hydroxide to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Potassium Hydroxide TS, Alcoholic—Use *0.5 N Potassium Hydroxide, Alcoholic* (see in the section *Volumetric Solutions*).

Potassium Hydroxide TS 2, Alcoholic—Dissolve 130 g of potassium hydroxide, with cooling, in 200 mL of water. Add alcohol to 1000 mL. Store in a well-stoppered dark glass bottle.

Potassium Iodide TS—Dissolve 16.5 g of potassium iodide in water to make 100 mL. Store in light-resistant containers.

20% Potassium Iodide TS—Transfer 20 g of potassium iodide to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Potassium Iodide and Starch TS: Dissolve 0.75 g of potassium iodide in 100 mL of water. Heat to boiling, and add, with stirring, a solution of 0.5 g of soluble starch in 35 mL of water. Boil for 2 minutes, and allow to cool.

Sensitivity: Mix 15 mL in 0.05 mL of glacial acetic acid and 0.3 mL of diluted iodine TS: a blue color is produced.

Potassium Iodoplatinate TS—Dissolve 200 mg of platinic chloride in 2 mL of water, mix with 25 mL of potassium iodide solution (1 in 25), and add water to make 50 mL.

Potassium Permanganate TS—Use *0.1 N Potassium Permanganate* (see in the section *Volumetric Solutions*).

0.02 M Monobasic Potassium Phosphate TS—Transfer 2.72 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

0.2 M Dibasic Potassium Phosphate TS—Transfer 34.83 g of dibasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

Potassium Pyroantimonate TS—Dissolve 2 g of potassium pyroantimonate in 85 mL of hot water. Cool quickly, and add 50 mL of a solution containing 50 mg/mL of potassium hydroxide in water and 1 mL of sodium hydroxide solution (8.5 in 100). Allow to stand for 24 h, filter, and dilute with water to 150 mL.

Potassium Sulfate TS—Dissolve 1 g of potassium sulfate in water to make 100 mL.

Potassium Thiocyanate TS: Dissolve 9.7 g of potassium thiocyanate in water to make 100 mL.

Pyridine–Pyrazolone TS—To 100 mL of a saturated solution of 1-phenyl-3-methyl-2-pyrazoline-5-one add 20 mL of a 1 in 1000 solution of 3,3′-dimethyl-1,1′-diphenyl-[4,4′-bi-2-pyrazoline]-5,5′-dione in pyridine. Store in a dark bottle, and use within 3 days.

Pyrogallol TS, Alkaline—Dissolve 500 mg of pyrogallol in 2 mL of water. Dissolve 12 g of potassium hydroxide in 8 mL of water. The solutions should be freshly prepared and mixed immediately before use.

Quinaldine Red TS—Dissolve 100 mg of quinaldine red in 100 mL of alcohol.

Quinone TS—Dissolve 500 mg of *p*-benzoquinone in 2.5 mL of glacial acetic acid, and dilute with alcohol to 50 mL. Prepare this solution fresh daily.

Resorcinol TS—Dissolve 1 g of resorcinol in hydrochloric acid to make 100 mL.

Ruthenium Red TS—Dissolve 10 g of lead acetate in water, dilute with water to 100 mL, and add 80 mg of ruthenium red. The solution is wine-red in color. [NOTE—If necessary, add additional ruthenium red to obtain a wine-red color.]

Saline TS—Dissolve 9.0 g of sodium chloride in water to make 1000 mL.

[NOTE—Where pyrogen-free saline TS is specified in this Pharmacopeia, saline TS that has met the requirements of the *Pyrogen Test* ⟨151⟩ is to be used.]

Saline TS, Pyrogen-Free—See *Saline TS*.

Schweitzer's Reagent—See *Cupric Oxide, Ammoniated, TS*.

Silver–Ammonia–Nitrate TS—Dissolve 1 g of silver nitrate in 20 mL of water. Add ammonia TS, dropwise, with constant stirring, until the precipitate is almost but not entirely dissolved. Filter, and store in tight, light-resistant containers.

Silver–Ammonium Nitrate TS—See *Silver–Ammonia–Nitrate TS*.

Silver Diethyldithiocarbamate TS—Dissolve 1 g of silver diethyldithiocarbamate in 200 mL of pyridine from a freshly opened bottle or that which has been recently distilled. Store in light-resistant containers, and use within 30 days.

Silver Nitrate TS—Use *0.1 N Silver Nitrate* (see in the section *Volumetric Solutions*).

Simulated Gastric Fluid TS—See *Gastric Fluid, Simulated, TS*.

Simulated Intestinal Fluid TS—See *Intestinal Fluid, Simulated, TS*.

Sodium Acetate TS—Dissolve 13.6 g of sodium acetate in water to make 100 mL.

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Sodium Alizarinsulfonate TS—Dissolve 100 mg of sodium alizarinsulfonate in 100 mL of water, and filter.

Sodium Aminoacetate TS ***(Sodium Glycinate TS)***—Dissolve 3.75 g of aminoacetic acid in about 500 mL of water, add 2.1 g of sodium hydroxide, and dilute with water to 1000 mL. Mix 9 mL of the resulting solution with 1 mL of dilute glacial acetic acid (1 in 300). This test solution has a pH between 10.4 and 10.5.

Sodium Bisulfite TS—Dissolve 10 g of sodium bisulfite in water to make 30 mL. Prepare this solution fresh.

Sodium Bitartrate TS—Dissolve 1 g of sodium bitartrate in water to make 10 mL. Prepare this solution fresh.

Sodium Carbonate TS—Dissolve 10.6 g of anhydrous sodium carbonate in water to make 100 mL.

Add the following:

▲Sodium Chloride TS—9 g/L of sodium chloride in water▲ (USP 1-May-2019)

0.5 M Sodium Chloride TS—Transfer 29.2 g of sodium chloride to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

0.1 N Sodium Chloride TS—Transfer 5.84 g of sodium chloride to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

Sodium Chloride TS, Alkaline—Dissolve 2 g of sodium hydroxide in 100 mL of water, saturate the solution with sodium chloride, and filter.

Sodium Citrate TS—Dissolve 73.5 g of sodium citrate dihydrate in water to make 250 mL.

Sodium Citrate TS, Alkaline—Dissolve 50 g of sodium citrate dihydrate and 2.5 g of sodium hydroxide in water to make 250 mL.

Sodium Cobaltinitrite TS—Dissolve 10 g of sodium cobaltinitrite in water to make 50 mL, and filter if necessary.

Sodium Fluoride TS—Dry about 500 mg of sodium fluoride at 200° for 4 hours. Accurately weigh 222 mg of the dried material, and dissolve in water to make 100.0 mL. Pipet 10 mL of this solution into a 1-L volumetric flask, and dilute with water to volume. Each mL of this solution corresponds to 0.01 mg of fluorine (F).

Sodium Hydrosulfite TS, Alkaline—Dissolve 25 g of potassium hydroxide in 35 mL of water, and 50 g of sodium hydrosulfite in 250 mL of water. When the test solution is required, mix 40 mL of the hydroxide solution with the 250 mL of the hydrosulfite solution. Prepare this solution fresh.

Sodium Hydroxide TS—Dissolve 4.0 g of sodium hydroxide in water to make 100 mL.

0.0025 N Sodium Hydroxide TS—Transfer 12.5 mL of 0.2 N sodium hydroxide TS to a 1000-mL volumetric flask. Dilute with water to volume.

0.02 N Sodium Hydroxide TS—Transfer 10 mL of 2 N sodium hydroxide TS to a 1000-mL volumetric flask. Dilute with water to volume.

0.2 N Sodium Hydroxide TS—Transfer 10.0 mL of 2 N sodium hydroxide TS to a 100-mL volumetric flask and dilute with water to volume.

2 N Sodium Hydroxide TS—Dissolve 80 g of sodium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL.

2.5 N Sodium Hydroxide TS—Dissolve 100 g of sodium hydroxide in about 800 mL of water. Cool and dilute with water to 1000 mL.

5 N Sodium Hydroxide TS—Transfer 20 g of sodium hydroxide to a 100-mL volumetric flask. Dissolve in about 80 mL of water. Cool and dilute with water to volume.

10 N Sodium Hydroxide TS—Transfer 400 g of sodium hydroxide to a 1000-mL volumetric flask, and dissolve in about 800 mL of water. Cool and dilute with water to volume.

Sodium Hydroxide TS 2—Transfer 8.5 g of sodium hydroxide to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.

Sodium Hydroxide TS 3—Prepare a 420-mg/mL solution of sodium hydroxide in water.

Add the following:

▲50% Sodium Hydroxide TS—Transfer 500 g of sodium hydroxide to a 1000-mL volumetric flask. Dissolve in and dilute with carbon dioxide-free water to volume. Allow to stand. Decant the clear supernatant, taking precautions to avoid the introduction of carbon dioxide.▲ (USP 1-May-2020)

Sodium Hypobromite TS—To a solution of 20 g of sodium hydroxide in 75 mL of water add 5 mL of bromine. After solution has taken place, dilute with water to 100 mL. Prepare this solution fresh.

Sodium Hypochlorite TS—Use *Sodium Hypochlorite Solution* (see in the section *Reagent Specifications*).

Sodium Iodohydroxyquinolinesulfonate TS—Dissolve 8.8 g of iodohydroxyquinoline sulfonic acid in 200 mL of water, and add 6.5 mL of 4 N sodium hydroxide. Dilute with water to 250 mL, mix, and filter.

Sodium Nitroferricyanide TS—Dissolve 1 g of sodium nitroferricyanide in water to make 20 mL. Prepare this solution fresh.

Dibasic Sodium Phosphate TS—Dissolve 12 g of dibasic sodium phosphate in water to make 100 mL.

Sodium Phosphotungstate TS—To a solution of 20 g of sodium tungstate in 100 mL of water add sufficient phosphoric acid to impart a strongly acid reaction to litmus, and filter. When required for use, decant the clear solution from any sediment that may be present. Store in tight, light-resistant containers.

Sodium Sulfide TS—Dissolve 1 g of sodium sulfide in water to make 10 mL. Prepare this solution fresh.

Sodium Tartrate TS—Dissolve 11.5 g of sodium tartrate in water to make 100 mL.

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Sodium Tetraphenylboron TS—Dissolve 1.2 g of sodium tetraphenylboron in water to make 200 mL. If necessary, stir for 5 minutes with 1 g of aluminum oxide, and filter to clarify.

Sodium Thioglycolate TS—Dissolve 1.5 g of sodium thioglycolate in 450 mL of water, and add 50 mL of alcohol. Use within 3 days.

Sodium Thiosulfate TS—Use *0.1 N Sodium Thiosulfate* (see in the section *Volumetric Solutions*).

Standard Lead Solution TS: On the day of use, dilute 10.0 mL of lead nitrate stock solution TS with water to 100.0 mL. Each mL of standard lead solution TS contains the equivalent to 10 µg of lead.

Stannous Chloride, Acid, TS—Dissolve 8 g of stannous chloride in 500 mL of hydrochloric acid. Store in glass containers, and use within 3 months.

Stannous Chloride, Acid, Stronger, TS—Dissolve 40 g of stannous chloride in 100 mL of hydrochloric acid. Store in glass containers, and use within 3 months.

Starch TS—Mix 1 g of soluble starch with 10 mg of red mercuric iodide and sufficient cold water to make a thin paste. Add 200 mL of boiling water, and boil for 1 minute with continuous stirring. Cool, and use only the clear solution. [NOTE—Commercially available, stabilized starch indicator solutions may be used, including mercury-free solutions preserved with other compounds such as salicylic acid.]

Starch, Iodide-Free, TS—Mix 1 g of soluble starch with sufficient cold water to make a thin paste. While stirring, add 100 mL of boiling water, and allow to cool. Prepare this solution immediately before use. Iodide-free starch TS shows a blue color when 20 mL of potassium iodide solution (1 in 400) and 0.05 mL of an iodine–potassium iodide solution (prepared by dissolving 127 mg of iodine and 800 mg of potassium iodide in water and diluting with water to 100 mL) are added to 1 mL of the iodide-free starch TS.

Starch Iodide Paste TS—Heat 100 mL of water in a 250-mL beaker to boiling, add a solution of 0.75 g of potassium iodide in 5 mL of water, then add 2 g of zinc chloride dissolved in 10 mL of water, and, while the solution is boiling, add, with stirring, a smooth suspension of 5 g of soluble starch in 30 mL of cold water. Continue to boil for 2 minutes, then cool. Store in well-closed containers in a cold place.

Starch iodide paste TS must show a definite blue streak when a glass rod, dipped in a mixture of 1 mL of 0.1 M sodium nitrite, 500 mL of water, and 10 mL of hydrochloric acid, is streaked on a smear of the paste.

Starch–Potassium Iodide TS—Dissolve 500 mg of potassium iodide in 100 mL of freshly prepared starch TS. Prepare this solution fresh.

Stronger Cupric Acetate TS—See *Cupric Acetate TS, Stronger.*

Sudan III TS—Dissolve 0.05 g of Sudan III in 25 mL of alcohol, with warming if necessary. Cool, add 25 mL of glycerin, and mix. Filter if undissolved material persists.

Sudan IV TS—Dissolve 0.5 g of Sudan IV in chloroform to make 100 mL.

Sulfanilic Acid TS—Dissolve 800 mg of sulfanilic acid in 100 mL of acetic acid. Store in tight containers.

Diazotized Sulfanilic Acid TS—Dissolve 0.9 g of sulfanilic acid in 9 mL of hydrochloric acid with warming, and dilute with water to 100 mL. Cool 10 mL of this solution in iced water, and add 10 mL of a sodium nitrite solution (4.5 in 100) previously cooled in iced water. Allow to stand at 0° for at least 15 minutes (the solution may be kept for 3 days at this temperature). Immediately before use, add 20 mL of sodium carbonate solution (1 in 10).

Sulfanilic-1-Naphthylamine TS—Dissolve 500 mg of sulfanilic acid in 150 mL of acetic acid. Dissolve 100 mg of 1-naphthylamine hydrochloride in 150 mL of acetic acid, and mix the two solutions. The pink color that may develop on standing can be removed by treatment with zinc.

Sulfanilic-α-Naphthylamine TS—See *Sulfanilic-1-Naphthylamine TS.*

Sulfomolybdic Acid TS—Dissolve, with the aid of heat, 2.5 g of ammonium molybdate in 20 mL of water, add 50 mL of 12 N sulfuric acid, and dilute with water to 100 mL. Store this solution in a polyethylene container.

Sulfuric Acid TS—Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% (w/w) of H_2SO_4.

[NOTE—Since the acid concentration may change upon standing or upon intermittent use, the concentration should be checked frequently and solutions assaying more than 95.5% or less than 94.5% discarded.]

1 M Sulfuric Acid TS—Transfer 56.1 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.02 N Sulfuric Acid TS—Transfer 2.9 mL of 7 N sulfuric acid TS to a 1000-mL volumetric flask and dilute with water to volume.

0.2 N Sulfuric Acid TS—Transfer 5.6 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

0.5 N Sulfuric Acid TS—Transfer 14 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

2 N Sulfuric Acid TS—Transfer 56.2 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

6 N Sulfuric Acid TS—Slowly transfer 168.5 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

7 N Sulfuric Acid TS—Slowly transfer 196.5 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

10 N Sulfuric Acid TS—Transfer 281 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Sulfuric Acid–Formaldehyde TS—Add 1 drop of formaldehyde TS to each mL of sulfuric acid, and mix. Prepare this solution fresh.

Tannic Acid TS—Dissolve 1 g of tannic acid in 1 mL of alcohol, and dilute with water to 10 mL. Prepare this solution fresh.

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Tartaric Acid TS—Dissolve 3 g of tartaric acid in water to make 10 mL. Prepare this solution fresh.

Tetrabromophenolphthalein Ethyl Ester TS—Dissolve 100 mg of tetrabromophenolphthalein ethyl ester in 90 mL of glacial acetic acid, and dilute with glacial acetic acid to 100 mL. Prepare this solution fresh.

0.02 M Tetrabutylammonium Hydrogen Sulfate TS—Transfer 6.79 g of tetrabutylammonium hydrogen sulfate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

Add the following:

▲25% Tetrabutylammonium Hydroxide TS—Transfer about 34.82 g of tetrabutylammonium hydroxide to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.▲ (USP 1-Dec-2019)

Tetramethylammonium Hydroxide TS—Use an aqueous solution containing, in each 100 mL, the equivalent of 10 g of anhydrous tetramethylammonium hydroxide.

Thioacetamide TS—Dissolve 4 g of thioacetamide in 100 mL of water.

Thioacetamide–Glycerin Base TS—Mix 0.2 mL of thioacetamide TS and 1 mL of glycerin base TS, and heat in a boiling water bath for 20 seconds. Use the mixture immediately.

Thorium Nitrate TS—Dissolve 1 g of thorium nitrate in water to make 100 mL. Filter, if necessary.

Thymol Blue TS—Dissolve 100 mg of thymol blue in 100 mL of alcohol, and filter if necessary.

Thymolphthalein TS—Dissolve 100 mg of thymolphthalein in 100 mL of alcohol, and filter if necessary.

Titanium Trichloride TS—Dissolve 15 g of titanium trichloride in 100 mL of 10% hydrochloric acid solution.

Titanium Trichloride–Sulfuric Acid TS—Mix carefully 20 mL of titanium trichloride TS in 13 mL of sulfuric acid. Add sufficient 30% hydrogen peroxide to produce a yellow color. Heat until white fumes are evolved, allow to cool, and dilute with water. Repeat the evaporation and addition of water until a colorless solution is obtained. Dilute with water to 100 mL.

***p*-Toluenesulfonic Acid TS**—Dissolve 2 g of *p*-toluenesulfonic acid in 10 mL of a mixture of 7 parts of acetone and 3 parts of water.

0.1% Trifluoroacetic Acid TS—Transfer 1 mL of trifluoroacetic acid to a 1000-mL volumetric flask containing about 500 mL of water. Dilute with water to volume.

Triketohydrindene Hydrate TS *(Ninhydrin TS)*—Dissolve 200 mg of triketohydrindene hydrate in water to make 10 mL. Prepare this solution fresh.

Trinitrophenol TS *(Picric Acid TS)*—Dissolve the equivalent of 1 g of anhydrous trinitrophenol in 100 mL of hot water. Cool the solution, and filter if necessary.

Triphenyltetrazolium Chloride TS—Dissolve 500 mg of triphenyltetrazolium chloride in dehydrated alcohol to make 100 mL.

Change to read:

Xylenol Orange TS—▲If using xylenol orange in the free acid form,▲ (USP 1-May-2020) dissolve 100 mg of xylenol orange in 100 mL of alcohol. ▲If using xylenol orange in the salt form, dissolve 100 mg of xylenol orange in 100 mL of water.▲ (USP 1-May-2020)

Zinc Uranyl Acetate TS—Dissolve 50 g of uranyl acetate in a mixture of 15 mL of glacial acetic acid and water to make 500 mL. Then dissolve 150 g of zinc acetate in a mixture of 15 mL of glacial acetic acid and water to make 500 mL. Mix the two solutions, allow to stand overnight, and pass through a dry filter, if necessary.

VOLUMETRIC SOLUTIONS

1. DEFINITIONS

1.1 Normal solutions: Normal solutions are solutions that contain 1 gram equivalent weight of the compound in 1 L of solution.

$$N = \text{equivalent/L}$$

$$N = \text{weight (g)/equivalent weight (g)} \times L$$

Normal solutions and solutions bearing a specific relationship to normal solutions, and used in volumetric determinations, are designated as follows:

1 N = normal
2 N = double normal
0.5 N = half-normal
0.1 N = tenth-normal
0.02 N = fiftieth normal
0.01 N = hundredth-normal
0.001 N = thousandth-normal

1.2 Molar solutions: Molar solutions are solutions that contain 1 gram-molecule of the compound in 1 L of solution.

$$M = \text{mol/L}$$

$$M = \text{weight (g)/mol weight} \times L$$

Solutions containing one-tenth of a gram-molecule of the compound in 1 L are designated tenth-molar, 0.1 M; and other molarities are similarly indicated.

1.3 Correction factor: It is frequently difficult to prepare standard solutions of a desired theoretical normality or molarity, and this is not essential. A solution of approximately the desired normality or molarity is prepared and standardized by titration against a standard. The correction factor so obtained is used in all calculations where such solutions are used. If necessary, the concentration of the solution may be adjusted to a given normality or molarity, by dilution or by addition of the appropriate reagent.

The concentration of the volumetric solution does not differ from the prescribed one by more than 10%. The correction factor is determined by an appropriate number of replicates. The correction factor should be redetermined frequently.

1.4 Blank determinations: Where it is directed that "any necessary correction" be made by a blank determination, the determination is to be conducted with the use of the same

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quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

2. PREPARATION AND STANDARDIZATION

2.1 Scope

When solutions of a reagent are used in different concentrations, the details of the preparation and standardization are usually given for the concentration most frequently required. Stronger or weaker solutions are prepared and standardized in the same general manner as described, using proportionate amounts of the reagent.

2.2 Preparation by dilution

It is possible in many instances to prepare lower concentrations accurately by making an exact dilution of a stronger solution.

Volumetric solutions prepared by dilution should be restandardized as directed for the stronger solution, using proportionate amounts of reagents.

Dilute solutions that are not stable, as, for instance, potassium permanganate 0.01 N, are preferably prepared by exactly diluting the higher normality with thoroughly boiled and cooled water on the same day they are required for use.

2.3 Standardization

The following directions give only one method for standardization, but other methods of standardization, capable of yielding at least the same degree of accuracy, may be used.

The values obtained in the standardization of volumetric solutions are valid for all Pharmacopeial uses of these solutions, regardless of the instrumental or chemical indicators used in the individual monographs.

Where the apparent normality or molarity of a titrant depends upon the special conditions of its use, the individual monograph sets forth the directions for standardizing the reagent in the specified context.

Primary standards are reagents that are extremely pure, stable, and have no waters of hydration. They are established by organizations such as the National Institute of Standards and Technology (NIST, www.nist.gov) in the United States, National Physical Laboratory (NPL, www.npl.co.uk) in the United Kingdom, etc. Some examples of primary standards are sodium carbonate, tris-(hydroxymethyl)aminomethane (TRIS or THAM), sodium chloride, potassium dichromate, and sodium tartrate. Primary standards that are used for the standardization of volumetric solutions are also known as volumetric standards. It is acceptable to use volumetric standards provided by other organizations as far as they are traceable to the appropriate primary standard.

For those salts that usually are available as certified primary standards or that are available as highly purified salts of primary standard quality, it is permissible to prepare solutions by accurately weighing a suitable quantity of the salt and dissolving it to produce a specific volume of solution of known concentration.

2.4 Temperature

All volumetric solutions, if practicable, are to be prepared, standardized, and used at the standard temperature of 25°.

If a titration is carried out with the volumetric solution at a markedly different temperature, standardize the volumetric solution used as the titrant at that different temperature, or make a suitable temperature correction.

2 N Acetic Acid VS $C_2H_4O_2$**,— 60.05**

120.10 g in 1000 mL

Add 116 mL of glacial acetic acid to sufficient water to make 1000 mL after cooling to room temperature.

Add the following:

▲0.2 N Alcoholic Potassium Hydroxide VS, KOH**—56.11**

11.22 g in 1000 mL

Dissolve about 14 g of potassium hydroxide in 20 mL of water, and add aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant into a suitable, tight container. Store in tightly stoppered bottles, protected from light.

Standardization: Accurately measure 10 mL of 0.5 N hydrochloric acid VS. Dilute with 50 mL of water, add 2 drops of phenolphthalein TS, and titrate with the alcoholic potassium hydroxide solution until a permanent pale pink color is produced.

$$N = \frac{\text{mL HCl} \times \text{N HCl}}{\text{mL KOH}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not necessary.▲ (USP 1-May-2019)]

Change to read:

0.1 N Ammonium Thiocyanate VS NH_4SCN**,— 76.12**

7.612 g in 1000 mL

Dissolve about 8 g of ammonium thiocyanate in 1000 mL of water.

Standardization: Accurately measure about 30 mL of 0.1 N silver nitrate VS into a glass-stoppered flask. Dilute with 50 mL of water, then add 2 mL of nitric acid and ▲titrate with the ammonium thiocyanate solution potentiometrically using a combined silver electrode to the first endpoint.▲ (USP 1-Aug-2019)

$$N = \frac{\text{mL } AgNO_3 \times \text{N } AgNO_3}{\text{mL } NH_4SCN \text{ Solution}}$$

If desirable, 0.1 N ammonium thiocyanate may be replaced by 0.1 N potassium thiocyanate where the former is directed in various tests and assays.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.05 M Barium Perchlorate VS, $Ba(ClO_4)_2$**—336.2**

Diluted acetic acid solution: Transfer 28.5 mL of glacial acetic acid to a 100-mL volumetric flask. Dilute with water to volume.

Diluted ammonia solution: Transfer 75 mL of stronger ammonia water to a 100-mL volumetric flask. Dilute with water to volume.

Buffer solution pH 3.7: Transfer 15.0 mL of *Diluted acetic acid solution* to a 100-mL volumetric flask. Add 60 mL of alcohol and 20 mL of water. Adjust with *Diluted ammonia solution* to a pH of 3.7. Dilute with water to volume.

Diluted sulfuric acid: Transfer 2.8 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Barium perchlorate solution: Dissolve 15.8 g of barium hydroxide in a mixture of 7.5 mL of perchloric acid and 75 mL of water. Adjust with perchloric acid to a pH of 3 and filter if necessary. Add 150 mL of alcohol and dilute with water to 250 mL. Dilute with *Buffer solution pH 3.7* to 1000 mL.

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Standardization: To 5.0 mL of *Diluted sulfuric acid* add 5 mL of water, 50 mL of *Buffer solution pH 3.7*, and 0.5 mL of sodium alizarinsulfonate TS. Titrate with the *Barium perchlorate solution* until an orange–red color appears. Standardize immediately before use.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Benzethonium Chloride, Two Hundred Fiftieth-Molar (0.004 M) $C_{27}H_{42}ClNO_2$— **448.08**

Dissolve 1.792 g of benzethonium chloride, previously dried at 100°–105° to constant weight, in water to make 1000 mL.

Calculate the molarity of the solution from the content of benzethonium chloride in the dried benzethonium chloride determined as follows. Dissolve 0.350 g of the dried benzethonium chloride in 30 mL of glacial acetic acid and add 6 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS, using 0.05 mL of crystal violet TS as an indicator. Carry out a blank titration. One mL of 0.1 N perchloric acid VS is equivalent to 44.81 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

[NOTE—This solution is commercially available ready to be used. Use a suitable grade.]

0.01 M Bismuth Nitrate VS $Bi(NO_3)_3 \cdot 5H_2O$,—**485.07**

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate

Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of diluted nitric acid, and add 0.01 N nitric acid to make 1000 mL.

Standardization: Accurately measure 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS, and titrate the solution with 0.01 M edetate disodium VS until the red color changes to yellow. Calculate the molarity factor.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Bromine VS Br,— **79.90**

7.990 g in 1000 mL

Dissolve 3 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL.

Standardization: Accurately measure about 25 mL of the solution into a 500-mL iodine flask, and dilute with 120 mL of water. Add 5 mL of hydrochloric acid, insert the stopper in the flask, and shake it gently. Then add 5 mL of potassium iodide TS, again insert the stopper, shake the mixture, allow it to stand for 5 min, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached.

Preserve in dark amber-colored, glass-stoppered bottles.

$$N = \frac{mL\ Na_2S_2O_3 \times N Na_2S_2O_3}{mL\ Br_2\ Solution}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.05 N Ceric Ammonium Nitrate VS $Ce(NO_3)_4 \cdot 2NH_4NO_3$,— **548.22**

2.741 g in 100 mL

Dissolve 2.75 g of ceric ammonium nitrate in 1 N nitric acid to obtain 100 mL of solution, and filter.

Standardization: Accurately measure 10 mL of freshly standardized 0.1 N ferrous ammonium sulfate VS into a flask, and dilute with water to about 100 mL. Add 1 drop of nitrophenanthroline TS, and titrate with the ceric ammonium nitrate solution to a colorless endpoint.

$$N = \frac{mL\ Fe(NH_4)_2(SO_4)_2 \times N\ Fe(NH_4)_2(SO_4)_2}{mL\ Ce(NO_3)_4 \cdot 2NH_4NO_3}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Ceric Sulfate VS $Ce(SO_4)_2$,— **332.24**

Use commercially available volumetric standard solution.

Standardization: Accurately weigh about 0.2 g of sodium oxalate, primary standard, dried according to the label instructions or, if this information is not available, dried at 105° for 2 h, and dissolve in 75 mL of water. Add, with stirring, 2 mL of sulfuric acid that has previously been mixed with 5 mL of water, mix well, add 10 mL of hydrochloric acid, and heat to between 70° and 75°. Titrate with 0.1 N ceric sulfate to a permanent slight yellow color. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.1 N ceric sulfate.

$$N = \frac{mg\ Na_2C_2O_4}{67.00 \times mL\ Ce(SO_4)_2\ solution}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Cupric Nitrate VS $Cu(NO_3)_2 \cdot 2.5H_2O$,—**232.59**

23.26 g in 1000 mL

$Cu(NO_3)_2 \cdot 3H_2O$,—**241.60**

24.16 g in 1000 mL

Dissolve 23.3 g of cupric nitrate 2.5 hydrate, or 24.2 g of the trihydrate, in water to make 1000 mL.

Standardization: Transfer 20.0 mL of the solution to a 250-mL beaker. Add 2 mL of 5 M sodium nitrate, 20 mL of ammonium acetate TS, and sufficient water to make 100 mL. Titrate with 0.05 M edetate disodium VS. Determine the endpoint potentiometrically using a cupric ion-double junction reference electrode system. Perform a blank determination, and make any necessary correction.

$$N = \frac{mL\ edetate\ disodium\ (corrected\ for\ the\ blank) \times M\ edetate\ disodium}{20.0}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Cupric Tartrate, Alkaline, Solution (Fehling's Solution)

Copper solution (Solution A): Transfer 34.639 g of cupric sulfate to a 500-mL volumetric flask, and dissolve in and dilute with water to volume. Filter, if necessary.

Alkaline tartrate solution (Solution B): Transfer 173 g of potassium sodium tartrate and 50 g of sodium hydroxide to a 500-mL volumetric flask, and dissolve in and dilute with water to volume. Filter, if necessary.

Just before use, prepare *Cupric Tartrate, Alkaline, Solution* by mixing equal volumes of *Solution A* and *Solution B*.

• **STANDARDIZATION**

Standard stock solution: Transfer 9.5 g of *Sucrose* to a 1-L volumetric flask, dissolve in 100 mL of water, add 5 mL of hydrochloric acid, and store for 3 days at 20°–25°. Dilute

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with water to volume. This solution is stable for several months.

Invert sugar solution: Immediately before use in standardizing the *Cupric Tartrate, Alkaline, Solution*, transfer 25 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Procedure

Apparatus: Mount a ring support on a ring stand 1–2 inches above a gas burner, and mount a second ring 6–7 inches above the first. Place 6-inch open-wire gauze on the lower ring to support a 400-mL conical flask, and place a 4-inch watch glass with a center hole on the upper ring to deflect heat. Attach a 50-mL buret to the ring stand so that the tip just passes through the watch glass centered above the flask. Place an indirectly lighted white surface behind the assembly for observing the endpoint.

Standardization: Transfer 20.0 mL of the *Cupric Tartrate, Alkaline, Solution* to a 400-mL flask containing a few boiling chips, and add 15 mL of water and 39.0 mL of *Invert sugar solution*. Mix by swirling at ambient temperature, and immediately place the flask on the wire gauze of the *Apparatus*. Adjust the burner so that the boiling point of the solution is reached in about 2 min. Boil gently but steadily for 2 min. As boiling continues, add 3–4 drops of methylene blue solution (1 in 100). Complete the titration within 1 min by adding the *Invert sugar solution* dropwise until the blue color disappears. Allow a 5-s reaction time between drops at the end of titration. Adjust the *Cupric Tartrate, Alkaline, Solution* for the correct amount of copper (equivalent to 100 mg of invert sugar), and restandardize if the total volume of *Invert sugar solution* is more or less than 40.0 mL.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Standard Dichlorophenol–Indophenol Solution

To 50 mg of 2,6-dichlorophenol–indophenol sodium that has been stored in a desiccator over soda lime add 50 mL of water containing 42 mg of sodium bicarbonate, shake vigorously, and when the dye is dissolved, add water to make 200 mL. Filter into an amber, glass-stoppered bottle. Use within 3 days and standardize immediately before use.

Standardization: Accurately weigh 50 mg of USP Ascorbic Acid RS, and transfer to a glass-stoppered, 50-mL volumetric flask with the aid of a sufficient volume of metaphosphoric–acetic acids TS to make 50 mL. Immediately transfer 2 mL of the ascorbic acid solution to a 50-mL conical flask containing 5 mL of the metaphosphoric–acetic acids TS, and titrate rapidly with the dichlorophenol–indophenol solution until a distinct rose-pink color persists for at least 5 s. Perform a blank titration by titrating 7 mL of the metaphosphoric–acetic acids TS plus a volume of water equal to the volume of the dichlorophenol solution used in titrating the ascorbic acid solution. Express the concentration of the standard solution in terms of its equivalent in mg of ascorbic acid.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Add the following:

▲0.002 M Edetate Disodium VS $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$—372.24

0.0744 g in 1000 mL

Transfer 20 mL of 0.1 M edetate disodium VS to a 1000-mL volumetric flask. Dilute with water to volume.▲ (USP 1-May-2019)

Change to read:

0.01 M Edetate Disodium VS $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$—372.24

3.722 g in 1000 mL

Transfer 100 mL of 0.1 M edetate disodium VS to a 1000-mL volumetric flask. Dilute with water to volume.

Standardization: Accurately weigh about 40 mg of chelometric standard calcium carbonate, previously dried at ▲110°▲ (ERR 1-Jun-2019) for 2 h and cooled in a desiccator, or dried according to the label instructions, transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 4 mL of diluted hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with water, and dilute with water to about 200 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Adjust the solution with sodium hydroxide TS to a pH of 12–13, add 300 mg of hydroxy naphthol blue, and continue the titration with edetate disodium solution to a blue endpoint.

$$M = \frac{(g\ CaCO_3)(1000)}{100.09 \times mL\ EDTA}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.02 M Edetate Disodium VS $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$—372.24

0.7444 g in 1000 mL

Transfer 200 mL of 0.1 M edetate disodium VS to a 1000-mL volumetric flask. Dilute with water to volume.

Standardization: Accurately weigh about 80 mg of chelometric standard calcium carbonate, previously dried at ▲110°▲ (ERR 1-Jun-2019) for 2 h and cooled in a desiccator, or dried according to the label instructions, transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 4 mL of diluted hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with water, and dilute with water to about 200 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Adjust the solution to a pH of 12–13 with sodium hydroxide TS, add 300 mg of hydroxy naphthol blue, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(g\ CaCO_3)(1000)}{100.09 \times mL\ EDTA}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.05 M Edetate Disodium VS $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, —372.24

18.61 g in 1000 mL

Dissolve 18.6 g of edetate disodium in water to make 1000 mL.

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Standardization: Accurately weigh about 200 mg of chelometric standard calcium carbonate, previously dried according to the label instructions or, if this information is not available, at 110° for 2 h, transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of diluted hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with water, and dilute with water to about 100 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Add 15 mL of sodium hydroxide TS and 300 mg of hydroxy naphthol blue, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(g\ CaCO_3)(1000)}{100.09 \times mL\ EDTA}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Edetate Disodium VS $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ **—372.24**

37.22 g in 1000 mL

Dissolve 37.22 g of edetate disodium in water to make 1000 mL.

Standardization: Accurately weigh about 400 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 h and cooled in a desiccator, or dried according to the label instructions, transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 4 mL of diluted hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass with water, and dilute with water to about 200 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the edetate disodium solution from a 50-mL buret. Adjust the solution to a pH of 12–13 with sodium hydroxide TS, add 300 mg of hydroxy naphthol blue, and continue the titration with the edetate disodium solution to a blue endpoint.

$$M = \frac{(g\ CaCO_3)(1000)}{100.09 \times mL\ EDTA}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Ferric Ammonium Sulfate VS $FeNH_4(SO_4)_2 \cdot 12H_2O$, **—482.19**

48.22 g in 1000 mL

Dissolve 50 g of ferric ammonium sulfate in a mixture of 300 mL of water and 6 mL of sulfuric acid, dilute with water to 1000 mL, and mix.

Standardization: Accurately measure about 40 mL of the solution into a glass-stoppered flask, add 5 mL of hydrochloric acid, mix, and add a solution of 3 g of potassium iodide in 10 mL of water. Insert the stopper, allow to stand for 10 min, and then titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents.

Store in tight containers, protected from light.

$$N = \frac{mL\ Na_2S_2O_3 \times N\ Na_2S_2O_3}{mL\ FeNH_4(SO_4)_2}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Ferrous Ammonium Sulfate VS $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, **—392.14**

39.21 g in 1000 mL

Dissolve 40 g of ferrous ammonium sulfate in a previously cooled mixture of 40 mL of sulfuric acid and 200 mL of water, dilute with water to 1000 mL, and mix.

Standardization (carried out on the day of use): Accurately measure 25–30 mL of the solution into a flask, add 2 drops of orthophenanthroline TS, and titrate with 0.1 N ceric sulfate VS until the red color is changed to pale blue.

$$N = \frac{mL\ Ce^{IV} \times N\ Ce^{IV}}{mL\ Fe^{II}\ Solution}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.07 N Ferrous Ammonium Sulfate VS $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, **— 392.14**

27.5 g in 1000 mL

In a 1000-mL volumetric flask, dissolve 27.5 g of ferrous ammonium sulfate in 500 mL of water. Add 20 mL of sulfuric acid. Cool and dilute with water to volume.

Standardize this solution on the day of use as follows. Dilute 25 mL of 0.025 N potassium dichromate VS with water to 100 mL. Add 30 mL of sulfuric acid and cool to room temperature. Add 3 drops of ferroin TS and titrate with 0.07 N ferrous ammonium sulfate VS. The color change is sharp, going from blue-green to reddish brown.

$$N = \frac{mL\ K_2Cr_2O_7 \times NK_2Cr_2O_7}{mL\ Fe(NH_4)_2SO_4)_2}$$

Add the following:

▲0.01 N Hydrochloric Acid VS HCl**—36.46**

0.3646 g in 1000 mL

Transfer 100 mL of 0.1 N hydrochloric acid VS to a 1000-mL volumetric flask. Dilute with water to volume.

Standardization: Accurately weigh about 250 mg of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water. Determine the endpoint potentiometrically. Each 1.2114 mg of tromethamine is equivalent to 1 mL of 0.01 hydrochloric acid.

$$N = \frac{mg\ tromethamine}{121.14 \times mL\ HCl}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.▲ (USP 1-May-2019)]

0.02 N Hydrochloric Acid VS HCl, **—36.46**

729.2 mg in 1000 mL

Reagents

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Transfer 1.6 mL of hydrochloric acid to a 1000-mL volumetric flask containing about 250 mL of water. Cool and dilute to volume with water.

Alternatively, dilute 20 mL of 1 N hydrochloric acid VS to 1000 mL with water.

Standardization: Accurately weigh about 50 mg of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h, and dissolve in 50 mL of water. Determine the endpoint potentiometrically. Each 2.4228 mg of tromethamine is equivalent to 1 mL of 0.02 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Hydrochloric Acid VS, HCl, **—36.46**

3.646 g in 1000 mL

Dilute 8.5 mL of hydrochloric acid with water to 1000 mL.

Standardization: Accurately weigh about 0.5 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water, and add 2 drops of bromocresol green TS. Titrate with 0.1 N hydrochloric acid to a pale yellow endpoint. Each 12.114 mg of tromethamine is equivalent to 1 mL of 0.1 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.5 N Hydrochloric Acid VS HCl, **—36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of water slowly add 43 mL of hydrochloric acid. Cool, and add water to volume.

Standardization: Accurately weigh about 2.5 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water, and add 2 drops of bromocresol green TS. Titrate with 0.5 N hydrochloric acid to a pale yellow endpoint. Each 60.57 mg of tromethamine is equivalent to 1 mL of 0.5 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

1 N Hydrochloric Acid VS HCl, **—36.46**

36.46 g in 1000 mL

Dilute 85 mL of hydrochloric acid with water to 1000 mL.

Standardization: Accurately weigh about 5.0 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water, and add 2 drops of bromocresol green TS. Titrate with 1 N hydrochloric acid to a pale yellow endpoint. Each 121.14 mg of tromethamine is equivalent to 1 mL of 1 N hydrochloric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Alcoholic Hydrochloric Acid VS HCl,— **36.46**

Dilute 9.0 mL of hydrochloric acid to 1000 mL with aldehyde-free alcohol.

0.5 N Hydrochloric Acid in Methanol VS HCl, **—36.46**

18.23 g in 1000 mL

To a 1000-mL volumetric flask containing 40 mL of water slowly add 43 mL of hydrochloric acid. Cool, and add methanol to volume.

Standardization: Accurately weigh about 2.5 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water and add 2 drops of bromocresol green TS. Titrate with 0.5 N hydrochloric acid in methanol to a pale yellow endpoint. Each 60.57 mg of tromethamine is equivalent to 1 mL of 0.5 N hydrochloric acid in methanol.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL HCl}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.01 N Iodine VS I, **—126.90**

1.269 g in 1000 mL

Dissolve about 1.4 g of iodine in a solution of 3.6 g of potassium iodide in 100 mL of water, add 3 drops of hydrochloric acid, and dilute with water to 1000 mL.

Standardization: Transfer 100.0 mL of iodine solution to a 250-mL flask, add 1 mL of 1 N hydrochloric acid, swirl gently to mix, and titrate with 0.1 N sodium thiosulfate VS until the solution has a pale yellow color. Add 2 mL of starch TS, and continue titrating until the solution is colorless.

Preserve in amber-colored, glass-stoppered bottles.

$$N = \frac{\text{mL } Na_2S_2O_3 \times \text{N } Na_2S_2O_3}{100}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.05 N Iodine VS I, **—126.90**

6.33 g in 1000 mL

Dissolve about 6.5 g of iodine in a solution of 18 g of potassium iodide in 100 mL of water, add 3 drops of hydrochloric acid, and dilute with water to 1000 mL.

Standardization: Transfer 50.0 mL of the iodine solution to a 250-mL flask, dilute with water to 100 mL, add 1 mL of 1 N hydrochloric acid, swirl gently to mix, and titrate with 0.1 N sodium thiosulfate VS until the solution has a pale yellow color. Add 2 mL of starch TS, and continue titrating until the solution is colorless.

$$N = \frac{\text{mL } Na_2S_2O_3 \times \text{N } Na_2S_2O_3}{50}$$

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[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Iodine VS I, **—126.90**

12.69 g in 1000 mL

Dissolve about 14 g of iodine in a solution of 36 g of potassium iodide in 100 mL of water, add 3 drops of hydrochloric acid, and dilute with water to 1000 mL.

Standardization: Transfer 25.0 mL of the iodine solution to a 250-mL flask, dilute with water to 100 mL, add 1 mL of 1 N hydrochloric acid, swirl gently to mix, and titrate with 0.1 N sodium thiosulfate VS until the solution has a pale yellow color. Add 2 mL of starch TS and continue titrating until the solution is colorless.

Preserve in amber-colored, glass-stoppered bottles.

$$N = \frac{mL\ Na_2S_2O_3 \times N\ Na_2S_2O_3}{25}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.01 M Lead Nitrate VS, $Pb(NO_3)_2$, **—331.21**

3.312 g in 1000 mL

Xylenol Orange Triturate: Triturate 1 part of xylenol orange with 99 parts of potassium nitrate.

0.1 M Lead Nitrate: Dissolve 33 g of lead nitrate in 1000 mL of water.

Standardization: To 20.0 mL of the lead nitrate solution add 300 mL of water. Add about 50 mg of *Xylenol Orange Triturate*, ▲if the solution is not violet-pink,▲ (USP 1-May-2019) and add methenamine until the solution becomes violet-pink. Titrate with 0.1 M edetate disodium VS to the yellow endpoint. Calculate the molarity.

Dilute 50.0 mL of *0.1 M Lead Nitrate* to 500.0 mL with water.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.01 M Lead Perchlorate VS $Pb(ClO_4)_2$, **— 406.10**

Accurately pipet 100 mL of commercially available 0.1 M lead perchlorate solution into a 1000-mL volumetric flask, and dilute with water to volume.

Standardization: Accurately pipet 50 mL of 0.01 M lead perchlorate solution into a 250-mL conical flask. Add 3 mL of aqueous hexamethylenetetramine solution (2.0 g per 100 mL) and 4 drops of 0.5% xylenol orange indicator prepared by adding 500 mg of xylenol orange to 10 mL of alcohol and diluting with water to 100 mL. Titrate with 0.05 M edetate disodium VS to a yellow endpoint.

$$M = \frac{mL\ edetate\ disodium \times M\ edetate\ disodium}{50.0}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Lead Perchlorate VS $Pb(ClO_4)_2 \cdot 3H_2O$, **—460.15**

46.01 g in 1000 mL

Dissolve 46 g of lead perchlorate in water, and dilute with water to 1000.0 mL.

Standardization: Accurately weigh about 150 mg of sodium sulfate, previously dried at 105° for 4 h, and dissolve in 50 mL of water. Add 50 mL of a mixture of water and formaldehyde (1:1), and stir for about 1 min. Determine the endpoint potentiometrically using a lead ion selective electrode. Perform a blank determination, and make any necessary corrections. Each 14.204 mg of sodium sulfate is equivalent to 1 mL of 0.1 M lead perchlorate.

$$M = \frac{mg\ sodium\ sulfate}{142.04 \times mL\ lead\ perchlorate}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Lithium Methoxide in Chlorobenzene VS CH_3OLi, **— 37.97**

3.798 g in 1000 mL

Dissolve 700 mg of freshly cut lithium metal in 150 mL of methanol, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of chlorobenzene. If cloudiness or precipitation occurs, add sufficient methanol to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture.

Standardization: Accurately weigh about 400 mg of primary standard benzoic acid, and dissolve in 80 mL of dimethylformamide in a flask. Add 3 drops of a 1-in-100 solution of thymol blue in dimethylformamide, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the dimethylformamide. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide. [NOTE—Restandardize the solution frequently.]

$$N = \frac{mg\ benzoic\ acid}{122.12 \times mL\ lithium\ methoxide\ (corrected\ for\ the\ blank)}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.02 N Lithium Methoxide in Methanol VS CH_3LiO, **— 37.97**

759.6 mg in 1000 mL

Dissolve 0.12 g of freshly cut lithium metal in 150 mL of methanol, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of methanol, and mix. Store the solution preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture.

Standardization: Accurately weigh about 100 mg of primary standard benzoic acid, and dissolve in 80 mL of dimethylformamide in a flask. Add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the dimethylformamide.

Each 2.442 mg of benzoic acid is equivalent to 1 mL of 0.02 N lithium methoxide. [NOTE—Restandardize the solution frequently.]

$$N = \frac{mg\ benzoic\ acid}{122.12 \times mL\ lithium\ methoxide\ (corrected\ for\ the\ blank)}$$

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[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Lithium Methoxide in Methanol VS CH_3OLi, **—37.97**
3.798 g in 1000 mL

Dissolve 700 mg of freshly cut lithium metal in 150 mL of methanol, cooling the flask during addition of the metal. When the reaction is complete, add 850 mL of methanol. If cloudiness or precipitation occurs, add sufficient methanol to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture.

Standardization: Accurately weigh about 400 mg of primary standard benzoic acid, and dissolve in 80 mL of dimethylformamide in a flask. Add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the dimethylformamide. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide. [NOTE—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Lithium Methoxide in Toluene VS CH_3OLi, **—37.97**
3.798 g in 1000 mL

Dissolve 700 mg of freshly cut lithium metal in 150 mL of methanol, cooling the flask during addition of the metal. When reaction is complete, add 850 mL of toluene. If cloudiness or precipitation occurs, add sufficient methanol to clarify the solution. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture.

Standardization: Accurately weigh about 400 mg of primary standard benzoic acid, and dissolve in 80 mL of dimethylformamide in a flask. Add 3 drops of a 1-in-100 solution of thymol blue in dimethylformamide, and titrate with the lithium methoxide solution to a blue endpoint. Correct for the volume of the lithium methoxide solution consumed by 80 mL of the dimethylformamide. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N lithium methoxide.

[NOTE—Restandardize the solution frequently.]

$$N = \frac{\text{mg benzoic acid}}{122.12 \times \text{mL lithium methoxide (corrected for the blank)}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.01 M Magnesium Chloride VS $MgCl_2 \cdot 6H_2O$, **—203.30**
2.0330 g in 1000 mL

Dissolve about 2.04 g of magnesium chloride in 1000 mL of freshly boiled and cooled water.

Standardization: Accurately measure 25 mL of the prepared magnesium chloride solution. Add 50 mL of water, 3 mL of ammonia–ammonium chloride buffer TS and 0.04 g of eriochrome black T–sodium chloride reagent. Titrate with 0.05 M edetate disodium VS until the red-purple color of the solution changes to blue-purple.

$$M = \frac{\text{(mL edetate disodium VS) x (M edetate disodium)}}{\text{mL magnesium chloride}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Mercuric Nitrate VS $Hg(NO_3)_2$, **—324.60**
32.46 g in 1000 mL

Dissolve about 35 g of mercuric nitrate in a mixture of 5 mL of nitric acid and 500 mL of water, and dilute with water to 1000 mL.

Standardization: Transfer an accurately measured volume of about 20 mL of the solution to a conical flask, and add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate TS. Cool to below 20°, and titrate with 0.1 N ammonium thiocyanate VS to the first appearance of a permanent brownish color.

$$M = \frac{\text{mL } NH_4SCN \text{ x N } NH_4SCN}{\text{mL } Hg(NO_3)_2 \text{ x } 2}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Oxalic Acid VS $H_2C_2O_4 \cdot 2H_2O$, **—126.07**
6.303 g in 1000 mL

Dissolve 6.45 g of oxalic acid in water to make 1000 mL.

Standardization: Transfer 10 mL of oxalic acid solution to an appropriate flask and titrate with freshly standardized 0.1 N potassium permanganate VS until a pale pink color, which persists for 15 s, is produced.

Preserve in glass-stoppered bottles, protected from light.

$$N = \frac{\text{mL } KMnO_4 \text{ x N } KMnO_4}{\text{mL } H_2C_2O_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Perchloric Acid in Dioxane VS $HClO_4$, **—100.46**
10.05 g in 1000 mL

Mix 8.5 mL of perchloric acid with sufficient dioxane to make 1000 mL.

Standardization: Accurately weigh about 700 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to bluish green. Carry out a blank determination. Each 20.422 mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

$$N = \frac{\text{g } KHC_8H_4O_4}{0.20422 \times \text{mL } HClO_4 \text{ solution (corrected for the blank)}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Perchloric Acid in Glacial Acetic Acid VS $HClO_4$, **—100.46**
10.05 g in 1000 mL

[NOTE—Where called for in the tests and assays, this volumetric solution is specified as "0.1 N perchloric acid."

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Thus, where 0.1 N or other strength of this volumetric solution is specified, the solution in glacial acetic acid is to be used, unless the words "in dioxane" are stated. (See also 0.1 N Perchloric Acid in Dioxane VS.)]

Mix 8.5 mL of perchloric acid with 500 mL of glacial acetic acid and 21 mL of acetic anhydride, cool, and add glacial acetic acid to make 1000 mL. Alternatively, the solution may be prepared as follows. Mix 11 mL of 60% perchloric acid with 500 mL of glacial acetic acid and 30 mL of acetic anhydride, cool, and add glacial acetic acid to make 1000 mL.

Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by *Method I* (see *Water Determination* ⟨921⟩), except to use a test specimen of about 5 g of the 0.1 N perchloric acid that is expected to contain approximately 1 mg of water and the *Reagent* (see *Reagent* in *Water Determination* ⟨921⟩, *Method Ia*) diluted such that 1 mL is equivalent to about 1–2 mg of water. If the water content exceeds 0.5%, add more acetic anhydride. If the solution contains no titratable water, add sufficient water to obtain a content of between 0.02% and 0.5% of water. Allow the solution to stand for 1 day, and again titrate the water content. The solution so obtained contains between 0.02% and 0.5% of water, indicating freedom from acetic anhydride.

Standardization: Accurately weigh about 700 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve it in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to blue-green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid. Each 20.422 mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

$$N = \frac{\text{g } KHC_8H_4O_4}{0.20422 \times \text{mL } HClO_4 \text{ (corrected for the blank)}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.1 N Potassium Arsenite VS $KAsO_2$**,—146.02**

7.301 g in 1000 mL

▲▲ (USP 1-Aug-2019)

Dissolve 4.9455 g of arsenic trioxide primary standard, dried according to the label instructions or, if this information is not available, dried at 105° for 1 h, in 75 mL of 1 N potassium hydroxide. Add 40 g of potassium bicarbonate, dissolved in about 200 mL of water, and dilute with water to 1000.0 mL.

▲▲ (USP 1-Aug-2019)

0.1 N Potassium Bromate VS $KBrO_3$**, —167.00**

2.784 g in 1000 mL

Dissolve 2.784 g of potassium bromate in water to make 1000 mL.

Standardization: Transfer an accurately measured volume of about 40 mL of the solution to a glass-stoppered flask, add 3 g of potassium iodide, and follow with 3 mL of hydrochloric acid. Allow to stand for 5 min, then titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents, and calculate the normality.

$$N = \frac{\text{mL } Na_2S_2O_3 \text{ x N } Na_2S_2O_3}{\text{mL } KBrO_3 \text{ Solution}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Potassium Bromide–Bromate VS

Dissolve 2.78 g of potassium bromate ($KBrO_3$) and 12.0 g of potassium bromide (KBr) in water, and dilute with water to 1000 mL.

Standardization: Transfer an accurately measured volume of about 40 mL of the solution to a glass-stoppered flask, add 3 g of potassium iodide, and follow with 3 mL of hydrochloric acid. Allow to stand for 5 min, then titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents, and calculate the normality.

$$N = \frac{\text{mL } Na_2S_2O_3 \text{ x N } Na_2S_2O_3}{\text{mL } KBrO_3/KBr}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Potassium Dichromate VS $K_2Cr_2O_7$**, —294.18**

4.903 g in 1000 mL

Dissolve about 5 g of potassium dichromate in 1000 mL of water.

Standardization: Transfer 25.0 mL of this solution to a glass-stoppered, 500-mL flask, add 2 g of potassium iodide (free from iodate), dilute with 200 mL of water, add 5 mL of hydrochloric acid, allow to stand for 10 min in a dark place, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Carry out a blank determination.

$$N = \frac{\text{mL } Na_2S_2O_3 \text{ x N } Na_2S_2O_3}{25.0}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.025 N Potassium Dichromate VS $K_2Cr_2O_7$**— 294.18**

1.2259 g in 1000 mL

Transfer 12.259 g of potassium dichromate primary standard, previously dried according to the label instructions or, if this information is not available, dried at 103° for 2 h, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask and dilute with water to volume.

0.05 M Potassium Ferricyanide VS $K_3Fe(CN)_6$**, —329.24**

16.46 g in 1000 mL

Dissolve about 17 g of potassium ferricyanide in water to make 1000 mL.

Standardization: Transfer 50.0 mL of this solution to a glass-stoppered, 500-mL flask, dilute with 50 mL of water, add 10 mL of potassium iodide TS and 10 mL of dilute hydrochloric acid, and allow to stand for 1 min. Then add 15 mL of zinc sulfate solution (1 in 10), and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached.

Protect from light, and restandardize before use.

$$M = \frac{\text{mL } Na_2S_2O_3 \text{ x N } Na_2S_2O_3}{50.0}$$

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[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Alcoholic Potassium Hydroxide VS KOH, **—56.11**

Dilute 20 mL of 0.5 N alcoholic potassium hydroxide to 100.0 mL with aldehyde-free alcohol.

0.5 N Alcoholic Potassium Hydroxide VS

28.06 g in 1000 mL

Dissolve about 34 g of potassium hydroxide in 20 mL of water, and add aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant into a suitable, tight container.

Standardization: Accurately measure about 25 mL of 0.5 N hydrochloric acid VS. Dilute with 50 mL of water, add 2 drops of phenolphthalein TS, and titrate with the alcoholic potassium hydroxide solution until a permanent, pale pink color is produced. [NOTE—Store in tightly stoppered bottles, protected from light.]

$$N = \frac{\text{mL HCl x N HCl}}{\text{mL KOH}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Methanolic Potassium Hydroxide VS

5.612 g in 1000 mL

Dissolve about 6.8 g of potassium hydroxide in 4 mL of water, and add methanol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant into a suitable, tight container.

Standardization: Accurately measure about 25 mL of 0.1 N hydrochloric acid VS. Dilute with 50 mL of water, add 2 drops of phenolphthalein TS, and titrate with the methanolic potassium hydroxide solution until a permanent, pale pink color is produced. [NOTE—Store in tightly stoppered bottles, protected from light.]

$$N = \frac{\text{mL HCl x N HCl}}{\text{mL KOH}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.1 N Potassium Hydroxide VS

Transfer 100 mL of ▲*1 N Potassium Hydroxide VS*▲ (ERR 1-May-2018) to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.

Standardization: Add 2 drops of phenolphthalein TS to 20 mL of 0.1 N potassium hydroxide VS. Titrate with 0.1 N hydrochloric acid VS until a permanent pale-pink color is produced.

$$N = \frac{\text{mL HCl x N HCl}}{\text{mL KOH}}$$

[NOTE—It this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

1 N Potassium Hydroxide VS KOH, **—56.11**

56.11 g in 1000 mL

Dissolve 68 g of potassium hydroxide in about 950 mL of water. Add a freshly prepared saturated solution of barium hydroxide until no more precipitate forms. Shake the mixture thoroughly, and allow it to stand overnight in a stoppered bottle. Decant the clear liquid, or filter the solution in a tight, polyolefin bottle.

• **STANDARDIZATION:** Accurately weigh about 5 g of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve in 75 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with the potassium hydroxide solution to the production of a permanent pink color. Each 204.22 mg of potassium biphthalate is equivalent to 1 mL of 1 N potassium hydroxide

$$N = \frac{\text{g } KHC_8H_4O_4}{0.20422 \times \text{mL KOH}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.05 M Potassium Iodate VS KIO_3, **—214.00**

10.70 g in 1000 mL

Dissolve 10.700 g of potassium iodate, previously dried at 110° to constant weight, in water to make 1000.0 mL.

Standardization: To 15.0 mL of solution in a 250-mL iodine flask, add 3 g of potassium iodide and 3 mL of hydrochloric acid previously diluted with 10 mL of water. Stopper immediately, and allow to stand in the dark for 5 min. Then add 50 mL of cold water, and titrate the liberated iodine with freshly standardized 0.1 N sodium thiosulfate. Add 3 mL of starch indicator solution near the end of the titration, and continue to the absence of the blue-starch-iodine complex.

$$M = \frac{\text{mL x N } Na_2S_2O_3}{\text{mL } KIO_3 \text{ x } 6}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Potassium Permanganate VS $KMnO_4$, **—158.03**

3.161 g in 1000 mL

Dissolve about 3.3 g of potassium permanganate in 1000 mL of water in a flask, and boil the solution for about 15 min. Insert the stopper in the flask, allow it to stand for at least 2 days, and filter through a fine-porosity, sintered-glass crucible. If necessary, the bottom of the sintered-glass crucible may be lined with a pledget of glass wool.

Standardization: Accurately weigh about 200 mg of primary standard sodium oxalate, dried according to the instructions on its label, and dissolve it in 250 mL of water. Add 7 mL of sulfuric acid, heat to about 70°, and then slowly add the permanganate solution from a buret, with constant stirring, until a pale pink color, which persists for 15 s, is produced. The temperature at the conclusion of the titration should be NLT 60°. Calculate the normality. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.1 N potassium permanganate.

Since potassium permanganate is reduced on contact with organic substances such as rubber, the solution must be handled in an apparatus made entirely of glass or other suitably inert material. It should be frequently restandardized. Store in glass-stoppered, amber-colored bottles.

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$$N = \frac{g\ Na_2C_2O_4}{mL\ KMnO_4\ solution \times 0.06700}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.02 M Potassium Permanganate VS—Use *0.1 N Potassium Permanganate VS*.

0.1 N Potassium Thiocyanate VS KSCN, **— 97.18**
9.72 g in 1000 mL
Weigh exactly 9.72 g of potassium thiocyanate, previously dried at 110° for 2 h, transfer to a 1-L volumetric flask, dilute with water to volume, and mix well.
Standardization: Transfer 40.0 mL of freshly standardized 0.1 N silver nitrate VS to a 250-mL Erlenmeyer flask; add 100 mL of water, 1 mL of nitric acid, and 2 mL of ferric ammonium sulfate TS. Titrate with the potassium thiocyanate solution, with agitation, to a permanent light pinkish-brown color of the supernatant.

$$N = \frac{mL \times N\ AgNO_3}{mL\ KSCN}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Silver Nitrate VS $AgNO_3$,**—169.87**
16.99 g in 1000 mL
Dissolve about 17.5 g of silver nitrate in 1000 mL of water.
Standardization: Transfer about 100 mg, accurately weighed, of primary or volumetric standard sodium chloride, dried according to the label instructions or, if this information is not available, dried at 110° for 2 h, to a 150-mL beaker, dissolve in 5 mL of water, and add 5 mL of acetic acid, 50 mL of methanol, and about 0.5 mL of eosin Y TS. Stir, preferably with a magnetic stirrer, and titrate with the silver nitrate solution.

$$N = \frac{mg\ NaCl}{mL\ AgNO_3 \times 58.44}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.05 N Silver Nitrate VS, $AgNO_3$,**—169.87**
8.49 in 1000 mL
Dissolve 8.75 g of silver nitrate in 1000 mL of water.
Standardization: Transfer about 50 mg, accurately weighed, of ▲primary or volumetric standard▲ (USP 1-May-2019) sodium chloride, ▲dried according to the label instructions or, if this information is not available,▲ (USP 1-May-2019) dried at 110° for 2 h, to a 150-mL beaker. Dissolve in 5 mL of water and add 5 mL of acetic acid, 50 mL of methanol, and about 0.5 mL of eosin Y TS. Stir, preferably with a magnetic stirrer, and titrate with the silver nitrate solution.

$$N = \frac{mg\ NaCl}{mL\ AgNO_3 \times 58.44}$$

0.002 N Silver Nitrate in Isopropyl Alcohol VS $AgNO_3$**—169.87**
0.3397 g in 1000 mL
Transfer 20.0 mL of 0.1 N silver nitrate VS to a 1000-mL volumetric flask, and dilute with isopropyl alcohol to volume. Store in a low-actinic flask.

0.05 M Sodium Arsenite VS $NaAsO_2$, **—129.91**
6.496 g in 1000 mL
Transfer 4.9455 g of arsenic trioxide, which has been pulverized and dried at 100° to constant weight, to a 1000-mL volumetric flask, dissolve it in 40 mL of 1 N sodium hydroxide, and add 1 N sulfuric acid or 1 N hydrochloric acid until the solution is neutral or only slightly acid to litmus. Add 30 g of sodium bicarbonate, dilute with water to volume, and mix.

Add the following:

▲0.025 N Sodium Hydroxide VS, NaOH,**—40**
1 g in 1000 mL
Transfer 25 mL of 1 N sodium hydroxide VS to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.
Standardization: Accurately weigh about 125 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve in 75 mL of carbon dioxide-free water. Determine the endpoint potentiometrically. Each 5.1055 mg of potassium biphthalate is equivalent to 1 mL of 0.025 N sodium hydroxide.

$$N = \frac{g\ KHC_8H_4O_4}{0.20422 \times mL\ NaOH\ solution}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.▲ (USP 1-May-2019)]

Change to read:

0.01 N Sodium Hydroxide VS
Transfer 10 mL of 1 N sodium hydroxide VS to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.
Standardization: Dissolve about 20 mg of benzoic acid in 15 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with 0.01 N sodium hydroxide VS until a permanent pale pink color is produced. ▲

$$N = \frac{mg\ benzoic\ acid}{122.1 \times mL\ sodium\ hydroxide}$$

▲ (ERR 1-Mar-2019)
[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.5 N Sodium Hydroxide VS
Transfer 500 mL of 1 N sodium hydroxide VS to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.
Standardization: Dissolve about 200 mg of benzoic acid in 15 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS until a permanent pale pink color is produced.

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$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL sodium hydroxide}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

1 N Sodium Hydroxide VS NaOH, **—40.00**
40.00 g in 1000 mL

Dissolve 162 g of sodium hydroxide in 150 mL of carbon dioxide-free water, cool the solution to room temperature, and filter through hardened filter paper. Transfer 54.5 mL of the clear filtrate to a tight, polyolefin container, and dilute with carbon dioxide-free water to 1000 mL.

Standardization: Accurately weigh about 5 g of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve in 75 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with the sodium hydroxide solution to the production of a permanent pink color. Each 204.22 mg of potassium biphthalate is equivalent to 1 mL of 1 N sodium hydroxide.

$$N = \frac{g\ KHC_8H_4O_4}{0.20422 \times \text{mL NaOH solution}}$$

[NOTE—(1) Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should be preserved in bottles having well-fitted, suitable stoppers, provided with a tube filled with a mixture of sodium hydroxide and lime (soda-lime tubes) so that air entering the container must pass through this tube, which will absorb the carbon dioxide. (2) Prepare solutions of lower concentration (e.g., 0.1 N, 0.01 N) by quantitatively diluting accurately measured volumes of the 1 N solution with sufficient carbon dioxide-free water to yield the desired concentration.]

Restandardize the solution frequently.

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Alcoholic Sodium Hydroxide VS NaOH, **—40.00**

To 250 mL of alcohol add 2 mL of a 50% (w/v) solution of sodium hydroxide.

Standardization: Dissolve about 200 mg of benzoic acid, accurately weighed, in 10 mL of alcohol and 2 mL of water. Add 2 drops of phenolphthalein TS, and titrate with the alcoholic sodium hydroxide solution until a permanent pale pink color is produced.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL sodium hydroxide}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.1 N Sodium Hydroxide VS NaOH,**—40.00**
4.0 g in 1000 mL

Transfer 100 mL of 1 N sodium hydroxide VS to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.

Standardization: ▲Standardize by one of the following procedures.

[NOTE—Other standardization procedures may be used. See Volumetric Solutions, Preparation and Standardization, Standardization.]

▲ (USP 1-Aug-2019) Dissolve about 200 mg of benzoic acid in ▲100 mL of carbon dioxide-free water, warm until completely dissolved and cool down to room temperature. ▲ (USP 1-Aug-2019) Add 2 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS until a permanent pale pink color is produced.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL sodium hydroxide}}$$

▲Standardization with potassium biphthalate: Accurately weigh about 500 mg of potassium biphthalate, previously crushed lightly and dried at 120° for 2 h, and dissolve in 75 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to the production of a permanent pink color.

$$N = \frac{g\ KHC_8H_4O_4}{0.20422 \times \text{mL NaOH solution}}$$

▲ (USP 1-Aug-2019)

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.5 N Sodium Methoxide in Methanol VS CH_3ONa, **—54.02**
27.01 g in 1000 mL

Weigh 11.5 g of freshly cut sodium metal, and cut into small cubes. Place about 0.5 mL of anhydrous methanol in a round-bottom, 250-mL flask equipped with a ground-glass joint, add 1 cube of the sodium metal, and, when the reaction has ceased, add the remaining sodium metal to the flask. Connect a water-jacketed condenser to the flask, and slowly add 250 mL of anhydrous methanol, in small portions, through the top of the condenser. Regulate the addition of the methanol so that the vapors are condensed and do not escape through the top of the condenser. After addition of the methanol is complete, connect a drying tube to the top of the condenser, and allow the solution to cool. Transfer the solution to a 1-L volumetric flask, dilute with anhydrous methanol to volume, and mix.

Standardization: Accurately measure about 20 mL of freshly standardized 1 N hydrochloric acid VS into a 250-mL conical flask, add 0.25 mL of phenolphthalein TS, and titrate with the sodium methoxide solution to the first appearance of a permanent pink color.

$$N = \frac{\text{mL HCl} \times \text{N HCl}}{\text{mL sodium methoxide}}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Sodium Methoxide in Toluene VS CH_3ONa, **—54.02**
5.402 g in 1000 mL

Cool in ice water 150 mL of methanol contained in a 1000-mL volumetric flask, and add, in small portions, about 2.5 g of freshly cut sodium metal. When the metal has dissolved, add toluene to make 1000 mL, and mix. Store preferably in the reservoir of an automatic delivery buret suitably protected from carbon dioxide and moisture.

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Standardization: Accurately weigh about 400 mg of primary standard benzoic acid, and dissolve in 80 mL of dimethylformamide in a flask. Add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with the sodium methoxide to a blue endpoint. Correct for the volume of the sodium methoxide solution consumed by 80 mL of the dimethylformamide. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N sodium methoxide.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL sodium methoxide (corrected for the blank)}}$$

[NOTE—(1) To eliminate any turbidity that may form following dilution with toluene, add methanol (25–30 mL usually suffices) until the solution is clear. (2) Restandardize the solution frequently.]

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Sodium Nitrite VS $NaNO_2$, **—69.00**

6.900 g in 1000 mL

Dissolve 6.9 g of sodium nitrite in water to make 1000 mL.

Standardization: Accurately weigh about 500 mg of USP Sulfanilamide RS, previously dried at 105° for 3 h, and transfer to a suitable beaker. Add 20 mL of hydrochloric acid and 50 mL of water, stir until dissolved, and cool to 15°. Maintaining the temperature at about 15°, titrate slowly with the sodium nitrite solution, placing the buret tip below the surface of the solution to preclude air oxidation of the sodium nitrite, and stir the solution gently with a magnetic stirrer, but avoid pulling a vortex of air beneath the surface. Use the indicator specified in the individual monograph, or, if a potentiometric procedure is specified, determine the endpoint electrometrically using platinum–calomel or platinum–platinum electrodes. When the titration is within 1 mL of the endpoint, add the titrant in 0.1-mL portions, and allow 1 min between additions. Each 17.22 mg of sulfanilamide is equivalent to 1 mL of 0.1000 M sodium nitrite.

$$M = \frac{\text{mg of sulfanilamide}}{172.20 \times \text{mL } NaNO_2}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.02 M Sodium Tetraphenylboron VS $NaB(C_6H_5)_4$, **—342.22**

6.845 g in 1000 mL

Dissolve an amount of sodium tetraphenylboron, equivalent to 6.845 g of $NaB(C_6H_5)_4$, in water to make 1000 mL.

Standardization: Pipet two 75-mL portions of the solution into separate beakers, and to each add 1 mL of acetic acid and 25 mL of water. To each beaker add, slowly and with constant stirring, 25 mL of potassium biphthalate solution (1 in 20), and allow to stand for 2 h. Filter one of the mixtures through a filtering crucible, and wash the precipitate with cold water. Transfer the precipitate to a container, add 50 mL of water, shake intermittently for 30 min, filter, and use the filtrate as the saturated potassium tetraphenylborate solution in the following standardization procedure. Filter the second mixture through a tared filtering crucible, and wash the precipitate with three 5-mL portions of saturated potassium tetraphenylborate solution. Dry the precipitate at 105° for 1 h. Each g of potassium tetraphenylborate (KTPB) is equivalent to 955.1 mg of sodium tetraphenylboron.

$$M = \frac{\text{g KTPB} \times 0.9551}{342.22 \times 0.075}$$

[NOTE—Prepare this solution just before use.]

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Add the following:

▲0.002 N Sodium Thiosulfate VS, $Na_2S_2O_3 \cdot 5H2O$**—248.19**

0.49 g in 1000 mL

Transfer 20.0 mL of 0.1 N sodium thiosulfate VS to a 1000-mL volumetric flask. Dilute with water to volume. ▲ (USP 1-May-2019)

0.1 N Sodium Thiosulfate VS $Na_2S_2O_3 \cdot 5H_2O$, **—248.19**

24.82 g in 1000 mL

Dissolve about 26 g of sodium thiosulfate and 200 mg of sodium carbonate in 1000 mL of recently boiled and cooled water.

Standardization: Accurately weigh about 210 mg of primary standard potassium dichromate, previously pulverized and dried according to the label instructions or, if this information is not available, dried at 120° for 4 h, and dissolve in 100 mL of water in a glass-stoppered, 500-mL flask. Swirl to dissolve the solid, remove the stopper, and quickly add 3 g of potassium iodide, 2 g of sodium bicarbonate, and 5 mL of hydrochloric acid. Insert the stopper gently in the flask, swirl to mix, and allow to stand in the dark for exactly 10 min. Rinse the stopper and the inner walls of the flask with water, and titrate the liberated iodine with the sodium thiosulfate solution until the solution is yellowish green in color. Add 3 mL of starch TS, and continue the titration until the blue color is discharged. Perform a blank determination.

Restandardize the solution as frequently as supported by laboratory stability data. In the absence of such data, restandardize the solution weekly.

$$N = \frac{\text{mg } K_2Cr_2O_7}{49.04 \times \text{mL } Na_2S_2O_3}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.01 M Sodium Thiosulfate VS

Transfer 100 mL of 0.1 N sodium thiosulfate VS to a 1000-mL volumetric flask. Dilute with carbon dioxide-free water to volume.

Standardization: Accurately weigh about 21.0 mg of primary standard potassium dichromate, previously pulverized and dried according to the label instructions or, if this information is not available, dried at 120° for 4 h, and dissolve in 100 mL of water in a glass-stoppered, 500-mL flask. Swirl to dissolve the solid, remove the stopper, and quickly add 1 g of potassium iodide, 2 g of sodium bicarbonate, and 5 mL of hydrochloric acid. Insert the stopper gently in the flask, swirl to mix, and allow to stand in the dark for exactly 10 min. Rinse the stopper and the inner walls of the flask with water, and titrate the liberated iodine with 0.01 M sodium thiosulfate VS until the solution is yellowish green in color. Add 3 mL of starch TS, and continue the titration until the blue color is discharged. Perform a blank determination. Restandardize the solution

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as frequently as supported by laboratory stability data. In the absence of such data, restandardize the solution weekly.

$$\text{▲M} = \frac{\text{mg } K_2Cr_2O_7}{49.04 \times \text{mL } Na_2S_2O_3}\text{▲ (ERR 1-May-2018)}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Add the following:

▲0.01 N Sulfuric Acid VS, H_2SO_4,—98.08

0.4904 g in 1000 mL

Transfer 10 mL of 1 N sulfuric acid VS to a 1000-mL volumetric flask. Dilute with water to volume.

Standardization: Accurately weigh about 50 mg of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water. Titrate with 0.01 N sulfuric acid solution. Determine the endpoint potentiometrically. Each 1.2114 mg of tromethamine is equivalent to 1 mL of 0.01 N sulfuric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL } H_2SO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application, such as pH adjustment, dissolution medium, or diluent, its standardization is not required.▲ (USP 1-May-2019)]

0.02 N Sulfuric Acid VS H_2SO_4, —98.08

Transfer 0.56 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

Standardization: Accurately weigh about 50 mg of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water and add 2 drops of bromocresol green TS. Titrate with the sulfuric acid solution to a pale yellow endpoint. Each 2.423 mg of tromethamine is equivalent to 1 mL of 0.02 N sulfuric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \text{ x mL } H_2SO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

0.05 N Sulfuric Acid VS, H_2SO_4—98.08

2.452 g in 1000 mL

Transfer 1.4 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.

- **STANDARDIZATION:** Accurately weigh about ▲0.25 g▲ (USP 1-May-2019) of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water and add 2 drops of bromocresol green TS. Titrate with the sulfuric acid solution to a pale yellow endpoint. Each 6.057 mg of tromethamine is equivalent to 1 mL of 0.05 N sulfuric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \times \text{mL } H_2SO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

1 N Sulfuric Acid VS H_2SO_4, —98.08

49.04 g in 1000 mL

Add slowly, with stirring, 27 mL of sulfuric acid to a sufficient quantity of water to make 1000 mL.

Standardization: Accurately weigh about 5.0 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water and add 2 drops of bromocresol green TS. Titrate with 1 N sulfuric acid to a pale yellow endpoint. Each 121.14 mg of tromethamine is equivalent to 1 mL of 1 N sulfuric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \text{ x mL } H_2SO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.5 N Sulfuric Acid in Alcohol VS H_2SO_4, —98.08

24.52 g in 1000 mL

Transfer 13.9 mL of sulfuric acid to a 1000-mL volumetric flask containing about 500 mL of dehydrated alcohol. Cool and dilute with dehydrated alcohol to volume.

Standardization: Accurately weigh about 2.5 g of tromethamine, dried according to the label instructions or, if this information is not available, dried at 105° for 3 h. Dissolve in 50 mL of water and add 2 drops of bromocresol green TS. Titrate with the sulfuric acid solution to a pale yellow endpoint. Each 60.57 mg of tromethamine is equivalent to 1 mL of 0.5 N sulfuric acid.

$$N = \frac{\text{mg tromethamine}}{121.14 \text{ x mL } H_2SO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Tetrabutylammonium Hydroxide VS $(C_4H_9)_4NOH$, —259.47

25.95 g in 1000 mL

Dissolve 40 g of tetra-*n*-butylammonium iodide in 90 mL of anhydrous methanol in a glass-stoppered flask. Place in an ice bath, add 20 g of powdered silver oxide, insert the stopper in the flask, and agitate vigorously for 60 min. Centrifuge a few milliliters, and test the supernatant for iodide (see *Identification Tests—General* ⟨191⟩, *Iodide*). If the test is positive, add an additional 2 g of silver oxide, and continue to allow to stand for 30 min with intermittent agitation. When all of the iodide has reacted, filter through a fine-porosity, sintered-glass funnel. Rinse the flask and the funnel with three 50-mL portions of anhydrous toluene, adding the rinsings to the filtrate. Dilute with a mixture of three volumes of anhydrous toluene and one volume of anhydrous methanol to 1000 mL, and flush the solution for 10 min with dry, carbon dioxide–free nitrogen. [NOTE—If necessary to obtain a clear solution, further small quantities of anhydrous methanol may be added.] Store in a reservoir protected from carbon dioxide and moisture, and discard after 60 days. Alternatively, the solution may be prepared by diluting a suitable volume of commercially

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available tetrabutylammonium hydroxide solution in methanol with a mixture of four volumes of anhydrous toluene and one volume of anhydrous methanol.

[NOTE—If necessary to obtain a clear solution, further small quantities of methanol may be added.]

• **STANDARDIZATION:** On the day of use, dissolve about 400 mg of primary standard benzoic acid, accurately weighed, in 80 mL of dimethylformamide, add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate to a blue endpoint with the tetrabutylammonium hydroxide solution, delivering the titrant from a buret equipped with a carbon dioxide absorption trap. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 12.21 mg of benzoic acid.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL } (C_4H_9)_4NOH}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Tetrabutylammonium Hydroxide in Methanol/Isopropyl Alcohol VS

Dissolve 40 g of tetra-*n*-butylammonium iodide in 90 mL of anhydrous methanol in a glass-stoppered flask. Place in an ice bath, add 20 g of powdered silver oxide, insert the stopper in the flask, and agitate vigorously for 60 min. Centrifuge a few milliliters, and test the supernatant for iodide (see *Identification Tests—General* ⟨191⟩, *Iodide*). If the test is positive, add an additional 2 g of silver oxide, and continue to allow to stand for 30 min with intermittent agitation. When all of the iodide has reacted, filter through a fine-porosity, sintered-glass funnel. Rinse the flask and the funnel with three 50-mL portions of isopropyl alcohol, adding the rinsings to the filtrate. Dilute with a mixture of three volumes of isopropyl alcohol and one volume of anhydrous methanol to 1000 mL, and flush the solution for 10 min with dry, carbon dioxide-free nitrogen. [NOTE—If necessary to obtain a clear solution, further small quantities of anhydrous methanol may be added.] Store in a reservoir protected from carbon dioxide and moisture, and discard after 60 days. Alternatively, the solution may be prepared by diluting a suitable volume of commercially available tetrabutylammonium hydroxide solution in methanol with a mixture of four volumes of isopropyl alcohol and one volume of anhydrous methanol.

[NOTE—If necessary to obtain a clear solution, further small quantities of methanol may be added.]

Standardization: On the day of use, dissolve about 400 mg of primary standard benzoic acid, accurately weighed, in 80 mL of dimethylformamide, add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate to a blue endpoint with the tetrabutylammonium hydroxide solution, delivering the titrant from a buret equipped with a carbon dioxide absorption trap. Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 N tetrabutylammonium hydroxide is equivalent to 12.21 mg of benzoic acid.

$$N = \frac{\text{mg benzoic acid}}{122.1 \times \text{mL } (C_4H_9)_4NOH}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Tetramethylammonium Bromide VS $(CH_3)_4NBr$, **—154.05**

15.41 g in 1000 mL

Dissolve 15.41 g of tetramethylammonium bromide in water to make 1000 mL.

Standardization: Transfer an accurately measured volume of about 40 mL of the solution to a beaker, add 10 mL of diluted nitric acid and 50.0 mL of 0.1 N silver nitrate VS, and mix. Add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS.

$$M = \frac{\text{mL } AgNO_3 \times \text{N } AgNO_3}{\text{mL } (CH_3)_4NBr}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Tetramethylammonium Chloride VS $(CH_3)_4NCl$, **—109.60**

10.96 g in 1000 mL

Dissolve 10.96 g of tetramethylammonium chloride in water to make 1000 mL.

Standardization: Transfer an accurately measured volume of about 40 mL of the solution to a flask, add 10 mL of diluted nitric acid and 50.0 mL of 0.1 N silver nitrate VS, and mix. Add 5 mL of nitrobenzene and 2 mL of ferric ammonium sulfate TS, shake, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS.

$$M = \frac{\text{mL } AgNO_3 \times \text{N } AgNO_3}{\text{mL } (CH_3)_4NCl}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 N Titanium Trichloride VS $TiCl_3$, **—154.23**

15.42 g in 1000 mL

Add 75 mL of titanium trichloride solution (1 in 5) to 75 mL of hydrochloric acid, dilute to 1000 mL, and mix.

Standardization

Apparatus: Store the titanium trichloride solution in the reservoir of a closed-system titration apparatus in an atmosphere of hydrogen.

Use a wide-mouth, 500-mL conical flask as the titration vessel, and connect it by means of a tight-fitting rubber stopper to the titration buret, an inlet tube for carbon dioxide, and an exit tube. Arrange for mechanical stirring. All joints must be air-tight. Arrange to have both the hydrogen and the carbon dioxide pass through wash bottles containing titanium trichloride solution (approximately 1 in 50) to remove any oxygen.

If the solution to be titrated is to be heated before or during titration, connect the titration flask with an upright reflux condenser through the rubber stopper.

Procedure: Place an accurately measured volume of about 40 mL of 0.1 N ferric ammonium sulfate VS in the titration flask, and pass in a rapid stream of carbon dioxide until all the air has been removed. Add the titanium trichloride solution from the buret until near the calculated endpoint (about 35 mL), then add through the outlet tube 5 mL of ammonium thiocyanate TS, and continue the titration until the solution is colorless.

$$N = \frac{\text{mL } FeNH_4(SO_4)_2 \times \text{N } FeNH_4(SO_4)_2}{\text{mL } TiCl_3}$$

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[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Change to read:

▲0.02 M Zinc Sulfate VS $ZnSO_4 \cdot 7H_2O$**—287.56**

5.76 g in 1000 mL

Dissolve 5.76 g of zinc sulfate in water to make 1 L.

Standardization: Accurately measure about 10 mL of 0.05 M edetate disodium VS ▲or of 0.02 M edetate disodium VS▲ (USP 1-Aug-2019) into a 125-mL conical flask, and add, in the order given, 10 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 2 mL of dithizone TS. Titrate with the zinc sulfate solution to a clear, rose-pink color.

$$M = \frac{\text{mL edetate disodium} \times \text{M edetate disodium}}{\text{mL } ZnSO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

▲ (USP 1-May-2019)

0.05 M Zinc Sulfate VS $ZnSO_4 \cdot 7H_2O$**, —287.56**

14.4 g in 1000 mL

Dissolve 14.4 g of zinc sulfate in water to make 1 L.

Standardization: Accurately measure about 10 mL of 0.05 M edetate disodium VS into a 125-mL conical flask, and add, in the order given, 10 mL of acetic acid-ammonium acetate buffer TS, 50 mL of alcohol, and 2 mL of dithizone TS. Titrate with the zinc sulfate solution to a clear, rose-pink color.

$$M = \frac{\text{mL edetate disodium} \times \text{M edetate disodium}}{\text{mL } ZnSO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

0.1 M Zinc Sulfate VS $ZnSO_4 \cdot 7H_2O$**, —287.54**

28.8 g in 1000 mL

Dissolve 28.8 g of zinc sulfate heptahydrate in water to make 1 L.

Standardization: Accurately measure about 10 mL of 0.1 M edetate disodium VS into a 125-mL conical flask, and add, in the order given, 10 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 2 mL of dithizone TS. Titrate with the zinc sulfate solution to a clear, rose-pink color.

$$M = \frac{\text{mL edetate disodium} \times \text{M edetate disodium}}{\text{mL } ZnSO_4}$$

[NOTE—If this volumetric solution is used in a qualitative application such as pH adjustment, dissolution medium, or diluent, its standardization is not required.]

Chromatographic Columns

The following list of packings (L), phases (G), and supports (S) is intended to be a convenient reference for the chromatographer.

[NOTE—Particle sizes given in this listing are those generally provided. Where other, usually finer, sizes are required, the individual monograph specifies the desired particle size. Within any category of packings or phases listed below, there may be a wide range of columns available. Where it is necessary to define more specifically the chromatographic conditions, the individual monograph so indicates.]

Packings

L1—Octadecyl silane chemically bonded to porous or nonporous silica or ceramic microparticles, 1.5 to 10 µm in diameter, or a monolithic silica rod.

L2—Octadecyl silane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50 µm in diameter.

L3—Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.

L4—Silica gel of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter.

L5—Alumina of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter.

L6—Strong cation-exchange packing–sulfonated fluorocarbon polymer coated on a solid spherical core, 30 to 50 µm in diameter.

L7—Octylsilane chemically bonded to totally porous or superficially porous silica particles, 1.5–10 µm in diameter, or a monolithic silica rod.

L8—An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5–10 µm in diameter, or a monolithic silica rod.

L9—Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.

L10—Nitrile groups chemically bonded to porous silica particles, 1.5–10 µm in diameter, or a monolithic silica rod.

L11—Phenyl groups chemically bonded to porous silica particles, 1.5–10 µm in diameter, or a monolithic silica rod.

L12—A strong anion-exchange packing made by chemically bonding a quaternary amine to a solid silica spherical core, 30 to 50 µm in diameter.

L13—Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.

L14—Silica gel having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm in diameter.

L15—Hexylsilane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

L16—Dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm in diameter.

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L17—Strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the hydrogen form, 6 to 12 µm in diameter.

L18—Amino and cyano groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.

L19—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, 5–15 µm in diameter.

L20—Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5–10 µm in diameter, or a monolithic silica rod.

L21—A rigid, spherical styrenedivinylbenzene copolymer 3 to 30 µm in diameter.

L22—A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, 5–15 µm in diameter.

L23—An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, 7–12 µm in size.

L24—Polyvinylalcohol chemically bonded to porous silica particle, 5 µm in diameter. [NOTE—Available as YMC-Pack PVA-SIL manufactured by YMC Co., Ltd. and distributed by Waters Corp. (www.waters.com).]

L25—Packing having the capacity to separate compounds with a molecular weight range from 100–5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contained some residual carboxyl functional groups) was found suitable.

L26—Butyl silane chemically bonded to totally porous or superficially porous silica particles, 1.5–10 µm in diameter.

L27—Porous silica particles, 30 to 50 µm in diameter.

L28—A multifunctional support, which consists of a high purity, 100 Å, spherical silica substrate that has been bonded with anionic exchanger, amine functionality in addition to a conventional reversed phase C8 functionality.

L29—Gamma alumina, reverse-phase, low carbon percentage by weight, alumina-based polybutadiene spherical particles, 5 µm in diameter with a pore volume of 80 Å.

L30—Ethyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

L31—A hydroxide-selective, strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 8.5-µm macroporous particles having a pore size of 2000 Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene.

L32—A chiral ligand-exchange packing–L-proline copper complex covalently bonded to irregularly shaped silica particles, 5 to 10 µm in diameter.

L33—Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability. [NOTE—Available as TSK-GEL G4000SWxl from Tosoh Bioscience (www.tosohbioscience.com).]

L34—Strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the lead form, 7 to 9 µm in diameter.

L35—A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase having a pore size of 150 Å.

L36—A 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5-µm aminopropyl silica.

L37—Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 Da. It is a polymethacrylate gel.

L38—A methacrylate-based size-exclusion packing for water-soluble samples.

L39—A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.

L40—Cellulose tris-3,5-dimethylphenylcarbamate-coated, porous silica particles, 3–20 µm in diameter.

L41—Immobilized α_1-acid glycoprotein on spherical silica particles, 5 µm in diameter.

L42—Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5 µm in diameter.

L43—Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm in diameter.

L44—A multifunctional support, which consists of a high purity, 60 Å, spherical silica substrate that has been bonded with a cationic exchanger, sulfonic acid functionality in addition to a conventional reversed phase C8 functionality.

L45—Beta cyclodextrin, *R,S*-hydroxypropyl ether derivative, bonded to porous silica particles, 3–10 µm in diameter.

L46—Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, about 9 to 11 µm in diameter.

L47—High-capacity anion-exchange microporous substrate, fully functionalized with trimethlyamine groups, 8 µm in diameter. [NOTE—Available as CarboPac MA1 and distributed by Dionex Corp. (www.dionex.com).]

L48—Sulfonated, cross-linked polystyrene with an outer layer of submicron, porous, anion-exchange microbeads, 5 to 15 µm in diameter.

L49—A reversed-phase packing made by coating a thin layer of polybutadiene onto spherical porous zirconia particles, 3 to 10 µm in diameter. [NOTE—Available as Zirchrom PBD from www.zirchrom.com.]

L50—Multifunction resin with reversed-phase retention and strong anion-exchange functionalities. The resin consists of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15 µm in diameter, and a surface area not less than 350 m^2 per g. Substrate is coated with quaternary ammonium functionalized latex particles consisting of styrene cross-linked with divinylbenzene. [NOTE—Available as OmniPac PAX-500 and distributed by Dionex Corp. (www.dionex.com).]

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L51—Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 3–10 µm in diameter. [NOTE—Available as Chiralpak AD from Chiral Technologies, Inc. (www.chiraltech.com).]

L52—A strong cation-exchange resin made of porous silica with sulfopropyl or sulfoethyl groups, 1–10 µm in diameter.

L53—Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15 µm diameter. Substrate is surface grafted with carboxylic acid and/or phosphoric acid functionalized monomers. Capacity not less than 500 µEq/column. [NOTE—Available as IonPac CS14 distributed by Dionex Corp. (www.dionex.com).]

L54—A size exclusion medium made of covalent bonding of dextran to highly cross-linked porous agarose beads, 5–15 µm in diameter.
[NOTE—Available as Superdex Peptide HR 10/30 from www.gelifesciences.com.]

L55—A strong cation-exchange resin made of porous silica coated with polybutadiene–maleic acid copolymer, about 5 µm in diameter. [NOTE—Available as IC-Pak C M/D from Waters Corp. (www.waters.com).]

L56—Propyl silane chemically bonded to totally or superficially porous silica particles, 3–10 µm in diameter. [NOTE—Available as Zorbax SB-C3 from Agilent Technologies (www.agilent.com/chem).]

L57—A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5 µm in diameter, with a pore size of 120 Å. [NOTE—Available as Ultron ES-OVM from Agilent Technologies (www.agilent.com/chem).]

L58—Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30 µm in diameter. [NOTE—Available as Aminex HPX-87N from Bio-Rad Laboratories, (2000/01 catalog, #125-0143) www.bio-rad.com.]

L59—Packing for the size-exclusion separation of proteins (separation by molecular weight) over the range of 5 to 7000 kDa. The packing is a spherical 1.5- to 10-µm silica or hybrid packing with a hydrophilic coating.

L60—Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped. [NOTE—Available as Supelcosil LC-ABZ from Supelco (www.sigmaaldrich.com/supelco).]

L61—A hydroxide selective strong anion-exchange resin consisting of a highly cross-linked core of 13-µm microporous particles having a pore size less than 10 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 85-nm diameter microbeads bonded with alkanol quaternary ammonium ions (6%). [NOTE—Available as Ion Pac AS-11 and AG-11 from Dionex (www.dionex.com).]

L62—C30 silane bonded phase on a fully porous spherical silica, 3 to 15 µm in diameter.

L63—Glycopeptide teicoplanin linked through multiple covalent bonds to a 100-Å units spherical silica.
[NOTE—Available as Astec Chirobiotic T from Supelco (www.sigmaaldrich.com).]

L64—Strongly basic anion-exchange resin consisting of 8% cross-linked styrene-divinylbenzene copolymer with a quaternary ammonium group in the chloride form, 45 to 180 µm in diameter.
[NOTE—A suitable grade is available as AG 1-X8 resin chloride form from www.discover.bio-rad.com.]

L65—Strongly acidic cation-exchange resin consisting of 2% sulfonated cross-linked styrene-divinylbenzene copolymer with a sulfonic acid group in the hydrogen form, 45–250 µm in diameter.
[NOTE—A suitable grade is available as AG 50W-X2 resin hydrogen form from www.bio-rad.com.]

L66—A crown ether coated on a 5-µm particle size silica gel substrate. The active site is (*S*)-18-crown-6-ether.
[NOTE—Available as Crownpak CR(+) from www.chiraltech.com.]

L67—Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10 µm in diameter.
[NOTE—Available as apHera C18 from Supelco (www.sigmaaldrich.com).]

L68—Spherical, porous silica, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped.
[NOTE—Available as SUPELCOSIL SUPLEX pKb-100 from Supelco (www.sigmaaldrich.com).]

L69—Ethylvinylbenzene/divinylbenzene substrate agglomerated with quaternary amine functionalized 130-nm latex beads, about 6.5 µm in diameter.
[NOTE—Available as CarboPac PA20 from www.dionex.com.]

L70—Cellulose tris(phenyl carbamate) coated on 5-µm silica.
[NOTE—Available as Chiralcel OC-H from www.chiraltech.com.]

L71—A rigid, spherical polymetacrylate, 4 to 6 µm in diameter.
[NOTE—Available as RSpak DE-613 from www.shodex.com.]

L72—(*S*)-phenylglycine and 3,5-dinitroanaline urea linkage covalently bonded to silica.
[NOTE—Available as Sumichiral OA-3300, distributed by www.phenomenex.com.]

Change to read:

L73—A rigid spherical polydivinylbenzene particle, 5 to 10 µm in diameter. ▲▲ (ERR 1-Aug-2018)

L74—A strong anion-exchange resin consisting of a highly cross-linked core of 7- µm macroporous particles having a 100-Å average pore size and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and an anion-exchange layer grafted to the surface, which is functionalized with alkyl quaternary ammonium ions. [NOTE—Available as IonPac AS14A from Dionex (www.dionex.com).]

L75—A chiral-recognition protein, bovine serum albumine (BSA), chemically bonded to silica particles, about 7 µm in diameter, with a pore size of 300 Å.

L76—Silica-based, weak cation-exchange material, 5 µm in diameter. Substrate is surface polymerized polybutadiene–

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maleic acid to provide carboxylic acid functionalities. Capacity not less than 29 µEq/column. [NOTE—Available as Metrosep C4-250 from Metrohm (www.metrohm.com).]

L77—Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 6- to 9-µm diameter. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity is not less than 500 µEq/column (4 mm × 25 cm).

L78—A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and anion-exchange (primary, secondary, or tertiary amino groups) functional groups chemically bonded to porous or nonporous silica or ceramic microparticles, 1.0–50 µm in diameter, or a monolithic rod.
[NOTE—Available as Acclaim Mixed-Mode WAX-1 from Thermo Fisher (www.thermofisher.com).]

L79—A chiral-recognition protein, human serum albumin (HSA), chemically bonded to silica particles, about 5 µm in diameter.

L80—Cellulose tris(4-methylbenzoate)-coated, porous, spherical, silica particles, 5–20 µm in diameter.

L81—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 9-µm porous particles having a pore size of 2000 Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 70-nm diameter microbeads (6% cross-linked) bonded with alkanol quaternary ammonium ions.
[NOTE—A suitable column is Dionex IonPac AS11-HC available from www.thermofisher.com.]

L82—Polyamine chemically bonded to cross-linked polyvinyl alcohol polymer, 5 µm in diameter.
[NOTE—Available as Asahipak NH2P-50 from www.shodex.com.]

L83—A hydroxide-selective, strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 10.5-µm microporous particles of 10-Å pore size, and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene.

L84—Weak cation-exchange resin consisting of ethyvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 5 µm in diameter. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity NLT 8400 µEq/column (5-mm × 25-cm).

L85—A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and weak cation-exchange (carboxyl groups) functional groups chemically bonded to porous or nonporous particles, 1.0–50 µm in diameter.

Change to read:

L86—Fused core particles with a highly polar ligand possessing five hydroxyl groups tethered to the silica gel outer layer, 1.5–5 µm in diameter.▲ ▲ (USP 1-Aug-2019)

Change to read:

L87—Dodecyl silane chemically bonded to porous ▲or superficially porous▲ (USP 1-Aug-2019) silica particles, 1.5–10 µm in diameter.

L88—Glycopeptide vancomycin linked through multiple covalent bonds to 100 Å spherical silica.

L89—Packing having the capacity to separate compounds with a molecular range from 100 to 3000 (as determined by polyethylene oxide), applied to neutral and anionic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contains some residual cationic functional groups).

L90—Amylose tris-[(*S*)-α-methylbenzyl]carbamate coated on porous, spherical silica particles, 3–10 µm in diameter.

L91—Strong anion-exchange resin consisting of monodisperse polystyrene/divinyl benzene beads coupled with quartenary amine. Bead size is 3–10 µm.
[NOTE—A suitable column is available as Mono Q 5/50 GL from www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-us/17516601.]

L92—A strong anion-exchange resin consisting of highly cross-linked 5- to 9-µm macroporous particles having a 100-Å average pore size and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and an anion-exchange layer grafted to the surface, which is functionalized with alkanol quarternary ammonium ions.

L93—Cellulose tris(3,5-dimethylphenylcarbamate) reversed-phase chiral stationary-phase coated on 3- or 5-µm silica gel particles.

L94—A strong anion-exchange resin consisting of highly cross-linked 15-µm microporous particles functionalized with very low cross-linked latex (0.5%) to provide alkanol quartenary ammonium ion exchange sites.

L95—A highly polar alkyl ligand comprising five hydroxyl groups that are chemically bonded to totally porous or superficially porous silica or a monolithic silica rod.

L96—Alkyl chain, reversed-phase bonded totally or superficially to porous silica designed to retain hydrophilic and other polar compounds when using highly aqueous mobile phase, including 100% aqueous, 1.5–10 µm in diameter.

L97—Weak cation-exchange resin consisting of a highly cross-linked core of 5.5-µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity NLT 2400 µEq/column (4-mm × 25-cm).

L98—Weak cation-exchange resin consisting of a highly cross-linked core of 8.0-µm microporous particles having an average pore size of 10 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene. Substrate is surface grafted with carboxylic acid functionalized groups. Capacity of NLT 46 µEq/column (4-mm × 5-cm).

L99—Amylose tris-(3,5-dimethylphenylcarbamate), immobilized on porous, spherical, silica particles, 3–5 µm in diameter.

L100—A 55% crosslinked, microporous, hydrophobic resin core (9-µm microporous particles having a pore size of 10 Å) that consists of a bilayer of anion and cation exchange latex. The first layer is fully sulfonated latex (140 nm) and the second layer is fully aminated latex (76 nm).

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L101—Cholesteryl groups chemically bonded to porous or nonporous silica or ceramic microparticles, 1.5–10 µm in diameter, or a monolithic rod.

L102—1-(3,5-Dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene covalently bonded to porous spherical silica particles, 5–10 µm in diameter.

L103—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 7.5-µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene electrostatically bonded with hyperbranched alkanol quaternary ammonium ions.

L104—Triazol groups chemically bonded to porous silica particles, 1.5–10 µm in diameter.

L105—A strong anion-exchange resin consisting of highly cross-linked 9-µm supermacroporous (2000 Å) particles functionalized with very low cross-linked latex (0.2%) to provide alkyl quarternary ammonium ion sites.

L107 (Rosuvastatin Calcium, CHIRALCEL OJ-RH)—Cellulose tris(4-methylbenzoate)-coated porous spherical particles, 3–5 µm in diameter, for use with reversed-phase mobile phases. [NOTE—A suitable column is Chiralcel OJ-RH from www.chiraltech.com.]

L108—A chiral-recognition protein, cellobiohydrolase (CBH), chemically bonded to silica particles, about 5 µm in diameter.

L109—Spherical particles of porous graphitic carbon, 3–30 µm in diameter.

L110—A strong anion-exchange resin consisting of highly cross-linked 13-µm (less than 10 Å) particles coated with very low cross-linked latex (0.5%) to provide alkanol quaternary ammonium ion exchange sites.

Add the following:

▲L111—Polyamine chemically bonded to porous, spherical, silica particles, 5 µm in diameter.▲ 2S (USP41)

L112—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 8.5-µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 65-nm diameter microbeads (5% crosslinked) bonded with alkanol quaternary ammonium ions.

Add the following:

▲L113—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 7.5-µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 65-nm diameter microbeads (8% crosslinked) bonded with alkanol quaternary ammonium ions.▲ (USP 1-Dec-2019)

Add the following:

▲L115 (Eptacog Alfa, Dionex CarboPac PA100)—Ethylvinylbenzene/divinylbenzene substrate (55% cross-linked) agglomerated with quaternary amine functionalized 275-nm latex microbeads (6% cross-linked), about 8.5-µm in diameter.▲ (USP 1-Dec-2019)

Add the following:

▲L116 (Eptacog Alfa, Dionex DNAPac PA100)—Sulfonated ethylvinylbenzene/divinylbenzene substrate approximately 12–14 µm in diameter agglomerated with hydrophilic quaternary amine functionalized glycidyl-derivative methacrylate microbeads.▲ (USP 1-Dec-2019)

Add the following:

▲L117—A crown ether coated on a 5-µm particle size silica gel substrate. The active site is (*R*)-18-crown-6-ether.▲ (USP 1-Aug-2019)

Add the following:

▲L118—Aqueous polymerized C18 groups on silica particles, 1.2–5 µm in diameter.▲ (USP 1-Aug-2019)

Phases

G1—Dimethylpolysiloxane oil.

G2—Dimethylpolysiloxane gum.

G3—50% Phenyl-50% methylpolysiloxane.

G4—Diethylene glycol succinate polyester.

G5—3-Cyanopropylpolysiloxane.

G6—Trifluoropropylmethylpolysiloxane.

G7—50% 3-Cyanopropyl-50% phenylmethylsilicone.

G8—80% Bis(3-cyanopropyl)-20% 3-cyanopropylphenylpoly-siloxane (percentages refer to molar substitution).

G9—Methylvinylpolysiloxane.

G10—Polyamide formed by reacting a C_{36} dicarboxylic acid with 1,3-di-4-piperidylpropane and piperidine in the respective mole ratios of 1.00:0.90:0.20.

G11—Bis(2-ethylhexyl) sebacate polyester.

G12—Phenyldiethanolamine succinate polyester.

G13—Sorbitol.

G14—Polyethylene glycol (av. mol. wt. of 950 to 1050).

G15—Polyethylene glycol (av. mol. wt. of 3000 to 3700).

G16—Polyethylene glycol compound (av. mol. wt. about 15,000). A high molecular weight compound of polyethylene glycol with a diepoxide linker. [NOTE—Available commercially as Polyethylene Glycol Compound 20M, or as Carbowax 20M, from suppliers of chromatographic reagents.]

G17—75% Phenyl-25% methylpolysiloxane.

G18—Polyalkylene glycol.

G19—25% Phenyl-25% cyanopropyl-50% methylsilicone.

G20—Polyethylene glycol (av. mol. wt. of 380 to 420).

G21—Neopentyl glycol succinate.

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G22—Bis(2-ethylhexyl) phthalate.

G23—Polyethylene glycol adipate.

G24—Diisodecyl phthalate.

G25—Polyethylene glycol compound TPA. A high molecular weight compound of a polyethylene glycol and a diepox ide that is esterified with terephthalic acid.
[NOTE—Available commercially as Carbowax 20M-TPA from suppliers of chromatographic reagents.]

G26—25% 2-Cyanoethyl-75% methylpolysiloxane.

G27—5% Phenyl-95% methylpolysiloxane.

G28—25% Phenyl-75% methylpolysiloxane.

G29—3,3′-Thiodipropionitrile.

G30—Tetraethylene glycol dimethyl ether.

G31—Nonylphenoxypoly(ethyleneoxy)ethanol (av. ethyleneoxy chain length is 30); Nonoxynol 30.

G32—20% Phenylmethyl-80% dimethylpolysiloxane.

G33—20% Carborane-80% methylsilicone.

G34—Diethylene glycol succinate polyester stabilized with phosphoric acid.

G35—A high molecular weight compound of a polyethylene glycol and a diepoxide that is esterified with nitroterephthalic acid.

G36—1% Vinyl-5% phenylmethylpolysiloxane.

G37—Polyimide.

G38—Phase G1 containing a small percentage of a tailing inhibitor.
[NOTE—A suitable grade is available commercially as "SP2100/0.1% Carbowax 1500" from Supelco, Inc. (www.sigmaaldrich.com/supelco).]

G39—Polyethylene glycol (av. mol. wt. about 1500).

G40—Ethylene glycol adipate.

G41—Phenylmethyldimethylsilicone (10% phenyl-substituted).

G42—35% phenyl-65% dimethylpolysiloxane (percentages refer to molar substitution).

G43—6% cyanopropylphenyl-94% dimethylpolysiloxane (percentages refer to molar substitution).

G44—2% low molecular weight petrolatum hydrocarbon grease and 1% solution of potassium hydroxide.

G45—Divinylbenzene-ethylene glycol-dimethylacrylate.

G46—14% Cyanopropylphenyl-86% methylpolysiloxane.

G47—Polyethylene glycol (av. mol. wt. of about 8000).

G48—Highly polar, partially cross-linked cyanopolysiloxane.

G49—Dimethylpolysiloxane with chiral building block containing D- or L-valine as chiral agent (for amino acids).

Add the following:

▲**G50**—Base-deactivated Carbowax.▲ (USP 1-Aug-2019)

Add the following:

▲**G51**—50% phenyl–50% dimethylpolysiloxane phase, which is modified with an aromatic selector for optimized separation of polycyclic aromatic hydrocarbons (PAH).▲ (USP 1-Aug-2019)

Supports

[NOTE—Unless otherwise specified, mesh sizes of 80 to 100 or, alternatively, 100 to 120 are intended.]

S1A—Siliceous earth for gas chromatography has been flux-calcined by mixing diatomite with Na_2CO_3 flux and calcining above 900°. The siliceous earth is acid-washed, then water-washed until neutral, but not base-washed. The siliceous earth may be silanized by treating with an agent such as dimethyldichlorosilane [NOTE—Unless otherwise specified in the individual monograph, silanized support is intended.] to mask surface silanol groups.

S1AB—The siliceous earth as described above is both acid- and base-washed. [NOTE—Unless otherwise specified in the individual monograph, silanized support is intended.]

S1C—A support prepared from crushed firebrick and calcined or burned with a clay binder above 900° with subsequent acid-wash. It may be silanized.

S1D—A support prepared from crushed firebrick and calcinated or burned with a clay binder above 900°, not acid washed. It may be silanized.

S1NS—The siliceous earth is untreated.

S2—Styrene-divinylbenzene copolymer having a nominal surface area of less than 50 m^2 per g and an average pore diameter of 0.3 to 0.4 µm.

S3—Copolymer of ethylvinylbenzene and divinylbenzene having a nominal surface area of 500 to 600 m^2 per g and an average pore diameter of 0.0075 µm.

S4—Styrene-divinylbenzene copolymer with aromatic –O and –N groups, having a nominal surface area of 400 to 600 m^2 per g and an average pore diameter of 0.0076 µm.

S5—40- to 60-mesh, high-molecular weight tetrafluorethylene polymer.

S6—Styrene-divinylbenzene copolymer having a nominal surface area of 250 to 350 m^2 per g and an average pore diameter of 0.0091 µm.

S7—Graphitized carbon having a nominal surface area of 12 m^2 per g.

S8—Copolymer of 4-vinyl-pyridine and styrene-divinylbenzene.

S9—A porous polymer based on 2,6-diphenyl-*p*-phenylene oxide.

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S10—A highly polar cross-linked copolymer of acrylonitrite and divinylbenzene.

S11—Graphitized carbon having a nominal surface area of 100 m^2 per g modified with small amounts of petrolatum and polyethylene glycol compound.

S12—Graphitized carbon having a nominal surface area of 100 m^2 per g.

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Reference Tables

CONTAINERS FOR DISPENSING CAPSULES AND TABLETS

The following table is provided as a reminder for the pharmacist engaged in the typical dispensing situation who already is acquainted with the *Packaging and Storage* requirements set forth in the individual monographs. It lists the capsules and tablets that are official in the *United States Pharmacopeia* and indicates the relevant tight (T), well-closed (W), and light-resistant (LR) specifications applicable to containers in which the drug that is repackaged should be dispensed.

This table is not intended to replace, nor should it be interpreted as replacing, the definitive requirements stated in the individual monographs.

Container Specifications for Capsules and Tablets

Monograph Title	Container Specification
Abacavir Tablets	W
Abacavir and Lamivudine Tablets	T, LR
Abiraterone Acetate Tablets	T
Acarbose Tablets	T, LR
Acebutolol Hydrochloride Capsules	T
Acepromazine Maleate Tablets	T, LR
Acetaminophen Capsules	T
Acetaminophen Tablets, Extended-Release	T
Acetaminophen Tablets	T
Acetaminophen and Aspirin Tablets	T
Acetaminophen, Aspirin, and Caffeine Tablets	T
Acetaminophen and Caffeine Tablets	T
Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine, Capsules Containing at Least Three of the Following—	T
Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine, Tablets Containing at Least Three of the Following—	T
Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets	T
Acetaminophen and Codeine Phosphate Capsules	T, LR
Acetaminophen and Codeine Phosphate Tablets	T, LR
Acetaminophen and Diphenhydramine Citrate Tablets	T
Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets	T
Acetaminophen and Pseudoephedrine Hydrochloride Tablets	T
Acetaminophen and Tramadol Hydrochloride Tablets	T
Acetazolamide Tablets	T

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Acetohydroxamic Acid Tablets	T
Acitretin Capsules	W, LR
Acyclovir Capsules	T
Acyclovir Tablets	T
Albendazole Tablets	T
Albuterol Tablets	T, LR
Albuterol Tablets, Extended-Release	T, LR
Alendronate Sodium Tablets	T
Alfuzosin Hydrochloride Tablets, Extended-Release	LR
Allopurinol Tablets	W
Almotriptan Tablets	T, LR
Add the following:	
▲Alosetron Tablets	T ▲(USP 1-Dec-2019)
Alprazolam Tablets	T, LR
Alprazolam Tablets, Extended-Release	T, LR
Alprazolam Tablets, Orally Disintegrating	T
Altretamine Capsules	T, LR
Alumina and Magnesia Tablets	W
Alumina, Magnesia, and Calcium Carbonate Tablets	W
Alumina, Magnesia, Calcium Carbonate, and Simethicone Tablets	W
Alumina, Magnesia, and Simethicone Tablets	W
Alumina and Magnesium Carbonate Tablets	T
Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets	T
Alumina and Magnesium Trisilicate Tablets	W
Aluminum Carbonate Gel, Dried Basic, Capsules	W
Aluminum Carbonate Gel, Dried Basic, Tablets	W
Aluminum Hydroxide Gel, Dried, Capsules	W
Aluminum Hydroxide Gel, Dried, Tablets	W
Amantadine Hydrochloride Capsules	T
Amiloride Hydrochloride Tablets	W
Amiloride Hydrochloride and Hydrochlorothiazide Tablets	W
Aminobenzoate Potassium Capsules	W

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Delete the following:	
▲Aminobenzoate Potassium Tablets	W▲ (USP 1-Dec-2019)
Aminocaproic Acid Tablets	T
Aminopentamide Sulfate Tablets	W
Aminophylline Tablets	T
Aminophylline Tablets, Delayed-Release	T
Add the following:	
▲Amiodarone Hydrochloride Tablets	T, LR▲ (USP 1-Dec-2019)
Amitriptyline Hydrochloride Tablets	W
Amlodipine and Atorvastatin Tablets	W
Amlodipine and Benazepril Hydrochloride Capsules	W
Amlodipine Besylate Tablets	T, LR
Add the following:	
▲Amlodipine and Olmesartan Medoxomil Tablets	T▲ (USP 1-Dec-2019)
Amlodipine and Valsartan Tablets	T
Amlodipine, Valsartan, and Hydrochlorothiazide Tablets	T
Ammonium Chloride Tablets, Delayed-Release	T
Amodiaquine Hydrochloride Tablets	T
Amoxapine Tablets	W
Amoxicillin Capsules	T
Amoxicillin Tablets	T
Amoxicillin and Clavulanate Potassium Tablets	T
Amoxicillin and Clavulanic Acid Tablets, Extended-Release	T
Amphetamine Sulfate Tablets	W
Ampicillin Capsules	T
Ampicillin Tablets	T
Anagrelide Capsules	T, LR
Anastrozole Tablets	T
Anileridine Hydrochloride Tablets	T, LR
Delete the following:	
▲Apomorphine Hydrochloride Tablets	T, LR▲ (USP 1-Dec-2019)
Aprepitant Capsules	T
Arginine Capsules	T, LR
Arginine Tablets	T, LR
Aripiprazole Tablets	T
Aripiprazole Tablets, Orally Disintegrating	T
Ascorbic Acid Tablets	T, LR
Aspirin Capsules	T
Aspirin Capsules, Delayed-Release	T
Aspirin Tablets	T
Aspirin Tablets, Buffered	T
Aspirin Tablets, Delayed-Release	T

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Aspirin Tablets, Effervescent for Oral Solution	T
Aspirin Tablets, Extended-Release	T
Aspirin, Alumina, and Magnesia Tablets	T
Aspirin, Alumina, and Magnesium Oxide Tablets	T
Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules	T
Aspirin and Codeine Phosphate Tablets	W, LR
Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets	W, LR
Delete the following:	
▲Astemizole Tablets	T▲ (USP 1-Dec-2019)
Atenolol Tablets	W
Atenolol and Chlorthalidone Tablets	W
Atomoxetine Capsules	W
Atorvastatin Calcium Tablets	T
Delete the following:	
▲Atropine Sulfate Tablets	W▲ (USP 1-Dec-2019)
Delete the following:	
▲Azatadine Maleate Tablets	W▲ (USP 1-Dec-2019)
Azathioprine Tablets	LR
Azithromycin Capsules	W
Azithromycin Tablets	T
Bacampicillin Hydrochloride Tablets	T
Bacillus coagulans Capsules	W
Baclofen Tablets	W
Balsalazide Disodium Capsules	T
Barium Sulfate Tablets	W
Belladonna Extract Tablets	T, LR
Benazepril Hydrochloride Tablets	W
Bendroflumethiazide Tablets	T
Benzonatate Capsules	T, LR
Benzphetamine Hydrochloride Tablets	T
Benztropine Mesylate Tablets	W
Beta Carotene Capsules	T, LR
Betaxolol Tablets	T
Bethanechol Chloride Tablets	T
Bicalutamide Tablets	T
Biotin Capsules	T, LR
Biotin Tablets	T, LR
Bisacodyl Tablets	T
Bisacodyl Tablets, Delayed-Release	W
Bismuth Subsalicylate Tablets	T
Bisoprolol Fumarate Tablets	T, LR
Bisoprolol Fumarate and Hydrochlorothiazide Tablets	W
Black Cohosh Tablets	T, LR

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Borage Seed Oil Capsules	T, LR
Bromocriptine Mesylate Capsules	T, LR
Bromocriptine Mesylate Tablets	T, LR
Brompheniramine Maleate Tablets	T
Bumetanide Tablets	T, LR
Buprenorphine and Naloxone Sublingual Tablets	T
Bupropion Hydrochloride Tablets, Extended-Release	W
Buspirone Hydrochloride Tablets	T, LR
Busulfan Tablets	W
Butabarbital Sodium Tablets	T
Butalbital, Acetaminophen, and Caffeine Capsules	T
Butalbital, Acetaminophen, and Caffeine Tablets	T
Butalbital and Aspirin Tablets	T
Butalbital, Aspirin, and Caffeine Capsules	T
Butalbital, Aspirin, and Caffeine Tablets	T
Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules	T, LR
Cabergoline Tablets	T, LR
Calcifediol Capsules	T, LR
Calcium with Vitamin D Tablets	T, LR
Calcium Acetate Capsules	W
Calcium Acetate Tablets	W
Calcium Carbonate Tablets	W
Calcium Carbonate and Magnesia Tablets	W
Calcium Carbonate and Magnesia Chewable Tablets	W
Calcium Citrate Tablets	W
Calcium and Magnesium Carbonates Tablets	W
Calcium Gluconate Tablets	W
Calcium L-5-Methyltetrahydrofolate Capsules	T, LR
Calcium L-5-Methyltetrahydrofolate Tablets	T, LR
Calcium Lactate Tablets	T
Calcium Pantothenate Tablets	T
Calcium Phosphate, Dibasic Tablets	W
Candesartan Cilexetil Tablets	T, LR
Candesartan Cilexetil and Hydrochlorothiazide Tablets	T, LR
Capecitabine Tablets	T
Captopril Tablets	T
Captopril and Hydrochlorothiazide Tablets	T
Carbamazepine Tablets	T
Carbamazepine Tablets, Extended-Release	T
Carbenicillin Indanyl Sodium Tablets	T
Carbidopa and Levodopa Tablets	T, LR
Carbidopa and Levodopa Tablets, Extended-Release	W, LR

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Carbidopa and Levodopa Tablets, Orally Disintegrating	W, LR
Carbinoxamine Maleate Tablets	T, LR
Urea C14 Capsules	T
Carboxymethylcellulose Sodium Tablets	T
Carisoprodol Tablets	W
Carisoprodol and Aspirin Tablets	W
Carisoprodol, Aspirin, and Codeine Phosphate Tablets	W
Carprofen Tablets	T
Carteolol Hydrochloride Tablets	T
Carvedilol Tablets	T, LR
Cascara Tablets	T, W
Castor Oil Capsules	T
Cat's Claw Capsules	T, LR
Cat's Claw Tablets	T, LR
Cefaclor Capsules	T
Cefaclor Tablets, Extended-Release	T, LR
Cefadroxil Capsules	T
Cefadroxil Tablets	T
Cefdinir Capsules	T, LR
Cefixime Tablets	T
Cefpodoxime Proxetil Tablets	T
Cefprozil Tablets	T
Cefuroxime Axetil Tablets	W
Cephalexin Capsules	T
Cephalexin Tablets	T
Cephradine Capsules	T
Cephradine Tablets	T
Cetirizine Hydrochloride Tablets	W
Cetirizine Hydrochloride Tablets, Orally Disintegrating	T
Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Tablets, Extended-Release	W
Chloral Hydrate Capsules	T
Chlorambucil Tablets	W, LR
Chloramphenicol Capsules	T
Chloramphenicol Tablets	T
Chlordiazepoxide Tablets	T, LR
Chlordiazepoxide and Amitriptyline Hydrochloride Tablets	T, LR
Chlordiazepoxide Hydrochloride Capsules	T, LR
Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules	T, LR
Chloroquine Phosphate Tablets	W
Chlorothiazide Tablets	W, LR
Chlorpheniramine Maleate Capsules, Extended-Release	T

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Chlorpheniramine Maleate Tablets	T
Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Capsules, Extended-Release	T, LR
Chlorpromazine Hydrochloride Tablets	W, LR
Chlorpropamide Tablets	W
Chlortetracycline Hydrochloride Tablets	T, LR
Chlorthalidone Tablets	W
Chlorzoxazone Tablets	T
Chlorzoxazone and Acetaminophen Capsules	T
Cholecalciferol Capsules	T, LR
Cholecalciferol Tablets	T, LR
Chondroitin Sulfate Sodium Tablets	T, LR
Chromium Picolinate Tablets	W
Cimetidine Tablets	T, LR
Cilostazol Tablets	T, LR
Ciprofloxacin Tablets	W
Ciprofloxacin Tablets, Extended-Release	T
Citalopram Tablets	W
Clarithromycin Tablets	T
Clarithromycin Tablets, Extended-Release	W
Clemastine Fumarate Tablets	W
Clindamycin Hydrochloride Capsules	T
Clofazimine Capsules	W
Clofibrate Capsules	W, LR
Clomiphene Citrate Tablets	W
Clomipramine Hydrochloride Capsules	T, LR
Clonazepam Tablets	T, LR
Clonazepam Tablets, Orally Disintegrating	W, LR
Clonidine Hydrochloride Tablets	W
Clonidine Hydrochloride and Chlorthalidone Tablets	W
Clopidogrel Tablets	W
Clorazepate Dipotassium Tablets	T, LR
Clotrimazole Tablets, Vaginal	W
Red Clover Tablets	T, LR
Cloxacillin Sodium Capsules	T
Clozapine Tablets	W
Cyanocobalamin Co 57 Capsules	W, LR
Cyanocobalamin Co 58 Capsules	W, LR
Cocaine Hydrochloride Tablets for Topical Solution	W, LR
Codeine Phosphate Tablets	W, LR
Codeine Sulfate Tablets	W
Colestipol Hydrochloride Tablets	T
Cortisone Acetate Tablets	W
Cromolyn Sodium for Inhalation (in Capsules)	T, LR

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Crypthecodinium cohnii Oil Capsules	T, LR
Curcuminoids Capsules	W, LR
Curcuminoids Tablets	W, LR
Cyanocobalamin Tablets	T, LR
Cyclizine Hydrochloride Tablets	T, LR
Cyclobenzaprine Hydrochloride Capsules, Extended-Release	T, LR
Cyclobenzaprine Hydrochloride Tablets	W
Cyclophosphamide Tablets	T
Cycloserine Capsules	T
Cyclosporine Capsules	T
Cyproheptadine Hydrochloride Tablets	W
Danazol Capsules	W
Dantrolene Sodium Capsules	T
Dapsone Tablets	W, LR
Delete the following:	
▲Dehydrocholic Acid Tablets	W▲ (USP 1-Dec-2019)
Delete the following:	
▲Demeclocycline Hydrochloride Capsules	T, LR▲ (USP 1-Dec-2019)
Demeclocycline Hydrochloride Tablets	T, LR
Desipramine Hydrochloride Tablets	T
Desloratadine Tablets	T
Desloratadine Tablets, Orally Disintegrating	T
Desogestrel and Ethinyl Estradiol Tablets	W
Dexamethasone Tablets	W
Dexchlorpheniramine Maleate Tablets	T
Delete the following:	
▲Dextroamphetamine Sulfate Capsules	T▲ (USP 1-Dec-2019)
Dextroamphetamine Sulfate Tablets	W
Delete the following:	
▲Diazepam Capsules	T, LR▲ (USP 1-Dec-2019)
Delete the following:	
▲Diazepam Capsules, Extended-Release	T, LR▲ (USP 1-Dec-2019)
Diazepam Tablets	T, LR
Delete the following:	
▲Diazoxide Capsules	W▲ (USP 1-May-2020)
Dichlorphenamide Tablets	W
Diclofenac Potassium Tablets	T, LR
Diclofenac Sodium Tablets, Delayed-Release	T, LR
Diclofenac Sodium Tablets, Extended-Release	W
Diclofenac Sodium and Misoprostol Delayed-Release Tablets	T
Dicloxacillin Sodium Capsules	T

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Container Specifications for Capsules and Tables *(continued)*

Monograph Title	Container Specification
Dicyclomine Hydrochloride Capsules	W
Dicyclomine Hydrochloride Tablets	W
Didanosine Capsules, Delayed-Release	W
Didanosine Tablets for Oral Suspension	T
Diethylcarbamazine Citrate Tablets	T
Diethylpropion Hydrochloride Tablets	W
Delete the following:	
▲Diethylstilbestrol Tablets	W▲ (USP 1-Dec-2019)
Diflunisal Tablets	W
Digitalis Capsules	T
Digitalis Tablets	T
Digitoxin Tablets	W
Digoxin Tablets	T
Dihydrotachysterol Capsules	W, LR
Dihydrotachysterol Tablets	W, LR
Dihydroxyaluminum Sodium Carbonate Tablets	W
Diltiazem Hydrochloride Tablets	T, LR
Diltiazem Hydrochloride Capsules, Extended-Release	T
Dimenhydrinate Tablets	W
Diphenhydramine Citrate and Ibuprofen Tablets	T
Diphenhydramine Hydrochloride Capsules	T
Diphenhydramine Hydrochloride and Ibuprofen Capsules	T
Diphenhydramine and Phenylephrine Hydrochlorides Tablets	T
Diphenhydramine and Pseudoephedrine Capsules	T
Diphenoxylate Hydrochloride and Atropine Sulfate Tablets	W, LR
Dipyridamole Tablets	T, LR
Dirithromycin Tablets, Delayed-Release	T
Disopyramide Phosphate Capsules	W
Disopyramide Phosphate Capsules, Extended-Release	W
Disulfiram Tablets	T, LR
Divalproex Sodium Capsules, Delayed-Release	T, LR
Divalproex Sodium Tablets, Delayed-Release	T, LR
Divalproex Sodium Tablets, Extended-Release	W
Docusate Calcium Capsules	T
Docusate Potassium Capsules	T
Docusate Sodium Capsules	T
Docusate Sodium Tablets	W
Donepezil Hydrochloride Tablets	W
Donepezil Hydrochloride Tablets, Orally Disintegrating	W
Doxazosin Tablets	T
Doxepin Hydrochloride Capsules	W

Container Specifications for Capsules and Tables *(continued)*

Monograph Title	Container Specification
Doxycycline Capsules	T, LR
Doxycycline Capsules, Extended-Release	T, LR
Doxycycline Hyclate Capsules	T, LR
Doxycycline Hyclate Capsules, Delayed-Release	T, LR
Doxycycline Hyclate Tablets	T, LR
Doxycycline Hyclate Tablets, Delayed-Release	T, LR
Doxycycline Tablets	T, LR
Doxylamine Succinate Tablets	T, LR
Dronabinol Capsules	W, LR
Drospirenone and Ethinyl Estradiol Tablets	W
Duloxetine Capsules, Delayed-Release	T
Dydrogesterone Tablets	W
Dyphylline Tablets	T
Dyphylline and Guaifenesin Tablets	T
Echinacea Species Dry Extract Capsules	W
Echinacea Species Dry Extract Tablets	W
Echinacea Species Powder Capsules	W
Efavirenz Capsules	W
Efavirenz Tablets	W
Eleuthero Root and Rhizome Dry Extract Capsules	W
Eleuthero Root and Rhizome Dry Extract Tablets	W
Eleuthero Root and Rhizome Powder Capsules	W
Enalapril Maleate Tablets	W
Enalapril Maleate and Hydrochlorothiazide Tablets	W
Entacapone Tablets	LR
Entecavir Tablets	T
Ephedrine Sulfate Capsules	T, LR
Ergocalciferol Capsules	T, LR
Ergocalciferol Tablets	T, LR
Ergoloid Mesylates Capsules	T, LR
Ergoloid Mesylates Tablets	T, LR
Delete the following:	
▲Ergoloid Mesylates Tablets, Sublingual	T, LR▲ (USP 1-Dec-2019)
Ergonovine Maleate Tablets	W
Ergotamine Tartrate Tablets	W, LR
Ergotamine Tartrate Tablets, Sublingual	W, LR
Ergotamine Tartrate and Caffeine Tablets	W, LR
Erythromycin Capsules, Delayed-Release	T
Erythromycin Tablets	T
Erythromycin Tablets, Delayed-Release	T
Erythromycin Estolate Capsules	T
Erythromycin Estolate Tablets	T

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Erythromycin Ethylsuccinate Tablets	T
Erythromycin Stearate Tablets	T
Escitalopram Tablets	W
Esomeprazole Magnesium Capsules, Delayed-Release	T
Estazolam Tablets	T, LR
Estradiol Tablets	T, LR
Estradiol and Norethindrone Acetate Tablets	W
Estrogens Tablets, Conjugated	W
Estrogens Tablets, Esterified	W
Estropipate Tablets	W
Eszopiclone Tablets	T
Ethacrynic Acid Tablets	W
Ethambutol Hydrochloride Tablets	W
Ethchlorvynol Capsules	T, LR
Ethinyl Estradiol Tablets	W
Ethionamide Tablets	W
Ethosuximide Capsules	T
Ethotoin Tablets	T
Ethynodiol Diacetate and Ethinyl Estradiol Tablets	W
Ethynodiol Diacetate and Mestranol Tablets	W
Etidronate Disodium Tablets	T
Etodolac Capsules	T
Etodolac Tablets	T
Etodolac Tablets, Extended-Release	W
Evening Primrose Oil Capsules	T, LR
Famciclovir Tablets	T
Famotidine Tablets	W, LR
Felbamate Tablets	W
Felodipine Tablets, Extended-Release	T
Fenofibrate Capsules	W
Fenofibrate Tablets	W
Fenoprofen Calcium Capsules	W
Fenoprofen Calcium Tablets	W
Ferrous Fumarate Tablets	T
Ferrous Fumarate and Docusate Sodium Tablets, Extended-Release	W
Ferrous Gluconate Capsules	T
Ferrous Gluconate Tablets	T
Ferrous Sulfate Tablets	T
Fexofenadine Hydrochloride Capsules	T, LR
Fexofenadine Hydrochloride Tablets	W
Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Tablets, Extended-Release	W
Finasteride Tablets	T, LR
Fish Oil Containing Omega-3 Acids Capsules	T, LR

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Fish Oil Containing Omega-3 Acids Capsules, Delayed-Release	T, LR
Flavoxate Hydrochloride Tablets	W, LR
Flax Seed Oil Capsules	T, LR
Flecainide Acetate Tablets	W
Fluconazole Tablets	W
Flucytosine Capsules	T, LR
Fludrocortisone Acetate Tablets	T, LR
Fluoxetine Capsules	T, LR
Fluoxetine Capsules, Delayed-Release	T
Fluoxetine Tablets	T
Fluoxymesterone Tablets	W
Fluphenazine Hydrochloride Tablets	T, LR
Flurazepam Hydrochloride Capsules	T, LR
Flurbiprofen Tablets	W
Flutamide Capsules	W, LR
Fluvastatin Capsules	T, LR
Fluvoxamine Maleate Tablets	T
Folic Acid Tablets	W
Fosinopril Sodium Tablets	T
Fosinopril Sodium and Hydrochlorothiazide Tablets	T
Furazolidone Tablets	T, LR
Furosemide Tablets	W, LR
Gabapentin Capsules	W
Gabapentin Tablets	W
Galantamine Tablets	W
Galantamine Extended-Release Capsules	T, LR
Garlic Tablets, Delayed-Release	T
Gemfibrozil Capsules	T
Gemfibrozil Tablets	T
Ginger Capsules	W
Ginkgo Capsules	T, LR
Ginkgo Tablets	T, LR
American Ginseng Capsules	T, LR
American Ginseng Tablets	T, LR
Asian Ginseng Capsules	T, LR
Asian Ginseng Tablets	T, LR
Glimepiride Tablets	W
Glipizide Tablets	T
Glipizide and Metformin Hydrochloride Tablets	W
Glucosamine Tablets	T, LR
Glucosamine and Chondroitin Sulfate Tablets	T, LR
Glucosamine and Methylsulfonylmethane Tablets	T, LR
Glucosamine, Chondroitin Sulfate Sodium, and Methylsulfonylmethane Tablets	T, LR

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Glyburide Tablets	W
Glyburide and Metformin Hydrochloride Tablets	T, LR
Glycopyrrolate Tablets	T
Granisetron Hydrochloride Tablets	W, LR
Griseofulvin Capsules	T
Griseofulvin Tablets	T
Griseofulvin Tablets, Ultramicrosize	W
Guaifenesin Capsules	T
Guaifenesin Tablets	T
Guaifenesin and Pseudoephedrine Hydrochloride Capsules	T, LR
Guaifenesin, Pseudoephedrine Hydrochloride, and Dextromethorphan Hydrobromide Capsules	T, LR
Guanabenz Acetate Tablets	T, LR
Guanethidine Monosulfate Tablets	W
Guanfacine Tablets	T, LR
Guggul Tablets	W, LR
Halazone Tablets for Solution	T, LR
Haloperidol Tablets	T, LR
Hexylresorcinol Lozenges	W
Homatropine Methylbromide Tablets	T, LR
Hydralazine Hydrochloride Tablets	T, LR
Hydrochlorothiazide Capsules	W
Hydrochlorothiazide Tablets	W
Hydrocodone Bitartrate Tablets	T, LR
Hydrocodone Bitartrate and Acetaminophen Tablets	T, LR
Hydrocodone Bitartrate and Homatropine Methylbromide Tablets	T, LR
Hydrocortisone Tablets	W
Hydroflumethiazide Tablets	T
Hydromorphone Hydrochloride Tablets	T, LR
Hydroxychloroquine Sulfate Tablets	T, LR
Hydroxyurea Capsules	T
Hydroxyzine Hydrochloride Tablets	T
Hydroxyzine Pamoate Capsules	W
Hyoscyamine Tablets	W, LR
Hyoscyamine Sulfate Tablets	T, LR
Ibuprofen Tablets	W
Ibuprofen and Pseudoephedrine Hydrochloride Tablets	T
Imipramine Hydrochloride Tablets	T
Imipramine Pamoate Capsules	T, LR
Indapamide Tablets	W
Indomethacin Capsules	W
Indomethacin Capsules, Extended-Release	W
Sodium Iodide I 123 Capsules	W
Sodium Iodide I 131 Capsules	W

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Iodoquinol Tablets	W
Irbesartan Tablets	W
Irbesartan and Hydrochlorothiazide Tablets	W
Isoniazid Tablets	W, LR
Isopropamide Iodide Tablets	W
Isoproterenol Hydrochloride Tablets	W, LR
Isosorbide Dinitrate Capsules, Extended-Release	W
Isosorbide Dinitrate Tablets	W
Isosorbide Dinitrate Tablets, Chewable	W
Isosorbide Dinitrate Tablets, Extended-Release	W
Isosorbide Dinitrate Tablets, Sublingual	W
Isosorbide Mononitrate Tablets	T
Isosorbide Mononitrate Tablets, Extended-Release	T
Isotretinoin Capsules	T
Isoxsuprine Hydrochloride Tablets	T
Isradipine Capsules	T
Itraconazole Capsules	T, LR
Ivermectin Tablets	W
Ivermectin and Pyrantel Pamoate Tablets	T, LR
Kanamycin Sulfate Capsules	T
Ketoconazole Tablets	W
Ketoprofen Capsules	T
Ketoprofen Capsules, Extended-Release	T
Ketorolac Tromethamine Tablets	W
Krill Oil Capsules	T
Krill Oil Capsules, Delayed-Release	T
Labetalol Hydrochloride Tablets	T, LR
Lamivudine Tablets	T, LR
Lamivudine and Zidovudine Tablets	W, LR
Lamotrigine Tablets	W
Lamotrigine Tablets, Extended-Release	W
Lamotrigine Tablets for Oral Suspension	T, LR
Lansoprazole Capsules, Delayed-Release	T
Leflunomide Tablets	T, LR
Letrozole Tablets	T
Leucovorin Calcium Tablets	W, LR
Levamisole Hydrochloride Tablets	W
Levetiracetam Tablets	T
Levetiracetam Tablets, Extended-Release	W
Levocarnitine Tablets	T
Levocetirizine Dihydrochloride Tablets	W
Delete the following:	
▲Levodopa Capsules	T, L R▲ (USP 1-Dec-2019)

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Delete the following:	
▲Levodopa Tablets	T, L R▲ (USP 1-Dec-2019)
Levofloxacin Tablets	T
Levonorgestrel and Ethinyl Estradiol Tablets	W
Levorphanol Tartrate Tablets	W
Levothyroxine Sodium Tablets	T, LR
Lincomycin Hydrochloride Capsules	T
Alpha Lipoic Acid Capsules	W
Alpha Lipoic Acid Tablets	W
Liothyronine Sodium Tablets	T
Liotrix Tablets	T
Lisinopril Tablets	T
Lisinopril and Hydrochlorothiazide Tablets	W
Lithium Carbonate Capsules	W
Lithium Carbonate Tablets	T
Lithium Carbonate Tablets, Extended-Release	W
Lomustine Capsules	W
Loperamide Hydrochloride Capsules	W
Loratadine Tablets	T
Loratadine Tablets, Chewable	T
Loratadine Tablets, Orally Disintegrating	T
Lorazepam Tablets	T, LR
Losartan Potassium Tablets	T, LR
Losartan Potassium and Hydrochlorothiazide Tablets	T, LR
Lovastatin Tablets	W
Loxapine Capsules	T
Lutein Capsules	T, LR
Lysine Hydrochloride Tablets	W
Delete the following:	
▲Magaldrate Tablets	W▲ (USP 1-Dec-2019)
Magaldrate and Simethicone Tablets	W
Magnesia Tablets	W
Magnesia and Alumina Tablets	W
Magnesium Gluconate Tablets	W
Magnesium Oxide Capsules	W
Magnesium Oxide Tablets	W
Magnesium Salicylate Tablets	T
Magnesium Trisilicate Tablets	W
Maprotiline Hydrochloride Tablets	W
Delete the following:	
▲Mazindol Tablets	T▲ (USP 1-Dec-2019)
Mebendazole Tablets	W
Mecamylamine Hydrochloride Tablets	W

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Meclizine Hydrochloride Tablets	W
Meclofenamate Sodium Capsules	T, LR
Medroxyprogesterone Acetate Tablets	W
Mefenamic Acid Capsules	T
Mefloquine Hydrochloride Tablets	T, LR
Megestrol Acetate Tablets	W
Melatonin Tablets	T, LR
Meloxicam Tablets	W
Melphalan Tablets	W, LR
Memantine Hydrochloride Tablets	T
Delete the following:	
▲Menadiol Sodium Diphosphate Tablets	W, L R▲ (USP 1-Dec-2019)
Menaquinone-7 Capsules	T, LR
Menaquinone-7 Tablets	T, LR
Meperidine Hydrochloride Tablets	W, LR
Mephobarbital Tablets	W
Meprobamate Tablets	W
Mercaptopurine Tablets	W
Mesalamine Capsules, Extended-Release	T, LR
Mesalamine Tablets, Delayed-Release	T
Mesna Tablets	T
Metaproterenol Sulfate Tablets	W, LR
Metaxalone Tablets	W, LR
Metformin Hydrochloride Tablets	T
Metformin Hydrochloride Tablets, Extended-Release	W, LR
Methadone Hydrochloride Tablets	W
Methamphetamine Hydrochloride Tablets	T, LR
Methazolamide Tablets	W
Methdilazine Hydrochloride Tablets	T, LR
Methenamine Tablets	W
Methenamine Hippurate Tablets	W
Methenamine Mandelate Tablets	W
Methimazole Tablets	W, LR
Methocarbamol Tablets	T
Methotrexate Tablets	W
Methoxsalen Capsules	T, LR
Methscopolamine Bromide Tablets	T
Methsuximide Capsules	T
Methyclothiazide Tablets	W
Methylcellulose Tablets	W
Methylcobalamin Tablets	T, LT
Methyldopa Tablets	W
Methyldopa and Hydrochlorothiazide Tablets	W

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Methylergonovine Maleate Tablets	T, LR
Methylphenidate Hydrochloride Tablets	T
Methylphenidate Hydrochloride Tablets, Extended-Release	T
Methylprednisolone Tablets	T
Methylsulfonylmethane Tablets	T, LR
Methyltestosterone Capsules	W
Methyltestosterone Tablets	W
Metoclopramide Tablets	T, LR
Metolazone Tablets	T, LR
Metoprolol Succinate Tablets, Extended-Release	T
Metoprolol Tartrate Tablets	T, LR
Metoprolol Tartrate and Hydrochlorothiazide Tablets	T, LR
Metronidazole Capsules	W, LR
Metronidazole Tablets	W, LR
Metronidazole Tablets, Extended-Release	W
Metyrosine Capsules	W
Mexiletine Hydrochloride Capsules	T
Midodrine Hydrochloride Tablets	W
Minerals Capsules	T, LR
Minerals Tablets	T, LR
Minocycline Hydrochloride Capsules	T, LR
Minocycline Hydrochloride Tablets	T, LR
Minocycline Hydrochloride Tablets, Extended-Release	T
Minoxidil Tablets	T
Mirtazapine Tablets	T, LR
Mirtazapine Tablets, Orally Disintegrating	LR
Mitotane Tablets	T, LR
Modafinil Tablets	T
Moexipril Hydrochloride and Hydrochlorothiazide Tablets	W
Moexipril Hydrochloride Tablets	T, W
Molindone Hydrochloride Tablets	T, LR
Montelukast Sodium Tablets	T
Montelukast Sodium Tablets, Chewable	T
Morphine Sulfate Capsules, Extended-Release	T, LR
Moxifloxacin Tablets	T
Mycophenolate Mofetil Capsules	W, LR
Mycophenolate Mofetil Tablets	W, LR
Mycophenolic Acid Tablets, Delayed-Release	T
Nabumetone Tablets	W
Nadolol Tablets	T
Nadolol and Bendroflumethiazide Tablets	T
Naltrexone Hydrochloride Tablets	T

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Naproxen Tablets	W
Naproxen Tablets, Delayed-Release	W
Naproxen Sodium Tablets	W
Naratriptan Tablets	T
Nateglinide Tablets	T
Nefazodone Hydrochloride Tablets	T
Neomycin Sulfate Tablets	T
Neostigmine Bromide Tablets	T
Nevirapine Tablets	W
Nevirapine Tablets, Extended-Release	W
Niacin Tablets	W
Niacin Tablets, Extended-Release	T
Niacinamide Tablets	T
Nifedipine Capsules	T, LR
Nifedipine Tablets, Extended-Release	T, LR
Nitrofurantoin Capsules	T
Nitrofurantoin Tablets	T, LR
Nitroglycerin Tablets	T
Nitroglycerin Tablets, Sublingual	T
Norethindrone Tablets	W
Norethindrone and Ethinyl Estradiol Tablets	W
Norethindrone and Mestranol Tablets	W
Norethindrone Acetate Tablets	W
Norethindrone Acetate and Ethinyl Estradiol Tablets	W
Norgestimate and Ethinyl Estradiol Tablets	W
Norgestrel Tablets	W
Norgestrel and Ethinyl Estradiol Tablets	W
Nortriptyline Hydrochloride Capsules	T
Nystatin Tablets	T, LR
Nystatin Tablets, Vaginal	W, LR
Ofloxacin Tablets	W
Olanzapine Tablets	T, LR
Olanzapine Tablets, Orally Disintegrating	W
Olanzapine and Fluoxetine Capsules	T
Oleovitamin A and D Capsules	T, LR
Olmesartan Medoxomil Tablets	W
Omega-3 Acids Ethyl Esters Capsules	T, LR
Omeprazole Capsules, Delayed-Release	T, LR
Ondansetron Tablets	T, LR
Ondansetron Tablets, Orally Disintegrating	LR
Orbifloxacin Tablets	T
Orlistat Capsules	T
Orphenadrine Citrate, Aspirin, and Caffeine Tablets	T

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Orphenadrine Citrate Tablets, Extended-Release	W, LR
Oseltamivir Phosphate Capsules	W
Oxandrolone Tablets	T, LR
Oxaprozin Tablets	T, LR
Oxazepam Capsules	W
Oxcarbazepine Tablets	W
Delete the following:	
▲Oxprenolol Hydrochloride Tablets	W, L R▲ (USP 1-Dec-2019)
Delete the following:	
▲Oxprenolol Hydrochloride Tablets, Extended-Release	W, L R▲ (USP 1-May-2020)
Delete the following:	
▲Oxtriphylline Tablets	T▲ (USP 1-Dec-2019)
Oxtriphylline Tablets, Extended-Release	T
Oxybutynin Chloride Tablets	T, LR
Oxybutynin Chloride Tablets, Extended-Release	T
Oxycodone Hydrochloride Tablets	T, LR
Oxycodone Hydrochloride Tablets, Extended-Release	T, LR
Delete the following:	
▲Oxycodone and Acetaminophen Capsules	T, L R▲ (USP 1-May-2020)
Oxycodone and Acetaminophen Tablets	T, LR
Oxycodone and Aspirin Tablets	T, LR
Oxymetholone Tablets	W
Oxymorphone Hydrochloride Tablets	T
Oxymorphone Hydrochloride Tablets, Extended-Release	T
Oxytetracycline Tablets	T, LR
Delete the following:	
▲Oxytetracycline and Nystatin Capsules	T, L R▲ (USP 1-Dec-2019)
Oxytetracycline Hydrochloride Capsules	T, LR
Oxytetracycline Hydrochloride and Polymyxin B Sulfate Tablets, Vaginal	W
Pancreatin Tablets	T
Pancrelipase Capsules	T
Pancrelipase Capsules, Delayed-Release	T
Pancrelipase Tablets	T
Pantoprazole Sodium Tablets, Delayed-Release	W
Papain Tablets for Topical Solution	T, LR
Papaverine Hydrochloride Tablets	T
Paricalcitol Capsules	T
Paromomycin Sulfate Capsules	T
Paroxetine Tablets	T
Paroxetine Tablets, Extended-Release	W

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Penbutolol Sulfate Tablets	W, LR
Penicillamine Capsules	T
Penicillamine Tablets	T
Penicillin G Benzathine Tablets	T
Penicillin G Potassium Tablets	T
Penicillin V Tablets	T
Penicillin V Potassium Tablets	T
Pentazocine and Acetaminophen Tablets	T, LR
Pentazocine and Aspirin Tablets	T, LR
Pentazocine and Naloxone Tablets	T, LR
Pentoxifylline Tablets, Extended-Release	W
Perindopril Erbumine Tablets	T
Perphenazine Tablets	T, LR
Perphenazine and Amitriptyline Hydrochloride Tablets	W
Phenazopyridine Hydrochloride Tablets	T
Phendimetrazine Tartrate Capsules	T
Phendimetrazine Tartrate Tablets	W
Phenelzine Sulfate Tablets	T
Phenobarbital Tablets	W
Phenoxybenzamine Hydrochloride Capsules	W
Delete the following:	
▲Phensuximide Capsules	T▲ (USP 1-Dec-2019)
Phentermine Hydrochloride Capsules	T
Phentermine Hydrochloride Tablets	T
Phenylbutazone Tablets	T
Phenylephrine Hydrochloride Tablets	T
Phenytoin Chewable Tablets	W
Phenytoin Sodium Capsules, Extended	T
Phenytoin Sodium Capsules, Prompt	T
Phytonadione Tablets	W, LR
Pilocarpine Hydrochloride Tablets	T
Pimozide Tablets	T, LR
Pindolol Tablets	W, LR
Pioglitazone and Glimepiride Tablets	T
Pioglitazone and Metformin Hydrochloride Tablets	T
Pioglitazone Tablets	T
Delete the following:	
▲Piperazine Citrate Tablets	T▲ (USP 1-May-2020)
Piroxicam Capsules	T, LR
Potassium Bicarbonate Effervescent Tablets for Oral Solution	T
Potassium Bicarbonate and Potassium Chloride Effervescent Tablets for Oral Solution	T
Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution	T

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Potassium Chloride Capsules, Extended-Release	T
Potassium Chloride Tablets, Extended-Release	T
Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution	T
Potassium Citrate Tablets	W
Potassium Citrate Tablets, Extended-Release	T
Potassium Gluconate Tablets	T
Potassium Iodide Tablets	T
Potassium Iodide Tablets, Delayed-Release	T
Delete the following:	
▲Potassium Perchlorate Capsules	T, L R▲ (USP 1-Dec-2019)
Pravastatin Sodium Tablets	T
Praziquantel Tablets	T
Prazosin Hydrochloride Capsules	W, LR
Prednisolone Tablets	W
Prednisone Tablets	W
Primaquine Phosphate Tablets	W, LR
Primidone Tablets	W
Probenecid and Colchicine Tablets	W, LR
Procainamide Hydrochloride Capsules	T
Procainamide Hydrochloride Tablets	T
Procarbazine Hydrochloride Capsules	T, LR
Prochlorperazine Maleate Tablets	W
Procyclidine Hydrochloride Tablets	T
Promazine Hydrochloride Tablets	T, LR
Promethazine Hydrochloride Tablets	T, LR
Propafenone Hydrochloride Capsules, Extended-Release	T
Propafenone Hydrochloride Tablets	T
Propantheline Bromide Tablets	W
Propranolol Hydrochloride Capsules, Extended-Release	W
Propranolol Hydrochloride Tablets	W, LR
Propranolol Hydrochloride and Hydrochlorothiazide Capsules, Extended-Release	W
Propranolol Hydrochloride and Hydrochlorothiazide Tablets	W
Propylthiouracil Tablets	W
Protriptyline Hydrochloride Tablets	T
Pseudoephedrine Hydrochloride Tablets	T
Pseudoephedrine Hydrochloride Tablets, Extended-Release	T
Pygeum Capsules	T
Pyrazinamide Tablets	W
Pyridostigmine Bromide Tablets	T, LR
Pyridoxine Hydrochloride Tablets	W
Pyrilamine Maleate Tablets	W

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Pyrimethamine Tablets	T, LR
Delete the following:	
▲Pyrvinium Pamoate Tablets	T, L R▲ (USP 1-May-2020)
Quazepam Tablets	W
Quetiapine Tablets	W
Quetiapine Tablets, Extended-Release	W
Quinapril Tablets	W
Quinapril and Hydrochlorothiazide Tablets	W, LR
Quinidine Gluconate Tablets, Extended-Release	W, LR
Quinidine Sulfate Capsules	T, LR
Quinidine Sulfate Tablets	W, LR
Quinidine Sulfate Tablets, Extended-Release	W, LR
Quinine Sulfate Capsules	T
Quinine Sulfate Tablets	W
Raloxifene Hydrochloride Tablets	T
Raltegravir Tablets	T
Raltegravir Tablets, Chewable	T
Ramipril Capsules	W
Ramipril Tablets	W
Ranitidine Tablets	T, LR
Rauwolfia Serpentina Tablets	T, LR
Reserpine Tablets	T, LR
Reserpine and Chlorothiazide Tablets	T, LR
Reserpine, Hydralazine Hydrochloride, and Hydrochlorothiazide Tablets	T, LR
Rhodiola rosea Capsules	W
Rhodiola rosea Tablets	W
Ribavirin Capsules	W
Ribavirin Tablets	T
Riboflavin Tablets	T, LR
Rifabutin Capsules	W
Rifampin Capsules	T, LR
Rifampin and Isoniazid Capsules	T, LR
Rifampin, Isoniazid, and Pyrazinamide Tablets	T, LR
Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride Tablets	T, LR
Riluzole Tablets	W, LR
Rimantadine Hydrochloride Tablets	T, LR
Risedronate Sodium Tablets	W
Risperidone Tablets, Orally Disintegrating	W, LR
Risperidone Tablets	T, LR
Delete the following:	
▲Ritodrine Hydrochloride Tablets	T▲ (USP 1-May-2020)
Ritonavir Capsules	T, LR
Ritonavir Tablets	T

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Rivastigmine Tartrate Capsules	T
Rizatriptan Benzoate Tablets	W, LR
Rizatriptan Benzoate Tablets, Orally Disintegrating	W, LR
Ropinirole Tablets	W
Ropinirole Tablets, Extended-Release	W
Rosuvastatin Tablets	W
Rufinamide Tablets	T
Saccharin Sodium Tablets	W
St. John's Wort Flowering Top Dry Extract Capsules	W
St. John's Wort Flowering Top Dry Extract Tablets	W
Salsalate Capsules	T
Salsalate Tablets	T
Saquinavir Capsules	T
Saw Palmetto Capsules	T, LR
Schizochytrium Oil Capsules	T, LR
Delete the following:	
▲Scopolamine Hydrobromide Tablets	T, L R▲ (USP 1-May-2020)
Secobarbital Sodium Capsules	T
Secobarbital Sodium and Amobarbital Sodium Capsules	W
Selegiline Hydrochloride Capsules	T, LR
Selegiline Hydrochloride Tablets	T, LR
Sennosides Tablets	W
Sertraline Tablets	W
Sildenafil Tablets	W
Simethicone Capsules	W
Simethicone Tablets	W
Simvastatin Tablets	T
Sitagliptin Tablets	W
Sodium Bicarbonate Tablets	W
Sodium Chloride Tablets	W
Sodium Chloride Tablets for Solution	W
Sodium Chloride and Dextrose Tablets	W
Sodium Fluoride Tablets	T
Sodium Salicylate Tablets	W
Sotalol Hydrochloride Tablets	W, LR
Soy Isoflavones Capsules	T, LR
Soy Isoflavones Tablets	T, LR
Spironolactone Tablets	T, LR
Spironolactone and Hydrochlorothiazide Tablets	T, LR
Spirulina Tablets	W, LR
Stanozolol Tablets	T, LR
Stavudine Capsules	T

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Sulfadiazine Tablets	W, LR
Sulfadimethoxine Tablets	T, LR
Sulfadoxine and Pyrimethamine Tablets	W, LR
Sulfamethizole Tablets	W
Sulfamethoxazole Tablets	W, LR
Sulfamethoxazole and Trimethoprim Tablets	W, LR
Sulfapyridine Tablets	W, LR
Sulfasalazine Tablets	W
Sulfasalazine Tablets, Delayed-Release	W
Sulfinpyrazone Capsules	W
Sulfinpyrazone Tablets	W
Sulfisoxazole Tablets	W, LR
Sulindac Tablets	W
Sumatriptan Tablets	W
Tacrolimus Capsules	T
Tadalafil Tablets	T
Tamoxifen Citrate Tablets	W, LR
Tamsulosin Hydrochloride Capsules	T
Telmisartan Tablets	W
Telmisartan and Amlodipine Tablets	T
Telmisartan and Hydrochlorothiazide Tablets	W
Temazepam Capsules	W, LR
Temozolomide Capsules	T
Terazosin Capsules	W, LR
Terazosin Tablets	W, LR
Terbinafine Tablets	W, LR
Terbutaline Sulfate Tablets	T
Testolactone Tablets	T
Tetracycline Hydrochloride Capsules	T, LR
Tetracycline Hydrochloride Tablets	T, LR
Tetracycline Hydrochloride and Novobiocin Sodium Tablets	T
Tetracycline Hydrochloride, Novobiocin Sodium, and Prednisolone Tablets	T
Tetracycline Hydrochloride and Nystatin Capsules	T, LR
Thalidomide Capsules	W
Theophylline Capsules	W
Theophylline Capsules, Extended-Release	W
Theophylline Tablets	W
Theophylline, Ephedrine Hydrochloride, and Phenobarbital Tablets	T
Theophylline and Guaifenesin Capsules	T
Theophylline Sodium Glycinate Tablets	W
Thiabendazole Tablets	T
Thiamine Hydrochloride Tablets	T, LR
Thiethylperazine Maleate Tablets	T, LR

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Thioguanine Tablets	T
Thioridazine Hydrochloride Tablets	T, LR
Thiothixene Capsules	W, LR
Thyroid Tablets	T
Ticlopidine Hydrochloride Tablets	W
Tienchi Ginseng Root and Rhizome Dry Extract Capsules	W
Tienchi Ginseng Root and Rhizome Dry Extract Tablets	W
Tienchi Ginseng Root and Rhizome Powder Capsules	W
Tienchi Ginseng Root and Rhizome Powder Tablets	W
Timolol Maleate Tablets	W
Timolol Maleate and Hydrochlorothiazide Tablets	W, LR
Tizanidine Tablets	T
Tolazamide Tablets	T
Tolazoline Hydrochloride Tablets	W
Tolbutamide Tablets	W
Tolcapone Tablets	T
Tolmetin Sodium Capsules	T
Tolmetin Sodium Tablets	W
Topiramate Capsules	T
Topiramate Tablets	T
Torsemide Tablets	T
Tramadol Hydrochloride Tablets	T
Tramadol Hydrochloride Tablets, Extended-Release	T
Trandolapril Tablets	T
Trandolapril and Verapamil Hydrochloride Tablets, Extended-Release	W
Tranexamic Acid Tablets	W
Tranylcypromine Tablets	W
Trazodone Hydrochloride Tablets	T, LR
Triamcinolone Tablets	W
Triamterene Capsules	T, LR
Triamterene and Hydrochlorothiazide Capsules	T, LR
Triamterene and Hydrochlorothiazide Tablets	T, LR
Triazolam Tablets	T, LR
Trientine Hydrochloride Capsules	T
Trifluoperazine Hydrochloride Tablets	W, LR
Triflupromazine Hydrochloride Tablets	W, LR
Trihexyphenidyl Hydrochloride Capsules, Extended-Release	T
Trihexyphenidyl Hydrochloride Tablets	T
Trimeprazine Tartrate Tablets	W, LR
Trimethobenzamide Hydrochloride Capsules	W
Trimethoprim Tablets	T, LR
Trioxsalen Tablets	W, LR

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Tripelennamine Hydrochloride Tablets	W
Triple Sulfa Tablets, Vaginal	W, LR
Triprolidine Hydrochloride Tablets	T, LR
Triprolidine and Pseudoephedrine Hydrochlorides Tablets	T, LR
Trisulfapyrimidines Tablets	W
Troleandomycin Capsules	T
Trospium Chloride Tablets	T, LR
Ubidecarenone Capsules	T, LR
Ubidecarenone Tablets	T, LR
Ubiquinol Capsules	T, LR
Ursodiol Capsules	W
Ursodiol Tablets	W
Valacyclovir Tablets	T
Valerian Tablets	T, LR
Valerian Root Dry Extract Capsules	W
Valerian Root Powder Capsules	W
Valganciclovir Tablets	T
Valproic Acid Capsules	T
Valsartan Tablets	T
Valsartan and Hydrochlorothiazide Tablets	T
Vancomycin Hydrochloride Capsules	T
Venlafaxine Tablets	W
Verapamil Hydrochloride Capsules, Extended-Release	T, LR
Verapamil Hydrochloride Tablets	T, LR
Verapamil Hydrochloride Tablets, Extended-Release	T, LR
Vigabatrin Tablets	T
Vinpocetine Capsules	T, LR
Vinpocetine Tablets	T, LR
Vitamin A Capsules	T, LR
Vitamin A Tablets	T, LR
Vitamin E Capsules	T
Oil-Soluble Vitamins Capsules	T, LR
Oil-Soluble Vitamins Tablets	T, LR
Oil-Soluble Vitamins with Minerals Capsules	T, LR
Oil-Soluble Vitamins with Minerals Tablets	T, LR
Oil- and Water-Soluble Vitamins Capsules	T, LR
Oil- and Water-Soluble Vitamins Tablets	T, LR
Oil- and Water-Soluble Vitamins with Minerals Capsules	T, LR
Oil- and Water-Soluble Vitamins with Minerals Tablets	T, LR
Water-Soluble Vitamins Capsules	T, LR
Water-Soluble Vitamins Tablets	T, LR
Water-Soluble Vitamins with Minerals Capsules	T, LR
Water-Soluble Vitamins with Minerals Tablets	T, LR
Warfarin Sodium Tablets	T, LR

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Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Zalcitabine Tablets	T, LR
Zaleplon Capsules	LR
Zidovudine Capsules	T, LR
Zidovudine Tablets	T, LR
Zinc Citrate Tablets	W
Zinc Gluconate Tablets	T, LR
Zinc Sulfate Tablets	W

Container Specifications for Capsules and Tablets *(continued)*

Monograph Title	Container Specification
Ziprasidone Capsules	W
Zolmitriptan Tablets	W, LR
Zolmitriptan Tablets, Orally Disintegrating	W, LR
Zolpidem Tartrate Tablets	W
Zolpidem Tartrate Tablets, Extended-Release	W
Zonisamide Capsules	T, LR

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DESCRIPTION AND RELATIVE SOLUBILITY

Description and Relative Solubility of USP and NF Articles

The "description" and "solubility" statements pertaining to an article (formerly included in the individual monograph) are general in nature. The information is provided for those who use, prepare, and dispense drugs, solely to indicate descriptive and solubility properties of an article complying with monograph standards. The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article.

Taste and Odor

Organoleptic characteristics are indicated in many instances because they may be useful and descriptive properties of substances. However, they are not meant to be applied as tests for identifying materials.

The inclusion of odor or taste among other descriptive properties may aid in identifying the causative agent following accidental exposure to or contact with a substance. This information is provided as a warning or to make an individual aware of sensations that may be encountered. The use of odor or taste as a test for identification or content is strongly discouraged.

The characteristic odor of a volatile substance becomes apparent immediately on opening a container of it. The odor may be agreeable (e.g., Peppermint Oil), unpleasant (e.g., Sulfur Dioxide), or potentially hazardous on prolonged exposure (e.g., Coal Tar). Moreover, an unexpected odor may be encountered if the characteristics of a substance are not known or if a container is incorrectly labeled. Consequently, containers of such substances should be opened cautiously, preferably in a well-ventilated fume hood. A characteristic taste or sensation produced in the oral cavity likewise is apparent if traces of residue materials on fingers are inadvertently brought into contact with the tongue or adjacent mucosal tissues.

Solubility

Only where a special, quantitative solubility test is given in the individual monograph, and is designated by a test heading, is it a test for purity.

The approximate solubilities of Pharmacopeial and National Formulary substances are indicated by the descriptive terms in the accompanying table. The term "miscible" as used in this Pharmacopeia pertains to a substance that yields a homogeneous mixture when mixed in any proportion with the designated solvent.

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble, or Insoluble	10,000 and over

Soluble Pharmacopeial and National Formulary articles, when brought into solution, may show traces of physical impurities, such as minute fragments of filter paper, fibers, and other particulate matter, unless limited or excluded by definite tests or other specifications in the individual monographs.

Abacavir Sulfate: White to off-white powder. Soluble in water, in ethyl acetate, in absolute alcohol, and in methanol.

Abiraterone Acetate: White to off-white powder. Freely soluble in methylene chloride, in tetrahydrofuran, and in toluene; soluble in methanol, in ethanol, in ethyl acetate, in isobutyl methyl ketone, in *N,N*-dimethylformamide, and in acetone; sparingly soluble in acetonitrile and in dimethyl sulfoxide; slightly soluble in hexane; very slightly soluble in 0.1 N hydrochloric acid; practically insoluble in aqueous media over a wide range of pH values.

Acacia: Is practically odorless and produces a mucilaginous sensation on the tongue. Insoluble in alcohol. Optical rotation varies depending on the source of Acacia. For example, specific rotation values, calculated on the anhydrous basis and determined on a 1.0% (w/v) solution, usually are between −25° and −35° for *Acacia senegal* and between +35° and +60° for *Acacia seyal*. *NF category:* Emulsifying agent; suspending and/or viscosity-increasing agent; wet binder.

Acamprosate Calcium: A white or almost-white powder. Freely soluble in water; practically insoluble in alcohol and in methylene chloride.

Acebutolol Hydrochloride: White or almost white, crystalline powder. Soluble in alcohol and in water; very slightly soluble in acetone and in methylene chloride; practically insoluble in ether. Melts at about 141° to 144°.

Acesulfame Potassium: A white, crystalline powder or colorless crystals. Soluble in water; very slightly soluble in acetone and in alcohol. *NF category:* Sweetening agent.

Acetaminophen: White, odorless, crystalline powder, having a slightly bitter taste. Freely soluble in alcohol; soluble in boiling water and in 1 N sodium hydroxide.

Acetazolamide: White to faintly yellowish-white, crystalline, odorless powder. Sparingly soluble in practically boiling water; slightly soluble in alcohol; very slightly soluble in water.

Acetic Acid: Clear, colorless liquid, having a strong, characteristic odor, and a sharply acid taste. Specific gravity is about 1.045. Miscible with water, with alcohol, and with glycerin. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Glacial Acetic Acid: Clear, colorless liquid, having a pungent, characteristic odor and, when well diluted with water, an acid taste. Boils at about 118°. Specific gravity is about 1.05. Miscible with water, with alcohol, and with glycerin. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Acetohydroxamic Acid: White, slightly hygroscopic, crystalline powder. Melts, after drying at about 80° for 2 to 4 hours, at about 88°. Freely soluble in water and in alcohol; very slightly soluble in chloroform.

Acetone: Transparent, colorless, mobile, volatile liquid, having a characteristic odor. A solution (1 in 2) is neutral to litmus. Miscible with water, with alcohol, with ether, with chloroform, and with most volatile oils. *NF category:* Solvent.

Acetylcholine Chloride: White or off-white crystals or crystalline powder. Very soluble in water; freely soluble in alcohol; insoluble in ether. Is decomposed by hot water and by alkalies.

Acetylcysteine: White, crystalline powder, having a slight acetic odor. Freely soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Acetyltributyl Citrate: Clear, practically colorless, oily liquid. Freely soluble in alcohol, in isopropyl alcohol, in acetone, and in toluene; insoluble in water. *NF category:* Plasticizer.

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Acetyltriethyl Citrate: Clear, practically colorless, oily liquid. Freely soluble in alcohol, in isopropyl alcohol, in acetone, and in toluene; insoluble in water. *NF category:* Plasticizer.

Acitretin: Yellow or greenish, crystalline powder. Sparingly soluble in tetrahydrofuran; slightly soluble in acetone and in alcohol; very slightly soluble in cyclohexane; practically insoluble in water.

Acyclovir: White to off-white, crystalline powder. Melts at temperatures higher than 250°, with decomposition. Soluble in diluted hydrochloric acid; slightly soluble in water; insoluble in alcohol.

Adapalene: White or almost white powder. Soluble in tetrahydrofuran; sparingly soluble in ethanol; practically insoluble in water.

Ademetionine Disulfate Tosylate: White powder. Freely soluble in water.

Adenine: White crystals or crystalline powder. Is odorless and tasteless. Sparingly soluble in boiling water; slightly soluble in alcohol; very slightly soluble in water; practically insoluble in ether and in chloroform.

Adenosine: White, odorless, crystalline powder. Slightly soluble in water; practically insoluble in alcohol. Melts at about 235°.

Adipic Acid: A white, crystalline powder. Freely soluble in alcohol and in methanol; soluble in boiling water and in acetone; slightly soluble in water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); flavors and fragrance.

Agar: Odorless or has a slight odor, and produces a mucilaginous sensation on the tongue. Soluble in boiling water; insoluble in cold water. *NF category:* Suspending and/or viscosity-increasing agent; emulsifying agent; suppository base; wet binder.

Alamic Acid: *NF category:* Suspending and/or viscosity-increasing agent.

Alanine: White, odorless crystals or crystalline powder, having a slightly sweet taste. Freely soluble in water; slightly soluble in 80% alcohol; insoluble in ether.

Albendazole: White to faintly yellowish powder. Freely soluble in anhydrous formic acid; very slightly soluble in ether and in methylene chloride; practically insoluble in alcohol and in water.

Albumin Human: Practically odorless, moderately viscous, clear, brownish fluid.

rAlbumin Human: Clear, slightly viscous, and colorless to yellow amber in color. *NF category:* Vehicle (sterile).

Albuterol: White, crystalline powder. Soluble in alcohol; sparingly soluble in water. Melts at about 156°.

Albuterol Sulfate: White or practically white powder. Freely soluble in water; slightly soluble in alcohol, in chloroform, and in ether.

Alcohol: Clear, colorless, mobile, volatile liquid. Has a characteristic odor and produces a burning sensation on the tongue. Is readily volatilized even at low temperatures, and boils at about 78°. Is flammable. Miscible with water and with practically all organic solvents. *NF category:* Solvent; antimicrobial preservative.

Dehydrated Alcohol: Clear, colorless, mobile, volatile liquid. Has a characteristic odor and produces a burning sensation on the tongue. Is readily volatilized even at low temperatures, and boils at about 78°. Is flammable. Miscible with water and with practically all organic solvents.

Diluted Alcohol: Clear, colorless, mobile liquid, having a characteristic odor and producing a burning sensation on the tongue. *NF category:* Solvent.

Rubbing Alcohol: Transparent, colorless, or colored as desired, mobile, volatile liquid. Has an extremely bitter taste and, in the absence of added odorous constituents, a characteristic odor. Is flammable.

Alendronate Sodium: White, free-flowing powder. Soluble in water; very slightly soluble in dimethyl sulfoxide, in methyl alcohol, and in propylene glycol; practically insoluble in acetone, in acetonitrile, in alcohol, in chloroform, and in isopropyl alcohol.

Alfadex: White or almost white, amorphous or crystalline powder. Freely soluble in water and in propylene glycol; practically insoluble in ethanol and in methylene chloride. *NF category:* Chelating and/or complexing agent.

Alfentanil Hydrochloride: White to almost white powder. Freely soluble in methanol, in alcohol, and in chloroform; soluble in water; sparingly soluble in acetone. Melting point range, crystals from acetone: 136°–143° (anhydrous) and reported as crystals from aqueous hydrochloric acid: 116°–126° (monohydrate).

Alfentanil Injection: Clear, colorless solution.

Alfuzosin Hydrochloride: White to almost white powder, slightly hygroscopic. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in methylene chloride.

Alginic Acid: White to yellowish white, fibrous powder. Is odorless, or practically odorless, and is tasteless. Soluble in alkaline solutions; insoluble in water and in organic solvents. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; disintegrant; film-forming agent; release-modifying agent.

Alkyl (C12-15) Benzoate: Clear, practically colorless, oily liquid. Soluble in acetone, in alcohol, in isopropyl alcohol, in ethyl acetate, in isopropyl myristate, in isopropyl palmitate, in lanolin, in mineral oil, in vegetable oils, and in volatile silicones; insoluble in water, in glycerin, and in propylene glycol. *NF category:* Vehicle (oleaginous); emollient.

Allantoin: White, crystalline powder. Slightly soluble in water; very slightly soluble in alcohol. Melts at about 225°, with decomposition.

Allopurinol: Fluffy white to off-white powder, having only a slight odor. Soluble in solutions of potassium and sodium hydroxides; very slightly soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Allyl Isothiocyanate: Colorless to pale yellow, very refractive, liquid. Pungent irritating odor, acrid taste. [**CAUTION**—*Lachrymator.*] Miscible with alcohol, with carbon disulfide, and with ether. Slightly soluble in water.

Almond Oil: Clear, pale straw-colored or colorless, oily liquid, having a bland taste. Remains clear at –10°, and does not congeal until cooled to almost –20°. Slightly soluble in alcohol. Miscible with ether, with chloroform, with benzene, and with solvent hexane. *NF category:* Flavors and fragrance; vehicle (oleaginous); emollient; solvent.

Almotriptan Malate: White to slightly yellow, crystalline powder. Soluble in water; slightly soluble in methanol and in *N,N*-dimethylformamide; very slightly soluble in acetone, in acetonitrile, in ethanol, in ethyl acetate, in propanol, in tetrahydrofuran, and in toluene; practically insoluble in chloroform and in methylene chloride.

Aloe: Has a characteristic, somewhat sour and disagreeable, odor.

Alprazolam: A white to off-white, crystalline powder. Melts at about 225°. Freely soluble in chloroform; soluble in alcohol; sparingly soluble in acetone; slightly soluble in ethyl acetate; insoluble in water.

Alprostadil: A white to off-white, crystalline powder. Melts at about 110°. Freely soluble in alcohol; soluble in water and in acetone; slightly soluble in ethyl acetate; very slightly soluble in chloroform and in ether.

Altretamine: White, crystalline powder. Soluble in chloroform; insoluble in water.

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Ammonium Alum: Large, colorless crystals, crystalline fragments, or white powder. Is odorless, and has a sweetish, strongly astringent taste. Its solutions are acid to litmus. Very soluble in boiling water; freely soluble in water and in glycerin; insoluble in alcohol.

Potassium Alum: Large, colorless crystals, crystalline fragments, or white powder. Is odorless, and has a sweetish, strongly astringent taste. Its solutions are acid to litmus. Very soluble in boiling water; freely soluble in water and in glycerin; insoluble in alcohol.

Aluminum Acetate Topical Solution: Clear, colorless liquid having a faint odor of acetic acid, and a sweetish, astringent taste. Specific gravity is about 1.02.

Aluminum Chloride: White, or yellowish-white, deliquescent, crystalline powder. Is practically odorless, and has a sweet, very astringent taste. Its solutions are acid to litmus. Very soluble in water; freely soluble in alcohol; soluble in glycerin.

Aluminum Hydroxide Gel: White, viscous suspension, from which small amounts of clear liquid may separate on standing.

Dried Aluminum Hydroxide Gel: White, odorless, tasteless, amorphous powder. Soluble in dilute mineral acids and in solutions of fixed alkali hydroxides; insoluble in water and in alcohol.

Aluminum Monostearate: Fine, white to yellowish-white, bulky powder, having a faint, characteristic odor. Insoluble in water, in alcohol, and in ether. *NF category:* Suspending and/or viscosity-increasing agent; emollient.

Aluminum Oxide: Occurs as a white or almost white, amorphous powder. It is very slightly soluble in dilute mineral acids and in solutions of alkali hydroxides. It is practically insoluble in water. *NF category:* Coloring agent.

Aluminum Phosphate Gel: White, viscous suspension from which small amounts of water separate on standing.

Aluminum Subacetate Topical Solution: Clear, colorless or faintly yellow liquid, having an odor of acetic acid and an acid reaction to litmus. Gradually becomes turbid on standing, through separation of a more basic salt.

Aluminum Sulfate: White, crystalline powder, shining plates, or crystalline fragments. Is stable in air. Is odorless, and has a sweet taste, becoming mildly astringent. Freely soluble in water; insoluble in alcohol.

Amantadine Hydrochloride: White or practically white, crystalline powder, having a bitter taste. Freely soluble in water; soluble in alcohol and in chloroform.

Amifostine: White, crystalline powder. Freely soluble in water.

Amikacin: White, crystalline powder. Sparingly soluble in water.

Amikacin Sulfate: White, crystalline powder. Freely soluble in water.

Amiloride Hydrochloride: Yellow to greenish-yellow, odorless or practically odorless powder. Freely soluble in dimethyl sulfoxide; sparingly soluble in methanol; slightly soluble in water; insoluble in ether, in ethyl acetate, in acetone, and in chloroform.

Amino Methacrylate Copolymer: Colorless to yellowish granules. Soluble in acetone, in isopropyl alcohol, and in diluted acids; practically insoluble in water. The solutions are clear to slightly cloudy. *NF category:* Coating agent; polymer membrane; wet binder; diluent; film-forming agent.

Aminobenzoate Potassium: White, crystalline powder. The pH of a 1 in 100 solution in water is about 7. Very soluble in water; soluble in alcohol; practically insoluble in ether.

Aminobenzoic Acid: White or slightly yellow, odorless crystals or crystalline powder. Discolors on exposure to air or light. Freely soluble in alcohol and in solutions of alkali hydroxides and carbonates; sparingly soluble in ether; slightly soluble in water and in chloroform. *NF category:* Free radical scavenger.

Aminobenzoic Acid Topical Solution: Straw-colored solution having the odor of alcohol.

Aminocaproic Acid: Fine, white, crystalline powder. Is odorless, or practically odorless. Its solutions are neutral to litmus. Melts at about 205°. Freely soluble in water, in acids, and in alkalies; slightly soluble in methanol and in alcohol; practically insoluble in chloroform and in ether.

Aminoglutethimide: Fine, white, or creamy white, crystalline powder. Soluble in most organic solvents; very slightly soluble in water. Forms water-soluble salts with strong acids.

Aminohippuric Acid: White, crystalline powder. Discolors on exposure to light. Melts at about 195°, with decomposition. Freely soluble in alkaline solutions, with some decomposition, and in diluted hydrochloric acid; sparingly soluble in water and in alcohol; very slightly soluble in benzene, in carbon tetrachloride, in chloroform, and in ether.

Incommunicable Acid Hydrochloride: White to off-white, odorless, crystalline solid. Very soluble in water; slightly soluble in methanol and in ethanol; practically insoluble in chloroform, in hexane, and in mineral oil.

Acetaminophen Sulfate: White, crystalline powder. Freely soluble in water and in alcohol; very slightly soluble in chloroform; practically insoluble in ether.

Aminophylline: White or slightly yellowish granules or powder, having a slight ammoniacal odor and a bitter taste. Upon exposure to air, it gradually loses methamphetamine and absorbs carbon dioxide with the liberation of free theophylline. Its solutions are alkaline to litmus. One g dissolves in 25 mL of water to give a clear solution; 1 g dissolved in 5 mL of water crystallizes upon standing, but redissolves when a small amount of ethylenediamine is added. Insoluble in alcohol and in ether.

Aminophylline Tablets: May have a faint ammoniacal odor.

Aminosalicylate Sodium: White to cream-colored, crystalline powder. Is practically odorless, and has a sweet, saline taste. Its solutions decompose slowly and darken in color. Freely soluble in water; sparingly soluble in alcohol; very slightly soluble in ether and in chloroform.

Aminosalicylic Acid: White or practically white, bulky powder, that darkens on exposure to light and air. Is odorless, or has a slight acetous odor. Soluble in alcohol; slightly soluble in water and in ether; practically insoluble in benzene.

Amiodarone Hydrochloride: White or almost white, fine, crystalline powder. Freely soluble in methylene chloride; soluble in methanol; sparingly soluble in alcohol; very slightly soluble in water.

Amitriptyline Hydrochloride: White or practically white, odorless or practically odorless, crystalline powder or small crystals. Freely soluble in water, in alcohol, in chloroform, and in methanol; insoluble in ether.

Amlodipine Besylate: A white or almost white powder. Freely soluble in methanol; sparingly soluble in alcohol; slightly soluble in 2-propanol and in water.

Strong Ammonia Solution: Clear, colorless liquid, having an exceedingly pungent, characteristic odor. Specific gravity is about 0.90. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Aromatic Ammonia Spirit: Practically colorless liquid when recently prepared, but gradually acquiring a yellow color on standing. Has the taste of ammonia, has an aromatic and pungent odor, and is affected by light. Specific gravity is about 0.90.

Ammonio Methacrylate Copolymer: Colorless, clear to white-opaque granules or a white powder, both with a faint amine-like odor. Soluble to freely soluble in methanol, in

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alcohol, and in isopropyl alcohol, each of which contains small amounts of water; soluble to freely soluble in acetone, in ethyl acetate, and in methylene chloride. The solutions are clear to slightly cloudy. Insoluble in petroleum ether and in water. *NF category:* Coating agent; wet binder; polymer membrane; diluent; film-forming agent.

Ammonio Methacrylate Copolymer Dispersion: Milky-white liquids of low viscosity with a faint characteristic odor. Miscible with water in any proportion, the milky-white appearance being retained. A clear or slightly cloudy solution is obtained on mixing one part with five parts of acetone, alcohol, or isopropyl alcohol. When mixed with methanol in a ratio of 1:5, Ammonio Methacrylate Copolymer Dispersion Type A dissolves completely, and Ammonio Methacrylate Copolymer Dispersion Type B dissolves only partially. *NF category:* Coating agent; polymer membrane; wet binder; diluent; film-forming agent.

Ammonium Carbonate: White powder, or hard, white or translucent masses, having a strong odor of ammonia, without empyreuma, and a sharp, ammoniacal taste. Its solutions are alkaline to litmus. On exposure to air, it loses ammonia and carbon dioxide, becoming opaque, and is finally converted into friable porous lumps or a white powder of ammonium bicarbonate. Freely soluble in water, but is decomposed by hot water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Ammonium Chloride: Colorless crystals or white, fine or coarse, crystalline powder. Has a cool, saline taste, and is somewhat hygroscopic. Freely soluble in water and in glycerin, and even more so in boiling water; sparingly soluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Ammonium Glycyrrhizate: White or yellowish-white, hygroscopic powder. It is slightly soluble in water; very slightly soluble in anhydrous ethanol; practically insoluble in acetone. It dissolves in dilute solutions of acids and of alkali hydroxides. *NF category:* Flavors and fragrance; wetting and/or solubilizing agent.

Ammonium Molybdate: Colorless or slightly greenish or yellowish crystals. Soluble in water; practically insoluble in alcohol.

Ammonium Phosphate: Colorless or white granules or powder, having a saline taste. Freely soluble in water; practically insoluble in acetone and in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Ammonium Sulfate: Colorless or white crystals or granules that decompose at temperatures above 280°. One g is soluble in about 1.5 mL of water. It is insoluble in alcohol. The pH of a 0.1 M solution is between 4.5 and 6.0.

Amobarbital Sodium: White, friable, granular powder. Is odorless, has a bitter taste, and is hygroscopic. Its solutions decompose on standing, heat accelerating the decomposition. Very soluble in water; soluble in alcohol; practically insoluble in ether and in chloroform.

Amodiaquine: Very pale yellow to light tan-yellow, odorless powder. Sparingly soluble in 1.0 N hydrochloric acid; slightly soluble in alcohol; practically insoluble in water.

Amodiaquine Hydrochloride: Yellow, crystalline powder. Is odorless and has a bitter taste. Soluble in water; sparingly soluble in alcohol; very slightly soluble in benzene, in chloroform, and in ether.

Amoxapine: White to yellowish crystalline powder. Freely soluble in chloroform; soluble in tetrahydrofuran; sparingly soluble in methanol and in toluene; slightly soluble in acetone; practically insoluble in water.

Amoxicillin: White, practically odorless, crystalline powder. Slightly soluble in water and in methanol; insoluble in benzene, in carbon tetrachloride, and in chloroform.

Amphetamine Sulfate: White, odorless, crystalline powder, having a slightly bitter taste. Its solutions are acid to litmus, having a pH of 5 to 6. Freely soluble in water; slightly soluble in alcohol; practically insoluble in ether.

Amphotericin B: Yellow to orange powder; odorless or practically so. Soluble in dimethylformamide, in dimethyl sulfoxide, and in propylene glycol; slightly soluble in methanol; insoluble in water, in anhydrous alcohol, in ether, in benzene, and in toluene.

Amphotericin B for Injection: It yields a colloidal dispersion in water.

Ampicillin: White, practically odorless, crystalline powder. Slightly soluble in water and in methanol; insoluble in benzene, in carbon tetrachloride, and in chloroform.

Ampicillin Sodium: White to off-white, odorless or practically odorless, crystalline powder. Is hygroscopic. Very soluble in water and in isotonic sodium chloride and dextrose solutions.

Amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$): White to light yellow powder. Freely soluble in water, in methanol, in alcohol, and in dimethylformamide; sparingly soluble in dehydrated alcohol; practically insoluble in isopropyl alcohol, in butyl alcohol, and in acetone.

Amyl Nitrite: Clear, yellowish liquid, having a peculiar, ethereal, fruity odor. Is volatile even at low temperatures, and is flammable. Boils at about 96°. Practically insoluble in water. Miscible with alcohol and with ether.

Amylene Hydrate: Clear, colorless liquid, having a camphoraceous odor. Its solutions are neutral to litmus. Freely soluble in water. Miscible with alcohol, with chloroform, with ether, and with glycerin. *NF category:* Solvent.

Anagrelide Hydrochloride: Off-white to pale pinkish powder. Sparingly soluble in dimethylsulfoxide and in dimethylformamide; very slightly soluble in water.

Anastrozole: White to off-white crystalline powder. Very soluble in acetonitrile; freely soluble in methanol, in acetone, in alcohol, and in tetrahydrofuran.

Anethole: Colorless or faintly yellow liquid at or above 23°. Has a sweet taste and the aromatic odor of anise. Is affected by light. Freely soluble in alcohol; very slightly soluble in water. Readily miscible with ether and with chloroform. *NF category:* Flavors and fragrance.

Anileridine: White to yellowish-white, odorless to practically odorless, crystalline powder. Is oxidized on exposure to air and light, becoming darker in color. It exhibits polymorphism, and of two crystalline forms observed, one melts at about 80° and the other at about 89°. Freely soluble in alcohol and in chloroform; soluble in ether, although it may show turbidity; very slightly soluble in water.

Anileridine Hydrochloride: White or nearly white, odorless, crystalline powder. Is stable in air. Melts at about 270°, with decomposition. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in ether, and in chloroform.

Add the following:

▲**Anise Oil:** Clear, colorless to light yellow in the liquid state having a powerful and characteristic licorice-like scent. Congeals between 15° and 19°. Miscible with alcohol. *NF category:* Flavors and fragrance. ▲ (NF 1-May-2020)

Antazoline Phosphate: White to off-white, crystalline powder, having a bitter taste. Soluble in water; sparingly soluble in methanol; practically insoluble in benzene and in ether.

Anthralin: Yellowish-brown, crystalline powder. Is odorless and tasteless. Soluble in chloroform, in acetone, in benzene, and in solutions of alkali hydroxides; slightly soluble

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in alcohol, in ether, and in glacial acetic acid; insoluble in water.

Anticoagulant Citrate Dextrose Solution: Clear, colorless, odorless liquid. Is dextrorotatory.

Anticoagulant Citrate Phosphate Dextrose Solution: Clear, colorless to slightly yellow, odorless liquid. Is dextrorotatory.

Anticoagulant Sodium Citrate Solution: Clear and colorless liquid.

Antihemophilic Factor: White or yellowish powder. On constitution is opalescent with a slight blue tinge or is a yellowish liquid.

Cryoprecipitated Antihemophilic Factor: Yellowish, frozen solid. On thawing becomes a very viscous, yellow, gummy liquid.

Antimony Potassium Tartrate: Colorless, odorless, transparent crystals, or white powder. The crystals effloresce upon exposure to air and do not readily rehydrate even on exposure to high humidity. Its solutions are acid to litmus. Freely soluble in boiling water; soluble in water and in glycerin; insoluble in alcohol.

Antimony Sodium Tartrate: Colorless, odorless, transparent crystals, or white powder. The crystals effloresce upon exposure to air. Freely soluble in water; insoluble in alcohol.

Antipyrine: Colorless crystals, or white, crystalline powder. Is odorless and has a slightly bitter taste. Its solutions are neutral to litmus. Very soluble in water; freely soluble in alcohol and in chloroform; sparingly soluble in ether.

Antivenin (Crotalidae) Polyvalent: Solid exhibiting the characteristic structure of a freeze-dried solid; light cream in color.

Antivenin (Micrurus Fulvius): Solid exhibiting the characteristic structure of a freeze-dried solid; light cream in color.

Apomorphine Hydrochloride: Minute, white or grayish-white, glistening crystals or white powder. Is odorless. It gradually acquires a green color on exposure to light and air. Its solutions are neutral to litmus. Soluble in water at 80°; sparingly soluble in water and in alcohol; very slightly soluble in chloroform and in ether.

Apraclonidine Hydrochloride: White to off-white, odorless to practically odorless powder. Soluble in methanol; sparingly soluble in water and in alcohol; insoluble in chloroform, in ethyl acetate, and in hexanes.

Aprepitant: White to off-white powder. Sparingly soluble in alcohol; slightly soluble in acetonitrile; practically insoluble in water.

Arginine: White, practically odorless crystals. Freely soluble in water; sparingly soluble in alcohol; insoluble in ether.

Arginine Hydrochloride: White crystals or crystalline powder, practically odorless. Freely soluble in water.

Aripiprazole: A white to off-white, crystalline powder. Freely soluble in dichloromethane; sparingly soluble in toluene; insoluble in methanol and in water.

Aromatic Elixir: *NF category:* Vehicle (flavored and/or sweetened).

Arsanilic Acid: White to off-white, crystalline powder. Melts at about 232°. Soluble in hot water, in amyl alcohol, and in solutions of alkali carbonates; sparingly soluble in concentrated mineral acids; slightly soluble in cold water, in alcohol, and in acetic acid; insoluble in acetone, in benzene, in chloroform, in ether, and in dilute mineral acids.

Articaine Hydrochloride: White or almost white, crystalline powder. Freely soluble in water and in alcohol.

Ascorbic Acid: White or slightly yellow crystals or powder. On exposure to light it gradually darkens. In the dry state, is reasonably stable in air, but in solution rapidly oxidizes. Melts at about 190°. Freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, in ether, and in benzene. *NF category:* Antioxidant.

Ascorbyl Palmitate: White to yellowish white powder, having a characteristic odor. Soluble in alcohol; very slightly soluble in water and in vegetable oils. *NF category:* Antioxidant.

Asparagine: White crystals or a crystalline powder. Soluble in water; practically insoluble in alcohol and in ether. Its solutions are acid to litmus. It melts at about 234°.

Aspartame: White, odorless, crystalline powder, having a sweet taste. Sparingly soluble in water; slightly soluble in alcohol. Melts at about 246°. The pH of an 8 in 1000 solution is about 5. *NF category:* Sweetening agent.

Aspartame Acesulfame: White, odorless, crystalline powder. Slightly soluble in water and in ethanol. *NF category:* Sweetening agent.

Aspartic Acid: White or almost white, crystalline powder, or colorless crystals. Soluble in dilute solutions of alkali hydroxides and in dilute mineral acids; slightly soluble in water; practically insoluble in alcohol and in ether.

Aspirin: White crystals, commonly tabular or needle-like, or white, crystalline powder. Is odorless or has a faint odor. Is stable in dry air; in moist air it gradually hydrolyzes to salicylic and acetic acids. Freely soluble in alcohol; soluble in chloroform and in ether; sparingly soluble in absolute ether; slightly soluble in water.

Atenolol: White or practically white, odorless powder. Melting point 146°–148° (crystals from ethyl acetate). Freely soluble in methanol; sparingly soluble in alcohol; slightly soluble in water and in isopropanol.

Atomoxetine Hydrochloride: White to practically white solid. Sparingly soluble in water.

Atorvastatin Calcium: White to off-white powder. Soluble to freely soluble in methanol; slightly soluble in alcohol; insoluble to very slightly soluble in distilled water, in pH 7.4 phosphate buffer, and in acetonitrile; insoluble in aqueous solutions of pH 4 and below.

Atovaquone: Yellow powder. Freely soluble in *N*-methyl-2-pyrrolidone and in tetrahydrofuran; soluble in chloroform; sparingly soluble in acetone, in di- *n*-butyl adipate, in dimethyl sulfoxide, and in polyethylene glycol 400; slightly soluble in alcohol, in 1,3-butanediol, in ethyl acetate, in glycerin, in octanol, and in polyethylene glycol 200; very slightly soluble in 0.1 N sodium hydroxide; insoluble in water.

Atracurium Besylate: White to yellowish white powder, slightly hygroscopic. Very soluble in acetonitrile, in alcohol, and in methylene chloride; soluble in water.

Atropine: White crystals, usually needle-like, or white, crystalline powder. Its saturated solution is alkaline to phenolphthalein TS. Is optically inactive, but usually contains some levorotatory hyoscyamine. Freely soluble in alcohol and in chloroform; soluble in glycerin and in ether; slightly soluble in water; sparingly soluble in water at 80°.

Atropine Sulfate: Colorless crystals, or white, crystalline powder. Odorless; effloresces in dry air; is slowly affected by light. Very soluble in water; freely soluble in alcohol and even more so in boiling alcohol; freely soluble in glycerin.

Activated Attapulgite: Cream-colored, micronized, nonswelling powder, free from gritty particles. The high heat treatment used in its preparation causes it to yield only moderately viscous aqueous suspensions, its dispersion consisting mainly of particle groups. Insoluble in water. *NF category:* Suspending and/or viscosity-increasing agent.

Colloidal Activated Attapulgite: Cream-colored, micronized, nonswelling powder, free from gritty particles. Yields viscous aqueous suspensions, as a result of dispersion into its constituent ultimate particles. Insoluble in water. *NF category:* Suspending and/or viscosity-increasing agent.

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Aurothioglucose: Yellow, odorless or practically odorless powder. Is stable in air. An aqueous solution is unstable on long standing. The pH of its 1 in 100 solution is about 6.3. Freely soluble in water; practically insoluble in acetone, in alcohol, in chloroform, and in ether.

Azatadine Maleate: White to light cream-colored, odorless powder. Melts at about 153°. Freely soluble in water, in alcohol, in chloroform, and in methanol; practically insoluble in benzene and in ether.

Azathioprine: Pale yellow, odorless powder. Soluble in dilute solutions of alkali hydroxides; sparingly soluble in dilute mineral acids; very slightly soluble in alcohol and in chloroform; insoluble in water.

Azathioprine Sodium for Injection: Bright yellow, hygroscopic, amorphous mass or cake.

Azelastine Hydrochloride: White or almost white, crystalline powder. Soluble in ethanol and in methylene chloride; sparingly soluble in water.

Azithromycin: White or almost white powder. Freely soluble in anhydrous ethanol and in methylene chloride; practically insoluble in water.

Aztreonam: White, odorless, crystalline powder. Soluble in dimethylformamide and in dimethyl sulfoxide; slightly soluble in methanol; very slightly soluble in dehydrated alcohol; practically insoluble in ethyl acetate, in chloroform, and in toluene.

Bacampicillin Hydrochloride: White or practically white powder. Is hygroscopic. Freely soluble in alcohol and in chloroform; soluble in methylene chloride and in water; very slightly soluble in ether.

Bacitracin: White to pale buff powder, odorless or having a slight odor. Is hygroscopic. Its solutions deteriorate rapidly at room temperature. Is precipitated from its solutions and is inactivated by salts of many of the heavy metals. Freely soluble in water; soluble in alcohol, in methanol, and in glacial acetic acid, the solution in the organic solvents usually showing some insoluble residue; insoluble in acetone, in chloroform, and in ether.

Bacitracin Zinc: White to pale tan powder, odorless or having a slight odor. Is hygroscopic. Sparingly soluble in water.

Baclofen: White to off-white, crystalline powder. Is odorless or practically so. Slightly soluble in water; very slightly soluble in methanol; insoluble in chloroform.

Balsalazide Disodium: Orange to yellow powder. Freely soluble in water and in isotonic saline; sparingly soluble in methanol and in alcohol; practically insoluble in all other organic solvents.

Adhesive Bandage: The compress of Adhesive Bandage is substantially free from loose threads or ravelings. The adhesive strip may be perforated, and the back may be coated with a water-repellent film.

Gauze Bandage: One continuous piece, tightly rolled, in various widths and lengths and substantially free from loose threads and ravelings.

Barium Hydroxide Lime: White or grayish-white granules. May have a color if an indicator has been added. *NF category:* Sorbent, carbon dioxide.

Barium Sulfate: Fine, white, odorless, tasteless, bulky powder, free from grittiness. Practically insoluble in water, in organic solvents, and in solutions of acids and of alkalies.

Barium Sulfate for Suspension: White or colored, bulky or granular powder.

BCG Vaccine: White to creamy white, dried mass, having the characteristic texture of material dried in the frozen state.

Beclomethasone Dipropionate: White to cream white, odorless powder. Very soluble in chloroform; freely soluble in acetone and in alcohol; very slightly soluble in water.

Behenoyl Polyoxylglycerides: Waxy solid or fine powder. Soluble in methylene chloride; insoluble in alcohol; dispersible in water. *NF category:* Lubricant; emulsifying agent; wetting and/or solubilizing agent.

Belladonna Leaf: When moistened, its odor is slight, somewhat tobacco-like. Its taste is somewhat bitter and acrid.

Benazepril Hydrochloride: White to off-white, crystalline powder. Soluble in water, in methanol, and in alcohol.

Bendamustine Hydrochloride: White or almost-white powder. Freely soluble in methanol; slightly soluble in isopropyl alcohol, in acetonitrile, and in tetrahydrofuran.

Bendroflumethiazide: White to cream-colored, finely divided, crystalline powder. Is odorless, or has a slight odor. Melts at about 220°. Freely soluble in alcohol and in acetone; practically insoluble in water.

Benoxinate Hydrochloride: White, or slightly off-white, crystals or crystalline powder. Is odorless, or has a slight characteristic odor, has a salty taste, and exhibits local anesthetic properties when placed upon the tongue. Its solutions are neutral to litmus, and it melts at about 158°. Very soluble in water; freely soluble in chloroform and in alcohol; insoluble in ether.

Bentonite: Very fine, odorless, pale buff or cream-colored to grayish powder, free from grit. Has a slightly earthy taste. Is hygroscopic. Insoluble in water, but swells to approximately twelve times its volume when added to water; insoluble in, and does not swell in, organic solvents. *NF category:* Suspending and/or viscosity-increasing agent.

Purified Bentonite: Odorless, tasteless, fine (micronized) powder or small flakes that are creamy when viewed on their flat surfaces and tan to brown when viewed on their edges. Insoluble in water and in alcohol. Swells when added to water or glycerin. *NF category:* Suspending and/or viscosity-increasing agent.

Bentonite Magma: *NF category:* Suspending and/or viscosity-increasing agent.

Benzaldehyde: Colorless, strongly refractive liquid. Is affected by light. Slightly soluble in water. Miscible with alcohol, with ether, and with fixed and volatile oils. The specific gravity is 1.041–1.046 at 25° (see *Specific Gravity* ⟨841⟩), and the refractive index is 1.544–1.546 at 20° (see *Refractive Index* ⟨831⟩). *NF category:* Flavors and fragrance.

Benzaldehyde Elixir, Compound: *NF category:* Flavored and/or sweetened vehicle.

Benzalkonium Chloride: White or yellowish-white, thick gel or gelatinous pieces. Usually has a mild, aromatic odor. Its aqueous solution has a bitter taste, foams strongly when shaken, and usually is slightly alkaline. Very soluble in water and in alcohol. Anhydrous form freely soluble in benzene, and slightly soluble in ether. *NF category:* Antimicrobial preservative; wetting and/or solubilizing agent; emulsifying agent.

Benzalkonium Chloride Solution: Clear liquid; colorless or slightly yellow unless a color has been added. Has an aromatic odor and a bitter taste. *NF category:* Antimicrobial preservative.

Benzethonium Chloride: White crystals, having a mild odor. Its solution (1 in 100) is slightly alkaline to litmus. Soluble in water, in alcohol, and in chloroform; slightly soluble in ether. *NF category:* Antimicrobial preservative; wetting and/or solubilizing agent.

Benzethonium Chloride Solution: Odorless, clear liquid, slightly alkaline to litmus.

Benzethonium Chloride Tincture: Clear liquid, having the characteristic odor of acetone and of alcohol.

Benzocaine: Small, white crystals or white, crystalline powder. Is odorless, is stable in air, and exhibits local anesthetic properties when placed upon the tongue. Freely soluble in alcohol, in chloroform, and in ether; sparingly

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soluble in almond oil and in olive oil; very slightly soluble in water. Dissolves in dilute acids.

Benzoic Acid: White crystals, scales, or needles. Has a slight odor, usually suggesting benzaldehyde or benzoin. Somewhat volatile at moderately warm temperatures. Freely volatile in steam. Freely soluble in alcohol, in chloroform, and in ether; slightly soluble in water. *NF category:* Antimicrobial preservative.

Benzoin: Sumatra Benzoin has an aromatic and balsamic odor. When heated it does not emit a pinaceous odor. When Sumatra Benzoin is digested with boiling water, the odor suggests cinnamates or storax. Its taste is aromatic and slightly acrid. Siam Benzoin has an agreeable, balsamic, vanilla-like odor. Its taste is aromatic and slightly acrid.

Benzonatate: Clear, pale yellow, viscous liquid, having a faint, characteristic odor. Has a bitter taste, and exhibits local anesthetic properties when placed upon the tongue. Miscible with water in all proportions. Freely soluble in chloroform, in alcohol, and in benzene.

Hydrous Benzoyl Peroxide: White, granular powder, having a characteristic odor. Soluble in acetone, in chloroform, and in ether; sparingly soluble in water and in alcohol.

Benzoyl Peroxide Gel: A soft, white gel, having a characteristic odor.

Benzoyl Peroxide Lotion: White, viscous, creamy lotion, having a characteristic odor.

Benzphetamine Hydrochloride: White to off-white, crystalline powder. Freely soluble in alcohol and soluble in chloroform.

Benztropine Mesylate: White, slightly hygroscopic, crystalline powder. Very soluble in water; freely soluble in alcohol; very slightly soluble in ether.

Benzyl Alcohol: Clear, colorless, oily liquid. Boils at about 206°, without decomposition. Is neutral to litmus. Freely soluble in 50% alcohol; sparingly soluble in water. Miscible with alcohol, with ether, and with chloroform. The specific gravity is between 1.042 and 1.047. *NF category:* Antimicrobial preservative; solvent.

Benzyl Benzoate: Clear, colorless, oily liquid having a slight aromatic odor and producing a sharp, burning sensation on the tongue. Practically insoluble in water and in glycerin. Miscible with alcohol, with ether, and with chloroform. *NF category:* Solvent; emulsifying agent; plasticizer.

Beta Carotene: Red or reddish-brown to violet-brown crystals or crystalline powder. Soluble in carbon disulfide, in benzene, and in chloroform; sparingly soluble in ether, in solvent hexane, and in vegetable oils; practically insoluble in methanol and in alcohol; insoluble in water, in acids, and in alkalies.

Betadex: White, practically odorless, fine crystalline powder having a slightly sweet taste. Sparingly soluble in water. *NF category:* Sequestering agent; chelating and/or complexing agent.

Betadex Sulfobutyl Ether Sodium: White to off-white, amorphous powder. Freely soluble in water; sparingly soluble in methanol; practically insoluble in ethanol, in *n*-hexane, in 1-butanol, in acetonitrile, in 2-propanol, and in ethyl acetate. *NF category:* Chelating and/or complexing agent; sequestering agent; wetting and/or solubilizing agent.

Betahistine Hydrochloride: White to almost yellow, crystalline powder. Very hygroscopic. Melts between 151° and 154°. Very soluble in water; freely soluble in alcohol; practically insoluble in isopropyl alcohol.

Betaine Hydrochloride: White, crystalline powder. Soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Betamethasone: White to practically white, odorless, crystalline powder. Melts at about 240°, with some decomposition. Sparingly soluble in acetone, in alcohol, in dioxane, and in methanol; very slightly soluble in chloroform and in ether; insoluble in water.

Betamethasone Acetate: White to creamy white, odorless powder. Sinters and resolidifies at about 165°, and remelts at about 200° or 220°, with decomposition (see *Melting Range or Temperature* ⟨741⟩). Freely soluble in acetone; soluble in alcohol and in chloroform; practically insoluble in water.

Betamethasone Benzoate: White to practically white, practically odorless powder. Melts at about 220°, with decomposition. Soluble in alcohol, in methanol, and in chloroform; insoluble in water.

Betamethasone Dipropionate: White to cream-white, odorless powder. Freely soluble in acetone and in chloroform; sparingly soluble in alcohol; insoluble in water.

Betamethasone Sodium Phosphate: White to practically white, odorless powder. Is hygroscopic. Freely soluble in water and in methanol; practically insoluble in acetone and in chloroform.

Betamethasone Valerate: White to practically white, odorless powder. Melts at about 190°, with decomposition. Freely soluble in acetone and in chloroform; soluble in alcohol; slightly soluble in benzene and in ether; practically insoluble in water.

Betaxolol Hydrochloride: White, crystalline powder. Freely soluble in water, in alcohol, in chloroform, and in methanol.

Bethanechol Chloride: Colorless or white crystals or white, crystalline powder, usually having a slight, amine-like odor. Is hygroscopic. Exhibits polymorphism, and of two crystalline forms observed, one melts at about 211° and the other melts at about 219°. Freely soluble in water and in alcohol; insoluble in chloroform and in ether.

Bicalutamide: Fine, white to off-white powder. Freely soluble in tetrahydrofuran and in acetone; soluble in acetonitrile; sparingly soluble in methanol; slightly soluble in alcohol.

Biotin: Practically white, crystalline powder. Very slightly soluble in water and in alcohol; insoluble in other common organic solvents.

Biperiden: White, practically odorless, crystalline powder. Freely soluble in chloroform; sparingly soluble in alcohol; practically insoluble in water.

Biperiden Hydrochloride: White, practically odorless, crystalline powder. Melts at about 275°, with decomposition. Is optically inactive. Sparingly soluble in methanol; slightly soluble in water, in ether, in alcohol, and in chloroform.

Bisacodyl: White to off-white, crystalline powder, in which the number of particles having a longest diameter smaller than 50 µm predominate. Soluble in chloroform and in benzene; sparingly soluble in alcohol and in methanol; slightly soluble in ether; practically insoluble in water.

Milk of Bismuth: Thick, white, opaque suspension that separates on standing. Is odorless and practically tasteless. Miscible with water and with alcohol.

Bismuth Citrate: White, amorphous or crystalline powder. Stable in air. Melts at about 300°, with decomposition. Soluble in ammonia TS and in solutions of alkali citrates; insoluble in water and in alcohol.

Bismuth Subcarbonate: White or almost white powder. Practically insoluble in water, in alcohol, and in ether. Dissolves in dilute acids with effervescence.

Bismuth Subgallate: Amorphous, bright yellow powder. Is odorless and tasteless. Is stable in air, but is affected by light. Dissolves readily with decomposition in warm, moderately dilute hydrochloric, nitric, or sulfuric acid; readily dissolved by solutions of alkali hydroxides, forming a clear, yellow liquid that rapidly assumes a deep red color. Practically insoluble in water, in alcohol, in chloroform, and in ether; insoluble in very dilute mineral acids.

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Bismuth Subnitrate: White, slightly hygroscopic powder. Practically insoluble in water and in alcohol; readily dissolved by hydrochloric acid or by nitric acid.

Bismuth Subsalicylate: Fine to off-white, microcrystalline, odorless, tasteless powder. Practically insoluble in water, in alcohol, and in ether. Reacts with alkalies and mineral acids.

Bisoprolol Fumarate: White, crystalline powder. Very soluble in water and in methanol; freely soluble in chloroform, in glacial acetic acid, and in alcohol; slightly soluble in acetone and in ethyl acetate.

Bleomycin Sulfate: Cream-colored, amorphous powder. Very soluble in water.

Anti-A Blood Grouping Serum: Liquid Serum is a clear or slightly opalescent fluid unless artificially colored blue. Dried Serum is light yellow to deep cream color, unless artificially colored as indicated for liquid Serum. The liquid Serum may develop slight turbidity on storage. The dried Serum may show slight turbidity upon reconstitution for use.

Anti-B Blood Grouping Serum: Liquid Serum is a clear or slightly opalescent fluid unless artificially colored yellow. Dried Serum is light yellow to deep cream color, unless artificially colored as indicated for liquid Serum. The liquid Serum may develop a slight turbidity on storage. The dried Serum may show slight turbidity upon reconstitution for use.

Blood Grouping Serums Anti-D, Anti-C, Anti-E, Anti-c, Anti-e: The liquid Serums are clear, slightly yellowish fluids, that may develop slight turbidity on storage. The dried Serums are light yellow to deep cream color.

Blood Group Specific Substances A, B, and AB: Clear solution that may have a slight odor because of the preservative.

Red Blood Cells: Dark red in color when packed. May show a slight creamy layer on the surface and a small supernatant layer of yellow or opalescent plasma. Also supplied in deep-frozen form with added cryophylactic substance to extend storage time.

Whole Blood: Deep red, opaque liquid from which the corpuscles readily settle upon standing for 24 to 48 hours, leaving a clear, yellowish or pinkish supernatant layer of plasma.

Boric Acid: Colorless, odorless scales of a somewhat pearly luster, or crystals, or white powder that is slightly unctuous to the touch. Is stable in air. Freely soluble in glycerin, in boiling water, and in boiling alcohol; soluble in water and in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative.

Botulism Antitoxin: Transparent or slightly opalescent liquid, practically colorless, and practically odorless or having an odor because of the antimicrobial agent.

Bretylium Tosylate: White, crystalline powder. Is hygroscopic. Freely soluble in water, in methanol, and in alcohol; practically insoluble in ether, in ethyl acetate, and in hexane.

Brimonidine Tartrate: White, or slightly yellowish, or slightly brownish powder. Soluble in water; practically insoluble in anhydrous ethanol and in toluene.

Brinzolamide: White or almost white powder. Slightly soluble in alcohol and in methanol; insoluble in water.

Bromocriptine Mesylate: White or slightly colored, fine crystalline powder, odorless or having a weak, characteristic odor.

Bromodiphenhydramine Hydrochloride: White to pale buff, crystalline powder, having no more than a faint odor. Freely soluble in water and in alcohol; soluble in isopropyl alcohol; insoluble in ether and in solvent hexane.

Brompheniramine Maleate: White, odorless, crystalline powder. Freely soluble in water; soluble in alcohol and in chloroform; slightly soluble in ether and in benzene.

Budesonide: White to off-white, odorless, crystalline powder. Freely soluble in chloroform; sparingly soluble in alcohol; practically insoluble in water and in heptane.

Bumetanide: Practically white powder. Soluble in alkaline solutions; slightly soluble in water.

Bupivacaine Hydrochloride: White, odorless, crystalline powder. Melts at about 248°, with decomposition. Freely soluble in water and in alcohol; slightly soluble in chloroform and in acetone.

Bupivacaine Hydrochloride Injection: Clear, colorless solution.

Bupivacaine Hydrochloride and Epinephrine Injection: Clear, colorless solution.

Bupropion Hydrochloride: White powder. Soluble in water, in 0.1 N hydrochloric acid, and in alcohol.

Buspirone Hydrochloride: White crystalline powder. Very soluble in water; freely soluble in methanol and in methylene chloride; sparingly soluble in ethanol and in acetonitrile; very slightly soluble in ethyl acetate; practically insoluble in hexanes.

Busulfan: White, crystalline powder. Sparingly soluble in acetone; slightly soluble in alcohol; very slightly soluble in water.

Butabarbital: White, odorless, crystalline powder. Soluble in alcohol, in chloroform, in ether, and in solutions of alkali hydroxides and carbonates; very slightly soluble in water.

Butabarbital Sodium: White powder, having a bitter taste. Freely soluble in water and in alcohol; practically insoluble in absolute ether.

Butalbital: White, crystalline, odorless powder, having a slightly bitter taste. Is stable in air. Its saturated solution is acid to litmus. Freely soluble in alcohol, in ether, and in chloroform; soluble in boiling water, and in solutions of fixed alkalies and alkali carbonates; slightly soluble in cold water.

Butamben: White, crystalline powder. Is odorless and tasteless. Soluble in dilute acids, in alcohol, in chloroform, in ether, and in fixed oils; very slightly soluble in water. Is slowly hydrolyzed when boiled with water.

Butane: Colorless, flammable gas (boiling temperature is about –0.5°). One volume of water dissolves 0.15 volume, and 1 volume of alcohol dissolves 18 volumes at 17° and 770 mm; 1 volume of ether or chloroform at 17° dissolves 25 or 30 volumes, respectively. Vapor pressure at 21° is about 1620 mm of mercury (17 psig). *NF category:* Propellant.

Butoconazole Nitrate: White to off-white, crystalline powder. Melts at about 160°. Sparingly soluble in methanol; slightly soluble in acetonitrile, in acetone, in dichloromethane, and in tetrahydrofuran; very slightly soluble in ethyl acetate; practically insoluble in water.

Butorphanol Tartrate: White powder. Its solutions are slightly acidic. Melts between 217° and 219°, with decomposition. Soluble in dilute acids; sparingly soluble in water; slightly soluble in methanol; insoluble in alcohol, in chloroform, in ethyl acetate, in ethyl ether, and in hexane.

Butyl Alcohol: Clear, colorless, mobile liquid, having a characteristic, penetrating vinous odor. Soluble in water. Miscible with alcohol, with ether, and with many other organic solvents. *NF category:* Solvent.

Butylene Glycol: Clear, colorless, hygroscopic, viscous liquid. It is miscible with water, with acetone, and with ether in all proportions; immiscible with fixed oils. It dissolves most essential oils and synthetic flavoring substances. *NF category:* Solvent; wetting and/or solubilizing agent.

Butyl Palmitostearate: Colorless or pale yellowish waxy solid at temperatures below 17°–24° or colorless or pale yellowish liquid at temperatures of 17°–24° or above. Soluble in acetone, in alcohol, in ether, in mineral oils, and in vegetable oils; practically insoluble in water and in propylene glycol. *NF*

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category: Emulsifying and/or solubilizing agent; flavors and fragrance; plasticizer.

Butyl Stearate: Colorless or pale yellowish waxy solid at temperatures below 19°–24° or colorless or pale yellowish liquid at temperatures of 19°–24° or above. Soluble in acetone, in alcohol, in ether, in mineral oils, and in vegetable oils; practically insoluble in water and in propylene glycol. *NF category:* Emulsifying and/or solubilizing agent; flavors and fragrance; plasticizer.

Butylated Hydroxyanisole: White or slightly yellow, waxy solid, having a faint, characteristic odor. Freely soluble in alcohol, in propylene glycol, in chloroform, and in ether; insoluble in water. *NF category:* Antioxidant.

Butylated Hydroxytoluene: White, crystalline solid, having a faint, characteristic odor. Freely soluble in alcohol, in chloroform, and in ether; insoluble in water and in propylene glycol. *NF category:* Antioxidant.

Butylparaben: Small, colorless crystals or white powder. Freely soluble in acetone, in alcohol, in ether, and in propylene glycol; very slightly soluble in water and in glycerin. *NF category:* Antimicrobial preservative.

Cabergoline: White or almost white, crystalline or amorphous powder. Freely soluble in alcohol (96%); slightly soluble in 0.1 M hydrochloric acid; very slightly soluble in hexane; practically insoluble in water.

Caffeine: White powder or white, glistening needles, usually matted together. Is odorless and has a bitter taste. Its solutions are neutral to litmus. The hydrate is efflorescent in air. Freely soluble in chloroform; sparingly soluble in water and in alcohol; slightly soluble in ether.

Calamine: Pink, odorless, practically tasteless, fine powder. Soluble in mineral acids; insoluble in water.

Calcipotriene: White or almost white crystalline powder. Freely soluble in alcohol; slightly soluble in methylene chloride; practically insoluble in water.

Calcitriol: White or almost white crystals. Freely soluble in alcohol; soluble in ether and in fatty oils; practically insoluble in water. It is sensitive to air, heat, and light.

Calcium Acetate: White, odorless or almost odorless, hygroscopic, crystalline powder. When heated to above 160°, it decomposes to calcium carbonate and acetone. Freely soluble in water; slightly soluble in methanol; practically insoluble in acetone, in dehydrated alcohol, and in benzene. *NF category:* Antimicrobial preservative; sequestering agent.

Calcium Ascorbate: White to slightly yellow, practically odorless powder. Freely soluble in water (approximately 50 g per 100 mL); slightly soluble in alcohol; insoluble in ether.

Calcium Carbonate: Fine, white, odorless, tasteless, microcrystalline powder. Is stable in air. Practically insoluble in water. Its solubility in water is increased by the presence of any ammonium salt or of carbon dioxide. The presence of any alkali hydroxide reduces its solubility. Insoluble in alcohol. Dissolves with effervescence in 1 N acetic acid, in 3 N hydrochloric acid, and in 2 N nitric acid. *NF category:* Diluent; pH modifier (acidifying agent/alkalizing agent/buffering agent); coating agent; wet binder.

Calcium Chloride: White, hard, odorless fragments or granules. Is deliquescent. Very soluble in boiling water; freely soluble in water, in alcohol, and in boiling alcohol. *NF category:* Desiccant; antimicrobial preservative.

Calcium Citrate: White, odorless, crystalline powder. Freely soluble in diluted 3 N hydrochloric acid and in diluted 2 N nitric acid; slightly soluble in water; insoluble in alcohol.

Calcium Gluceptate: White to faintly yellow, amorphous powder. Is stable in air, but the hydrous forms may lose part of their water of hydration on standing. Freely soluble in water; insoluble in alcohol and in many other organic solvents.

Calcium Gluconate: White, crystalline, odorless, tasteless granules or powder. Is stable in air. Its solutions are neutral to litmus. Freely soluble in boiling water; sparingly (and slowly) soluble in water; insoluble in alcohol.

Calcium Hydroxide: White powder. Has an alkaline, slightly bitter taste. Soluble in glycerin and in syrup; slightly soluble in water; very slightly soluble in boiling water; insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Calcium Hydroxide Solution: Clear, colorless liquid having an alkaline taste. Is alkaline to litmus.

Calcium Lactate: White, practically odorless granules or powder. The pentahydrate is somewhat efflorescent and at 120° becomes anhydrous. The pentahydrate is soluble in water; it is practically insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative; wet binder.

Calcium Levulinate: White, crystalline or amorphous, powder, having a faint odor suggestive of burnt sugar. Has a bitter, salty taste. Freely soluble in water; slightly soluble in alcohol; insoluble in ether and in chloroform.

Calcium Pantothenate: Slightly hygroscopic, white powder. Is odorless and has a bitter taste. Freely soluble in water; soluble in glycerin; practically insoluble in alcohol, in chloroform, and in ether.

Racemic Calcium Pantothenate: White, slightly hygroscopic powder, having a faint, characteristic odor, and a bitter taste. Is stable in air. Its solutions are neutral or alkaline to litmus. Is optically inactive. Freely soluble in water; soluble in glycerin; practically insoluble in alcohol, in chloroform, and in ether.

Dibasic Calcium Phosphate: White, odorless, tasteless powder. Is stable in air. Soluble in 3 N hydrochloric acid and in 2 N nitric acid; practically insoluble in water; insoluble in alcohol. *NF category:* Diluent.

Tribasic Calcium Phosphate: White, odorless, tasteless powder. Is stable in air. Freely soluble in 3 N hydrochloric acid and in 2 N nitric acid; practically insoluble in water; insoluble in alcohol. *NF category:* Diluent; pH modifier (acidifying agent/alkalizing agent/buffering solution); glidant and/or anticaking agent.

Calcium Polycarbophil: White to creamy white powder. Insoluble in water, in dilute acids, in dilute alkalies, and in common organic solvents.

Calcium Propionate: Occurs as a white crystalline solid. One g dissolves in about 3 mL of water. *NF category:* Antimicrobial preservative.

Calcium Saccharate: White, odorless, tasteless, crystalline powder. Soluble in dilute mineral acids and in solutions of calcium gluconate; slightly soluble in boiling water; very slightly soluble in alcohol, and in cold water; practically insoluble in ether and in chloroform.

Calcium Silicate: White to off-white, free-flowing powder that remains so after absorbing relatively large amounts of water or other liquids. Insoluble in water. Forms a gel with mineral acids. *NF category:* Glidant and/or anticaking agent.

Calcium Stearate: Fine, white to yellowish-white, bulky powder having a slight, characteristic odor. Is unctuous, and is free from grittiness. Insoluble in water, in alcohol, and in ether. *NF category:* Lubricant.

Calcium Sulfate: Fine, white to slightly yellow-white, odorless powder. Soluble in 3 N hydrochloric acid; slightly soluble in water. *NF category:* Desiccant; diluent.

Calcium Undecylenate: Fine, white powder, having a characteristic odor and no grit. Slightly soluble in hot alcohol; practically insoluble in water, in ether, in chloroform, in acetone, and in cold alcohol.

Camphor: Colorless or white crystals, granules, or crystalline masses; or colorless to white, translucent, tough masses. Has a penetrating, characteristic odor and a pungent, aromatic taste. Specific gravity is about 0.99. Slowly volatilizes

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at ordinary temperatures. Slightly soluble in water; very soluble in alcohol, in chloroform, and in ether; freely soluble in carbon disulfide, in solvent hexane, and in fixed and volatile oils.

Candelilla Wax: A hard, yellowish-brown-opaque to translucent wax. Its specific gravity is about 0.983. Soluble in chloroform and in toluene; insoluble in water.

Candesartan Cilexetil: White to off-white powder. Sparingly soluble in methanol; practically insoluble in water.

Canola Oil: Clear, pale yellow, slightly viscous liquid. Practically insoluble in water and in alcohol. Miscible with light petroleum (bp: 40° to 60°). *NF category:* Solvent; emollient; vehicle (oleaginous).

Capecitabine: White to off-white crystalline powder. Freely soluble in methanol; soluble in acetonitrile and in alcohol; sparingly soluble in water.

Capreomycin Sulfate: White to practically white, amorphous powder. Freely soluble in water; practically insoluble in most organic solvents.

Caprylic Acid: Clear, colorless or slightly yellowish, oily liquid. Very soluble in acetone and in alcohol; very slightly soluble in water. It dissolves in dilute solutions of alkali hydroxides. *NF category:* Emulsifying agent.

Caprylocaproyl Polyoxylglycerides: Pale yellow, oily liquids. Dispersible in hot water; freely soluble in methylene chloride. *NF category:* Ointment base; solvent; wetting and/or solubilizing agent; emulsifying agent.

Capsaicin: Off-white powder. Melts at about 65°. Soluble in alcohol, in benzene, in chloroform; slightly soluble in carbon disulfide; practically insoluble in cold water.

Capsicum Oleoresin: Dark red, oily liquid. Soluble in alcohol, in acetone, in ether, in chloroform, and in volatile oils; soluble with opalescence in fixed oils.

Captopril: White to off-white, crystalline powder, which may have a characteristic, sulfide-like odor. Melts in the range of 104° to 110°. Freely soluble in water, in methanol, in alcohol, and in chloroform.

Caramel: Thick, dark brown liquid having the characteristic odor of burnt sugar, and a pleasant, bitter taste. One part dissolved in 1000 parts of water yields a clear solution having a distinct yellowish-orange color. The color of this solution is not changed and no precipitate is formed after exposure to sunlight for 6 hours. When spread in a thin layer on a glass plate, it appears homogeneous, reddish-brown, and transparent. Miscible with water. Soluble in dilute alcohol up to 55% (v/v). Immiscible with ether, with chloroform, with acetone, with benzene, and with solvent hexane. *NF category:* Coloring agent.

Carbachol: White powder. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in chloroform and in ether.

Carbamazepine: White to off-white powder. Soluble in alcohol and in acetone; practically insoluble in water.

Carbamide Peroxide Topical Solution: Clear, colorless, viscous liquid, having a characteristic odor and taste.

Carbenicillin Disodium: White to off-white, crystalline powder. Freely soluble in water; soluble in alcohol; practically insoluble in chloroform and in ether.

Carbenicillin Indanyl Sodium: White to off-white powder. Soluble in water and in alcohol.

Carbidopa: White to creamy white, odorless or practically odorless, powder. Freely soluble in 3 N hydrochloric acid; slightly soluble in water, and in methanol; practically insoluble in alcohol, in acetone, in chloroform, and in ether.

Carbinoxamine Maleate: White, odorless, crystalline powder. Very soluble in water; freely soluble in alcohol and in chloroform; very slightly soluble in ether.

Carbol-Fuchsin Topical Solution: Dark purple liquid, which appears purplish red when spread in a thin film.

Carbomer 910: White, fluffy powder, having a slight, characteristic odor. Is hygroscopic. The pH of a 1 in 100 dispersion is about 3. When neutralized with alkali hydroxides or with amines, it dissolves in water, in alcohol, and in glycerin. *NF category:* Suspending and/or viscosity-increasing agent.

Carbomer 934: See *Carbomer 910.*

Carbomer 934P: See *Carbomer 910.*

Carbomer 940: See *Carbomer 910.*

Carbomer 941: See *Carbomer 910.*

Carbomer 1342: See *Carbomer 910.*

Carbomer Copolymer: White, hygroscopic powder. It swells in water when a dispersion of it is neutralized with sodium hydroxide to a pH within the range of 7.3 to 7.8. *NF category:* Emulsifying agent; suspending and/or viscosity-increasing agent; wet binder; polymers for ophthalmic use; release-modifying agent.

Carbomer Homopolymer: White, fluffy hygroscopic powder, having a slight, characteristic odor. The pH of a 1 in 100 dispersion in water is about 3. When neutralized with alkali hydroxides or with amines, it swells giving the appearance of dissolving in water; when neutralized with lower amines and alkanolamines, it swells giving the appearance of dissolving in methanol or glycerin; when neutralized with ethoxylated long-chain (C_{14}–C_{18}) amines, it swells giving the appearance of dissolving in ethanol. *NF category:* Wet binder; suspending and/or viscosity-increasing agent; emulsifying agent; polymers for ophthalmic use; release-modifying agent.

Carbomer Interpolymer: White, hygroscopic powder. It swells in water when a dispersion of it is neutralized with sodium hydroxide to a pH within the range of 5.5 to 9. *NF category:* Emulsifying agent; suspending and/or viscosity increasing agent; wet binder; polymers for ophthalmic use; release-modifying agent.

Carbon Dioxide: Odorless, colorless gas. Its solutions are acid to litmus. One L at 0° and at a pressure of 760 mm of mercury weighs 1.977 g. One volume dissolves in about 1 volume of water. *NF category:* Air displacement; propellant.

Carboprost Tromethamine: White to off-white powder. Soluble in water.

Carboxymethylcellulose Calcium: White to yellowish-white powder. Is hygroscopic. Practically insoluble in alcohol, in acetone, in ether, in chloroform, and in benzene. It swells with water to form a suspension; the pH of the suspension, obtained by shaking 1 g with 100 mL of water, is between 4.5 and 6.0. *NF category:* Suspending and/or viscosity-increasing agent; coating agent; emulsifying agent; film-forming agent; disintegrant.

Carboxymethylcellulose Sodium: White to cream-colored powder or granules. The powder is hygroscopic. Is easily dispersed in water to form colloidal solutions. Insoluble in alcohol, in ether, and in most other organic solvents. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; wet binder; film-forming agent; release-modifying agent; disintegrant.

Carboxymethylcellulose Sodium 12: Colorless or white to off-white powder or granules. Is odorless. Water solubility depends on degree of substitution (easily dispersed in water at all temperatures, forming a clear, colloidal solution). Insoluble in acetone, in alcohol, in ether, and in toluene. *NF category:* Suspending and/or viscosity-increasing agent.

Enzymatically-Hydrolyzed Carboxymethylcellulose Sodium: White or slightly yellowish or grayish, odorless, slightly hygroscopic granular or fibrous powder. Soluble in water; insoluble in alcohol. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; film-forming agent.

Low-Substituted Carboxymethylcellulose Sodium: A white or almost white powder or short fibers. Practically

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insoluble in acetone, in alcohol, and in toluene. It swells in water to form a gel.

Carisoprodol: White, crystalline powder, having a mild, characteristic odor and a bitter taste. Freely soluble in alcohol, in chloroform, and in acetone; very slightly soluble in water.

Carmellose: White powder. Practically insoluble in ethanol (99.5%). Swells with water to form a suspension. Becomes viscous in sodium hydroxide TS. Is hygroscopic. *NF category:* Suspending and/or viscosity-increasing agent; polymers for ophthalmic use.

Carmustine: Light yellow powder. Freely soluble in ether.

Carprofen: White crystalline powder. Freely soluble in ether, in acetone, in ethyl acetate, and in sodium hydroxide TS or sodium carbonate TS; practically insoluble in water.

Carrageenan: Yellowish or tan to white, coarse to fine powder. Is practically odorless and has a mucilaginous taste. Soluble in water at a temperature of about 80°, forming a viscous, clear or slightly opalescent solution that flows readily. Disperses in water more readily if first moistened with alcohol, with glycerin, or with a saturated solution of sucrose in water. *NF category:* Suspending and/or viscosity-increasing agent; release-modifying agent.

Carvedilol: White or nearly white, crystalline powder. Slightly soluble in alcohol; practically insoluble in water and in dilute acids.

Casanthranol: Light tan to brown, amorphous, hygroscopic powder. Freely soluble in water, with some residue; partially soluble in methanol and in hot isopropyl alcohol; practically insoluble in acetone.

Cascara Sagrada: Has a distinct odor and a bitter and slightly acrid taste.

Castor Oil: Pale yellowish or almost colorless, transparent, viscid liquid. Has a faint, mild odor; is free from foreign and rancid odor; and has a bland, characteristic taste. Soluble in alcohol. Miscible with dehydrated alcohol, with glacial acetic acid, with chloroform, and with ether. *NF category:* Plasticizer; emollient; solvent; vehicle.

Hydrogenated Castor Oil: White, crystalline wax. Insoluble in water and in most common organic solvents. *NF category:* Stiffening agent; lubricant.

Cefaclor: White to off-white, crystalline powder. Slightly soluble in water; practically insoluble in methanol, in chloroform, and in benzene.

Cefadroxil: White to off-white, crystalline powder. Slightly soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Cefamandole Nafate: White, odorless, crystalline solid. Soluble in water and in methanol; practically insoluble in ether, in chloroform, in benzene, and in cyclohexane.

Cefazolin: White to slightly off-white, odorless, crystalline powder. Melts at about 198° to 200°, with decomposition. Soluble in dimethylformamide and in pyridine; sparingly soluble in acetone; slightly soluble in alcohol, in methanol, and in water; very slightly soluble in ethyl acetate, in isopropyl alcohol, and in methyl isobutyl ketone; practically insoluble in benzene, in chloroform, in ether, and in methylene chloride.

Cefazolin Sodium: White to off-white, practically odorless, crystalline powder, or white to off-white solid. Freely soluble in water, in saline TS, and in dextrose solutions; very slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Cefdinir: White to light-yellow crystalline powder. Sparingly soluble in 0.1 M phosphate buffer (pH 7) solution; practically insoluble in water, in alcohol, and in diethyl ether.

Cefepime Hydrochloride: White to off-white, crystalline, nonhygroscopic solid. Freely soluble in water.

Cefepime for Injection: White to pale yellow powder. Freely soluble in water.

Cefixime: White to light yellow, crystalline powder. Soluble in methanol and in propylene glycol; slightly soluble in alcohol, in acetone, and in glycerin; very slightly soluble in 70% sorbitol and in octanol; practically insoluble in ether, in ethyl acetate, in hexane, and in water.

Cefmenoxime Hydrochloride: White to light orange-yellow crystals or crystalline powder. Freely soluble in formamide; slightly soluble in methanol; very slightly soluble in water; practically insoluble in dehydrated alcohol and in ether.

Cefmetazole Sodium: White solid. Very soluble in water and in methanol; soluble in acetone; practically insoluble in chloroform.

Cefonicid Sodium: White to off-white solid. Freely soluble in water, in 0.9% sodium chloride solution, and in 5% dextrose solution; soluble in methanol; very slightly soluble in dehydrated alcohol.

Cefoperazone Sodium: White to pale buff crystalline powder. Freely soluble in water; soluble in methanol; slightly soluble in dehydrated alcohol; insoluble in acetone, in ethyl acetate, and in ether.

Ceforanide: White to off-white powder. Very soluble in 1 N sodium hydroxide; practically insoluble in water, in methanol, in chloroform, and in ether.

Cefotaxime Sodium: Off-white to pale yellow crystalline powder. Freely soluble in water; practically insoluble in organic solvents.

Cefoxitin Sodium: White to off-white, granules or powder, having a slight characteristic odor. Is somewhat hygroscopic. Very soluble in water; soluble in methanol; sparingly soluble in dimethylformamide; slightly soluble in acetone; insoluble in ether and in chloroform.

Cefpodoxime Proxetil: White to light brownish-white powder. Odorless or having a faint odor, and has a bitter taste. Freely soluble in dehydrated alcohol; soluble in acetonitrile and in methanol; slightly soluble in ether; very slightly soluble in water.

Ceftazidime: White to cream-colored, crystalline powder. Soluble in alkali and in dimethyl sulfoxide; slightly soluble in dimethylformamide, in methanol, and in water; insoluble in acetone, in alcohol, in chloroform, in dioxane, in ether, in ethyl acetate, and in toluene.

Ceftiofur Hydrochloride: White to beige powder. Sparingly soluble in dimethylformamide and in ethanol; slightly soluble in 0.1 N hydrochloric acid; very slightly soluble in tetrahydrofuran; practically insoluble in ethyl acetate.

Ceftiofur Sodium: White to beige powder. Freely soluble in propylene glycol; soluble in water; slightly soluble in tetrahydrofuran, in dehydrated alcohol, and in acetone; insoluble in methylene chloride.

Ceftizoxime Sodium: White to pale yellow crystalline powder. Freely soluble in water.

Ceftriaxone Sodium: White to yellowish-orange crystalline powder. Freely soluble in water; sparingly soluble in methanol; very slightly soluble in alcohol.

Cefuroxime Axetil: White to almost white powder. The amorphous form is freely soluble in acetone; soluble in chloroform, in ethyl acetate, and in methanol; slightly soluble in dehydrated alcohol; insoluble in ether and in water. The crystalline form is freely soluble in acetone; sparingly soluble in chloroform, in ethyl acetate, and in methanol; slightly soluble in dehydrated alcohol; insoluble in ether and in water.

Cefuroxime Sodium: White or faintly yellow powder. Freely soluble in water; soluble in methanol; very slightly soluble in alcohol, in ether, in ethyl acetate, and in chloroform.

Celecoxib: White or almost white, crystalline or amorphous powder. Soluble to freely soluble in ethanol; soluble in methylene chloride; practically insoluble in water.

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Cellaburate: Fine white or almost white powder or granules. Available in a range of viscosities, acetyl and butyl contents. Slightly hygroscopic; soluble in acetone, in methylene chloride, in pyridine, and in dimethyl sulfoxide; practically insoluble in water and in alcohol. *NF category:* Coating agent; polymer membrane; film-forming agent; release-modifying agent; diluent.

Cellacefate: Free-flowing, white powder. May have a slight odor of acetic acid. Soluble in acetone and in dioxane; insoluble in water and in alcohol. *NF category:* Coating agent; film-forming agent.

Cellulose Acetate: Fine, white powder or free-flowing pellets. Available in a range of viscosities and acetyl contents. High viscosity, which reflects high molecular weight, decreases solubility slightly. High acetyl content cellulose acetates generally have more limited solubility in commonly used organic solvents than low acetyl content cellulose acetates, but are more soluble in methylene chloride. All acetyl content cellulose acetates are soluble in dioxane and in dimethylformamide; insoluble in alcohol and in water. *NF category:* Coating agent; polymer membrane; film-forming agent; diluent.

Microcrystalline Cellulose: Fine, white or almost white powder. It consists of free-flowing, nonfibrous particles. Practically insoluble in sodium hydroxide solution (1 in 20); insoluble in water, in dilute acids, and in most organic solvents. *NF category:* Diluent; disintegrant; wet binder; suspending and/or viscosity-increasing agent.

Silicified Microcrystalline Cellulose: White or almost white, very fine to moderately fine powder. It is a free-flowing material that may be compacted into self-binding tablets that disintegrate rapidly in water. Slightly soluble in sodium hydroxide solution (1 in 20); practically insoluble in water, in acetone, in ethanol, in toluene, and in diluted acid. *NF category:* Diluent; disintegrant; wet binder.

Microcrystalline Cellulose and Carboxymethylcellulose Sodium: Tasteless, odorless, white to off-white, coarse to fine powder. Swells in water, producing, when dispersed, a white, opaque dispersion or gel. Insoluble in organic solvents and in dilute acids. *NF category:* Suspending and/or viscosity-increasing agent.

Oxidized Cellulose: In the form of gauze or lint. Is slightly off-white in color, is acidic to the taste, and has a slight, charred odor. Soluble in dilute alkalies; insoluble in water and in acids.

Oxidized Regenerated Cellulose: A knit fabric, usually in the form of sterile strips. Slightly off-white, having a slight odor. Soluble in dilute alkalies; insoluble in water and in dilute acids.

Powdered Cellulose: White or almost white powder. Exhibits degrees of fineness ranging from a free-flowing dense powder to a coarse, fluffy, nonflowing material. Slightly soluble in sodium hydroxide solution (1 in 20); insoluble in water, in dilute acids, and in nearly all organic solvents. *NF category:* Filtering aid; sorbent; diluent; disintegrant; glidant and/or anticaking agent; suspending and/or viscosity-increasing agent.

Cellulose Sodium Phosphate: Free-flowing cream-colored, odorless, tasteless powder. Insoluble in water, in dilute acids, and in most organic solvents.

Cephalexin: White to off-white, crystalline powder. Slightly soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Cephalexin Hydrochloride: White to off-white, crystalline powder. Soluble to the extent of 10 mg per mL in water, in acetone, in acetonitrile, in alcohol, in dimethylformamide, and in methanol; practically insoluble in chloroform, in ether, in ethyl acetate, and in isopropyl alcohol.

Cephalothin Sodium: White to off-white, practically odorless, crystalline powder. Freely soluble in water, in saline TS, and in dextrose solutions; insoluble in most organic solvents.

Cephapirin Benzathine: White, crystalline powder. Soluble in 0.1 N hydrochloric acid; practically insoluble in water, in ether, and in toluene; insoluble in alcohol.

Cephapirin Sodium: White to off-white, crystalline powder, odorless or having a slight odor. Very soluble in water; insoluble in most organic solvents.

Cephradine: White to off-white, crystalline powder. Sparingly soluble in water; very slightly soluble in alcohol and in chloroform; practically insoluble in ether.

Cetirizine Hydrochloride: White to almost white powder. Freely soluble in water; practically insoluble in acetone and in methylene chloride.

Cetostearyl Alcohol: Unctuous, white flakes or granules, having a faint, characteristic odor, and a bland, mild taste. Soluble in alcohol and in ether; insoluble in water. *NF category:* Stiffening agent; emollient; emulsifying agent; suspending and/or viscosity-increasing agent.

Cetrimonium Bromide: A white to creamy white, voluminous, free-flowing powder, with a characteristic faint odor and bitter, soapy taste. Freely soluble in water and in alcohol; practically insoluble in ether. *NF category:* Antimicrobial preservative.

Cetyl Alcohol: Unctuous, white flakes, granules, cubes, or castings. Has a faint characteristic odor and a bland, mild taste. Usually melts in the range between 45° and 50°. Soluble in alcohol and in ether, the solubility increasing with an increase in temperature; insoluble in water. *NF category:* Stiffening agent; coating agent; emulsifying agent.

Cetyl Esters Wax: White to off-white, somewhat translucent flakes, having a crystalline structure and a pearly luster when caked. Has a faint odor and a bland, mild taste, free from rancidity, and has a specific gravity of about 0.83 at 50°. Soluble in boiling alcohol, in ether, in chloroform, and in fixed and volatile oils; slightly soluble in cold solvent hexane; practically insoluble in cold alcohol; insoluble in water. *NF category:* Stiffening agent; emollient.

Cetyl Palmitate: White crystals or flakes. Freely soluble in alcohol and in ether; practically insoluble in water. *NF category:* Stiffening agent.

Cetylpyridinium Chloride: White to almost-white powder, having a slight, characteristic odor. Very soluble in water, in alcohol, and in chloroform; slightly soluble in benzene and in ether. *NF category:* Antimicrobial preservative; wetting and/or solubilizing agent; emulsifying agent.

Cetylpyridinium Chloride Topical Solution: Clear liquid. Is colorless unless a color has been added; has an aromatic odor and a bitter taste.

Activated Charcoal: Fine, black, odorless, tasteless powder, free from gritty matter. *NF category:* Sorbent.

Chitosan: White or almost white powder or granules. Soluble in aqueous solutions of glycolic acid, of formic acid, of acetic acid, of hydrochloric acid, and of lactic acid; practically insoluble in organic solvents and in water. *NF category:* Coating agent; film-forming agent; suspending and/or viscosity-increasing agent; vehicle (solid carrier).

Chloral Hydrate: Colorless, transparent, or white crystals having an aromatic, penetrating, and slightly acrid odor, and a slightly bitter, caustic taste. Melts at about 55°, and slowly volatilizes when exposed to air. Very soluble in water and in olive oil; freely soluble in alcohol, in chloroform, and in ether.

Chlorambucil: Off-white, slightly granular powder. Freely soluble in acetone; soluble in dilute alkali; very slightly soluble in water.

Chloramphenicol: Fine, white to grayish-white or yellowish-white, needle-like crystals or elongated plates. Its

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solutions are practically neutral to litmus. Is reasonably stable in neutral or moderately acid solutions. Its alcohol solution is dextrorotatory and its ethyl acetate solution is levorotatory. Freely soluble in alcohol, in propylene glycol, in acetone, and in ethyl acetate; slightly soluble in water.

Chloramphenicol Palmitate: Fine, white, unctuous, crystalline powder, having a faint odor and a bland, mild taste. Freely soluble in acetone and in chloroform; soluble in ether; sparingly soluble in alcohol; very slightly soluble in solvent hexane; insoluble in water.

Chloramphenicol Sodium Succinate: Light yellow powder. Freely soluble in water and in alcohol.

Chlordiazepoxide: Yellow, practically odorless, crystalline powder. Is sensitive to sunlight. Melts at about 240°. Sparingly soluble in chloroform and in alcohol; insoluble in water.

Chlordiazepoxide Hydrochloride: White or practically white, odorless, crystalline powder. Is affected by sunlight. Soluble in water; sparingly soluble in alcohol; insoluble in solvent hexane.

Chlorhexidine Acetate: A white or almost white, microcrystalline powder. Soluble in alcohol; sparingly soluble in water; slightly soluble in glycerol and in propylene glycol.

Chlorhexidine Gluconate Solution: Almost colorless or pale yellow, clear liquid. Miscible with glacial acetic acid and with water; miscible with three times its volume of acetone and with five times its volume of dehydrated alcohol; further addition of acetone or dehydrated alcohol yields a white turbidity.

Chlorhexidine Hydrochloride: White or almost white, crystalline powder. Sparingly soluble in propylene glycol and in water; very slightly soluble in alcohol.

Chlorobutanol: Colorless to white crystals, having a characteristic, somewhat camphoraceous, odor and taste. Anhydrous form melts at about 95°, and hydrous form melts at about 76°. Freely soluble in alcohol, in ether, in chloroform, and in volatile oils; soluble in glycerin; slightly soluble in water. *NF category:* Antimicrobial preservative; plasticizer.

Chlorocresol: Colorless or practically colorless crystals or crystalline powder, having a characteristic, nontarry odor. Is volatile in steam. Very soluble in alcohol; soluble in ether, in terpenes, in fixed oils, and in solutions of alkali hydroxides; slightly soluble in water and more soluble in hot water. *NF category:* Antimicrobial preservative.

Chloroprocaine Hydrochloride: White, crystalline powder. Is odorless, and is stable in air. Its solutions are acid to litmus. Exhibits local anesthetic properties when placed upon the tongue. Soluble in water; slightly soluble in alcohol; very slightly soluble in chloroform; practically insoluble in ether.

Chloroquine: White or slightly yellow, crystalline powder. Is odorless, and has a bitter taste. Soluble in dilute acids, in chloroform, and in ether; very slightly soluble in water.

Chloroquine Hydrochloride Injection: Colorless liquid.

Chloroquine Phosphate: White, crystalline powder. Is odorless, has a bitter taste, and is discolored slowly on exposure to light. Its solutions have a pH of about 4.5. Exists in two polymorphic forms, one melting between 193° and 195° and the other between 210° and 215° (see *Melting Range or Temperature* ⟨741⟩); mixture of the forms melts between 193° and 215°. Freely soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Chlorothiazide: White or practically white, crystalline, odorless powder. Melts at about 340°, with decomposition. Freely soluble in dimethylformamide and in dimethyl sulfoxide; slightly soluble in methanol and in pyridine; practically insoluble in ether, in benzene, and in chloroform; very slightly soluble in water.

Chloroxylenol: White crystals or crystalline powder, having a characteristic odor. Is volatile in steam. Freely soluble in alcohol, in ether, in terpenes, in fixed oils, and in solutions of alkali hydroxides; very slightly soluble in water. *NF category:* Antimicrobial preservative.

Chlorpheniramine Maleate: White, odorless, crystalline powder. Its solutions have a pH between 4 and 5. Freely soluble in water; soluble in alcohol and in chloroform; slightly soluble in ether and in benzene.

Chlorpromazine: White, crystalline solid, having an amine-like odor. Darkens on prolonged exposure to light. Melts at about 60°. Freely soluble in alcohol, in benzene, in chloroform, in ether, and in dilute mineral acids; practically insoluble in water and in dilute alkali hydroxides.

Chlorpromazine Hydrochloride: White or slightly creamy white, odorless, crystalline powder. Darkens on prolonged exposure to light. Very soluble in water; freely soluble in alcohol and in chloroform; insoluble in ether and in benzene.

Chlorpropamide: White, crystalline powder, having a slight odor. Soluble in alcohol; sparingly soluble in chloroform; practically insoluble in water.

Chlortetracycline Hydrochloride: Yellow, crystalline powder. Is odorless, and has a bitter taste. Is stable in air, but is slowly affected by light. Soluble in solutions of alkali hydroxides and carbonates; sparingly soluble in water; slightly soluble in alcohol; practically insoluble in acetone, in chloroform, in dioxane, and in ether.

Chlorthalidone: White to yellowish-white, crystalline powder. Melts at a temperature above 215°, with decomposition. Soluble in methanol; slightly soluble in alcohol; practically insoluble in water, in ether, and in chloroform.

Chlorzoxazone: White or practically white, practically odorless, crystalline powder. Soluble in solutions of alkali hydroxides and ammonia; sparingly soluble in alcohol, in isopropyl alcohol, and in methanol; slightly soluble in water.

Cholecalciferol: White, odorless crystals. Is affected by air and by light. Melts at about 85°. Soluble in alcohol, in chloroform, and in fatty oils; insoluble in water.

Cholesterol: White or faintly yellow, practically odorless, pearly leaflets, needles, powder, or granules. Acquires a yellow to pale tan color on prolonged exposure to light. Soluble in acetone, in chloroform, in dioxane, in ether, in ethyl acetate, in solvent hexane, and in vegetable oils; sparingly soluble in dehydrated alcohol; slightly (and slowly) soluble in alcohol; insoluble in water. *NF category:* Emulsifying agent; emollient.

Cholestyramine Resin: White to buff-colored, hygroscopic, fine powder. Is odorless or has not more than a slight amine-like odor. Insoluble in water, in alcohol, in chloroform, and in ether.

Choline Bitartrate: White, hygroscopic, crystalline powder. Clear, colorless liquid in solution. Melts between 148° and 153°. Is odorless, or may have a faint trimethylamine odor. Freely soluble in water; slightly soluble in alcohol; insoluble in ether and in chloroform.

Choline Chloride: Colorless or white crystals or crystalline powder, usually having a slight odor of trimethylamine. Clear and colorless in solution. Hygroscopic. Soluble in alcohol and in water.

Sodium Chromate Cr 51 Injection: Clear, slightly yellow solution.

Chromic Chloride: Dark green, odorless, slightly deliquescent crystals. Soluble in water and in alcohol; slightly soluble in acetone; practically insoluble in ether.

Chymotrypsin: White to yellowish-white, crystalline or amorphous, odorless, powder. An amount equivalent to 100,000 USP Units is soluble in 10 mL of water and in 10 mL of saline TS.

Ciclopirox: White to slightly yellowish-white, crystalline powder. Freely soluble in ethanol and in methylene chloride; soluble in ether; slightly soluble in water.

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Ciclopirox Olamine: White to slightly yellowish-white, crystalline powder. Very soluble in alcohol and in methylene chloride; slightly soluble in water; practically insoluble in cyclohexane.

Cidofovir: White to off-white, crystalline powder. Soluble in 0.1 M sodium hydroxide; sparingly soluble in water; practically insoluble in methanol and in ethanol.

Cilastatin Sodium: White to tan-colored powder. Soluble in water and in methanol.

Cilostazol: White to off-white crystals. Freely soluble in chloroform; slightly soluble in methanol and in alcohol; practically insoluble in water.

Cimetidine: White to off-white, crystalline powder; odorless, or having a slight mercaptan odor. Freely soluble in methanol; soluble in alcohol and in polyethylene glycol 400; sparingly soluble in isopropyl alcohol; slightly soluble in water and in chloroform; practically insoluble in ether.

Cinoxacin: White to yellowish-white, crystalline solid. Is odorless, and has a bitter taste and a lingering aftertaste. Soluble in alkaline solution; insoluble in water and in most common organic solvents.

Ciprofloxacin Hydrochloride: Faintly yellowish to light yellow crystals. Sparingly soluble in water; slightly soluble in acetic acid and in methanol; very slightly soluble in dehydrated alcohol; practically insoluble in acetone, in acetonitrile, in ethyl acetate, in hexane, and in methylene chloride.

Cisapride: White or almost white powder. Freely soluble in dimethylformamide; soluble in methylene chloride; sparingly soluble in methanol; practically insoluble in water.

Cisatracurium Besylate: White to pale yellow powder. Soluble in water; slightly soluble in acetonitrile, in chloroform, in methanol, and in methylene chloride.

Citalopram Hydrobromide: White to almost white, crystalline powder. Soluble in alcohol; sparingly soluble in water and in dehydrated alcohol.

Anhydrous Citric Acid: Colorless, translucent crystals, or white, granular to fine, crystalline powder. Melts at about 153°, with decomposition. Very soluble in water; freely soluble in alcohol; very slightly soluble in ether. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Citric Acid Monohydrate: Colorless, translucent crystals, or white, granular to fine, crystalline powder. Efflorescent in dry air. Very soluble in water; freely soluble in alcohol; very slightly soluble in ether. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antioxidant; chelating and/or complexing agent.

Clarithromycin: White to off-white, crystalline powder. Soluble in acetone; slightly soluble in dehydrated alcohol, in methanol, and in acetonitrile, and in phosphate buffer at pH values of 2 to 5; practically insoluble in water.

Clavulanate Potassium: White to off-white powder. Is moisture-sensitive. Freely soluble in water, but stability in aqueous solution is not good, optimum stability at a pH of 6.0 to 6.3; soluble in methanol, with decomposition.

Clemastine Fumarate: White to off-white, odorless powder. Its solutions are acid to litmus. Slightly soluble in methanol; very slightly soluble in water, and in chloroform.

Clenbuterol Hydrochloride: White or almost white, crystalline powder. Soluble in water and in alcohol; slightly soluble in acetone. It melts with decomposition at 173°.

Clidinium Bromide: White to nearly white, practically odorless, crystalline powder. Is optically inactive. Melts at about 242°. Soluble in water and in alcohol; slightly soluble in benzene and in ether.

Clindamycin Hydrochloride: White or practically white, crystalline powder. Is odorless or has a faint mercaptan-like odor. Is stable in the presence of air and light. Its solutions are acidic and are dextrorotatory. Freely soluble in water, in dimethylformamide, and in methanol; soluble in alcohol; practically insoluble in acetone.

Clindamycin Palmitate Hydrochloride: White to off-white amorphous powder, having a characteristic odor. Very soluble in ethyl acetate and in dimethylformamide; freely soluble in water, in benzene, in ether, in chloroform, and in alcohol.

Clindamycin Phosphate: White to off-white, hygroscopic, crystalline powder. Is odorless or practically odorless, and has a bitter taste. Freely soluble in water; slightly soluble in dehydrated alcohol; very slightly soluble in acetone; practically insoluble in chloroform, in benzene, and in ether.

Clioquinol: Voluminous, spongy, yellowish-white to brownish-yellow powder, having a slight, characteristic odor. Darkens on exposure to light. Melts at about 180°, with decomposition. Soluble in hot ethyl acetate and in hot glacial acetic acid; practically insoluble in water and in alcohol.

Clobetasol Propionate: White to cream, crystalline powder. Soluble in acetone, in dimethyl sulfoxide, in chloroform, in methanol, and in dioxane; sparingly soluble in ethanol; slightly soluble in benzene and in diethyl ether; practically insoluble in water.

Clocortolone Pivalate: White to yellowish-white, odorless powder. Melts at about 230°, with decomposition. Freely soluble in chloroform and in dioxane; soluble in acetone; sparingly soluble in alcohol; slightly soluble in benzene and in ether.

Clofazimine: Dark red crystals. Melts at about 217°, with decomposition. Soluble in chloroform and in benzene; sparingly soluble in alcohol, in acetone, and in ethyl acetate; practically insoluble in water.

Clofibrate: Colorless to pale yellow liquid having a characteristic odor. Soluble in acetone, in alcohol, in benzene, and in chloroform; insoluble in water.

Clomiphene Citrate: White to pale yellow, essentially odorless powder. Freely soluble in methanol; sparingly soluble in alcohol; slightly soluble in water and in chloroform; insoluble in ether.

Clomipramine Hydrochloride: White to faintly yellow, crystalline powder. Freely soluble in water and in methylene chloride; soluble in alcohol; practically insoluble in ethyl ether and in hexane.

Clonazepam: Light yellow powder, having a faint odor. Sparingly soluble in acetone and in chloroform; slightly soluble in alcohol and in ether; insoluble in water.

Clonidine: White to almost white, crystalline powder. Melting point is about 130°. Freely soluble in methanol and in alcohol.

Clopidogrel Bisulfate: White to off-white powder. Freely soluble at pH 1; practically insoluble at neutral pH.

Cloprostenol Sodium: White or almost white, amorphous powder. Is hygroscopic. Freely soluble in water, in alcohol, and in methanol; practically insoluble in acetone.

Clorazepate Dipotassium: Light yellow, crystalline powder. Darkens on exposure to light. Soluble in water but, upon standing, may precipitate from the solution; slightly soluble in alcohol and in isopropyl alcohol; practically insoluble in acetone, in benzene, in chloroform, in ether, and in methylene chloride.

Clorsulon: White to off-white powder. Freely soluble in acetonitrile and in methanol; slightly soluble in water; very slightly soluble in methylene chloride.

Clotrimazole: White to pale yellow, crystalline powder. Melts at about 142°, with decomposition. Freely soluble in methanol, in acetone, in chloroform, and in alcohol; practically insoluble in water.

Cloxacillin Benzathine: White or almost white, almost odorless, crystals or crystalline powder. Soluble in chloroform

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and in methanol; sparingly soluble in acetone; slightly soluble in water, in alcohol, and in isopropyl alcohol.

Cloxacillin Sodium: White, odorless, crystalline powder. Freely soluble in water; soluble in alcohol; slightly soluble in chloroform.

Clozapine: Yellow, crystalline powder. Soluble in chloroform, in acetone, and in alcohol; sparingly soluble in acetonitrile; insoluble in water.

Coal Tar: Nearly black, viscous liquid, heavier than water, having a characteristic, naphthalene-like odor, and producing a sharp, burning sensation on the tongue. Slightly soluble in water, to which it imparts its characteristic odor and taste and a faintly alkaline reaction; partially soluble in acetone, in alcohol, in carbon disulfide, in chloroform, in ether, in methanol, and in solvent hexane; soluble in benzene and nitrobenzene.

Cyanocobalamin Co 57 Capsules: May contain a small amount of solid or solids, or may appear empty.

Cyanocobalamin Co 57 Oral Solution: Clear, colorless to pink solution.

Cocaine: Colorless to white crystals or white, crystalline powder. Is levorotatory in 3 N hydrochloric acid solution. Its saturated solution is alkaline to litmus. Very soluble in warm alcohol; freely soluble in alcohol, in chloroform, and in ether; soluble in olive oil; sparingly soluble in mineral oil; slightly soluble in water.

Cocaine Hydrochloride: Colorless crystals or white, crystalline powder. Very soluble in water; freely soluble in alcohol; soluble in chloroform and in glycerin; insoluble in ether.

Coccidioidin: Clear, practically colorless or amber-colored liquid.

Cocoa Butter: Yellowish-white solid, having a faint, agreeable odor, and a bland, chocolate-like taste if the cocoa butter is obtained by pressing. If obtained by extraction, the taste is bland. Is usually brittle at temperatures below 25°. Freely soluble in ether and in chloroform; soluble in boiling dehydrated alcohol; slightly soluble in alcohol. *NF category:* Suppository base.

Coconut Oil: Clear, white to light yellow-tan, viscous liquid. Freely soluble in methylene chloride and in light petroleum (bp: 65° to 70°); very slightly soluble in alcohol; practically insoluble in water. *NF category:* Coating agent; emulsifying agent; emollient; ointment base.

Hydrogenated Coconut Oil: White to yellowish, fatty solid to semi-solid. Freely soluble in ether; very slightly soluble in alcohol; practically insoluble in water. *NF category:* Coating agent; lubricant; wet binder.

Cod Liver Oil: Thin, oily liquid, having a characteristic, slightly fishy but not rancid odor, and a fishy taste. Freely soluble in ether, in chloroform, in carbon disulfide, and in ethyl acetate; slightly soluble in alcohol.

Codeine: Colorless or white crystals or white, crystalline powder. It effloresces slowly in dry air, and is affected by light. In acid or alcohol solutions it is levorotatory. Its saturated solution is alkaline to litmus. Very soluble in chloroform; freely soluble in alcohol; sparingly soluble in ether; slightly soluble in water. When heated in an amount of water insufficient for complete solution, it melts to oily drops that crystallize on cooling.

Codeine Phosphate: Fine, white, needle-shaped crystals, or white, crystalline powder. Is odorless. Is affected by light. Its solutions are acid to litmus. Very soluble in hot water; freely soluble in water; slightly soluble in alcohol but more so in boiling alcohol.

Codeine Sulfate: White crystals, usually needle-like, or white, crystalline powder. Is affected by light. Freely soluble in water at 80°; soluble in water; very slightly soluble in alcohol; insoluble in chloroform and in ether.

Colchicine: Pale yellow to pale greenish-yellow, amorphous scales, or powder or crystalline powder. Is odorless or nearly so, and darkens on exposure to light. Freely soluble in alcohol and in chloroform; soluble in water; slightly soluble in ether.

Colestipol Hydrochloride: Yellow to orange beads. Swells but does not dissolve in water or dilute aqueous solutions of acid or alkali. Insoluble in the common organic solvents.

Colistimethate Sodium: White to slightly yellow, odorless, fine powder. Freely soluble in water; soluble in methanol; insoluble in acetone and in ether.

Colistin Sulfate: White to slightly yellow, odorless, fine powder. Freely soluble in water; slightly soluble in methanol; insoluble in acetone and in ether.

Collodion: Clear, or slightly opalescent, viscous liquid. Is colorless, or slightly yellowish, and has the odor of ether.

Flexible Collodion: Clear, or slightly opalescent, viscous liquid. Is colorless or slightly yellow, and has the odor of ether. The strong odor of camphor becomes noticeable as the ether evaporates.

Copovidone: White to yellowish-white powder or flakes. Is hygroscopic. Freely soluble in water, in alcohol, and in methylene chloride; practically insoluble in ether. *NF category:* Wet binder; coating agent; film-forming agent.

Corn Oil: Clear, light yellow, oily liquid, having a faint, characteristic odor and taste. Slightly soluble in alcohol. Miscible with ether, with chloroform, with benzene, and with solvent hexane. *Specific Gravity* ⟨841⟩: Between 0.914 and 0.921. *NF category:* Solvent; vehicle (oleaginous).

Corn Syrup: Clear, white to light yellow, viscous liquid. Is miscible in all proportions with water. *NF category:* Suspending and/or viscosity-increasing agent; sweetening agent; diluent; wet binder; tonicity agent.

Corn Syrup Solids: Sweet, white to light yellow powder or granules. Soluble in water. *NF category:* Coating agent; vehicle (flavored and/or sweetened and solid carrier); humectant; suspending and/or viscosity-increasing agent; sweetening agent; diluent; wet binder; tonicity agent.

Corticotropin Injection: Colorless or light straw-colored liquid.

Corticotropin for Injection: White or practically white, soluble, amorphous solid having the characteristic appearance of substances prepared by freeze-drying.

Repository Corticotropin Injection: Colorless or light straw-colored liquid, which may be quite viscid at room temperature. Is odorless or has an odor of an antimicrobial agent.

Corticotropin Zinc Hydroxide Injectable Suspension: Flocculent, white, aqueous suspension, free from large particles following moderate shaking.

Cortisone Acetate: White or practically white, odorless, crystalline powder. Is stable in air. Melts at about 240°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Freely soluble in chloroform; soluble in dioxane; sparingly soluble in acetone; slightly soluble in alcohol; insoluble in water.

Purified Cotton: White, soft, fine filament-like hairs appearing under the microscope as hollow, flattened, and twisted bands, striate and slightly thickened at the edges. Is practically odorless and practically tasteless. Soluble in ammoniated cupric oxide TS; insoluble in ordinary solvents.

Cottonseed Oil: Pale yellow, oily liquid. Is odorless or nearly so, and has a bland taste. At temperatures below 10° particles of solid fat may separate from the Oil, and at about 0° to −5° the Oil becomes a solid or nearly so. *Specific Gravity* ⟨841⟩: Between 0.915 and 0.921. Slightly soluble in alcohol. Miscible with ether, with chloroform, with solvent hexane, and with carbon disulfide. *NF category:* Solvent; vehicle (oleaginous).

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Hydrogenated Cottonseed Oil: A white mass or powder that melts to a clear, pale yellow liquid when heated. Freely soluble in methylene chloride and in toluene; very slightly soluble in alcohol; practically insoluble in water.

Creatinine: White crystals or crystalline powder; odorless. Soluble in water; slightly soluble in alcohol; practically insoluble in acetone, in ether, and in chloroform. *NF category:* Bulking agent.

Cresol: Colorless, or yellowish to brownish-yellow, or pinkish, highly refractive liquid, becoming darker with age and on exposure to light. Has a phenol-like, sometimes empyreumatic odor. A saturated solution of it is neutral or only slightly acid to litmus. Sparingly soluble in water, usually forming a cloudy solution; dissolves in solutions of fixed alkali hydroxides. Miscible with alcohol, with ether, and with glycerin. *NF category:* Antimicrobial preservative.

Cromolyn Sodium: White, odorless, crystalline powder. Is tasteless at first, with a slightly bitter aftertaste. Is hygroscopic. Soluble in water; insoluble in alcohol and in chloroform.

Cromolyn Sodium for Inhalation: White to creamy white, odorless, hygroscopic, and very finely divided powder.

Croscarmellose Sodium: White, free-flowing powder. Partially soluble in water; insoluble in alcohol, in ether, and in other organic solvents. *NF category:* Disintegrant.

Crospovidone: White to creamy-white, hygroscopic powder, having a faint odor. Insoluble in water and in ordinary organic solvents. *NF category:* Disintegrant.

Crotamiton: Colorless to slightly yellowish oil, having a faint amine-like odor. Soluble in alcohol and in methanol.

Cupric Chloride: Bluish green, deliquescent crystals. Freely soluble in water; soluble in alcohol; slightly soluble in ether.

Cupric Sulfate ($CuSO_4 \cdot 5H_2O$): Deep blue, triclinic crystals or blue, crystalline granules or powder. It effloresces slowly in dry air. Its solutions are acid to litmus. Very soluble in boiling water; freely soluble in water and in glycerin; slightly soluble in alcohol.

Cupric Sulfate (anhydrous): A white or grayish-white powder. Soluble in water; insoluble in alcohol.

Cyanocobalamin: Dark red crystals or amorphous or crystalline red powder. In the anhydrous form, it is very hygroscopic and when exposed to air it may absorb about 12% of water. Soluble in alcohol; sparingly soluble in water; insoluble in acetone, in chloroform, and in ether.

Cyclandelate: White, crystalline powder. Very soluble in acetonitrile, in alcohol, and in ether; practically insoluble in water. Melts at about 58°.

Cyclizine Hydrochloride: White, crystalline powder or small, colorless crystals. Is odorless or nearly so, and has a bitter taste. Melts indistinctly at about 285°, with decomposition. Sparingly soluble in chloroform; slightly soluble in water and in alcohol; insoluble in ether.

Cyclobenzaprine Hydrochloride: White to off-white, odorless, crystalline powder. Freely soluble in water, in alcohol, and in methanol; sparingly soluble in isopropanol; slightly soluble in chloroform and in methylene chloride; insoluble in hydrocarbons.

Cyclopentolate Hydrochloride: White, crystalline powder, which upon standing develops a characteristic odor. Its solutions are acid to litmus. Melts at about 138°, the melt appearing opaque. Very soluble in water; freely soluble in alcohol; insoluble in ether.

Cyclophosphamide: White, crystalline powder. Liquefies upon loss of its water of crystallization. Soluble in water and in alcohol.

Cyclopropane: Colorless gas having a characteristic odor. Has a pungent taste. One L at a pressure of 760 mm and a temperature of 0° weighs about 1.88 g. One volume dissolves in about 2.7 volumes of water at 15°. Freely soluble in alcohol; soluble in fixed oils.

Cycloserine: White to pale yellow, crystalline powder. Is odorless or has a faint odor. Is hygroscopic and deteriorates upon absorbing water. Its solutions are dextrorotatory. Freely soluble in water.

Cyclosporine: White to almost white powder. Soluble in acetone, in alcohol, in methanol, in ether, in chloroform, and in methylene chloride; slightly soluble in saturated hydrocarbons; practically insoluble in water.

Cyproheptadine Hydrochloride: White to slightly yellow, odorless or practically odorless, crystalline powder. Freely soluble in methanol; soluble in chloroform; sparingly soluble in alcohol; slightly soluble in water; practically insoluble in ether.

Cyromazine: White or off-white, odorless, crystalline powder. Slightly soluble in methanol and in water.

Cysteine Hydrochloride: White crystals or crystalline powder. Soluble in water, in alcohol, and in acetone.

Cytarabine: Odorless, white to off-white, crystalline powder. Freely soluble in water; slightly soluble in alcohol and in chloroform.

Dactinomycin: Bright red, crystalline powder. Is somewhat hygroscopic and is affected by light and by heat. Freely soluble in alcohol; soluble in water at 10° and slightly soluble in water at 37°; very slightly soluble in ether.

Dalfampridine: White powder. Soluble in water, in methanol, in acetone, in tetrahydrofuran, in isopropanol, in acetonitrile, in *N,N*-dimethylformamide, in dimethylsulfoxide, and in alcohol.

Danazol: White to pale yellow, crystalline powder. Melts at about 225°, with some decomposition. Freely soluble in chloroform; soluble in acetone; sparingly soluble in alcohol and in benzene; slightly soluble in ether; practically insoluble or insoluble in water and in hexane.

Dantrolene Sodium: Fine orange to orange-brown powder. Sparingly soluble in dimethylformamide and in glycerine; sparingly soluble to practically insoluble in acetone.

Dapsone: White or creamy white, crystalline powder. Is odorless and has a slightly bitter taste. Soluble in acetone and in dilute mineral acids; sparingly soluble in alcohol; very slightly soluble in water.

Daunorubicin Hydrochloride: Orange-red, crystalline, hygroscopic powder. Freely soluble in water and in methanol; slightly soluble in alcohol; very slightly soluble in chloroform; practically insoluble in acetone.

Deferoxamine Mesylate: White to off-white powder. Freely soluble in water; slightly soluble in methanol.

Dehydroacetic Acid: White or nearly white, crystalline powder. Soluble in aqueous solutions of alkalies; very slightly soluble in water. One g of sample dissolves in about 35 mL of alcohol and in 5 mL of acetone. *NF category:* Antimicrobial preservative.

Dehydrocholic Acid: White, fluffy, odorless powder, having a bitter taste. Soluble in glacial acetic acid and in solutions of alkali hydroxides and carbonates; sparingly soluble in chloroform (the solutions in alcohol and in chloroform usually are slightly turbid); slightly soluble in alcohol and in ether; practically insoluble in water.

Demecarium Bromide: White or slightly yellow, slightly hygroscopic, crystalline powder. Freely soluble in water and in alcohol; soluble in ether; sparingly soluble in acetone.

Demeclocycline: Yellow, crystalline, odorless powder, having a bitter taste. Soluble in alcohol; sparingly soluble in water. Dissolves readily in 3 N hydrochloric acid and in alkaline solutions.

Demeclocycline Hydrochloride: Yellow, crystalline, odorless powder, having a bitter taste. Sparingly soluble in water and in solutions of alkali hydroxides and carbonates;

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slightly soluble in alcohol; practically insoluble in acetone and in chloroform.

Denatonium Benzoate: Very soluble in chloroform and in methanol; freely soluble in water and in alcohol; very slightly soluble in ether. *NF category:* Alcohol denaturant; flavors and fragrance.

Desipramine Hydrochloride: White to off-white, crystalline powder. Melts at about 213°. Freely soluble in methanol and in chloroform; soluble in water and in alcohol; insoluble in ether.

Desloratadine: White to light pink-colored powder. Very soluble in ethanol and in propylene glycol; soluble in dichloromethane; slightly soluble in water.

Desmopressin Acetate: White, fluffy powder. Soluble in water, in alcohol, and in acetic acid.

Desogestrel: White or almost-white, crystalline powder. Very soluble in methanol; freely soluble in anhydrous ethanol and in methylene chloride; practically insoluble in water.

Desonide: White powder or crystal. Soluble in chloroform; sparingly soluble in ethanol and in acetone; practically insoluble in water.

Desoximetasone: White to practically white, odorless, crystalline powder. Freely soluble in alcohol, in acetone, and in chloroform; insoluble in water.

Desoxycholic Acid: [(Title for this monograph is not to change until December 1, 2021.)

(Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)]

Deoxycholic Acid: Occurs as a white, crystalline powder. Freely soluble in alcohol; soluble in acetone and in solutions of alkali hydroxides and carbonates; slightly soluble in chloroform and in ether; practically insoluble in water. *NF category:* Emulsifying agent.

Desoxycorticosterone Acetate: White or creamy white, crystalline powder. Is odorless, and is stable in air. Sparingly soluble in alcohol, in acetone, and in dioxane; slightly soluble in vegetable oils; practically insoluble in water.

Desvenlafaxine: White to off-white, crystalline powder. Sparingly soluble in dimethyl sulfoxide.

Desvenlafaxine Fumarate: White to off-white powder. Soluble in methanol; slightly soluble in water.

Desvenlafaxine Succinate: White to creamish, crystalline powder. Sparingly soluble in dimethyl sulfoxide, in methanol, and in water.

Dexamethasone: White to practically white, odorless, crystalline powder. Is stable in air. Melts at about 250°, with some decomposition. Sparingly soluble in acetone, in alcohol, in dioxane, and in methanol; slightly soluble in chloroform; very slightly soluble in ether; practically insoluble in water.

Dexamethasone Acetate: Clear, white to off-white, odorless powder. Freely soluble in methanol, in acetone, and in dioxane; practically insoluble in water.

Dexamethasone Sodium Phosphate: White or slightly yellow, crystalline powder. Is odorless or has a slight odor of alcohol, and is exceedingly hygroscopic. Freely soluble in water; slightly soluble in alcohol; very slightly soluble in dioxane; insoluble in chloroform and in ether.

Dexbrompheniramine Maleate: White, odorless, crystalline powder. Exists in two polymorphic forms, one melting between 106° and 107° and the other between 112° and 113°. Mixtures of the forms may melt between 105° and 113°. The pH of a solution (1 in 100) is about 5. Freely soluble in water; soluble in alcohol and in chloroform.

Dexchlorpheniramine Maleate: White, odorless, crystalline powder. Freely soluble in water; soluble in alcohol and in chloroform; slightly soluble in benzene and in ether.

Dexmedetomidine Hydrochloride: White or almost white powder. Freely soluble in water, and has a pKa of 7.1.

Dexpanthenol: Clear, viscous, somewhat hygroscopic liquid, having a slight, characteristic odor. Some crystallization may occur on standing. Freely soluble in water, in alcohol, in methanol, and in propylene glycol; soluble in chloroform and in ether; slightly soluble in glycerin.

Dextran 1: A white to off-white powder. Is hygroscopic. Very soluble in water; sparingly soluble in alcohol.

Dextrates: Free-flowing, porous, white, odorless, spherical granules consisting of aggregates of microcrystals, having a sweet taste and producing a cooling sensation in the mouth. May be compressed directly into self-binding tablets. Freely soluble in water (heating increases its solubility in water); soluble in dilute acids and alkalies and in basic organic solvents such as pyridine; insoluble in the common organic solvents. *NF category:* Sweetening agent; diluent; wet binder.

Dextrin: Free-flowing, white, yellow, or brown powder. Its solubility in water varies; it is usually very soluble, but often contains an insoluble portion. *NF category:* Suspending and/or viscosity-increasing agent; diluent; wet binder; film-forming agent; stiffening agent.

Dextroamphetamine Sulfate: White, odorless, crystalline powder. Soluble in water; slightly soluble in alcohol; insoluble in ether.

Dextromethorphan: Practically white to slightly yellow, odorless, crystalline powder. Eleven mg of Dextromethorphan is equivalent to 15 mg of dextromethorphan hydrobromide monohydrate. Freely soluble in chloroform; practically insoluble in water.

Dextromethorphan Hydrobromide: Practically white crystals or crystalline powder, having a faint odor. Melts at about 126°, with decomposition. Freely soluble in alcohol and in chloroform; sparingly soluble in water; insoluble in ether.

Dextrose: Colorless crystals or white, crystalline or granular powder. Is odorless, and has a sweet taste. Very soluble in boiling water; freely soluble in water; soluble in boiling alcohol; slightly soluble in alcohol. *NF category:* Sweetening agent; tonicity agent; vehicle (flavored and/or sweetened); diluent.

Dextrose Excipient: Colorless crystals or white, crystalline or granular powder. Is odorless and sweet-tasting. Very soluble in boiling water; freely soluble in water; sparingly soluble in boiling alcohol; slightly soluble in alcohol. *NF category:* Sweetening agent; diluent.

Diacetylated Monoglycerides: Clear liquid. Very soluble in 80% (w/w) aqueous alcohol, in vegetable oils, and in mineral oils; sparingly soluble in 70% alcohol. *NF category:* Plasticizer.

Diatrizoate Meglumine: White, odorless powder. Freely soluble in water.

Diatrizoate Meglumine Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Diatrizoate Meglumine and Diatrizoate Sodium Injection: Clear, colorless to pale yellow, slightly viscous liquid. May crystallize at room temperature or below.

Diatrizoate Sodium: White, odorless powder. Soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in ether.

Diatrizoate Sodium Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Diatrizoate Sodium Solution: Clear, pale yellow to light brown liquid.

Diatrizoic Acid: White, odorless powder. Soluble in dimethylformamide and in alkali hydroxide solutions; very slightly soluble in water and in alcohol.

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Diazepam: Off-white to yellow, practically odorless, crystalline powder. Freely soluble in chloroform; soluble in alcohol; practically insoluble in water.

Diazoxide: White or cream-white crystals or crystalline powder. Very soluble in strong alkaline solutions; freely soluble in dimethylformamide; sparingly soluble to practically insoluble in water and in most organic solvents.

Dibucaine: White to off-white powder, having a slight, characteristic odor. Darkens on exposure to light. Soluble in 1 N hydrochloric acid and in ether; slightly soluble in water.

Dibucaine Hydrochloride: Colorless or white to off-white crystals or white to off-white, crystalline powder. Is odorless, is somewhat hygroscopic, and darkens on exposure to light. Its solutions have a pH of about 5.5. Freely soluble in water, in alcohol, in acetone, and in chloroform.

Dibutyl Phthalate: A clear, oily liquid, colorless or very slightly yellow. Practically insoluble in water. Miscible with alcohol and with ether. *NF category:* Film-forming agent; solvent.

Dibutyl Sebacate: Colorless, oily liquid of very mild odor. Soluble in alcohol, in isopropyl alcohol, and in mineral oil; very slightly soluble in propylene glycol; practically insoluble in water and in glycerin. *NF category:* Plasticizer.

Dichloralphenazone: White, microcrystalline powder. Has a slight odor characteristic of chloral hydrate. Decomposed by dilute alkali, liberating chloroform. Freely soluble in water, in alcohol, and in chloroform; soluble in dilute acids.

Dichlorodifluoromethane: Clear, colorless gas, having a faint, ethereal odor. Its vapor pressure at 25° is about 4880 mm of mercury (80 psig). *NF category:* Propellant.

Dichlorotetrafluoroethane: Clear, colorless gas, having a faint, ethereal odor. Its vapor pressure at 25° is about 1620 mm of mercury (17 psig). Usually contains between 6% and 10% of its isomer, $CCl_2F\text{-}CF_3$. *NF category:* Propellant.

Dichlorphenamide: White or practically white, crystalline compound. Soluble in dilute solutions of alkali hydroxides and carbonates; very slightly soluble in water.

Diclazuril: White to yellow powder. Sparingly soluble in dimethylformamide; practically insoluble in water, in alcohol, and in methylene chloride.

Diclofenac Potassium: White to off-white or slightly yellowish crystalline powder, slightly hygroscopic. Freely soluble in methanol; soluble in alcohol; sparingly soluble in water; slightly soluble in acetone.

Diclofenac Sodium: White to off-white, hygroscopic, crystalline powder. Melts at about 284°. Freely soluble in methanol; soluble in ethanol; sparingly soluble in water; practically insoluble in chloroform and in ether.

Dicloxacillin Sodium: White to off-white, crystalline powder. Freely soluble in water.

Dicyclomine Hydrochloride: Fine, white, crystalline powder. Is practically odorless and has a very bitter taste. Freely soluble in alcohol and in chloroform; soluble in water; very slightly soluble in ether.

Dicyclomine Hydrochloride Injection: Colorless solution, which may have the odor of a preservative.

Didanosine: White to off-white, crystalline powder. Very soluble in dimethyl sulfoxide; practically insoluble or insoluble in acetone and in methanol.

Dienestrol: Colorless, white or practically white, needle-like crystals, or white or practically white, crystalline powder. Is odorless. Soluble in alcohol, in acetone, in ether, in methanol, in propylene glycol, and in solutions of alkali hydroxides; slightly soluble in chloroform and in fatty oils; practically insoluble in water.

Diethanolamine: White or clear, colorless crystals, deliquescing in moist air; or colorless liquid. Miscible with water, with alcohol, with acetone, with chloroform, and with glycerin. Slightly soluble to insoluble in benzene, in ether, and in petroleum ether. *NF category:* Emulsifying agent; pH modifier (acidifying agent/alkalizing agent/buffering agent).

Diethylcarbamazine Citrate: White, crystalline powder. Melts at about 136°, with decomposition. Is odorless or has a slight odor; is slightly hygroscopic. Very soluble in water; sparingly soluble in alcohol; practically insoluble in acetone, in chloroform, and in ether.

Diethylene Glycol Monoethyl Ether: Clear, colorless liquid. Is hygroscopic. Miscible with water, with acetone, and with alcohol; partially miscible with vegetable oils; immiscible with mineral oils. Specific gravity about 0.991. *NF category:* Ointment base; solvent; emulsifying agent.

Diethylene Glycol Stearates: White or almost white, waxy solid. Soluble in acetone and in hot alcohol; practically insoluble in water. *NF category:* Emulsifying agent.

Diethyl Phthalate: Colorless, practically odorless, oily liquid. Insoluble in water. Miscible with alcohol, with ether, and with other usual organic solvents. *NF category:* Plasticizer; film-forming agent; solvent.

Diethyl Sebacate: A colorless to slightly yellow liquid. Miscible with alcohol, with ether, with other organic solvents, and with most fixed oils; insoluble or practically insoluble in water. *NF category*: Flavors and fragrance.

Diethylpropion Hydrochloride: White to off-white, fine crystalline powder. Is odorless, or has a slight characteristic odor. It melts at about 175°, with decomposition. Freely soluble in water, in chloroform, and in alcohol; practically insoluble in ether.

Diethylstilbestrol: White, odorless, crystalline powder. Soluble in alcohol, in chloroform, in ether, in fatty oils, and in dilute alkali hydroxides; practically insoluble in water.

Diethyltoluamide: Colorless liquid, having a faint, pleasant odor. Boils at about 111° under a pressure of 1 mm of mercury. Practically insoluble in water and in glycerin. Miscible with alcohol, with isopropyl alcohol, with ether, with chloroform, and with carbon disulfide.

Diflorasone Diacetate: White to pale yellow, crystalline powder. Soluble in methanol and in acetone; sparingly soluble in ethyl acetate; slightly soluble in toluene; very slightly soluble in ether; insoluble in water.

Diflunisal: White to off-white, practically odorless powder. Freely soluble in alcohol and in methanol; soluble in acetone and in ethyl acetate; slightly soluble in chloroform, in carbon tetrachloride, and in methylene chloride; insoluble in hexane and in water.

Digitoxin: White or pale buff, odorless, microcrystalline powder. Sparingly soluble in chloroform; slightly soluble in alcohol; very slightly soluble in ether; practically insoluble in water.

Digoxin: Clear to white, odorless crystals or white, odorless crystalline powder. Freely soluble in pyridine; slightly soluble in diluted alcohol and in chloroform; practically insoluble in water and in ether.

Dihydroergotamine Mesylate: White to slightly yellowish powder, or off-white to faintly red powder, having a faint odor. Soluble in alcohol; slightly soluble in water and in chloroform.

Dihydrostreptomycin Sulfate: White or almost white, amorphous or crystalline powder. Amorphous form is hygroscopic. Freely soluble in water; practically insoluble in acetone, in chloroform, and in methanol.

Dihydrotachysterol: Colorless or white, odorless crystals, or white, odorless, crystalline powder. Freely soluble in ether and in chloroform; soluble in alcohol; sparingly soluble in vegetable oils; practically insoluble in water.

Dihydroxyacetone: White to off-white crystalline powder. The monomeric form is freely soluble in water, in alcohol, and in ether. The dimeric form is freely soluble in water; soluble in alcohol; and sparingly soluble in ether.

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Dihydroxyaluminum Aminoacetate: White, odorless powder having a faintly sweet taste. Soluble in dilute mineral acids and in solutions of fixed alkalies; insoluble in water and in organic solvents.

Dihydroxyaluminum Aminoacetate Magma: White, viscous suspension, from which small amounts of water may separate on standing.

Dihydroxyaluminum Sodium Carbonate: Fine, white, odorless powder. Soluble in dilute mineral acids with the evolution of carbon dioxide; practically insoluble in water and in organic solvents.

Diloxanide Furoate: White or almost white, crystalline powder. Freely soluble in chloroform; slightly soluble in alcohol and in ether; very slightly soluble in water.

Diltiazem Hydrochloride: White, odorless, crystalline powder or small crystals. Freely soluble in chloroform, in formic acid, in methanol, and in water; sparingly soluble in dehydrated alcohol; insoluble in ether. Melts at about 210°, with decomposition.

Dimenhydrinate: White, crystalline, odorless powder. Freely soluble in alcohol and in chloroform; sparingly soluble in ether; slightly soluble in water.

Dimercaprol: Colorless or practically colorless liquid, having a disagreeable, mercaptan-like odor. Soluble in water, in alcohol, in benzyl benzoate, and in methanol.

Dimercaprol Injection: Yellow, viscous solution having a pungent, disagreeable odor. Specific gravity is about 0.978.

Dimethicone: A clear, colorless, and odorless liquid. Soluble in chlorinated hydrocarbons, in benzene, in toluene, in xylene, in *n*-hexane, in petroleum spirits, in ether, and in amyl acetate; very slightly soluble in isopropyl alcohol; insoluble in water, in methanol, in alcohol, and in acetone. *NF category:* Antifoaming agent; water-repelling agent; adhesive; emollient.

Dimethyl Sulfoxide: Clear, colorless, odorless, hygroscopic liquid. Melts at about 18.4°. Boils at about 189°. Soluble in water; practically insoluble in acetone, in alcohol, in benzene, in chloroform, and in ether. *NF category:* Solvent.

Dinoprostone: White to off-white, crystalline powder. Freely soluble in acetone, in alcohol, in ether, in ethyl acetate, in isopropyl alcohol, in methanol, and in methylene chloride; soluble in toluene and in diisopropyl ether; practically insoluble in hexanes.

Dinoprost Tromethamine: White to off-white, crystalline powder. Very soluble in water; freely soluble in dimethylformamide; soluble in methanol; slightly soluble in chloroform.

Dioxybenzone: Yellow powder. Freely soluble in alcohol and in toluene; practically insoluble in water.

Diphenhydramine Hydrochloride: White, odorless, crystalline powder. Slowly darkens on exposure to light. Its solutions are practically neutral to litmus. Freely soluble in water, in alcohol, and in chloroform; sparingly soluble in acetone; very slightly soluble in benzene and in ether.

Diphenoxylate Hydrochloride: White, odorless, crystalline powder. Its saturated solution has a pH of about 3.3. Freely soluble in chloroform; soluble in methanol; sparingly soluble in alcohol and in acetone; slightly soluble in water and in isopropanol; practically insoluble in ether and in solvent hexane.

Diphtheria and Tetanus Toxoids Adsorbed: Turbid, and white, slightly gray, or slightly pink suspension, free from evident clumps after shaking.

Dipivefrin Hydrochloride: White, crystalline powder or small crystals, having a faint odor. Very soluble in water.

Dipyridamole: Intensely yellow, crystalline powder or needles. Very soluble in methanol, in alcohol, and in chloroform; slightly soluble in water; very slightly soluble in acetone and in ethyl acetate.

Dirithromycin: White or practically white powder. Very soluble in methanol and in methylene chloride; very slightly soluble in water.

Disopyramide Phosphate: White or practically white, odorless powder. Melts at about 205°, with decomposition. Freely soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Disulfiram: White to off-white, odorless, crystalline powder. Soluble in acetone, in alcohol, in carbon disulfide, and in chloroform; very slightly soluble in water.

Divalproex Sodium: White to off-white powder. Very soluble in chloroform; freely soluble in methanol and in ethyl ether; soluble in acetone; practically insoluble in acetonitrile.

Dobutamine Hydrochloride: White to practically white, crystalline powder. Soluble in pyridine; sparingly soluble in water, in alcohol, and in methanol.

Docetaxel: White or almost white, crystalline powder. Freely soluble in acetone; soluble in methanol; practically insoluble in water.

Docusate Calcium: White, amorphous solid, having the characteristic odor of octyl alcohol. It is free of the odor of other solvents. Very soluble in alcohol, in polyethylene glycol 400, and in corn oil; very slightly soluble in water.

Docusate Potassium: White, amorphous solid, having a characteristic odor suggestive of octyl alcohol. Very soluble in solvent hexane; soluble in alcohol and in glycerin; sparingly soluble in water.

Docusate Sodium: White, wax-like, plastic solid, having a characteristic odor suggestive of octyl alcohol, but no odor of other solvents. Very soluble in solvent hexane; freely soluble in alcohol and in glycerin; sparingly soluble in water. *NF category:* Wetting and/or solubilizing agent.

Dofetilide: White to off-white powder. Soluble in 0.1 N sodium hydroxide, in acetone, and in 0.1 N hydrochloric acid; very slightly soluble in water and in isopropyl alcohol.

Dolasetron Mesylate: White to off-white powder. Freely soluble in water and in propylene glycol; slightly soluble in alcohol and in saline TS.

Donepezil Hydrochloride: White, crystalline powder. Some polymorphic forms are very hygroscopic. Freely soluble in chloroform; soluble in water and in glacial acetic acid; slightly soluble in alcohol and in acetonitrile; practically insoluble in ethyl acetate and in *n*-hexane.

Dopamine Hydrochloride: White to off-white, crystalline powder. May have a slight odor of hydrochloric acid. Melts at about 240°, with decomposition. Freely soluble in water and in aqueous solutions of alkali hydroxides; soluble in methanol; insoluble in ether and in chloroform.

Dorzolamide Hydrochloride: White to off-white, crystalline powder. Soluble in water.

Doxapram Hydrochloride: White to off-white, odorless, crystalline powder. Melts at about 220°. Soluble in water and in chloroform; sparingly soluble in alcohol; practically insoluble in ether.

Doxazosin Mesylate: White to tan-colored powder. Freely soluble in formic acid; very slightly soluble in methanol and in water.

Doxercalciferol: White to off-white powder or crystalline solid. Soluble in *N,N*-dimethylformamide; sparingly soluble in methanol; slightly soluble in acetonitrile; practically insoluble in water.

Doxorubicin Hydrochloride: Red-orange, hygroscopic, crystalline or amorphous powder. Soluble in water, in isotonic sodium chloride solution, and in methanol; practically insoluble in chloroform, in ether, and in other organic solvents.

Doxycycline: Yellow, crystalline powder. Freely soluble in dilute acid and in alkali hydroxide solutions; very slightly soluble in alcohol and in water; practically insoluble in chloroform and in ether.

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Doxycycline Hyclate: Yellow, crystalline powder. Soluble in water and in solutions of alkali hydroxides and carbonates; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Doxylamine Succinate: White or creamy white powder, having a characteristic odor. Very soluble in water and in alcohol; freely soluble in chloroform; very slightly soluble in ether and in benzene.

Dronabinol: Light yellow resinous oil that is sticky at room temperature and hardens upon refrigeration. Insoluble in water.

Dronedarone Hydrochloride: A white to off-white, fine powder. Freely soluble in methylene chloride and in methanol; practically insoluble in water.

Droperidol: White to light tan, amorphous or microcrystalline powder. Freely soluble in chloroform; slightly soluble in alcohol and in ether; practically insoluble in water. Melts at about 145°.

Drospirenone: White to off-white powder. Freely soluble in methylene chloride; soluble in acetone and in methanol; sparingly soluble in ethyl acetate and in alcohol; practically insoluble in hexane and in water.

Duloxetine Hydrochloride: White to brownish-white solid. Freely soluble in methanol; sparingly soluble in water; practically insoluble in hexane.

Absorbable Dusting Powder: White, odorless powder.

Dutasteride: White to pale-yellow powder. Soluble in ethanol and methanol; slightly soluble in polyethylene glycol 400; and insoluble in water.

Dyclonine Hydrochloride: White crystals or white crystalline powder, which may have a slight odor. Exhibits local anesthetic properties when placed upon the tongue. Soluble in water, in acetone, in alcohol, and in chloroform.

Dydrogesterone: White to pale yellow, crystalline powder. Sparingly soluble in alcohol; practically insoluble in water.

Dyphylline: White, odorless, extremely bitter, amorphous or crystalline solid. Freely soluble in water; sparingly soluble in alcohol and in chloroform; practically insoluble in ether.

Ecamsule Solution: Clear yellow liquid.

Echothiophate Iodide: White, crystalline, hygroscopic solid having a slight mercaptan-like odor. Its solutions have a pH of about 4. Freely soluble in water and in methanol; soluble in dehydrated alcohol; practically insoluble in other organic solvents.

Echothiophate Iodide for Ophthalmic Solution: White, amorphous powder.

Econazole Nitrate: White or practically white, crystalline powder, having not more than a slight odor. Soluble in methanol; sparingly soluble in chloroform; slightly soluble in alcohol; very slightly soluble in water and in ether.

Edetate Calcium Disodium: White, crystalline granules or white, crystalline powder. Is odorless, is slightly hygroscopic, and has a faint, saline taste. Is stable in air. Freely soluble in water. *NF category:* Chelating and/or complexing agent.

Edetate Disodium: White, crystalline powder. Soluble in water. *NF category:* Chelating and/or complexing agent; transfer ligand.

Edetic Acid: White, crystalline powder. Melts above 220°, with decomposition. Soluble in solutions of alkali hydroxides; very slightly soluble in water. *NF category:* Chelating and/or complexing agent.

Edrophonium Chloride: White, odorless, crystalline powder. Its solution (1 in 10) is practically colorless. Very soluble in water; freely soluble in alcohol; insoluble in chloroform and in ether.

Efavirenz: White to slightly pink crystalline powder. Soluble in methanol; practically insoluble in water.

Emedastine Fumarate: White to faintly yellow, crystalline powder. Soluble in water.

Emetine Hydrochloride: White or very slightly yellowish, odorless, crystalline powder. Is affected by light. Freely soluble in water and in alcohol.

Enalapril Maleate: Off-white, crystalline powder. Melts at about 144°. Freely soluble in methanol and in dimethylformamide; soluble in alcohol; sparingly soluble in water; slightly soluble in semipolar organic solvents; practically insoluble in nonpolar organic solvents.

Enalaprilat: White to nearly white, hygroscopic, crystalline powder. Sparingly soluble in methanol and in dimethylformamide; slightly soluble in water and in isopropyl alcohol; very slightly soluble in acetone, in alcohol, and in hexane; practically insoluble in acetonitrile and in chloroform.

Enflurane: Clear, colorless, stable, volatile liquid, having a mild, sweet odor. Is nonflammable. Slightly soluble in water. Miscible with organic solvents, with fats, and with oils.

Enrofloxacin: Pale yellow to light yellow crystalline powder. Very slightly soluble in water at pH 7.

Entacapone: Greenish yellow to yellow powder. Sparingly soluble in acetone and in methanol; slightly soluble in ethanol, chloroform, isopropanol, and ether; very slightly soluble in toluene; practically insoluble in water.

Entecavir: White to off-white powder. Practically insoluble to slightly soluble in water; practically insoluble in anhydrous ethanol and in heptane.

Ephedrine: Unctuous, practically colorless solid or white crystals or granules. Gradually decomposes on exposure to light. Melts between 33° and 40°, the variability in the melting point being the result of differences in the moisture content, anhydrous Ephedrine having a lower melting point than the hemihydrate of Ephedrine. Its solutions are alkaline to litmus. Soluble in water, in alcohol, in chloroform, and in ether; sparingly and slowly soluble in mineral oil, the solution becoming turbid if the Ephedrine contains more than about 1% of water.

Ephedrine Hydrochloride: Fine, white, odorless crystals or powder. Is affected by light. Freely soluble in water; soluble in alcohol; insoluble in ether.

Ephedrine Sulfate: Fine, white, odorless crystals or powder. Darkens on exposure to light. Freely soluble in water; sparingly soluble in alcohol.

Ephedrine Sulfate Nasal Solution: Clear, colorless solution. Is neutral or slightly acid to litmus.

Epinephrine: White to practically white, odorless, microcrystalline powder or granules, gradually darkening on exposure to light and air. With acids, it forms salts that are readily soluble in water, and the base may be recovered by the addition of ammonia water or alkali carbonates. Its solutions are alkaline to litmus. Very slightly soluble in water and in alcohol; insoluble in ether, in chloroform, and in fixed and volatile oils.

Epinephrine Injection: Practically colorless, slightly acid liquid. Gradually turns dark on exposure to light and air.

Epinephrine Inhalation Solution: Practically colorless, slightly acid liquid. Gradually turns dark on exposure to light and air.

Epinephrine Nasal Solution: Nearly colorless, slightly acid liquid. Gradually turns dark on exposure to light and air.

Epinephrine Ophthalmic Solution: Colorless to faint yellow solution. Gradually turns dark on exposure to light and air.

Epinephrine Bitartrate: White, or grayish-white or light brownish-gray, odorless, crystalline powder. Slowly darkens on exposure to air and light. Its solutions are acid to litmus, having a pH of about 3.5. Freely soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

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Epinephrine Bitartrate for Ophthalmic Solution: White to off-white solid.

Epinephryl Borate Ophthalmic Solution: Clear, pale yellow liquid, gradually darkening on exposure to light and air.

Epirubicin Hydrochloride: Orange-red powder. Soluble in water and in methanol; slightly soluble in anhydrous ethanol; practically insoluble in acetone.

Eprinomectin: White to off-white powder. Insoluble in cold water.

Ergocalciferol: White, odorless crystals. Is affected by air and by light. Soluble in alcohol, in chloroform, in ether, and in fatty oils; insoluble in water.

Ergocalciferol Oral Solution: Clear liquid having the characteristics of the solvent used in preparing the Solution.

Ergoloid Mesylates: White to off-white, microcrystalline or amorphous, practically odorless powder. Soluble in methanol and in alcohol; sparingly soluble in acetone; slightly soluble in water.

Ergonovine Maleate: White to grayish-white or faintly yellow, odorless, microcrystalline powder. Darkens with age and on exposure to light. Sparingly soluble in water; slightly soluble in alcohol; insoluble in ether and in chloroform.

Ergotamine Tartrate: Colorless crystals or white to yellowish-white, crystalline powder. Is odorless. Melts at about 180°, with decomposition. One g dissolves in about 3200 mL of water; in the presence of a slight excess of tartaric acid 1 g dissolves in about 500 mL of water. Slightly soluble in alcohol.

Erythorbic Acid: White or slightly yellow crystals or powder. It gradually darkens when exposed to light. In the dry state, it is reasonably stable in air, but in solution, it rapidly deteriorates in the presence of air. It melts between 164° and 171° with decomposition. One g is soluble in about 2.5 mL of water and in about 20 mL of alcohol. Slightly soluble in glycerin. *NF category:* Antimicrobial preservative; antioxidant.

Erythritol: White or almost white, crystalline powder or free-flowing granules. It is stable to heat and is nonhygroscopic. Freely soluble in water; very slightly soluble in alcohol. *NF category:* Humectant; sweetening agent; diluent.

Erythromycin: White or slightly yellow, crystalline powder. Is odorless or practically odorless. Soluble in alcohol, in chloroform, and in ether; slightly soluble in water.

Erythromycin Estolate: White, crystalline powder. Is odorless or practically odorless, and is practically tasteless. Soluble in alcohol, in acetone, and in chloroform; practically insoluble in water.

Erythromycin Ethylsuccinate: White or slightly yellow crystalline powder. Is odorless or practically odorless, and is practically tasteless. Freely soluble in alcohol, in chloroform, and in polyethylene glycol 400; very slightly soluble in water.

Erythromycin Gluceptate: Colorless to white crystals. Slightly hygroscopic. Freely soluble in water, in alcohol, in methanol, in dioxane, and in propylene glycol; slightly soluble in acetone and in chloroform; practically insoluble in ether, in carbon tetrachloride, in benzene, and in toluene.

Erythromycin Lactobionate for Injection: White or slightly yellow crystals or powder, having a faint odor. Its solution (1 in 20) is neutral or slightly alkaline. Freely soluble in water, in alcohol, and in methanol; slightly soluble in acetone and in chloroform; practically insoluble in ether.

Erythromycin Stearate: White or slightly yellow crystals or powder. Is odorless or may have a slight, earthy odor, and has a slightly bitter taste. Soluble in alcohol, in chloroform, in methanol, and in ether; practically insoluble in water.

Esmolol Hydrochloride: White to off-white crystalline powder. Very soluble in water; freely soluble in alcohol.

Escitalopram Oxalate: Fine, white to slightly yellow powder. Freely soluble in methanol and in dimethyl sulfoxide; sparingly soluble in water and in alcohol; very slightly soluble in ethyl acetate and in isopropyl alcohol; insoluble in heptane.

Esomeprazole Magnesium: White to slightly colored powder. Soluble in methanol; slightly soluble in water; practically insoluble in heptane.

Esomeprazole Strontium: White to almost-white, crystalline powder. Soluble in water.

Estazolam: White to pale yellowish-white crystal. Soluble in methanol and in acetic anhydride; sparingly soluble in ethanol; practically insoluble in water and in ether.

Estradiol: White or creamy white, small crystals or crystalline powder. Is odorless, and is stable in air. Is hygroscopic. Soluble in alcohol, in acetone, in dioxane, and in solutions of fixed alkali hydroxides; slightly soluble in chloroform; sparingly soluble in vegetable oils; practically insoluble in water.

Estradiol Benzoate: White to off-white, crystalline powder. Soluble in alcohol and in acetone; slightly soluble in diethyl ether; insoluble in water.

Estradiol Cypionate: White to practically white, crystalline powder. Is odorless or has a slight odor. Soluble in alcohol, in acetone, in chloroform, and in dioxane; sparingly soluble in vegetable oils; insoluble in water.

Estradiol Valerate: White, crystalline powder. Is usually odorless but may have a faint, fatty odor. Soluble in castor oil, in methanol, in benzyl benzoate, and in dioxane; sparingly soluble in sesame oil and in peanut oil; practically insoluble in water.

Estriol: White to practically white, odorless, crystalline powder. Melts at about 280°. Soluble in acetone, in chloroform, in dioxane, in ether, and in vegetable oils; sparingly soluble in alcohol; insoluble in water.

Conjugated Estrogens: Conjugated Estrogens obtained from natural sources is a buff-colored, amorphous powder, odorless or having a slight, characteristic odor. The synthetic form is a white to light buff, crystalline or amorphous powder, odorless or having a slight odor.

Synthetic Conjugated Estrogens: A white to light buff, crystalline or amorphous powder that is odorless or has a slight odor.

Esterified Estrogens: White or buff-colored, amorphous powder, odorless or having a slight, characteristic odor.

Estrone: Small, white crystals or white to creamy white, crystalline powder. Is odorless, and is stable in air. Melts at about 260°. Soluble in alcohol, in acetone, in dioxane, and in vegetable oils; slightly soluble in solutions of fixed alkali hydroxides; practically insoluble in water.

Estropipate: White to yellowish-white, fine, crystalline powder. Is odorless, or may have a slight odor. Melts at about 190° to a light brown, viscous liquid, which solidifies on further heating and finally melts at about 245°, with decomposition. Soluble in warm water; very slightly soluble in water, in alcohol, in chloroform, and in ether.

Eszopiclone: White or light-yellow crystalline solid. Freely soluble in methylene chloride; soluble in pH 3.2 phosphate buffer and in dilute mineral acids; slightly soluble in alcohol; very slightly soluble in water.

Ethacrynic Acid: White or practically white, odorless or practically odorless, crystalline powder. Freely soluble in alcohol, in chloroform, and in ether; very slightly soluble in water.

Ethambutol Hydrochloride: White, crystalline powder. Freely soluble in water; soluble in alcohol and in methanol; slightly soluble in ether and in chloroform.

Ethchlorvynol: Colorless to yellow, slightly viscous liquid, having a characteristic pungent odor. Darkens on exposure to light and air. Immiscible with water; miscible with most organic solvents.

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Ether: Colorless, mobile, volatile liquid, having a characteristic sweet, pungent odor. Is slowly oxidized by the action of air and light, with the formation of peroxides. It boils at about 35°. Soluble in water and in hydrochloric acid. Miscible with alcohol, with benzene, with chloroform, with solvent hexane, with methylene chloride, and with fixed and volatile oils.

Ethinyl Estradiol: White to creamy white, odorless, crystalline powder. Soluble in alcohol, in chloroform, in ether, in vegetable oils, and in solutions of fixed alkali hydroxides; insoluble in water.

Ethiodized Oil Injection: Straw-colored to amber-colored, oily liquid. It may possess an alliaceous odor. Soluble in acetone, in chloroform, in ether, and in solvent hexane; insoluble in water.

Ethionamide: Bright yellow powder, having a faint to moderate sulfide-like odor. Soluble in methanol; sparingly soluble in alcohol and in propylene glycol; slightly soluble in water, in chloroform, and in ether.

Ethopabate: White to pinkish-white, odorless or practically odorless powder. Soluble in acetonitrile, in acetone, in dehydrated alcohol, and in methanol; sparingly soluble in isopropyl alcohol, in dioxane, in ethyl acetate, and in methylene chloride; slightly soluble in ether; very slightly soluble in water.

Ethosuximide: White to off-white, crystalline powder or waxy solid, having a characteristic odor. Freely soluble in water and in chloroform; very soluble in alcohol and in ether; very slightly soluble in solvent hexane.

Ethotoin: White, crystalline powder. Freely soluble in dehydrated alcohol and in chloroform; soluble in ether; insoluble in water.

Ethyl Acetate: Transparent, colorless liquid, having a fragrant, refreshing, slightly acetous odor, and a peculiar, acetous, burning taste. Soluble in water. Miscible with alcohol, with ether, with fixed oils, and with volatile oils. *NF category:* Flavors and fragrance; solvent.

Ethyl Acrylate and Methyl Methacrylate Copolymer Dispersion: Milky-white liquid of low viscosity with a faint, characteristic odor. It is miscible with water in any proportion; the milky-white appearance is retained. A clear or slightly opalescent, viscous solution is obtained on mixing one part with five parts of acetone, alcohol, or isopropyl alcohol; the polymer substance first precipitates, but then dissolves in the excess organic solvent. When mixed with 1 N sodium hydroxide in a ratio of 1:2, the dispersion does not dissolve; the milky-white appearance is retained. *NF category:* Coating agent; polymer membrane; wet binder; film-forming agent; diluent.

Ethyl Chloride: Colorless, mobile, very volatile liquid at low temperatures or under pressure, having a characteristic, ethereal odor. It boils between 12° and 13°, and its specific gravity at 0° is about 0.921. When liberated at room temperature from its sealed container, it vaporizes immediately. It burns with a smoky, greenish flame, producing hydrogen chloride. Freely soluble in alcohol and in ether; slightly soluble in water.

Ethyl Maltol: White, crystalline powder having a cotton-candy odor and a sweet, fruit-like flavor in dilute solution. One g dissolves in about 55 mL of water, 10 mL of alcohol, 17 mL of propylene glycol, and 5 mL of chloroform. It melts at about 90°. *NF category:* Vehicle (flavored and/or sweetened); flavors and fragrance.

Ethyl Oleate: Mobile, practically colorless liquid, having an agreeable taste. Insoluble in water. Miscible with vegetable oils, with mineral oil, with alcohol, and with most organic solvents. *NF category:* Vehicle (oleaginous); solvent.

Ethyl Vanillin: Fine, white or slightly yellowish crystals. Its taste and odor are similar to the taste and odor of vanillin. Is affected by light. Its solutions are acid to litmus. Freely soluble in alcohol, in chloroform, in ether, and in solutions of alkali hydroxides; sparingly soluble in water at 50°. *NF category:* Flavors and fragrance.

Ethylcellulose: Free-flowing, white to light tan powder. It forms films that have a refractive index of about 1.47. Its aqueous suspensions are neutral to litmus. Ethylcellulose containing less than 46.5% of ethoxy groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in mixtures of aromatic hydrocarbons with alcohol; Ethylcellulose containing not less than 46.5% of ethoxy groups is freely soluble in alcohol, in methanol, in toluene, in chloroform, and in ethyl acetate; insoluble in water, in glycerin, and in propylene glycol. *NF category:* Coating agent; wet binder; flavors and fragrance; film-forming agent; polymer membrane; release-modifying agent; suspending and/or viscosity-increasing agent.

Ethylcellulose Dispersion Type B: Off-white and slightly viscous liquid. Soluble in alcohol, in methyl alcohol, in tetrahydrofuran, and in ethyl acetate; insoluble in water and in chloroform. *NF category:* Coating agent; film-forming agent; polymer membrane; release-modifying agent.

Ethylenediamine: Clear, colorless or only slightly yellow liquid, having an ammonia-like odor and a strong alkaline reaction. Miscible with water and with alcohol.

Ethylene Glycol Stearates: White or almost white, waxy solid. Soluble in acetone and in hot alcohol; practically insoluble in water. *NF category:* Emulsifying agent; emollient.

Ethylene Glycol and Vinyl Alcohol Graft Copolymer: White or slightly yellowish powder. Very soluble in water; practically insoluble in anhydrous alcohol, and in acetone. It dissolves in dilute acids and dilute solutions of alkali hydroxides. *NF category:* Coating agent; wet binder; film-forming agent.

Ethylparaben: Small, colorless crystals or white powder. Freely soluble in acetone, in alcohol, in ether, and in propylene glycol; slightly soluble in water and in glycerin. *NF category:* Antimicrobial preservative.

Ethylparaben Sodium: White or almost-white, hygroscopic, crystalline powder. Freely soluble in water; soluble in anhydrous alcohol; practically insoluble in methylene chloride. *NF category:* Antimicrobial preservative.

Ethynodiol Diacetate: White, odorless, crystalline powder. Is stable in air. Very soluble in chloroform; freely soluble in ether; soluble in alcohol; sparingly soluble in fixed oils; insoluble in water.

Etidronate Disodium: White powder, which may contain lumps. Freely soluble in water; practically insoluble in alcohol.

Etomidate: White or almost white powder. Freely soluble in alcohol and in methylene chloride; very slightly soluble in water.

Etoposide: Fine, white to off-white, crystalline powder. Sparingly soluble in methanol; slightly soluble in alcohol, in chloroform, in ethyl acetate, and in methylene chloride; very slightly soluble in water.

Eucalyptus Oil: Colorless or pale yellow liquid. One mL of eucalyptus oil dissolves in 5 mL of 70% alcohol. Insoluble in cold water. *NF category:* Flavors and perfumes.

Eugenol: Colorless or pale yellow liquid, having a strongly aromatic odor of clove and a pungent, spicy taste. Upon exposure to air, it darkens and thickens. Is optically inactive. Slightly soluble in water. Miscible with alcohol, with chloroform, with ether, and with fixed oils.

Exemestane: White to slightly yellow, crystalline powder. Freely soluble in *N,N*-dimethylformamide; soluble in methanol; insoluble in water.

Ezetimibe: White powder. Freely soluble in alcohol; soluble in acetonitrile; insoluble in aqueous solvents and nonpolar solvents like hexane.

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Famciclovir: A white to pale yellow solid. Freely soluble in methanol and in acetone; sparingly soluble in ethanol and in isopropyl alcohol.

Famotidine: White to pale yellowish-white, crystalline powder. Is sensitive to light. Freely soluble in dimethylformamide and in glacial acetic acid; slightly soluble in methanol; very slightly soluble in water; practically insoluble in acetone, in alcohol, in chloroform, in ether, and in ethyl acetate.

Hard Fat: White mass; almost odorless and free from rancid odor; greasy to the touch. On warming, melts to give a colorless or slightly yellowish liquid. When the molten material is shaken with an equal quantity of hot water, a white emulsion is formed. Freely soluble in ether; slightly soluble in alcohol; practically insoluble in water. *NF category:* Stiffening agent; suppository base.

Felbamate: White to off-white powder. Freely soluble in dimethyl sulfoxide; sparingly soluble in methanol; slightly soluble in acetonitrile; very slightly soluble in water.

Felodipine: Light yellow to yellow, crystalline powder. Freely soluble in acetone and in methanol; very slightly soluble in heptane; insoluble in water.

Fenbendazole: White to off-white powder. Sparingly soluble in dimethylformamide; very slightly soluble in methanol; practically insoluble in water.

Fenofibrate: White or almost white, crystalline powder. Very soluble in methylene chloride; slightly soluble in alcohol; practically insoluble in water.

Fenoldopam Mesylate: White to off-white powder. Soluble in water.

Fenoprofen Calcium: White, crystalline powder. Slightly soluble in *n*-hexanol, in methanol, and in water; practically insoluble in chloroform.

Fentanyl Citrate: White, crystalline powder or white, glistening crystals. Melts at about 150°, with decomposition. Soluble in methanol; sparingly soluble in water; slightly soluble in chloroform.

Ferric Oxide: Powder exhibiting two basic colors (red and yellow), or other shades produced on blending the basic colors. Insoluble in water and in organic solvents; dissolves in hydrochloric acid upon warming, a small amount of insoluble residue usually remaining. *NF category:* Coloring agent.

Ferric Subsulfate Solution: Reddish-brown liquid, odorless or nearly so. Acid to litmus, and is affected by light. Specific gravity is about 1.548.

Ferric Sulfate: Grayish-white or yellowish powder or fawn-colored pearls. Hygroscopic. Slightly soluble in water and in ethanol (96%); practically insoluble in acetone and in ethyl acetate. Hydrolyzes slowly in aqueous solution.

Ferrosoferric Oxide: Black powder. Dissolves in hydrochloric acid upon warming, a small amount of insoluble residue usually remaining; insoluble in water and in organic solvents. *NF category:* Coloring agent.

Ferrous Fumarate: Reddish-orange to red-brown, odorless powder. May contain soft lumps that produce a yellow streak when crushed. Slightly soluble in water; very slightly soluble in alcohol. Its solubility in dilute hydrochloric acid is limited by the separation of fumaric acid.

Ferrous Gluconate: Yellowish-gray or pale greenish-yellow, fine powder or granules, having a slight odor resembling that of burned sugar. Its solution (1 in 20) is acid to litmus. Soluble in water, with slight heating; practically insoluble in alcohol.

Ferrous Sulfate: Pale, bluish-green crystals or granules. Is odorless and is efflorescent in dry air. Oxidizes readily in moist air to form brownish yellow basic ferric sulfate. Its solution (1 in 10) is acid to litmus, having a pH of about 3.7. Very soluble in boiling water; freely soluble in water; insoluble in alcohol.

Dried Ferrous Sulfate: Grayish-white to buff-colored powder, consisting primarily of $FeSO_4 \cdot H_2O$ with varying amounts of $FeSO_4 \cdot 4H_2O$. Slowly soluble in water; insoluble in alcohol.

Ferumoxides Injection: Black to reddish-brown, aqueous colloid. It is stable for 24 hours after dilution.

Fexofenadine Hydrochloride: White to off-white powder. Freely soluble in methanol; very slightly to slightly soluble in water; very slightly soluble in acetone.

Finasteride: White to off-white, crystalline solid. Melts at about 257°. Freely soluble in chloroform and in alcohol; very slightly soluble in water.

Fingolimod Hydrochloride: White to practically white powder. Freely soluble in water and in alcohol. Soluble in propylene glycol.

Fish Oil Containing Omega-3 Acids: Pale yellow liquid. Very soluble in acetone and in heptane; slightly soluble in anhydrous alcohol; practically insoluble in water.

Flavoxate Hydrochloride: White or almost white, crystalline powder. Slightly soluble in alcohol, in water, and in methylene chloride.

Flecainide Acetate: White to slightly off-white, crystalline powder. Freely soluble in alcohol; soluble in water. pK_a is 9.3.

Fluconazole: White or almost white, crystalline powder. Freely soluble in methanol; soluble in alcohol and in acetone; sparingly soluble in isopropanol and in chloroform; slightly soluble in water; very slightly soluble in toluene.

Flucytosine: White to off-white, crystalline powder. Is odorless or has a slight odor. Sparingly soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Fludarabine Phosphate: White to off-white, crystalline, hygroscopic powder. Freely soluble in dimethylformamide; slightly soluble in water and in 0.1 M hydrochloric acid; practically insoluble in ethanol.

Fludrocortisone Acetate: White to pale yellow crystals or crystalline powder. Is odorless or practically odorless. Is hygroscopic. Sparingly soluble in alcohol and in chloroform; slightly soluble in ether; insoluble in water.

Flumazenil: White to off-white powder. Slightly soluble in acidic aqueous solutions; practically insoluble in water.

Flumethasone Pivalate: White to off-white, crystalline powder. Slightly soluble in methanol; very slightly soluble in chloroform and in methylene chloride; insoluble in water.

Flunisolide: White to creamy-white, crystalline powder. Melts at about 245°, with decomposition. Soluble in acetone; sparingly soluble in chloroform; slightly soluble in methanol; practically insoluble in water.

Flunixin Meglumine: White to off-white crystalline powder. Soluble in water, in alcohol, and in methanol; practically insoluble in ethyl acetate.

Fluocinolone Acetonide: White or practically white, odorless, crystalline powder. Is stable in air. Melts at about 270°, with decomposition. Soluble in methanol; slightly soluble in ether and in chloroform; insoluble in water.

Fluocinonide: White to cream-colored, crystalline powder, having not more than a slight odor. Sparingly soluble in acetone and in chloroform; slightly soluble in alcohol, in methanol, and in dioxane; very slightly soluble in ether; practically insoluble in water.

Fluorescein: Yellowish-red to red, odorless powder. Soluble in dilute alkali hydroxides; insoluble in water.

Fluorescein Sodium: Orange-red, hygroscopic, odorless powder. Freely soluble in water; sparingly soluble in alcohol.

Fluorescein Sodium Ophthalmic Strip: Each Strip is a dry, white piece of paper, one end of which is rounded and is uniformly orange-red in color because of the fluorescein sodium impregnated in the paper.

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Fluorometholone: White to yellowish-white, odorless, crystalline powder. Melts at about 280°, with some decomposition. Slightly soluble in alcohol; very slightly soluble in chloroform; practically insoluble in water and in ether.

Fluorouracil: White to practically white, practically odorless, crystalline powder. Decomposes at about 282°. Sparingly soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Fluoxetine Hydrochloride: White to off-white crystalline powder. Freely soluble in alcohol and in methanol; sparingly soluble in water and in dichloromethane; practically insoluble in ether.

Fluoxymesterone: White or practically white, odorless, crystalline powder. Melts at about 240°, with some decomposition. Sparingly soluble in alcohol; slightly soluble in chloroform; practically insoluble in water.

Fluphenazine Decanoate: Pale yellow to yellow-orange, viscous liquid or yellow solid. Slowly crystallizes at room temperature. Melts at about 30°–32°. Very soluble in chloroform, in ether, in cyclohexane, in dehydrated alcohol, and in methylene chloride; freely soluble in methanol; insoluble in water.

Fluphenazine Enanthate: Pale yellow to yellow-orange, clear to slightly turbid, viscous liquid, having a characteristic odor. Is unstable in strong light, but stable to air at room temperature. Freely soluble in alcohol, in chloroform, and in ether; insoluble in water.

Fluphenazine Hydrochloride: White or nearly white, odorless, crystalline powder. Melts, within a range of 5°, at a temperature above 225°. Freely soluble in water; slightly soluble in alcohol, in chloroform, and in methylene chloride; practically insoluble in benzene and in ether.

Flurandrenolide: White to off-white, fluffy, crystalline powder. Is odorless. Freely soluble in chloroform; soluble in methanol; sparingly soluble in alcohol; practically insoluble in water and in ether.

Flurazepam Hydrochloride: Off-white to yellow, crystalline powder. Is odorless, or has a slight odor, and its solutions are acid to litmus. Melts at about 212°, with decomposition. Freely soluble in water and in alcohol; slightly soluble in isopropyl alcohol and in chloroform.

Flurbiprofen: White, crystalline powder. Freely soluble in acetone, in dehydrated alcohol, in ether, and in methanol; soluble in acetonitrile; practically insoluble in water. Optically inactive (1 in 50 solution in dehydrated alcohol).

Flutamide: Pale yellow, crystalline powder. Freely soluble in acetone, in ethyl acetate, and in methanol; soluble in chloroform and in ether; practically insoluble in mineral oil, in petroleum ether, and in water.

Fluticasone Propionate (micronized): Fine, white powder.

Fluvastatin Sodium: White to pale yellow, brownish-pale yellow, or reddish-pale yellow, hygroscopic powder. Soluble in alcohol, in methanol, and in water.

Fluvoxamine Maleate: White to off-white, crystalline powder. Freely soluble in alcohol and in chloroform; sparingly soluble in water; and practically insoluble in diethyl ether.

Folic Acid: Yellow, yellow-brownish, or yellowish-orange, odorless, crystalline powder. It readily dissolves in dilute solutions of alkali hydroxides and carbonates. Soluble in hot, 3 N hydrochloric acid, in hot, 2 N sulfuric acid, in hydrochloric acid, and in sulfuric acid, yielding very pale yellow solutions; very slightly soluble in water; insoluble in alcohol, in acetone, in chloroform, and in ether.

Folic Acid Injection: Clear, yellow to orange-yellow, alkaline liquid.

Formaldehyde Solution: Clear, colorless or practically colorless liquid, having a pungent odor. The vapor from it irritates the mucous membrane of the throat and nose. On long standing, especially in the cold, it may become cloudy because of the separation of paraformaldehyde. This cloudiness disappears when the solution is warmed. Miscible with water and with alcohol.

Formoterol Fumarate Dihydrate: White or almost white or slightly yellow powder. Freely soluble in dimethyl sulfoxide and in acetic acid; soluble in methanol; slightly soluble in water and in 2-propanol; practically insoluble in acetonitrile and in diethyl ether.

Foscarnet Sodium: White to almost white, crystalline powder. Soluble in water; practically insoluble in alcohol.

Fosphenytoin Sodium: White to pale yellow solid. Freely soluble in water.

Fructose: Colorless crystals or as a white, crystalline powder. Is odorless, and has a sweet taste. Freely soluble in water; soluble in alcohol and in methanol. *NF category:* Sweetening agent; diluent; flavors and fragrance.

Basic Fuchsin: Dark green powder or greenish glistening crystalline fragments, having a bronze-like luster and not more than a faint odor. Soluble in water, in alcohol, and in amyl alcohol; insoluble in ether.

Fulvestrant: White powder. Freely soluble in alcohol.

Fumaric Acid: White, odorless granules or crystalline powder. Soluble in alcohol; slightly soluble in water and in ether; very slightly soluble in chloroform. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); flavors and fragrance; antioxidant.

Furazolidone: Yellow, odorless, crystalline powder. Is tasteless at first, then a bitter aftertaste develops. Practically insoluble in water, in alcohol, and in carbon tetrachloride.

Furosemide: White to slightly yellow, odorless, crystalline powder. Freely soluble in acetone, in dimethylformamide, and in solutions of alkali hydroxides; soluble in methanol; sparingly soluble in alcohol; slightly soluble in ether; very slightly soluble in chloroform; practically insoluble in water.

Furosemide Injection: Clear, colorless solution.

Gabapentin: White to off-white, crystalline solid. Freely soluble in water and in alkaline and acidic solutions.

Gadodiamide: White, odorless powder. Freely soluble in water and in methanol; soluble in ethyl alcohol; slightly soluble in acetone and in chloroform.

Gadoteridol: White to off-white, crystalline, odorless powder. Freely soluble in water and in methyl alcohol; soluble in isopropyl alcohol. Melts at about 300°.

Gadoversetamide: White, odorless powder. Freely soluble in water.

Galactose: A white, crystalline or finely granulated powder. Soluble in water; very slightly soluble in alcohol. *NF category:* Sweetening agent; chelating and/or complexing agent.

Galantamine Hydrobromide: White to almost white powder. Soluble in 0.1 N sodium hydroxide; sparingly soluble in water; very slightly soluble in alcohol; insoluble in *n*-propanol.

Gallamine Triethiodide: White, odorless, amorphous powder. Is hygroscopic. Very soluble in water; sparingly soluble in alcohol; very slightly soluble in chloroform.

Gamma Cyclodextrin: White or almost white, amorphous or crystalline powder. Freely soluble in water and in propylene glycol; very slightly soluble in alcohol. *NF category:* Sequestering agent; chelating and/or complexing agent.

Ganciclovir: White to off-white, crystalline powder.

Ganciclovir for Injection: White to off-white powder. Soluble in water.

Petrolatum Gauze: The petrolatum recovered by draining in the *Assay* is a white or faintly yellowish, unctuous mass, transparent in thin layers even after cooling to 0°.

Gelatin: Sheets, flakes, or shreds, or coarse to fine powder. Is faintly yellow or amber in color, the color varying in depth

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according to the particle size. Has a slight, characteristic bouillon-like odor in solution. Is stable in air when dry, but is subject to microbic decomposition when moist or in solution. Gelatin has any suitable strength that is designated by Bloom Gelometer number. Type A Gelatin exhibits an isoelectric point between pH 7 and pH 9, and Type B Gelatin exhibits an isoelectric point between pH 4.7 and pH 5.2. Soluble in hot water, in 6 N acetic acid, and in a hot mixture of glycerin and water; insoluble in cold water, but swells and softens when immersed in it, gradually absorbing from 5 to 10 times its own weight of water, in alcohol, in chloroform, in ether, and in fixed and volatile oils. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; wet binder; capsule shell; colloid stabilizing agent; film-forming agent.

Absorbable Gelatin Film: Light amber, transparent, pliable film which becomes rubbery when moistened. Insoluble in water.

Absorbable Gelatin Sponge: Light, nearly white, nonelastic, tough, porous, hydrophilic solid. Insoluble in water.

Gellan Gum: Off-white powder. Soluble in hot or in cold deionized water. *NF category:* Suspending and/or viscosity-increasing agent.

Gemcitabine Hydrochloride: White to off-white solid. Soluble in water; slightly soluble in methanol; practically insoluble in alcohol and in polar organic solvents.

Gemfibrozil: White, waxy, crystalline solid. Soluble in alcohol, in methanol, and in chloroform; practically insoluble in water.

Gentamicin Sulfate: White to buff powder. Freely soluble in water; insoluble in alcohol, in acetone, in chloroform, in ether, and in benzene.

Gentamicin Injection: Clear, slightly yellow solution, having a faint odor.

Gentian Violet: Dark green powder or greenish, glistening pieces having a metallic luster, and having not more than a faint odor. Soluble in alcohol, in glycerin, and in chloroform; sparingly soluble in water; insoluble in ether.

Gentian Violet Cream: Dark purple, water-washable cream.

Gentian Violet Topical Solution: Purple liquid, having a slight odor of alcohol. A dilution (1 in 100), viewed downward through 1 cm of depth, is deep purple in color.

Powdered Asian Ginseng Extract: Pale yellow-brown, hygroscopic, powdery or easily pulverizable mass. Soluble in water, forming a slightly cloudy solution.

Glaze, Pharmaceutical: Denatured alcohol solution. *NF category:* Coating agent; film-forming agent.

Glimepiride: White to almost white powder. Soluble in dimethylformamide; sparingly soluble in methylene chloride; slightly soluble in methanol; practically insoluble in water.

Glipizide: White to off-white powder. Freely soluble in dimethylformamide; soluble in 0.1 N sodium hydroxide; slightly soluble in methylene chloride.

Immune Globulin: Transparent or slightly opalescent liquid, either colorless or of a brownish color due to denatured hemoglobin. Is practically odorless. May develop a slight, granular deposit during storage.

Rh_0(D) Immune Globulin: Transparent or slightly opalescent liquid. Is practically colorless and odorless. May develop a slight, granular deposit during storage.

Glucagon: Fine, white or faintly colored, crystalline powder. Is practically odorless and tasteless. Soluble in dilute alkali and acid solutions; insoluble in most organic solvents.

Glucagon for Injection: White, odorless powder.

Gluconolactone: Fine, white, practically odorless, crystalline powder. Melts at about 153°, with decomposition. Freely soluble in water; sparingly soluble in alcohol; insoluble in ether.

Liquid Glucose: Colorless or yellowish, thick, syrupy liquid. Odorless or nearly odorless, and has a sweet taste. Sparingly soluble in alcohol. Miscible with water. *NF category:* Wet binder; coating agent; sweetening agent.

L-Glutamic Acid Hydrochloride: A white, crystalline powder. 1 g dissolves in about 3 mL of water. It is almost insoluble in alcohol and in ether. Its solutions are acid to litmus. *NF category:* Flavors and fragrance.

Glutamine: White crystals or crystalline powder. Soluble in water; practically insoluble in alcohol and in ether.

Glutaral Concentrate: Clear, colorless or faintly yellow liquid, having a characteristic, irritating odor.

Glycerin: Clear, colorless, syrupy liquid, having a sweet taste. Has not more than a slight characteristic odor, which is neither harsh nor disagreeable. Is hygroscopic. Its solutions are neutral to litmus. Insoluble in chloroform, in ether, and in fixed and volatile oils. Miscible with water and with alcohol. *NF category:* Humectant; plasticizer; solvent; tonicity agent; antimicrobial preservative; emollient; sweetening agent.

Glyceryl Behenate: [(Title for this monograph—not to change until December 1, 2019.)

(Prior to December 1, 2019, the current practice of labeling the article of commerce with the name Glyceryl Behenate may be continued. Use of the name Glyceryl Dibehenate will be permitted as of December 1, 2014; however, the use of this name will not be mandatory until December 1, 2019. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)]

Glyceryl Dibehenate: Fine powder, having a faint odor. Melts at about 70°. Soluble in chloroform; practically insoluble in water and in alcohol. *NF category:* Lubricant; coating agent; suspending and/or viscosity-increasing agent; wet binder.

Glyceryl Distearate: Hard, waxy mass or powder or white or almost white flakes. Soluble in methylene chloride and in tetrahydrofuran; slightly soluble in hot alcohol; insoluble in water. *NF category:* Emulsifying agent.

Add the following:

▲**Glyceryl Mono and Dicaprylate:** Colorless or slightly yellow, oily liquid or semisolid. Very soluble in alcohol; freely soluble in methylene chloride; practically insoluble in water. *NF category:* Lubricant; emulsifying agent.▲ (NF 1-May-2020)

Add the following:

▲**Glyceryl Mono and Dicaprylocaprate:** Colorless or slightly yellow, oily liquid or semisolid. Very soluble in alcohol; freely soluble in methylene chloride; practically insoluble in water. *NF category:* Lubricant; emulsifying agent.▲ (NF 1-May-2020)

Glyceryl Monocaprylate: Colorless or slightly yellow, oily liquid or semisolid. Very soluble in alcohol; freely soluble in methylene chloride; practically insoluble in water. *NF category:* Lubricant; emulsifying agent.

Glyceryl Monocaprylocaprate: Colorless or slightly yellow, oily liquid or semisolid. Very soluble in alcohol; freely soluble in methylene chloride; practically insoluble in water. *NF category:* Lubricant; emulsifying agent.

Glyceryl Monolinoleate: Amber, oily liquids that may be partially solidified at room temperature. Freely soluble in methylene chloride; soluble in tetrahydrofuran; practically insoluble in water. *NF category:* Emulsifying agent.

Glyceryl Monooleate: Amber, oily liquids that may be partially solidified at room temperature. Freely soluble in methylene chloride; soluble in tetrahydrofuran; practically insoluble in water. *NF category:* Emulsifying agent; emollient; release-modifying agent.

Glyceryl Monostearate: White to yellowish wax-like solid; or white to yellowish wax-like beads, flakes, or powder. Slight,

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agreeable, fatty odor and taste. Is affected by light. Dissolves in hot organic solvents such as alcohol, minerals or fixed oils, benzene, ether, and acetone. Insoluble in water, but it may be dispersed in hot water with the aid of a small amount of soap or other suitable surface-active agent. *NF category:* Emulsifying agent; emollient; release-modifying agent; lubricant.

Glyceryl Tricaprylate: Clear, colorless to pale-yellow liquid. Very soluble in alcohol; freely soluble in methylene chloride; practically insoluble in water. *NF category:* Lubricant; emulsifying agent; vehicle (solid carrier).

Glyceryl Tristearate: White, solid, microcrystalline powder. Soluble in hot alcohol, in acetone, and in chloroform; very slightly soluble in cold alcohol, in ether, and in petroleum ether; insoluble in water. *NF category:* Lubricant; emulsifying agent.

Glycine: White, odorless, crystalline powder, having a sweetish taste. Its solutions are acid to litmus. Freely soluble in water; very slightly soluble in alcohol and in ether. *NF category:* Bulking agent; disintegrant; wetting and/or solubilizing agent; pH modifier (acidifying agent/alkalizing agent/buffering agent).

Glycopyrrolate: White, odorless, crystalline powder. Soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Gonadorelin Acetate: White to slightly yellowish powder. Soluble in water; sparingly soluble in methanol.

Chorionic Gonadotropin: White or practically white, amorphous powder. Freely soluble in water.

Chorionic Gonadotropin for Injection: White or practically white, amorphous solid having the characteristic appearance of substances prepared by freeze-drying.

Gramicidin: White or practically white, odorless, crystalline powder. Soluble in alcohol; insoluble in water.

Granisetron: Off-white to pale-yellow powder. Soluble to freely soluble in methanol and in methylene chloride.

Granisetron Hydrochloride: White or almost white powder. Freely soluble in water; sparingly soluble in methylene chloride; slightly soluble in methanol.

Green Soap: Soft, unctuous, yellowish-white to brownish or greenish yellow, transparent to translucent mass. Has a slight, characteristic odor, often suggesting the oil from which it was prepared. Its solution (1 in 20) is alkaline to bromothymol blue TS.

Griseofulvin: White to creamy white, odorless powder, in which particles of the order of 4 µm in diameter predominate. Soluble in acetone, in dimethylformamide, and in chloroform; sparingly soluble in alcohol; very slightly soluble in water.

Guaifenesin: White to slightly gray, crystalline powder. May have a slight characteristic odor. Soluble in water, in alcohol, in chloroform, and in propylene glycol; sparingly soluble in glycerin.

Guanabenz Acetate: White or almost white powder having not more than a slight odor. Soluble in alcohol and in propylene glycol; sparingly soluble in water and in 0.1 N hydrochloric acid.

Guanadrel Sulfate: White to off-white, crystalline powder. Melts at about 235°, with decomposition. Soluble in water; sparingly soluble in methanol; slightly soluble in alcohol and in acetone.

Guanethidine Monosulfate: White to off-white, crystalline powder. Very soluble in water; sparingly soluble in alcohol; practically insoluble in chloroform.

Guar Gum: White to yellowish-white, practically odorless powder. Dispersible in hot or cold water, forming a colloidal solution. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; polymers for ophthalmic use; release-modifying agent; disintegrant.

Gutta Percha: Lumps or blocks of variable size; externally brown or grayish-brown to grayish-white in color; internally reddish yellow or reddish gray and having a laminated or fibrous appearance. Is flexible but only slightly elastic. Has a slight, characteristic odor and a slight taste. Soluble in chloroform; partly soluble in benzene, in carbon disulfide, and in turpentine oil; insoluble in water.

Halazone: White, crystalline powder, having a characteristic chlorine-like odor. Is affected by light. Melts at about 194°, with decomposition. Soluble in glacial acetic acid; very slightly soluble in water and in chloroform. Dissolves in solutions of alkali hydroxides and carbonates with the formation of a salt.

Halazone Tablets for Solution: Soluble in water.

Halcinonide: White to off-white, odorless, crystalline powder. Soluble in acetone and in chloroform; slightly soluble in alcohol and in ethyl ether; insoluble in water and in hexanes.

Halobetasol Propionate: White to off-white powder. Freely soluble in dichloromethane and in acetone; practically insoluble in water.

Haloperidol: White to faintly yellowish, amorphous or microcrystalline powder. Its saturated solution is neutral to litmus. Soluble in chloroform; sparingly soluble in alcohol; slightly soluble in ether; practically insoluble in water.

Haloperidol Decanoate: A white or almost white powder. Very soluble in alcohol, in methanol, and in methylene chloride; practically insoluble in water.

Halothane: Colorless, mobile, nonflammable, heavy liquid, having a characteristic odor resembling that of chloroform. Its taste is sweet and produces a burning sensation. Slightly soluble in water. Miscible with alcohol, with chloroform, with ether, and with fixed oils.

Helium: Colorless, odorless, tasteless gas, which is not combustible and does not support combustion. Very slightly soluble in water. At 0° and at a pressure of 760 mm of mercury, 1000 mL of the gas weighs about 180 mg.

Heparin Sodium: White or pale-colored, amorphous powder. Is odorless or practically so, and is hygroscopic. Soluble in water.

Hexachlorophene: White to light tan, crystalline powder. Is odorless or has only a slight, phenolic odor. Freely soluble in acetone, in alcohol, and in ether; soluble in chloroform and in dilute solutions of fixed alkali hydroxides; insoluble in water.

Hexachlorophene Liquid Soap: Clear, amber-colored liquid, having a slight, characteristic odor. Its solution (1 in 20) is clear and has an alkaline reaction.

Hexylene Glycol: Clear, colorless, viscous liquid. Absorbs moisture when exposed to moist air. Miscible with water and with many organic solvents, including alcohol, ether, chloroform, acetone, and hexanes. *NF category:* Humectant; solvent.

Histamine Phosphate: Colorless, odorless, long prismatic crystals. Is stable in air but is affected by light. Its solutions are acid to litmus. Freely soluble in water.

Histidine: White, odorless crystals, having a slightly bitter taste. Soluble in water; very slightly soluble in alcohol; insoluble in ether.

Histoplasmin: Clear, red liquid. Miscible with water.

Homatropine Hydrobromide: White crystals, or white, crystalline powder. Slowly darkens on exposure to light. Freely soluble in water; sparingly soluble in alcohol; slightly soluble in chloroform; insoluble in ether. Melts between 214° and 217°, with slight decomposition.

Homatropine Methylbromide: White, odorless powder. Slowly darkens on exposure to light. Melts at about 190°. Very soluble in water; freely soluble in alcohol and in acetone containing about 20% of water; practically insoluble in ether and in acetone.

Hydralazine Hydrochloride: White to off-white, odorless, crystalline powder. Melts at about 275°, with decomposition.

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Soluble in water; slightly soluble in alcohol; very slightly soluble in ether.

Hydrochloric Acid: Colorless, fuming liquid having a pungent odor. It ceases to fume when it is diluted with 2 volumes of water. Specific gravity is about 1.18. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Diluted Hydrochloric Acid: Colorless, odorless liquid. Specific gravity is about 1.05. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Hydrochlorothiazide: White, or practically white, practically odorless, crystalline powder. Freely soluble in sodium hydroxide solution, in *n*-butylamine, and in dimethylformamide; very slightly soluble in water; sparingly soluble in methanol; insoluble in ether, in chloroform, and in dilute mineral acids.

Hydrocodone Bitartrate: Fine, white crystals or a crystalline powder. Is affected by light. Soluble in water; slightly soluble in alcohol; insoluble in ether and in chloroform.

Hydrocortisone: White to practically white, odorless, crystalline powder. Melts at about 215°, with decomposition. Sparingly soluble in acetone and in alcohol; slightly soluble in chloroform; very slightly soluble in water and in ether.

Hydrocortisone Acetate: White to practically white, odorless, crystalline powder. Melts at about 220°, with decomposition. Slightly soluble in alcohol and in chloroform; insoluble in water.

Hydrocortisone Butyrate: White to practically white, practically odorless, crystalline powder. Freely soluble in chloroform; soluble in methanol, in alcohol, and in acetone; slightly soluble in ether; practically insoluble in water.

Hydrocortisone Sodium Phosphate: White to light yellow, odorless or practically odorless, powder. Is exceedingly hygroscopic. Freely soluble in water; slightly soluble in alcohol; practically insoluble in chloroform, in dioxane, and in ether.

Hydrocortisone Sodium Succinate: White or nearly white, odorless, hygroscopic, amorphous solid. Very soluble in water and in alcohol; very slightly soluble in acetone; insoluble in chloroform.

Hydroflumethiazide: White to cream-colored, finely divided, odorless, crystalline powder. Freely soluble in acetone; soluble in alcohol; very slightly soluble in water.

Hydrogen Peroxide Concentrate: Clear, colorless liquid. Is acid to litmus. Slowly decomposes, and is affected by light.

Hydrogen Peroxide Solution: Clear, colorless liquid, odorless, or having an odor resembling that of ozone. Is acid to litmus and to the taste and produces a froth in the mouth. Rapidly decomposes when in contact with many oxidizing as well as reducing substances. When rapidly heated, it may decompose suddenly. Is affected by light. Specific gravity is about 1.01.

Hydromorphone Hydrochloride: Fine, white or practically white, odorless, crystalline powder. Is affected by light. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in ether.

Hydroquinone: Fine white needles. Darkens upon exposure to light and air. Freely soluble in water, in alcohol, and in ether.

Hydroxocobalamin: Dark red crystals or red crystalline powder. Is odorless, or has not more than a slight acetone odor. The anhydrous form is very hygroscopic. Sparingly soluble in water, in alcohol, and in methanol; practically insoluble in acetone, in ether, in chloroform, and in benzene.

Hydroxyamphetamine Hydrobromide: White, crystalline powder. Its solutions are slightly acid to litmus, having a pH of about 5. Freely soluble in water and in alcohol; slightly soluble in chloroform; practically insoluble in ether.

Hydroxychloroquine Sulfate: White or practically white, crystalline powder. Is odorless, and has a bitter taste. Its solutions have a pH of about 4.5. Exists in two forms, the usual form melting at about 240° and the other form melting at about 198°. Freely soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Hydroxyethyl Cellulose: White to light tan, practically odorless and tasteless, hygroscopic powder. Soluble in hot water and in cold water, giving a colloidal solution; practically insoluble in alcohol and in most organic solvents. *NF category:* Suspending and/or viscosity-increasing agent; coating agent; film-forming agent; polymers for ophthalmic use; wet binder.

Hydroxyprogesterone Caproate: White or creamy white, crystalline powder. Is odorless or has a slight odor. Soluble in ether; slightly soluble in benzene; insoluble in water.

Hydroxypropyl Betadex: White or almost white, amorphous or crystalline powder. Freely soluble in water and in propylene glycol. *NF category:* Sequestering agent; chelating and/or complexing agent; release-modifying agent.

Hydroxypropyl Cellulose: White to cream-colored, practically odorless and tasteless, granular solid or powder. Is hygroscopic after drying. Soluble in cold water, in alcohol, in chloroform, and in propylene glycol, giving a colloidal solution; insoluble in hot water. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; emulsifying agent; film-forming agent; release-modifying agent; wet binder.

Low-Substituted Hydroxypropyl Cellulose: White to yellowish-white, practically odorless and tasteless, fibrous or granular powder. Is hygroscopic. Practically insoluble in alcohol and in ether. Dissolves in a solution of sodium hydroxide (1 in 10), and produces a viscous solution. Swells in water, in sodium carbonate TS, and in 2 N hydrochloric acid. The pH of the suspension, obtained by shaking 1.0 g with 100 mL of water, is between 5.0 and 7.5. *NF category:* Disintegrant; wet binder.

Hydroxyurea: White to off-white powder. Is somewhat hygroscopic, decomposing in the presence of moisture. Melts at a temperature exceeding 133°, with decomposition. Freely soluble in water and in hot alcohol.

Hydroxyzine Hydrochloride: White, odorless powder. Melts at about 200°, with decomposition. Very soluble in water; soluble in chloroform; slightly soluble in acetone; practically insoluble in ether.

Hydroxyzine Pamoate: Light yellow, practically odorless powder. Freely soluble in dimethylformamide; practically insoluble in water and in methanol.

Hymetellose: A white, yellowish-white or grayish-white powder or granules. Hygroscopic after drying. Dissolves in cold water, giving a colloidal solution. Insoluble in hot water, in acetone, in alcohol, in ether, and in toluene.

Hyoscyamine: White, crystalline powder. Is affected by light. Its solutions are alkaline to litmus. Freely soluble in alcohol, in chloroform, and in dilute acids; sparingly soluble in ether; slightly soluble in water and in benzene.

Hyoscyamine Hydrobromide: White, odorless crystals or crystalline powder. The pH of a solution (1 in 20) is about 5.4. Is affected by light. Freely soluble in water, in alcohol, and in chloroform; very slightly soluble in ether.

Hyoscyamine Sulfate: White or almost white, crystalline powder or colorless needles. Is deliquescent and is affected by light. The pH of a solution (1 in 100) is about 5.3. Very soluble in water; freely soluble in alcohol; practically insoluble in ether. Melts at a temperature not less than 200°.

Hypophosphorous Acid: Colorless or slightly yellow, odorless liquid. Specific gravity is about 1.13. *NF category:* Antioxidant.

Hypromellose: White to slightly off-white, fibrous or granular powder. Swells in water and produces a clear to opalescent, viscous, colloidal mixture. Insoluble in dehydrated alcohol, in ether, and in chloroform. *NF category:* Coating

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agent; suspending and/or viscosity-increasing agent; wet binder; polymers for ophthalmic use; capsule shell; emulsifying agent; film-forming agent; release-modifying agent.

Hypromellose 2208: White to slightly off-white, fibrous or granular powder. Swells in water and produces a clear to opalescent, viscous, colloidal mixture. Insoluble in dehydrated alcohol, in ether, and in chloroform. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; tablet binder.

Hypromellose 2906: White to slightly off-white, fibrous or granular powder. Swells in water and produces a clear to opalescent, viscous, colloidal mixture. Insoluble in dehydrated alcohol, in ether, and in chloroform. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; tablet binder.

Hypromellose 2910: White to slightly off-white, fibrous or granular powder. Swells in water and produces a clear to opalescent, viscous, colloidal mixture. Insoluble in dehydrated alcohol, in ether, and in chloroform. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; tablet binder.

Hypromellose Acetate Succinate: White to yellowish-white powder or pills. Odorless, or has a faint, acetic acid-like odor, and tasteless. Practically insoluble in water, in dehydrated alcohol, in xylene, and in hexane. On the addition of a mixture of dehydrated alcohol and dichloromethane (1:1) or acetone, a clear or turbid viscous liquid is produced. Dissolves in 1 N sodium hydroxide. Slightly hygroscopic. *NF category:* Coating agent; wet binder; film-forming agent.

Hypromellose Phthalate: White powder or granules. Is odorless and tasteless. Practically insoluble in water, in dehydrated alcohol, and in hexane. Produces a viscous solution in a mixture of methanol and dichloromethane (1:1), or in a mixture of dehydrated alcohol and acetone (1:1). Dissolves in 1 N sodium hydroxide. *NF category:* Coating agent; film-forming agent.

Ibuprofen: White to off-white, crystalline powder, having a slight, characteristic odor. Very soluble in alcohol, in methanol, in acetone, and in chloroform; slightly soluble in ethyl acetate; practically insoluble in water.

Ibutilide Fumarate: White to off-white powder. Freely soluble in water; sparingly soluble in methanol.

Ichthammol: Reddish-brown to brownish-black, viscous fluid, having a strong, characteristic, empyreumatic odor. Miscible with water, with glycerin, and with fixed oils and fats. Partially soluble in alcohol and in ether.

Idarubicin Hydrochloride: Red-orange to red-brown powder. Soluble in methanol; slightly soluble in water; insoluble in acetone and in ethyl ether.

Idoxuridine: White, crystalline, practically odorless powder. Slightly soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Ifosfamide: White, crystalline powder. Melts at about 40°. Very soluble in alcohol, in ethyl acetate, in isopropyl alcohol, in methanol, and in methylene chloride; freely soluble in water; very slightly soluble in hexanes.

Imidurea: White, odorless, tasteless powder. Soluble in water and in glycerin; sparingly soluble in propylene glycol; insoluble in most organic solvents. *NF category:* Antimicrobial preservative.

Imipenem: White to tan-colored crystalline powder. Slightly soluble in water and in methanol.

Imipramine Hydrochloride: White to pale yellow powder. Freely soluble in water and in alcohol; insoluble in ether and in benzene.

Imipramine Pamoate: Fine, yellow powder. Soluble in acetone, in chloroform, in carbon tetrachloride, in ethanol, and in ether; insoluble in water.

Imiquimod: White or almost white, powder or crystalline powder. Very slightly soluble in methanol; practically insoluble in water and in acetone.

Inamrinone: Pale yellow to tan powder. It is odorless or has a faint odor. Slightly soluble in methanol; practically insoluble or insoluble in chloroform and in water.

Indapamide: White to off-white, crystalline powder. Melts between 167° and 170°. Soluble in methanol, in alcohol, in acetonitrile, in glacial acetic acid, and in ethyl acetate; very slightly soluble in ether and in chloroform; practically insoluble in water.

Indigotindisulfonate Sodium: Dusky, purplish-blue powder, or blue granules having a coppery luster. Is affected by light. Its solutions have a blue or bluish purple color. Slightly soluble in water and in alcohol; practically insoluble in most other organic solvents.

Indinavir Sulfate: White or almost white, hygroscopic powder. Freely soluble in water; soluble in methanol; practically insoluble in heptane.

Indocyanine Green: Olive-brown, dark green, blue-green, dark blue, or black powder. Is odorless or has a slight odor. Its solutions are deep emerald-green in color. The pH of a solution (1 in 200) is about 6. Its aqueous solutions are stable for about 8 hours. Soluble in water and in methanol; practically insoluble in most other organic solvents.

Indomethacin: Pale yellow to yellow-tan, crystalline powder, having not more than a slight odor. Is sensitive to light. Melts at about 162°. Exhibits polymorphism. Sparingly soluble in alcohol, in chloroform, and in ether; practically insoluble in water.

Influenza Virus Vaccine: Slightly turbid liquid or suspension, which may have a slight yellow or reddish tinge and may have an odor because of the preservative.

Inositol: White or almost white, crystalline powder. Very soluble in water; practically insoluble in alcohol absolute and in ether. *NF category:* Humectant.

Insulin: White or practically white crystals. Soluble in solutions of dilute acids and alkalies.

Insulin Injection: The Injection containing, in each mL, not more than 100 USP Units is a clear, colorless or almost colorless liquid; the Injection containing, in each mL, 500 Units may be straw-colored. Contains between 0.1% and 0.25% (w/v) of either phenol or cresol. Contains between 1.4% and 1.8% (w/v) of glycerin.

Insulin Lispro: White or practically white crystals. Soluble in solutions of dilute acids and alkalies.

Isophane Insulin Suspension: White suspension of rod-shaped crystals, free from large aggregates of crystals following moderate agitation. Contains either (1) between 1.4% and 1.8% (w/v) of glycerin, between 0.15% and 0.17% (w/v) of metacresol, and between 0.06% and 0.07% (w/v) of phenol; or (2) between 1.4% and 1.8% (w/v) of glycerin and between 0.20% and 0.25% (w/v) of phenol. Contains between 0.15% and 0.25% (w/v) of dibasic sodium phosphate. When examined microscopically, the insoluble matter in the Suspension is crystalline, and contains not more than traces of amorphous material.

Insulin Zinc Suspension: Practically colorless suspension of a mixture of characteristic crystals predominantly between 10 and 40 µm in maximum dimension and many particles that have no uniform shape and do not exceed 2 µm in maximum dimension. Contains between 0.15% and 0.17% (w/v) of sodium acetate, between 0.65% and 0.75% (w/v) of sodium chloride, and between 0.09% and 0.11% (w/v) of methylparaben.

Extended Insulin Zinc Suspension: Practically colorless suspension of a mixture of characteristic crystals the maximum dimension of which is predominantly between 10 and 40 µm. Contains between 0.15% and 0.17% (w/v) of sodium acetate,

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between 0.65% and 0.75% (w/v) of sodium chloride, and between 0.09% and 0.11% (w/v) of methylparaben.

Prompt Insulin Zinc Suspension: Practically colorless suspension of particles that have no uniform shape and the maximum dimension of which does not exceed 2 µm. Contains between 0.15% and 0.17% (w/v) of sodium acetate, between 0.65% and 0.75% (w/v) of sodium chloride, and between 0.09% and 0.11% (w/v) of methylparaben.

Inulin: White, friable, chalk-like, amorphous, odorless, tasteless powder. Soluble in hot water; slightly soluble in cold water and in organic solvents. *NF category:* Sweetening agent; wet binder.

Invert Sugar: Colorless to pale yellow liquid. Miscible with water, with glycerin, and with glycols. *NF category:* Sweetening agent; diluent; wet binder.

Iodine: Heavy, grayish-black plates or granules, having a metallic luster and a characteristic odor. Freely soluble in carbon disulfide, in chloroform, in carbon tetrachloride, and in ether; soluble in alcohol and in solutions of iodides; sparingly soluble in glycerin; very slightly soluble in water.

Iodine Topical Solution: Transparent, reddish-brown liquid, having the odor of iodine.

Strong Iodine Solution: Transparent liquid having a deep brown color and having the odor of iodine.

Iodine Tincture: Transparent liquid having a reddish-brown color and the odor of iodine and of alcohol.

Sodium Iodide I 123 Capsules: Capsules may contain a small amount of solid or solids, or may appear empty.

Sodium Iodide I 123 Solution: Clear, colorless solution. Upon standing, both the Solution and the glass container may darken as a result of the effects of the radiation.

Iodinated I 125 Albumin Injection: Clear, colorless to slightly yellow solution. Upon standing, both the Albumin and the glass container may darken as a result of the effects of the radiation.

Iodinated I 131 Albumin Injection: Clear, colorless to slightly yellow solution. Upon standing, both the Albumin and the glass container may darken as a result of the effects of the radiation.

Iodinated I 131 Albumin Aggregated Injection: Dilute suspension of white to faintly yellow particles, which may settle on standing. The glass container may darken on standing, as a result of the effects of the radiation.

Sodium Rose Bengal I 131 Injection: Clear, deep-red solution.

Iodohippurate Sodium I 131 Injection: Clear, colorless solution. Upon standing, both the Injection and the glass container may darken as a result of the effects of the radiation.

Sodium Iodide I 131 Capsules: May contain a small amount of solid or solids, or may appear empty.

Sodium Iodide I 131 Solution: Clear, colorless solution. Upon standing, both the Solution and the glass container may darken as a result of the effects of the radiation.

Iodipamide: White, practically odorless, crystalline powder. Slightly soluble in alcohol; very slightly soluble in water, in chloroform, and in ether.

Iodipamide Meglumine Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Iodixanol: White to off-white, amorphous, odorless, hygroscopic powder. Freely soluble in water.

Iodoform: Lustrous greenish yellow powder, or lustrous crystals. It is slightly volatile even at ordinary temperatures, and distills slowly with steam. Freely soluble in ether and in chloroform; soluble in boiling alcohol; sparingly soluble in alcohol, in glycerin, and in olive oil; practically insoluble in water. Melts to a brown liquid at about 115°, and decomposes at a higher temperature, emitting vapors of iodine.

Iodoquinol: Light yellowish to tan, microcrystalline powder not readily wetted by water. Is odorless or has a faint odor; is stable in air. Melts with decomposition. Sparingly soluble in alcohol and in ether; practically insoluble in water.

Iohexol: White to off-white, hygroscopic, odorless powder. Very soluble in water and in methanol; practically insoluble or insoluble in ether and in chloroform.

Iohexol Injection: Clear, colorless to pale yellow liquid.

Iopamidol: Practically odorless, white to off-white powder. Very soluble in water; sparingly soluble in methanol; practically insoluble in alcohol and in chloroform.

Iopanoic Acid: Cream-colored powder. Is tasteless or practically so, and has a faint, characteristic odor. Is affected by light. Soluble in alcohol, in chloroform, and in ether, and in solutions of alkali hydroxides and carbonates; insoluble in water.

Iophendylate: Colorless to pale yellow, viscous liquid, the color darkening on long exposure to air. Is odorless or has a faintly ethereal odor. Freely soluble in alcohol, in benzene, in chloroform, and in ether; very slightly soluble in water.

Iophendylate Injection: Colorless to pale yellow, viscous liquid, the color darkening on long exposure to air. Is odorless or has a faintly ethereal odor. Freely soluble in alcohol, in benzene, in chloroform, and in ether; very slightly soluble in water.

Iopromide: White to slightly yellow powder. Freely soluble in water and in dimethyl sulfoxide; practically insoluble in alcohol, in acetone, and in ether.

Iothalamate Meglumine Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Iothalamate Meglumine and Iothalamate Sodium Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Iothalamate Sodium Injection: Clear, colorless to pale yellow, slightly viscous liquid.

Iothalamic Acid: White, odorless powder. Soluble in solutions of alkali hydroxides; slightly soluble in water and in alcohol.

Ioxilan: White to off-white, practically odorless powder. Soluble in water and in methanol.

Ioxilan Injection: Clear, colorless to pale yellow liquid.

Powdered Ipecac: Pale brown, weak yellow, or light olive-gray powder.

Ipodate Sodium: White to off-white, odorless, fine, crystalline powder. Freely soluble in water, in alcohol, and in methanol; very slightly soluble in chloroform.

Ipratropium Bromide: White to off-white, crystalline powder. Freely soluble in methanol; soluble in water; slightly soluble in alcohol.

Irbesartan: White to off-white, crystalline powder. Slightly soluble in alcohol and in methylene chloride; practically insoluble in water.

Irinotecan Hydrochloride: Pale yellow to yellow crystalline powder. Slightly soluble in water and in organic solvents.

Iron Dextran Injection: Dark brown, slightly viscous liquid.

Iron Sorbitex Injection: Clear liquid, having a dark brown color.

Isobutane: Colorless, flammable gas (boiling temperature is about –11°). Vapor pressure at 21° is about 2950 mm of mercury (31 psig). *NF category:* Propellant.

Isobutyl Alcohol: Clear, colorless, mobile liquid. Soluble in water. Miscible with alcohol, with ether, and with many other organic solvents. *NF category:* Flavors and fragrance; solvent.

Isoetharine Inhalation Solution: Colorless or slightly yellow, slightly acid liquid, gradually turning dark on exposure to air and light.

Isoetharine Hydrochloride: White to off-white, odorless, crystalline solid. Melts between 196° and 208°, with

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decomposition. Soluble in water; sparingly soluble in alcohol; practically insoluble in ether.

Isoetharine Mesylate: White or practically white, odorless crystals having a salty, bitter taste. Freely soluble in water; soluble in alcohol; practically insoluble in acetone and in ether.

Isoflurane: Clear, colorless, volatile liquid, having a slight odor. Boils at about 49°. Insoluble in water. Miscible with common organic solvents and with fats and oils.

Isoleucine: White, practically odorless crystals, having a slightly bitter taste. Soluble in water; slightly soluble in hot alcohol; insoluble in ether.

Isometheptene Mucate: White, crystalline powder. Freely soluble in water; soluble in alcohol; practically insoluble in chloroform and in ether.

Isoniazid: Colorless or white crystals or white, crystalline powder. Is odorless and is slowly affected by exposure to air and light. Freely soluble in water; sparingly soluble in alcohol; slightly soluble in chloroform; very slightly soluble in ether.

Isoniazid Injection: Clear, colorless to faintly greenish-yellow liquid. Gradually darkens on exposure to air and light. Tends to crystallize at low temperatures.

Isopropamide Iodide: White to pale yellow, crystalline powder, having a bitter taste. Freely soluble in chloroform and in alcohol; sparingly soluble in water; very slightly soluble in benzene and in ether.

Isopropyl Alcohol: Transparent, colorless, mobile, volatile liquid, having a characteristic odor and a slightly bitter taste. Is flammable. Miscible with water, with alcohol, with ether, and with chloroform. *NF category:* Solvent.

Azeotropic Isopropyl Alcohol: Transparent, colorless, mobile, volatile liquid, having a characteristic odor and a slightly bitter taste. Is flammable. Miscible with water, with alcohol, with ether, and with chloroform.

Isopropyl Isostearate: Clear, colorless to pale-yellow liquid. Miscible with alcohol and with fatty oils. Insoluble in water. *NF category*: Emollient; emulsifying agent.

Isopropyl Myristate: Clear, practically colorless, oily liquid. Is practically odorless, and congeals at about 5°. Freely soluble in 90% alcohol; insoluble in water, in glycerin, and in propylene glycol. Miscible with most organic solvents and with fixed oils. *NF category:* Vehicle (oleaginous); emollient; solvent.

Isopropyl Palmitate: Colorless, mobile liquid having a very slight odor. Soluble in acetone, in castor oil, in chloroform, in cottonseed oil, in ethyl acetate, in alcohol, and in mineral oil; insoluble in water, in glycerin, and in propylene glycol. *NF category:* Vehicle (oleaginous); emollient; solvent.

Isoproterenol Inhalation Solution: Colorless or practically colorless, slightly acid liquid, gradually turning dark on exposure to air and light.

Isoproterenol Hydrochloride: White to practically white, odorless, crystalline powder, having a slightly bitter taste. Gradually darkens on exposure to air and light. Its solutions become pink to brownish pink on standing exposed to air, doing so almost immediately when rendered alkaline. Its solution (1 in 100) has a pH of about 5. Freely soluble in water; sparingly soluble in alcohol and less soluble in dehydrated alcohol; insoluble in chloroform and in ether.

Isoproterenol Hydrochloride Injection: Colorless or practically colorless liquid, gradually turning dark on exposure to air and light.

Isoproterenol Sulfate: White to practically white, odorless, crystalline powder. It gradually darkens on exposure to air and light. Its solutions become pink to brownish pink on standing exposed to air, doing so almost immediately when rendered alkaline. A solution (1 in 100) has a pH of about 5. Freely soluble in water; very slightly soluble in alcohol, in benzene, and in ether.

Isosorbide Concentrate: Colorless to slightly yellow liquid. Soluble in water and in alcohol.

Diluted Isosorbide Dinitrate: Ivory-white, odorless powder. [NOTE—Undiluted isosorbide dinitrate occurs as white, crystalline rosettes.] Undiluted isosorbide dinitrate is very soluble in acetone; freely soluble in chloroform; sparingly soluble in alcohol; very slightly soluble in water.

Isostearyl Isostearate: Colorless to slightly yellow, oily liquid. *Specific Gravity* ⟨841⟩ : 0.840–0.880 at 20°. Soluble in methylene chloride and in mineral oil; insoluble in water and in alcohol. *NF category*: Emollient; emulsifying agent.

Isotretinoin: Yellow crystals. Soluble in chloroform; sparingly soluble in alcohol, in isopropyl alcohol, and in polyethylene glycol 400; practically insoluble in water.

Isoxsuprine Hydrochloride: White, odorless, crystalline powder, having a bitter taste. Melts at about 200°, with decomposition. Sparingly soluble in alcohol; slightly soluble in water.

Isradipine: Yellow, fine crystalline powder.

Itraconazole: A white or almost white powder. Freely soluble in methylene chloride; sparingly soluble in tetrahydrofuran; very slightly soluble in alcohol; practically insoluble in water.

Ivermectin: White to yellowish-white, crystalline powder. Slightly hygroscopic. Freely soluble in methanol and in methylene chloride; soluble in acetone and in acetonitrile; practically insoluble in hexane and in water.

Ixabepilone: White or almost white powder. Slightly soluble in acetonitrile.

Juniper Tar: Dark brown, clear, thick liquid, having a tarry odor and a faintly aromatic, bitter taste. Sparingly soluble in solvent hexane; very slightly soluble in water. One volume dissolves in 9 volumes of alcohol. Dissolves in 3 volumes of ether, leaving only a slight, flocculent residue. Miscible with amyl alcohol, with chloroform, and with glacial acetic acid.

Kanamycin Sulfate: White, odorless, crystalline powder. Freely soluble in water; insoluble in acetone, in ethyl acetate, and in benzene.

Kaolin: Soft, white or yellowish-white powder or lumps. Has an earthy or clay-like taste and, when moistened with water, assumes a darker color and develops a marked clay-like odor. Insoluble in water, in cold dilute acids, and in solutions of alkali hydroxides. *NF category:* Diluent; suspending and/or viscosity-increasing agent.

Ketamine Hydrochloride: White, crystalline powder, having a slight, characteristic odor. Freely soluble in water and in methanol; soluble in alcohol; sparingly soluble in chloroform.

Ketorolac Tromethamine: White to off-white, crystalline powder. Melts between 165° and 170°, with decomposition. Freely soluble in water and in methanol; slightly soluble in alcohol, in dehydrated alcohol, and in tetrahydrofuran; practically insoluble in acetone, in dichloromethane, in toluene, in ethyl acetate, in dioxane, in hexane, in butyl alcohol, and in acetonitrile.

Labetalol Hydrochloride: White to off-white powder. Melts at about 180°, with decomposition. Soluble in water and in alcohol; insoluble in ether and in chloroform.

Lacosamide: White to light yellow powder. Freely soluble in methanol; soluble in anhydrous ethanol; sparingly soluble in water; slightly soluble in acetonitrile; and practically insoluble in heptane.

Alpha-Lactalbumin: Free-flowing, slightly hygroscopic light cream-colored powder. Freely soluble in water; soluble in wide pH ranges; insoluble in methanol, in alcohol, in ether, and in acetone. *NF category:* Bulking agent; coating agent; emulsifying agent; stiffening agent; suspending and/or viscosity-increasing agent; diluent; wet binder; vehicle (solid

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carrier); pH modifier (acidifying agent/alkalizing agent/buffering agent); chelating and/or complexing agent.

Lactic Acid: Colorless or yellowish, practically odorless, syrupy liquid. Is hygroscopic. When it is concentrated by boiling, lactic acid lactate is formed. Specific gravity is about 1.20. Insoluble in chloroform. Miscible with water, with alcohol, and with ether. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Lactitol: A white or light brown, odorless crystal. Has a mild, sweet taste, and no aftertaste. *NF category:* Flavors and fragrance; diluent; sweetening agent.

Lactobionic Acid: White or almost white, crystalline powder with a melting point of about 125° with decomposition. Freely soluble in water; slightly soluble in glacial acetic acid, in anhydrous ethanol, and in methanol. *NF category:* Antioxidant.

Anhydrous Lactose: White or almost white powder. Freely soluble in water; practically insoluble in alcohol. *NF category:* Diluent; carrier.

Lactose Monohydrate: White, free-flowing powder. Freely but slowly soluble in water; practically insoluble in alcohol. *NF category:* Diluent; carrier; wet binder.

Lactulose Concentrate: Colorless to amber syrupy liquid, which may exhibit some precipitation and darkening upon standing. Miscible with water.

Lamivudine: White to off-white solid. Soluble in water. Melts at about 176°.

Lamotrigine: A white to pale cream-colored powder. Slightly soluble in 0.1 N hydrochloric acid, in acetone, in methanol, and in water.

Lanolin: Yellow, tenacious, unctuous mass, having a slight, characteristic odor. Freely soluble in ether and in chloroform; soluble in hot alcohol; sparingly soluble in cold alcohol; insoluble in water, but mixes without separation with about twice its weight of water. *NF category:* Emulsifying agent; ointment base.

Hydrogenated Lanolin: White or pale yellow, unctuous substance. Soluble in boiling dehydrated alcohol and in light petroleum; insoluble in water. *NF category*: Emulsifying agent; emollient; humectant; ointment base.

Lanolin Alcohols: Hard, waxy, amber solid, having a characteristic odor. Freely soluble in chloroform, in ether, and in petroleum ether; slightly soluble in alcohol; insoluble in water. *NF category:* Emulsifying agent; ointment base.

Lansoprazole: White to brownish-white powder. Freely soluble in dimethylformamide; practically insoluble in water. Melts at about 166°, with decomposition.

Latanoprost: Colorless to slightly yellow oil. Very soluble in acetonitrile; freely soluble in acetone, in ethanol, in ethyl acetate, in isopropanol, in methanol, and in octanol; practically insoluble in water.

Lauric Acid: White or faintly yellow, somewhat glossy, crystalline solid or powder. Very soluble in alcohol, in ether, and in methanol; soluble in acetone; practically insoluble in water. *NF category*: Antifoaming agent; emulsifying agent; lubricant.

Lauroyl Polyoxylglycerides: Pale yellow semi-solids. Freely soluble in methylene chloride. Dispersible in hot water. *NF category*: Ointment base; solvent; emulsifying agent; wetting and/or solubilizing agent.

Lecithin: The consistency of both natural grades and refined grades of lecithin may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents. Its color varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached. It is odorless or has a characteristic, slight nut-like odor and a bland taste. Practically insoluble in water, but it readily hydrates to form emulsions. The oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils. When all phosphatide fractions are present, lecithin is sparingly soluble in alcohol and practically insoluble in acetone. *NF category:* Emulsifying agent; emollient.

Leflunomide: White to almost white powder. Freely soluble in methanol, in alcohol, in 2-propanol, in ethyl acetate, in acetone, and in acetonitrile; practically insoluble in water.

Letrozole: White to yellowish, crystalline powder. Freely soluble in dichloromethane; slightly soluble in alcohol; practically insoluble in water.

Leucine: White, practically odorless, tasteless crystals. Sparingly soluble in water; insoluble in ether. *NF category:* Flavors and fragrance.

Leucovorin Calcium: Yellowish-white or yellow powder. Slightly soluble to freely soluble in water; practically insoluble in alcohol.

Leucovorin Calcium Injection: Clear, yellowish solution.

Levamisole Hydrochloride: White or almost white, crystalline powder. Freely soluble in water; soluble in alcohol; slightly soluble in methylene chloride; practically insoluble in ether.

Levetiracetam: White to almost white powder. Very soluble in water; soluble in acetonitrile; practically insoluble in hexane.

Levmetamfetamine: Clear, practically colorless liquid.

Levobunolol Hydrochloride: White crystalline, odorless powder. Soluble in water and in methanol; slightly soluble in alcohol and in chloroform.

Levocarnitine: White crystals or crystalline powder. Hygroscopic. Freely soluble in water, and in hot alcohol; practically insoluble in acetone, in ether, and in benzene.

Levocetirizine Dihydrochloride: White to almost white powder. Freely soluble in water and in methanol.

Levodopa: White to off-white, odorless, crystalline powder. In the presence of moisture, is rapidly oxidized by atmospheric oxygen and darkens. Freely soluble in 3 N hydrochloric acid; slightly soluble in water; insoluble in alcohol.

Levofloxacin: Light yellowish-white to yellow-white crystals or crystalline powder. Soluble in dimethylsulfoxide and in acetic acid; sparingly soluble in water, in acetone, and in methanol; practically insoluble in glycerin and in *n*-octanol.

Levonordefrin: White to buff-colored, odorless, crystalline solid. Melts at about 210°. Freely soluble in aqueous solutions of mineral acids; slightly soluble in acetone, in chloroform, in alcohol, and in ether; practically insoluble in water.

Levonorgestrel: White or practically white, odorless powder. Soluble in chloroform; slightly soluble in alcohol; practically insoluble in water.

Levorphanol Tartrate: Practically white, odorless, crystalline powder. Sparingly soluble in water; slightly soluble in alcohol; insoluble in chloroform and in ether. Melts, in a sealed tube, at about 110°, with decomposition.

Levothyroxine Sodium: Light yellow to buff-colored, odorless, tasteless, hygroscopic powder. Is stable in dry air but may assume a slight pink color upon exposure to light. The pH of a saturated solution is about 8.9. Soluble in solutions of alkali hydroxides and in hot solutions of alkali carbonates; slightly soluble in alcohol; very slightly soluble in water; insoluble in acetone, in chloroform, and in ether.

Lidocaine: White or slightly yellow, crystalline powder. Has a characteristic odor and is stable in air. Very soluble in alcohol and in chloroform; freely soluble in benzene and in ether; practically insoluble in water. Dissolves in oils.

Lidocaine Hydrochloride: White, odorless, crystalline powder, having a slightly bitter taste. Very soluble in water and in alcohol; soluble in chloroform; insoluble in ether.

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Lime: Hard, white or grayish-white masses or granules, or white or grayish white powder. Is odorless. Slightly soluble in water; very slightly soluble in boiling water.

Lincomycin Hydrochloride: White or practically white, crystalline powder. Is odorless or has a faint odor. Is stable in the presence of air and light. Its solutions are acid and dextrorotatory. Freely soluble in water; soluble in dimethylformamide; very slightly soluble in acetone.

Lincomycin Hydrochloride Injection: Clear, colorless to slightly yellow solution, having a slight odor.

Lincomycin Hydrochloride Soluble Powder: White to off-white, or light tan free-flowing, fine powder.

Lindane: White, crystalline powder, having a slight, musty odor. Freely soluble in chloroform; soluble in dehydrated alcohol; sparingly soluble in ether; slightly soluble in ethylene glycol; practically insoluble in water.

Linezolid: White to off-white or cream-colored, crystalline powder. Sparingly soluble in acetone, in ethanol, and in methanol; very slightly soluble in water.

Linoleoyl Polyoxylglycerides: Amber, oily liquids. May develop deposit after prolonged storage periods at 20°. Freely soluble in methylene chloride; practically insoluble but dispersible in water. *NF category:* Ointment base; solvent; emulsifying agent; wetting and/or solubilizing agent.

Liothyronine Sodium: Light tan, odorless, crystalline powder. Slightly soluble in alcohol; very slightly soluble in water; practically insoluble in most other organic solvents.

Lisinopril: White, crystalline powder. Melts at about 160°, with decomposition. Soluble in water; sparingly soluble in methanol; practically insoluble in alcohol, in acetone, in acetonitrile, and in chloroform.

Lithium Carbonate: White, granular, odorless powder. Sparingly soluble in water; very slightly soluble in alcohol. Dissolves, with effervescence, in dilute mineral acids.

Lithium Citrate: White, odorless, deliquescent powder or granules, having a cooling, faintly alkaline taste. Freely soluble in water; slightly soluble in alcohol.

Lomustine: Yellow crystalline powder. Freely soluble in acetone, and in methylene chloride; soluble in ethanol; practically insoluble in water.

Loperamide Hydrochloride: White to slightly yellow powder. Melts at about 225°, with some decomposition. Freely soluble in methanol and in chloroform; slightly soluble in water and in dilute acids; very slightly soluble in isopropyl alcohol.

Lopinavir: White powder. Freely soluble in methanol and alcohol; soluble in isopropanol; practically insoluble in water.

Loratadine: White to off-white powder. Freely soluble in acetone, in chloroform, in methanol, and in toluene; insoluble in water.

Lorazepam: White or practically white, practically odorless powder. Sparingly soluble in alcohol; slightly soluble in chloroform; insoluble in water.

Losartan Potassium: White to off-white powder. Freely soluble in water; sparingly soluble in isopropyl alcohol; slightly soluble in acetonitrile.

Lovastatin: White to off-white, crystalline powder. Freely soluble in chloroform; soluble in acetone, in acetonitrile, and in methanol; sparingly soluble in alcohol; practically insoluble in hexane; insoluble in water.

Loxapine Succinate: White to yellowish, crystalline powder. Is odorless.

Lufenuron: White or pale yellow powder. Freely soluble in acetonitrile; soluble in dehydrated alcohol; practically insoluble in water.

Lumefantrine: Yellow crystalline powder. Freely soluble in *N,N*-dimethylformamide, in chloroform, and in ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and in methanol; practically insoluble in water.

Lutein: Red, crystalline powder. Soluble in ethanol, in ethyl acetate, and in methylene chloride; partially soluble in hexane.

Lysine Acetate: White, odorless crystals or crystalline powder, having an acid taste. Freely soluble in water.

Lysine Hydrochloride: White, odorless powder. Freely soluble in water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Mafenide Acetate: White to pale yellow, crystalline powder. Freely soluble in water.

Magaldrate: White, odorless, crystalline powder. Soluble in dilute solutions of mineral acids; insoluble in water and in alcohol.

Milk of Magnesia: White, opaque, more or less viscous suspension from which varying proportions of water usually separate on standing. pH is about 10.

Magnesium Aluminometasilicate: White powder or granules with an amorphous structure. Very slightly soluble in acids; practically insoluble or insoluble in water, in alcohol, and in alkalies.

Magnesium Aluminosilicate: White powder or granules with an amorphous structure. Very slightly soluble in acids; practically insoluble or insoluble in water, in alcohol, and in alkalies.

Magnesium Aluminum Silicate: Odorless, tasteless, fine (micronized) powder, small cream to tan granules, or small flakes that are creamy when viewed on their flat surfaces and tan to brown when viewed on their edges. Insoluble in water and in alcohol. Swells when added to water or glycerin. *NF category:* Suspending and/or viscosity-increasing agent; disintegrant.

Magnesium Carbonate: Light, white, friable masses or bulky, white powder. Is odorless, and is stable in air. Practically insoluble in water to which, however, it imparts a slightly alkaline reaction; insoluble in alcohol, but is dissolved by dilute acids with effervescence. *NF category:* Diluent.

Magnesium Chloride: Colorless, odorless, deliquescent flakes or crystals, which lose water when heated to 100° and lose hydrochloric acid when heated to 110°. Very soluble in water; freely soluble in alcohol.

Magnesium Citrate Oral Solution: Colorless to slightly yellow, clear, effervescent liquid, having a sweet, acidulous taste and a lemon flavor.

Magnesium Gluconate: Colorless crystals or white powder or granules. Is odorless and tasteless. Freely soluble in water; very slightly soluble in alcohol; insoluble in ether.

Magnesium Hydroxide: Bulky, white powder. Soluble in dilute acids; practically insoluble in water and in alcohol.

Magnesium Oxide: Very bulky, white powder or relatively dense, white powder or granulated powder. Soluble in dilute acids; practically insoluble in water; insoluble in alcohol. *NF category:* Emulsifying agent; glidant and/or anticaking agent; diluent.

Magnesium Phosphate: White, odorless, tasteless powder. Soluble in diluted mineral acids; practically insoluble in water.

Magnesium Salicylate: White, odorless, efflorescent, crystalline powder. Freely soluble in methanol; soluble in alcohol and in water; slightly soluble in ether.

Magnesium Silicate: Fine, white, odorless, tasteless powder, free from grittiness. Insoluble in water and in alcohol. Is readily decomposed by mineral acids. *NF category:* Glidant and/or anticaking agent.

Magnesium Stearate: Very fine, light, white powder, slippery to touch. Insoluble in water, in alcohol, and in ether. *NF category:* Lubricant.

Magnesium Sulfate: Small, colorless crystals, usually needle-like, with a cooling, saline, bitter taste. It effloresces in warm, dry air. Very soluble in boiling water; freely soluble in

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water; freely (and slowly) soluble in glycerin; sparingly soluble in alcohol.

Magnesium Trisilicate: Fine, white, odorless, tasteless powder, free from grittiness. Insoluble in water and in alcohol. Is readily decomposed by mineral acids. *NF category:* Glidant and/or anticaking agent.

Malathion: Clear, colorless, or slightly yellowish liquid, having a characteristic odor. Congeals at about 2.9°. Slightly soluble in water. Miscible with alcohols, with esters, with ketones, with ethers, with aromatic and alkylated aromatic hydrocarbons, and with vegetable oils.

Maleic Acid: White, crystalline powder. Freely soluble in water and in alcohol; sparingly soluble in ether. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Malic Acid: White or practically white, crystalline powder or granules, having a strongly acid taste. Melts at about 130°. Very soluble in water; freely soluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); flavors and fragrance; antioxidant, chelating and/or complexing agent.

Maltitol: White, crystalline powder. Very soluble in water; practically insoluble in ethanol. *NF category:* Humectant; sweetening agent; diluent; coating agent.

Maltodextrin: White, hygroscopic powder or granules. Freely soluble or readily dispersible in water; slightly soluble to insoluble in anhydrous alcohol. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; diluent; wet binder.

Maltol: A white, crystalline powder having a characteristic caramel-butterscotch odor, suggestive of a fruity-strawberry aroma in dilute solution. One g dissolves in about 82 mL of water, in 21 mL of alcohol, in 80 mL of glycerin, and in 28 mL of propylene glycol. *NF category:* Flavors and fragrance.

Maltose: Maltose occurs in either the anhydrous state or as a monohydrate. It is a white, crystalline powder, odorless, and has a sweet taste. Very slightly soluble in ethanol; freely soluble in water; slightly soluble in methanol; practically insoluble in ether. *NF category:* Sweetening agent; diluent; disintegrant; wet binder.

Mandelic Acid: White to light yellow powder or crystalline solid. Soluble in alcohol, in isopropyl alcohol; sparingly soluble in water. *NF category:* Antimicrobial preservative.

Mangafodipir Trisodium: Pale yellow crystals or cystalline powder. Freely soluble in water; sparingly soluble in methanol; slightly soluble in chloroform; very slightly soluble in alcohol and in acetone.

Manganese Chloride: Large, irregular, pink, odorless, translucent crystals. Soluble in water and in alcohol; insoluble in ether.

Manganese Chloride for Oral Solution: Off-white to tan-colored powder with a strawberry odor. Soluble in water.

Manganese Sulfate: Pale red, slightly efflorescent crystals, or purple, odorless powder. Soluble in water; insoluble in alcohol.

Mannitol: White, crystalline powder or free-flowing granules. Is odorless and has a sweet taste. Freely soluble in water; soluble in alkaline solutions; slightly soluble in pyridine; very slightly soluble in alcohol; practically insoluble in ether. *NF category:* Sweetening agent; tonicity agent; bulking agent; diluent; plasticizer.

Maprotiline Hydrochloride: Fine, white to off-white, crystalline powder. Is practically odorless. Freely soluble in methanol and in chloroform; slightly soluble in water; practically insoluble in isooctane.

Mazindol: White to off-white, crystalline powder, having not more than a faint odor. Slightly soluble in methanol and in chloroform; insoluble in water.

Mebendazole: White to slightly yellow powder. Is almost odorless. Melts at about 290°. Freely soluble in formic acid; practically insoluble in water, in dilute solutions of mineral acids, in alcohol, in ether, and in chloroform.

Mechlorethamine Hydrochloride: White, crystalline powder. Is hygroscopic.

Meclizine Hydrochloride: White or slightly yellowish, crystalline powder. Has a slight odor and is tasteless. Slightly soluble in dilute acids and in alcohol; practically insoluble in water and in ether; freely soluble in chloroform, in pyridine, and in acid-alcohol-water mixtures.

Meclofenamate Sodium: A white to creamy white, odorless to almost odorless, crystalline powder. Freely soluble in water, the solution sometimes being somewhat turbid due to partial hydrolysis and absorption of carbon dioxide; soluble in methanol; slightly soluble in chloroform; practically insoluble in ether. The solution is clear above pH 11.5.

Medroxyprogesterone Acetate: White to off-white, odorless, crystalline powder. Melts at about 205°. Is stable in air. Freely soluble in chloroform; soluble in acetone and in dioxane; sparingly soluble in alcohol and in methanol; slightly soluble in ether; insoluble in water.

Mefenamic Acid: White to off-white, crystalline powder. Melts at about 230°, with decomposition. Soluble in solutions of alkali hydroxides; sparingly soluble in chloroform; slightly soluble in alcohol and in methanol; practically insoluble in water.

Mefloquine Hydrochloride: White or slightly yellow, crystalline powder. It exhibits polymorphism. Freely soluble in methanol; soluble in alcohol; very slightly soluble in water.

Megestrol Acetate: White to creamy white, tasteless and essentially odorless, crystalline powder. Very soluble in chloroform; soluble in acetone; sparingly soluble in alcohol; slightly soluble in ether and in fixed oils; insoluble in water. Is unstable under aqueous conditions at pH 7 or above.

Meglumine: White to faintly yellowish-white, odorless crystals or powder. Freely soluble in water; sparingly soluble in alcohol.

Melengestrol Acetate: White to light yellow, crystalline powder. Freely soluble in chloroform and in ethyl acetate; slightly soluble in alcohol; insoluble in water.

Meloxicam: Pale yellow powder. Soluble in dimethylformamide; slightly soluble in acetone; very slightly soluble in methanol and in alcohol; practically insoluble in water.

Melphalan: Off-white to buff powder, having a faint odor. Melts at about 180°, with decomposition. Soluble in dilute mineral acids; slightly soluble in alcohol and in methanol; practically insoluble in water, in chloroform, and in ether.

Memantine Hydrochloride: White to off-white, colored powder. Slightly soluble in water.

Menadiol Sodium Diphosphate: White to pink powder, having a characteristic odor. Is hygroscopic. Its solutions are neutral or slightly alkaline to litmus, having a pH of about 8. Very soluble in water; insoluble in alcohol.

Menadione: Bright yellow, crystalline, practically odorless powder. Is affected by sunlight. Soluble in vegetable oils; sparingly soluble in chloroform and in alcohol; practically insoluble in water.

Menthol: Colorless, hexagonal crystals, usually needle-like, or in fused masses, or crystalline powder. Has a pleasant, peppermint-like odor. Very soluble in alcohol, in chloroform, in ether, and in solvent hexane; freely soluble in glacial acetic acid, in mineral oil, and in fixed and volatile oils; slightly soluble in water. *NF category:* Flavors and fragrance.

Meperidine Hydrochloride: Fine, white, crystalline, odorless powder. The pH of a solution (1 in 20) is about 5. Very soluble in water; soluble in alcohol; sparingly soluble in ether.

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Mephobarbital: White, odorless, crystalline powder, having a bitter taste. Its saturated solution is acid to litmus. Soluble in chloroform and in solutions of fixed alkali hydroxides and carbonates; slightly soluble in water, in alcohol, and in ether.

Mepivacaine Hydrochloride: White, odorless, crystalline solid. The pH of a solution (1 in 50) is about 4.5. Freely soluble in water and in methanol; very slightly soluble in chloroform; practically insoluble in ether.

Meprobamate: White powder, having a characteristic odor and a bitter taste. Freely soluble in acetone and in alcohol; slightly soluble in water; practically insoluble or insoluble in ether.

Mercaptopurine: Yellow, odorless or practically odorless, crystalline powder. Melts at a temperature exceeding 308°, with decomposition. Soluble in hot alcohol and in dilute alkali solutions; slightly soluble in 2 N sulfuric acid; insoluble in water, in acetone, and in ether.

Ammoniated Mercury: White, pulverulent pieces or white, amorphous powder. Is odorless, and is stable in air, but darkens on exposure to light. Readily soluble in warm hydrochloric, nitric, and acetic acids; insoluble in water, and in alcohol.

Meropenem: Colorless to white or light yellow crystals or crystalline powder. Soluble in dimethylformamide and in 5% dibasic potassium phosphate solution; sparingly soluble in water and in 5% monobasic potassium phosphate solution; practically insoluble in alcohol, in acetone, in methylene chloride, and in ether.

Mesalamine: Light tan to pink colored, needle-shaped crystals. Color may darken on exposure to air. Is odorless or may have a slight characteristic odor. Soluble in dilute hydrochloric acid and in dilute alkali hydroxides; slightly soluble in water; very slightly soluble in methanol, in dehydrated alcohol, and in acetone; practically insoluble in *n*-butyl alcohol, in chloroform, in ether, in ethyl acetate, in *n*-hexane, in methylene chloride, and in *n*-propyl alcohol.

Mesna: White or slightly yellow crystalline powder; hygroscopic. Freely soluble in water; slightly soluble in alcohol; practically insoluble in cyclohexane.

Mesoridazine Besylate: White to pale yellowish powder, having not more than a faint odor. Melts at about 178°, with decomposition. Freely soluble in water, in chloroform, and in methanol.

Mestranol: White to creamy white, odorless, crystalline powder. Freely soluble in chloroform; soluble in dioxane; sparingly soluble in dehydrated alcohol; slightly soluble in methanol; insoluble in water.

Metaproterenol Sulfate: White to off-white, crystalline powder. Freely soluble in water.

Metaxalone: White to almost-white, crystalline powder. Freely soluble in chloroform; soluble in methanol and in alcohol; practically insoluble in ether and in water.

Metformin Hydrochloride: White, crystalline powder. Freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.

Methacholine Chloride: Colorless or white crystals, or white, crystalline powder. Is odorless or has a slight odor, and is very hygroscopic. Its solutions are neutral to litmus. Very soluble in water; freely soluble in alcohol and in chloroform.

Methacrylic Acid and Ethyl Acrylate Copolymer Dispersion: Milky-white liquid of low viscosity. It is miscible with water in any proportion; the milky-white appearance is retained. A clear or slightly opalescent, viscous solution is obtained on mixing one part with five parts of acetone, alcohol, or isopropyl alcohol; the polymer substance is first precipitated, but then dissolves in the excess organic solvent. A clear or slightly opalescent, viscous solution is obtained on mixing one part with two parts of 1 N sodium hydroxide. *NF category:* Coating agent; film-forming agent; diluent; wet binder.

Methacrylic Acid and Ethyl Acrylate Copolymer: White powder having a faint, characteristic odor. Soluble to freely soluble in methanol, in alcohol, in isopropyl alcohol, and in acetone, each of which contains not less than 3% of water; soluble in diluted alkali, in simulated intestinal fluid TS, and in buffer solutions of pH 7 and above; insoluble in water, in diluted acids, in simulated gastric fluid TS, and in buffer solutions of up to pH 5. The solubility between pH 5.5 and pH 7 depends on the content of methacrylic acid units in the copolymer. *NF category:* Coating agent; film-forming agent.

Partially-Neutralized Methacrylic Acid and Ethyl Acrylate Copolymer: White or almost white, free-flowing powder. Freely soluble in alcohol, in methanol, and in a 40 g/L solution of sodium hydroxide; soluble in solutions at pH values above pH 5.5 under salt formation; practically insoluble in ethyl acetate and in acidic aqueous solutions. *NF category:* Coating agent; film-forming agent.

Methacrylic Acid and Methyl Methacrylate Copolymer: White powder having a faint, characteristic odor. Soluble to freely soluble in methanol, in alcohol, in isopropyl alcohol, and in acetone, each of which contains not less than 3% of water; soluble in diluted alkali, in simulated intestinal fluid TS, and in buffer solutions of pH 7 and above; insoluble in water, in diluted acids, in simulated gastric fluid TS, and in buffer solutions of up to pH 5. The solubility between pH 5.5 and pH 7 depends on the content of methacrylic acid units in the copolymer. *NF category:* Coating agent; film-forming agent.

Methacycline Hydrochloride: Yellow to dark yellow, crystalline powder. Soluble in water.

Methadone Hydrochloride: Colorless crystals or white, crystalline, odorless powder. Freely soluble in alcohol and in chloroform; soluble in water; practically insoluble in ether and in glycerin.

Methadone Hydrochloride Oral Concentrate: Clear to slightly hazy, syrupy liquid.

Methamphetamine Hydrochloride: White crystals or white, crystalline powder. Is odorless or practically so. Its solutions have a pH of about 6. Freely soluble in water, in alcohol, and in chloroform; very slightly soluble in absolute ether.

Methazolamide: White or faintly yellow, crystalline powder having a slight odor. Melts at about 213°. Soluble in dimethylformamide; slightly soluble in acetone; very slightly soluble in water and in alcohol.

Methdilazine Hydrochloride: Light tan, crystalline powder, having a slight, characteristic odor. Freely soluble in water, in alcohol, and in chloroform.

Methenamine: Colorless, lustrous crystals or white, crystalline powder. Is practically odorless. When brought into contact with fire, it readily ignites, burning with a smokeless flame. It sublimes at about 260°, without melting. Its solutions are alkaline to litmus. Freely soluble in water; soluble in alcohol and in chloroform.

Methenamine Mandelate: White, crystalline powder. Has a sour taste and is practically odorless. Its solutions have a pH of about 4. Melts at about 127°, with decomposition. Very soluble in water; soluble in alcohol and in chloroform; slightly soluble in ether.

Methimazole: White to pale buff, crystalline powder, having a faint, characteristic odor. Its solutions are practically neutral to litmus. Freely soluble in water, in alcohol, and in chloroform; slightly soluble in ether.

Methionine: White crystals, having a characteristic odor and taste. Soluble in water, in warm dilute alcohol, and in dilute mineral acids; insoluble in ether, in absolute alcohol, in benzene, and in acetone (L-form). *NF category:* Flavors and

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fragrance; pH modifier (acidifying agent/alkalizing agent/buffering agent); antioxidant.

Methocarbamol: White powder, odorless, or having a slight characteristic odor. Melts at about 94°, or, if previously ground to a fine powder, melts at about 90°. Soluble in alcohol only with heating; sparingly soluble in water and in chloroform; insoluble in benzene and in *n*-hexane.

Methohexital: White to faintly yellowish-white, crystalline, odorless powder. Slightly soluble in alcohol, in chloroform, and in dilute alkalies; very slightly soluble in water.

Methohexital Sodium for Injection: White to off-white, hygroscopic powder. Is essentially odorless.

Methotrexate: Orange-brown, or yellow, crystalline powder. Freely soluble in dilute solutions of alkali hydroxides and carbonates; slightly soluble in 6 N hydrochloric acid; practically insoluble in water, in alcohol, in chloroform, and in ether.

Methotrimeprazine: Fine, white, practically odorless, crystalline powder. Melts at about 126°. Freely soluble in chloroform, in ether, and in boiling alcohol; sparingly soluble in methanol and in alcohol at 25°; practically insoluble in water.

Methoxsalen: White to cream-colored, fluffy, needle-like crystals. Is odorless. Freely soluble in chloroform; soluble in boiling alcohol, in acetone, in acetic acid, in propylene glycol, and in benzene; sparingly soluble in boiling water and in ether; practically insoluble in water.

Methoxsalen Topical Solution: Clear, colorless liquid.

Methoxyflurane: Clear, practically colorless, mobile liquid, having a characteristic odor. Boils at about 105°. Miscible with alcohol, with acetone, with chloroform, with ether, and with fixed oils.

Methsuximide: White to grayish white, crystalline powder. Is odorless, or has not more than a slight odor. Very soluble in chloroform; freely soluble in alcohol and in ether; slightly soluble in hot water.

Methyclothiazide: White or practically white, crystalline powder. Is odorless, or has a slight odor. Freely soluble in acetone and in pyridine; sparingly soluble in methanol; slightly soluble in alcohol; very slightly soluble in water, in chloroform, and in benzene.

Methyl Alcohol: Clear, colorless liquid, having a characteristic odor. Is flammable. Miscible with water, with alcohol, with ether, with benzene, and with most other organic solvents. *NF category:* Solvent.

Methyl Benzylidene Camphor: A white, fine crystalline powder. Very soluble in chloroform; freely soluble in alcohol; practically insoluble in water.

Methyl Isobutyl Ketone: Transparent, colorless, mobile, volatile liquid, having a faint ketonic and camphoraceous odor. Slightly soluble in water. Miscible with alcohol, with ether, and with benzene. *NF category:* Alcohol denaturant; solvent.

Methyl Salicylate: Colorless, yellowish, or reddish liquid, having the characteristic odor and taste of wintergreen. It boils between 219° and 224°, with some decomposition. Soluble in alcohol and in glacial acetic acid; slightly soluble in water. *NF category:* Flavors and fragrance.

Methylbenzethonium Chloride: White, hygroscopic crystals, having a mild odor. Its solutions are neutral or slightly alkaline to litmus. Very soluble in water, in alcohol, and in ether; practically insoluble in chloroform.

Methylcellulose: White, fibrous powder or granules. Its aqueous suspensions are neutral to litmus. It swells in water and produces a clear to opalescent, viscous, colloidal suspension. Soluble in glacial acetic acid and in a mixture of equal volumes of alcohol and chloroform; insoluble in alcohol, in ether, and in chloroform. *NF category:* Coating agent; suspending and/or viscosity-increasing agent; wet binder; emulsifying agent; film-forming agent; disintegrant.

Methyldopa: White to yellowish-white, odorless, fine powder, which may contain friable lumps. Very soluble in 3 N hydrochloric acid; sparingly soluble in water; slightly soluble in alcohol; practically insoluble in ether.

Methyldopate Hydrochloride: White or practically white, odorless or practically odorless, crystalline powder. Freely soluble in water, in alcohol, and in methanol; slightly soluble in chloroform; practically insoluble in ether.

Methylene Blue: Dark green or blue crystals or crystalline powder having a bronze-like luster. Is odorless or practically so, and is stable in air. Its solutions in water and in alcohol are deep blue in color. Slightly soluble in water and in alcohol. *NF category:* Free radical scavenger.

Methylene Chloride: Clear, colorless, mobile liquid, having an odor resembling that of chloroform. Miscible with alcohol, with ether, and with fixed and volatile oils. *NF category:* Solvent.

Methylergonovine Maleate: White to pinkish-tan, microcrystalline powder. Is odorless. Slightly soluble in water and in alcohol; very slightly soluble in chloroform and in ether.

Methylnaltrexone Bromide: White to almost-white crystalline powder. Freely soluble in dimethylsulfoxide; soluble in dimethylformamide and in water; slightly soluble in methanol; very slightly soluble in ethanol; practically insoluble in toluene.

Methylparaben: White, crystalline powder or colorless crystals. Freely soluble in alcohol and in methanol; slightly soluble in water. *NF category:* Antimicrobial preservative.

Methylparaben Sodium: White, hygroscopic powder. Freely soluble in water; sparingly soluble in alcohol; insoluble in fixed oils. *NF category:* Antimicrobial preservative.

Methylphenidate Hydrochloride: White, odorless, fine, crystalline powder. Its solutions are acid to litmus. Freely soluble in water and in methanol; soluble in alcohol; slightly soluble in chloroform and in acetone.

Methylprednisolone: White to practically white, odorless, crystalline powder. Melts at about 240°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Sparingly soluble in alcohol, in dioxane, and in methanol; slightly soluble in acetone and in chloroform; very slightly soluble in ether; practically insoluble in water.

Methylprednisolone Acetate: White or practically white, odorless, crystalline powder. Melts at about 225°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Soluble in dioxane; sparingly soluble in acetone, in alcohol, in chloroform, and in methanol; slightly soluble in ether; practically insoluble in water.

Methylprednisolone Hemisuccinate: White or nearly white, odorless or nearly odorless, hygroscopic solid. Freely soluble in alcohol; soluble in acetone; very slightly soluble in water.

Methylprednisolone Sodium Succinate: White or nearly white, odorless, hygroscopic, amorphous solid. Very soluble in water and in alcohol; very slightly soluble in acetone; insoluble in chloroform.

Methylpyrrolidone: A clear, colorless to very slightly yellow liquid. Miscible with water and with most organic solvents including alcohol, ketones, and aromatic and chlorinated hydrocarbons. Boiling point: about 202°. Refractive index: about 1.469. *NF category:* Solvent.

Methylsulfonylmethane: White powder or flake crystal. Melts at about 109°. Freely soluble in water, in methanol, in alcohol, and in acetone; sparingly soluble in ether.

Methyltestosterone: White or creamy white crystals or crystalline powder. Is odorless and is stable in air, but is slightly hygroscopic. Is affected by light. Soluble in alcohol, in

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methanol, in ether, and in other organic solvents; sparingly soluble in vegetable oils; practically insoluble in water.

Methysergide Maleate: White to yellowish-white or reddish-white, crystalline powder. Is odorless or has not more than a slight odor. Slightly soluble in water and in alcohol; very slightly soluble in chloroform; practically insoluble in ether.

Metoclopramide Hydrochloride: White or practically white, crystalline, odorless or practically odorless powder. Very soluble in water; freely soluble in alcohol; sparingly soluble in chloroform; practically insoluble in ether.

Metoprolol Succinate: White to off-white powder. Freely soluble in water; soluble in methanol; sparingly soluble in alcohol; slightly soluble in isopropyl alcohol.

Metoprolol Tartrate: White, crystalline powder. Very soluble in water; freely soluble in methylene chloride, in chloroform, and in alcohol; slightly soluble in acetone; insoluble in ether.

Metronidazole: White to pale yellow, odorless crystals or crystalline powder. Is stable in air, but darkens on exposure to light. Soluble in dilute hydrochloric acid (1 in 2); sparingly soluble in water and in alcohol; slightly soluble in ether and in chloroform.

Metronidazole Benzoate: White to slightly yellow, crystalline powder. Freely soluble in methylene chloride; soluble in acetone; slightly soluble in alcohol; very slightly soluble in ethyl ether; practically insoluble in water.

Metyrapone: White to light amber, fine, crystalline powder, having a characteristic odor. Darkens on exposure to light. Soluble in methanol and in chloroform; sparingly soluble in water. It forms water-soluble salts with acids.

Mexiletine Hydrochloride: White powder. Freely soluble in dehydrated alcohol and in water; slightly soluble in acetonitrile; practically insoluble in ether. Optically inactive (1 in 20 solution in water).

Mezlocillin Sodium: White to pale yellow, crystalline powder. Freely soluble in water.

Mibolerone: White to off-white powder. Slightly soluble in chloroform, in dioxane, and in methylene chloride; practically insoluble in water (0.0454 mg per mL at 37°).

Miconazole: White to pale cream powder. Melts in the range of 78° to 88°. May exhibit polymorphism. Freely soluble in alcohol, in methanol, in isopropyl alcohol, in acetone, in propylene glycol, in chloroform, and in dimethylformamide; soluble in ether; insoluble in water.

Miconazole Nitrate: White or practically white, crystalline powder, having not more than a slight odor. Melts in the range of 178° to 183°, with decomposition. Freely soluble in dimethyl sulfoxide; soluble in dimethylformamide; sparingly soluble in methanol; slightly soluble in alcohol, in chloroform, and in propylene glycol; very slightly soluble in water and in isopropyl alcohol; insoluble in ether.

Midazolam: White or yellowish powder. The hydrochloride salt of midazolam is soluble in aqueous solutions. Insoluble in water.

Midodrine Hydrochloride: White crystalline powder. Soluble in water; sparingly soluble in methanol.

Milrinone: White to tan, crystalline solid. Is hygroscopic. Freely soluble in dimethyl sulfoxide; very slightly soluble in methanol; practically insoluble in water and in chloroform.

Mineral Oil: Colorless, transparent, oily liquid, free or practically free from fluorescence. Is odorless and tasteless when cold, and develops not more than a faint odor of petroleum when heated. Soluble in volatile oils; insoluble in water and in alcohol. Miscible with most fixed oils but not with castor oil. *NF category:* Solvent; vehicle (oleaginous); emollient.

Light Mineral Oil: Colorless, transparent, oily liquid, free, or practically free, from fluorescence. Is odorless and tasteless when cold, and develops not more than a faint odor of petroleum when heated. Soluble in volatile oils; insoluble in water and in alcohol. Miscible with most fixed oils, but not with castor oil. *NF category:* Vehicle (oleaginous); lubricant; emollient; solvent.

Minocycline Hydrochloride: Yellow, crystalline powder. Soluble in solutions of alkali hydroxides and carbonates; sparingly soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Minoxidil: White to off-white, crystalline powder. Melts in the approximate range of between 248° and 268°, with decomposition. Soluble in alcohol and in propylene glycol; sparingly soluble in methanol; slightly soluble in water; practically insoluble in chloroform, in acetone, in ethyl acetate, and in hexane.

Mirtazapine: White to creamy white, crystalline powder. Freely soluble in methanol and in toluene; soluble in ethyl ether; sparingly soluble in *n*-hexane; practically insoluble in water.

Misoprostol: Clear, colorless or light yellow viscous liquid. Very slightly soluble in water.

Mitomycin: Blue-violet, crystalline powder. Soluble in acetone, in methanol, in butyl acetate, and in cyclohexanone; slightly soluble in water.

Mitotane: White, crystalline powder, having a slight, aromatic odor. Soluble in alcohol, in ether, in solvent hexane, and in fixed oils and fats; practically insoluble in water.

Mitoxantrone Hydrochloride: Dark blue powder. Sparingly soluble in water; slightly soluble in methanol; practically insoluble in acetone, in acetonitrile, and in chloroform.

Modafinil: White to off-white, crystalline powder. Sparingly soluble in methanol; slightly soluble in absolute alcohol; very slightly soluble in water.

Moexipril Hydrochloride: White to off-white powder. Soluble in water, in alcohol, and in methanol.

Mometasone Furoate: White to off-white powder. Melts at about 220°, with decomposition. Soluble in acetone and in methylene chloride.

Monensin Sodium: Off-white to tan, crystalline powder. Soluble in chloroform and in methanol; slightly soluble in water; practically insoluble in solvent hexane.

Mono- and Di-glycerides: Vary in consistency from yellow liquids, through ivory-colored plastics, to ivory white-colored solids (bead or flake forms). Soluble in alcohol, in ethyl acetate, in chloroform, and in other chlorinated hydrocarbons; insoluble in water. *NF category:* Emulsifying agent.

Monobenzone Ointment: Dispersible with, but not soluble in, water.

Monoethanolamine: Clear, colorless, moderately viscous liquid, having a distinctly ammoniacal odor. Miscible with water, with acetone, with alcohol, with glycerin, and with chloroform. Immiscible with ether, with solvent hexane, and with fixed oils, although it dissolves many essential oils. *NF category:* Emulsifying agent; pH modifier (acidifying agent/alkalizing agent/buffering agent).

Monoglyceride Citrate: Soft white to ivory-colored, waxy solid with a lard-like consistency and bland odor. Dispersible in most common fat solvents and in alcohol. Insoluble in water.

Monosodium Glutamate: White, practically odorless, free-flowing crystals or crystalline powder. Freely soluble in water; sparingly soluble in alcohol. May have either a slightly sweet or a slightly salty taste. *NF category:* Flavors and fragrance; pH modifier (acidifying agent/alkalizing agent/buffering agent).

Monothioglycerol: Colorless or pale yellow, viscous liquid, having a slight sulfidic odor. Is hygroscopic. Freely soluble in water; insoluble in ether. Miscible with alcohol. *NF category:* Antioxidant; antimicrobial preservative.

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Montelukast Sodium: White or almost white, hygroscopic powder. Freely soluble to very soluble in alcohol; freely soluble in water and in methylene chloride.

Morantel Tartrate: A white or pale yellow, crystalline powder. Very soluble in water and in alcohol; practically insoluble in ethyl acetate.

Moricizine Hydrochloride: White to off-white, crystalline powder. Melts at about 189°, with decomposition. Soluble in water and in alcohol.

Morphine Sulfate: White, feathery, silky crystals, cubical masses of crystals, or white, crystalline powder. Is odorless, and when exposed to air it gradually loses water of hydration. Darkens on prolonged exposure to light. Freely soluble in hot water; soluble in water; slightly soluble in alcohol but more so in hot alcohol; insoluble in chloroform and in ether.

Moxidectin: White to pale yellow powder. Very soluble in alcohol; slightly soluble in hexane; practically insoluble in water.

Moxifloxacin Hydrochloride: Slightly yellow to yellow powder or crystals. Soluble in 0.1 N sodium hydroxide; sparingly soluble in water and in methanol; slightly soluble in 0.1 N hydrochloric acid, in dimethylformamide, and in alcohol; practically insoluble in methylene chloride, in acetone, in ethyl acetate, and in toluene; insoluble in *tert*-butyl methyl ether and *n*-heptane.

Mumps Skin Test Antigen: Slightly turbid liquid.

Mumps Virus Vaccine Live: Solid having the characteristic appearance of substances dried from the frozen state. The Vaccine is to be constituted with a suitable diluent just prior to use. Constituted vaccine undergoes loss of potency on exposure to sunlight.

Mupirocin: White to off-white, crystalline solid. Freely soluble in acetone, in chloroform, in dehydrated alcohol, and in methanol; slightly soluble in ether; very slightly soluble in water.

Mycophenolate Mofetil: White or almost white, crystalline powder. Its melting range is between 94° and 98°. Freely soluble in acetone; soluble in methanol; sparingly soluble in dehydrated alcohol; slightly soluble in water.

Mycophenolate Sodium: White to off-white crystalline powder. Slightly soluble in water; practically insoluble in 0.1 N hydrochloric acid.

Myristic Acid: Hard, white or faintly yellow, somewhat glossy, crystalline solid or white or yellow-white powder. Soluble in alcohol, in chloroform, and in ether; practically insoluble in water. *NF category:* Antifoaming agent; emulsifying agent; lubricant.

Nabumetone: A white, or almost white, crystalline powder. Freely soluble in acetone; sparingly soluble in alcohol and in methanol; practically insoluble in water.

Nadolol: White to off-white, practically odorless, crystalline powder. Freely soluble in alcohol and in methanol; soluble in water at pH 2; slightly soluble in chloroform, in methylene chloride, in isopropyl alcohol, and in water (between pH 7 and pH 10); insoluble in acetone, in benzene, in ether, in hexane, and in trichloroethane.

Nafcillin Sodium: White to yellowish-white powder, having not more than a slight characteristic odor. Freely soluble in water and in chloroform; soluble in alcohol.

Nalidixic Acid: White to very pale yellow, odorless, crystalline powder. Soluble in chloroform, in methylene chloride, and in solutions of fixed alkali hydroxides and carbonates; slightly soluble in acetone, in alcohol, in methanol, and in toluene; very slightly soluble in ether and in water.

Naloxone Hydrochloride: White to slightly off-white powder. Its aqueous solution is acidic. Soluble in water, in dilute acids, and in strong alkali; slightly soluble in alcohol; practically insoluble in ether and in chloroform.

Naloxone Hydrochloride Injection: Clear, colorless liquid.

Nandrolone Decanoate: Fine, white to creamy white, crystalline powder. Is odorless, or may have a slight odor. Soluble in chloroform, in alcohol, in acetone, and in vegetable oils; practically insoluble in water.

Naphazoline Hydrochloride: White, crystalline powder. Freely soluble in water; soluble in alcohol.

Naproxen: White to off-white, practically odorless, crystalline powder. Soluble in chloroform, in dehydrated alcohol, and in alcohol; sparingly soluble in ether; practically insoluble in water.

Naproxen Sodium: White to creamy crystalline powder. Soluble in water and in methanol; sparingly soluble in alcohol; very slightly soluble in acetone; and practically insoluble in chloroform and in toluene. Melts at about 255°, with decomposition.

Narasin: White to off-white, crystalline powder. Melts at about 217°, with decomposition. Soluble in methanol and in water.

Naratriptan Hydrochloride: White to pale yellow solid. Soluble in water.

Natamycin: Off-white to cream-colored powder, which may contain up to 3 moles of water. Soluble in glacial acetic acid and in dimethylformamide; slightly soluble in methanol; practically insoluble in water.

Nateglinide: White powder. Freely soluble in methanol and in alcohol; soluble in ether; sparingly soluble in acetonitrile and in octanol; practically insoluble in water.

Nefazodone Hydrochloride: Nonhygroscopic, white powder. Freely soluble in chloroform; soluble in propylene glycol; slightly soluble in polyethylene glycol and in water.

Add the following:

▲**Neohesperidin Dihydrochalcone:** White or yellowish-white powder. Freely soluble in dimethyl sulfoxide; soluble in methanol; practically insoluble in water and in methylene chloride. *NF category:* Sweetening agent; flavors and fragrance.▲ (NF 1-May-2020)

Neomycin Sulfate: White to slightly yellow powder, or cryodesiccated solid. Is odorless or practically so and is hygroscopic. Its solutions are dextrorotatory. Freely soluble in water; very slightly soluble in alcohol; insoluble in acetone, in chloroform, and in ether.

Netilmicin Sulfate: White to pale yellowish-white powder. Freely soluble in water; practically insoluble in dehydrated alcohol and in ether.

Nevirapine: White to off-white, odorless to nearly odorless, crystalline powder. Slightly soluble in alcohol and in methanol; practically insoluble in water. Hydrous form also slightly soluble in propylene glycol.

Niacin: White crystals or crystalline powder. Is odorless, or has a slight odor. Melts at about 235°. Freely soluble in boiling water, in boiling alcohol, and in solutions of alkali hydroxides and carbonates; sparingly soluble in water; practically insoluble in ether.

Niacinamide: White, crystalline powder. Is odorless or practically so, and has a bitter taste. Its solutions are neutral to litmus. Freely soluble in water and in alcohol; soluble in glycerin.

Nifedipine: Yellow powder. Is affected by exposure to light. Freely soluble in acetone; practically insoluble in water.

Nilotinib: White to slightly yellowish or slightly greenish-yellowish solid. Decomposes prior to melting. Slightly soluble in ethanol, in methanol, and in isopropanol; very slightly soluble in water.

Nilutamide: White or almost-white powder. Freely soluble in acetone and in ethyl acetate; soluble in methanol and in

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dichloromethane; sparingly soluble in absolute alcohol; very slightly soluble in water.

Nimodipine: Light yellow or yellow, crystalline powder, affected by light. Freely soluble in ethyl acetate; sparingly soluble in alcohol; practically insoluble in water. Exhibits polymorphism.

Nitric Acid: Highly corrosive fuming liquid, having a characteristic, highly irritating odor. Stains animal tissues yellow. Boils at about 120°. Specific gravity is about 1.41. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Nitrofurantoin: Lemon-yellow, odorless crystals or fine powder. Has a bitter aftertaste. Soluble in dimethylformamide; very slightly soluble in water and in alcohol.

Nitrofurazone: Lemon yellow, odorless, crystalline powder. Darkens slowly on exposure to light. Melts at about 236°, with decomposition. Soluble in dimethylformamide; slightly soluble in propylene glycol and in polyethylene glycol mixtures; very slightly soluble in alcohol and in water; practically insoluble in chloroform and in ether.

Nitrofurazone Ointment: Yellow, opaque, and water-miscible, and has ointment-like consistency.

Nitrofurazone Topical Solution: Light yellow, clear, somewhat viscous liquid, having a faint characteristic odor. Miscible with water.

Nitrogen: Colorless, odorless, tasteless gas. Is nonflammable and does not support combustion. One L at 0° and at a pressure of 760 mm of mercury weighs about 1.251 g. One volume dissolves in about 65 volumes of water and in about 9 volumes of alcohol at 20° and at a pressure of 760 mm of mercury. *NF category:* Air displacement; propellant.

Diluted Nitroglycerin: When diluted with lactose, it is a white, odorless powder. When diluted with propylene glycol or alcohol, it is a clear, colorless, or pale yellow liquid. [NOTE—Undiluted nitroglycerin occurs as a white to pale yellow, thick, flammable, explosive liquid.] Undiluted nitroglycerin is soluble in methanol, in alcohol, in carbon disulfide, in acetone, in ethyl ether, in ethyl acetate, in glacial acetic acid, in benzene, in toluene, in nitrobenzene, in phenol, in chloroform, and in methylene chloride; slightly soluble in water.

Nitromersol: Brownish yellow to yellow granules or brownish yellow to yellow powder. Is odorless and tasteless and is affected by light. Soluble in solutions of alkalies and of ammonia by opening of the anhydride ring and the formation of a salt; very slightly soluble in water, in alcohol, in acetone, and in ether.

Nitromersol Topical Solution: Clear, reddish-orange solution. Is affected by light.

Nitrous Oxide: Colorless gas, without appreciable odor or taste. One L at 0° and at a pressure of 760 mm of mercury weighs about 1.97 g. One volume dissolves in about 1.4 volumes of water at 20° and at a pressure of 760 mm of mercury. Freely soluble in alcohol; soluble in ether and in oils. *NF category:* Propellant.

Nizatidine: Off-white to buff crystalline solid. Freely soluble in chloroform; soluble in methanol; sparingly soluble in water.

Nonoxynol 9: Clear, colorless to light yellow, viscous liquid. Soluble in water, in alcohol, and in corn oil. *NF category:* Wetting and/or solubilizing agent.

Norepinephrine Bitartrate: White or faintly gray, odorless, crystalline powder. Slowly darkens on exposure to air and light. Its solutions are acid to litmus, having a pH of about 3.5. Freely soluble in water; slightly soluble in alcohol; practically insoluble in chloroform and in ether. Melts between 98° and 104°, without previous drying of the specimen, the melt being turbid.

Norepinephrine Bitartrate Injection: Colorless or practically colorless liquid, gradually turning dark on exposure to air and light.

Norethindrone: White to creamy white, odorless, crystalline powder. Is stable in air. Soluble in chloroform and in dioxane; sparingly soluble in alcohol; slightly soluble in ether; practically insoluble in water.

Norethindrone Acetate: White to creamy white, odorless, crystalline powder. Very soluble in chloroform; freely soluble in dioxane; soluble in ether and in alcohol; practically insoluble in water.

Norfloxacin: White to pale yellow, crystalline powder. Sensitive to light and moisture. Freely soluble in acetic acid; sparingly soluble in chloroform; slightly soluble in acetone, in water, and in alcohol; very slightly soluble in methanol and in ethyl acetate; insoluble in ether.

Norgestimate: White to pale yellow powder. Very to freely soluble in methylene chloride; sparingly soluble in acetonitrile; insoluble in water.

Norgestrel: White or practically white, practically odorless, crystalline powder. Freely soluble in chloroform; sparingly soluble in alcohol; insoluble in water.

Norelgestromin: White to off-white powder. Freely soluble in acetone, in ethanol, in methanol, and in dimethylformamide; sparingly soluble in methylene chloride and in iso-propanol; slightly soluble in acetonitrile; practically insoluble in water.

Nortriptyline Hydrochloride: White to off-white powder, having a slight, characteristic odor. Its solution (1 in 100) has a pH of about 5. Soluble in water and in chloroform; sparingly soluble in methanol; practically insoluble in ether, in benzene, and in most other organic solvents.

Noscapine: Fine, white or practically white, crystalline powder. Freely soluble in chloroform; soluble in acetone; slightly soluble in alcohol and in ether; practically insoluble in water.

Novobiocin Calcium: White or yellowish-white, odorless, crystalline powder. Freely soluble in alcohol and in methanol; sparingly soluble in acetone and in butyl acetate; slightly soluble in water and in ether; very slightly soluble in chloroform.

Novobiocin Sodium: White or yellowish-white, odorless, hygroscopic, crystalline powder. Freely soluble in water, in alcohol, in methanol, in glycerin, and in propylene glycol; slightly soluble in butyl acetate; practically insoluble in acetone, in chloroform, and in ether.

Nystatin: Yellow to light tan powder. Is hygroscopic, and is affected by long exposure to light, heat, and air. Freely soluble in dimethylformamide and in dimethyl sulfoxide; very slightly soluble in methanol; practically insoluble or insoluble in water, in alcohol, in *n*-propyl alcohol, in *n*-butyl alcohol, in chloroform, and in ether.

Octoxynol 9: Clear, pale yellow, viscous liquid, having a faint odor and a bitter taste. Soluble in benzene and in toluene; practically insoluble in solvent hexane. Miscible with water, with alcohol, and with acetone. *NF category:* Wetting and/or solubilizing agent.

Octyldodecanol: Clear water-white, free-flowing liquid. Soluble in alcohol and in ether; insoluble in water. *NF category:* Vehicle (oleaginous); emollient; emulsifying agent.

Octyl Methoxycinnamate: Pale yellow oil. Insoluble in water.

Ofloxacin: Pale yellowish-white to light yellowish-white crystals or crystalline powder. Sparingly soluble in chloroform; slightly soluble in alcohol, in methanol, and in water.

Hydrophilic Ointment:*NF category:* Ointment base.

White Ointment:*NF category:* Ointment base.

Yellow Ointment:*NF category:* Ointment base.

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Olanzapine: A yellow crystalline solid. Soluble in *n*-propanol; sparingly soluble in acetonitrile; slightly soluble in methanol and in dehydrated alcohol; practically insoluble in water.

Oleic Acid: Colorless to pale yellow, oily liquid when freshly prepared, but on exposure to air it gradually absorbs oxygen and darkens. Has a characteristic, lard-like odor and taste. When strongly heated in air, it is decomposed with the production of acrid vapors. Practically insoluble in water. Miscible with alcohol, with chloroform, with ether, with benzene, and with fixed and volatile oils. *NF category:* Emulsifying agent.

Oleovitamin A and D: Yellow to red, oily liquid, practically odorless or having a fish-like odor, and having no rancid odor or taste. Is a clear liquid at temperatures exceeding 65°, and may crystallize on cooling. Is unstable in air and in light. Very soluble in ether and in chloroform; soluble in dehydrated alcohol and in vegetable oils; insoluble in water and in glycerin.

Oleovitamin A and D Capsules: The oil contained in Oleovitamin A and D Capsules is a yellow to red, oily liquid, practically odorless or having a fish-like odor, and having no rancid odor or taste. Is a clear liquid at temperatures exceeding 65°, and may crystallize on cooling. Is unstable in air and in light.

Oleoyl Polyoxylglycerides: Amber, oily liquids. May develop deposit after prolonged storage at 20°. Freely soluble in methylene chloride; practically insoluble but dispersible in water. *NF category:* Ointment base; solvent; emulsifying agent; wetting and/or solubilizing agent.

Oleyl Alcohol: Clear, colorless to light yellow, oily liquid. Has a faint characteristic odor and a bland taste. Soluble in alcohol, in ether, in isopropyl alcohol, and in light mineral oil; insoluble in water. *NF category:* Emollient; emulsifying agent.

Oleyl Oleate: Clear, colorless to light yellow liquid. Has a faint characteristic odor. Slightly soluble in alcohol. Miscible with chloroform and with ether. *NF category:* Emollient; emulsifying agent.

Olive Oil: Pale yellow, or light greenish-yellow, oily liquid, having a slight, characteristic odor and taste, with a faintly acrid aftertaste. Slightly soluble in alcohol. Miscible with ether, with chloroform, and with carbon disulfide. *Specific Gravity* ⟨841⟩: Between 0.910 and 0.915. *NF category:* Methacrylic Acid and Vehicle (oleaginous).

Olmesartan Medoxomil: White to off-white crystalline powder. Sparingly soluble in methanol; practically insoluble in water.

Olopatadine Hydrochloride: White crystalline powder. Very soluble in formic acid; sparingly soluble in water; very slightly soluble in dehydrated alcohol.

Omeprazole: White to off-white powder. Soluble in dichloromethane; sparingly soluble in methanol and in alcohol; very slightly soluble in water.

Omeprazole Magnesium: White to off-white powder. Sparingly soluble in methanol; slightly soluble in alcohol; very slightly soluble in water and in dichloromethane.

Ondansetron: White to off-white powder. Very soluble in acid solutions; sparingly soluble in water.

Ondansetron Hydrochloride: White to off-white powder. Soluble in methanol; sparingly soluble in water and in alcohol; slightly soluble in isopropyl alcohol and in dichloromethane; very slightly soluble in acetone, in chloroform, and in ethyl acetate.

Opium: Has a very characteristic odor and a very bitter taste.

Powdered Opium: Light brown or moderately yellowish-brown powder.

Orbifloxacin: White to pale yellow crystals or crystalline powder. Odorless. Soluble in acetic acid; very slightly soluble in methanol, in water, and in chloroform; practically insoluble in ethanol and in diethyl ether.

Orlistat: White to off-white fine powder or fine powder with lumps. Freely soluble in chloroform; very soluble in methanol and in alcohol; practically insoluble in water.

Orphenadrine Citrate: White, practically odorless, crystalline powder, having a bitter taste. Sparingly soluble in water; slightly soluble in alcohol; insoluble in chloroform, in benzene, and in ether.

Oseltamivir Phosphate: White to off-white powder. Freely soluble in water; soluble in methanol, in dimethyl sulfoxide, and in propylene glycol; sparingly soluble in dimethylformamide; slightly soluble in alcohol; very slightly soluble in isopropyl alcohol and in polyethylene glycol 400; practically insoluble in acetonitrile, in acetone, in dichloromethane, and in *n*-hexane.

Oxacillin Sodium: Fine, white, crystalline powder, odorless or having a slight odor. Freely soluble in water, in methanol, and in dimethyl sulfoxide; slightly soluble in absolute alcohol, in chloroform, in pyridine, and in methyl acetate; insoluble in ethyl acetate, in ether, in benzene, and in ethylene chloride.

Oxacillin Sodium for Injection: Fine, white, crystalline powder, odorless or having a slight odor. Freely soluble in water, in methanol, and in dimethyl sulfoxide; slightly soluble in absolute alcohol, in chloroform, in pyridine, and in methyl acetate; insoluble in ethyl acetate, in ether, in benzene, and in ethylene chloride.

Oxaliplatin: White to off-white crystalline powder. Slightly soluble in water; very slightly soluble in methanol; practically insoluble in alcohol.

Oxandrolone: White, odorless, crystalline powder. Is stable in air, but darkens on exposure to light. Melts at about 225°. Freely soluble in chloroform; sparingly soluble in alcohol and in acetone; practically insoluble in water.

Oxaprozin: White to yellowish-white, crystalline powder.

Oxazepam: Creamy white to pale yellow powder. Is practically odorless. Slightly soluble in alcohol and in chloroform; very slightly soluble in ether; practically insoluble in water.

Oxcarbazepine: Light orange to creamish white or off-white powder. Soluble in acetic acid; sparingly soluble in chloroform; practically insoluble in water.

Oxfendazole: White or almost white powder. Slightly soluble in alcohol and in methylene chloride; practically insoluble in water.

Oxprenolol Hydrochloride: White, crystalline powder. Freely soluble in alcohol, in chloroform, and in water; sparingly soluble in acetone; practically insoluble in ether.

Oxtriphylline: White, crystalline powder, having an amine-like odor. A solution (1 in 100) has a pH of about 10.3. Freely soluble in water and in alcohol; very slightly soluble in chloroform.

Oxybenzone: Pale yellow powder. Freely soluble in alcohol and in toluene; practically insoluble in water.

Oxybutynin Chloride: White, crystalline, practically odorless powder. Very soluble in methanol and in chloroform; freely soluble in water and in alcohol; soluble in acetone; slightly soluble in ether; very slightly soluble in hexane.

Oxycodone Hydrochloride: White to off-white, hygroscopic crystals or powder. Is odorless. Soluble in water; slightly soluble in alcohol.

Oxygen: Colorless, odorless, tasteless gas, which supports combustion more energetically than does air. One L at 0° and at a pressure of 760 mm of mercury weighs about 1.429 g. One volume dissolves in about 32 volumes of water and in about 7 volumes of alcohol at 20° and at a pressure of 760 mm of mercury.

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Oxymetazoline Hydrochloride: White to practically white, fine crystalline powder. Is hygroscopic. Melts at about 300°, with decomposition. Soluble in water and in alcohol; practically insoluble in benzene, in chloroform, and in ether.

Oxymetholone: White to creamy white, crystalline powder. Is odorless, and is stable in air. Freely soluble in chloroform; soluble in dioxane; sparingly soluble in alcohol; slightly soluble in ether; practically insoluble in water.

Oxymorphone Hydrochloride: White or slightly off-white, odorless powder. Darkens on exposure to light. Its aqueous solutions are slightly acidic. Freely soluble in water; sparingly soluble in alcohol and in ether.

Oxyquinoline Sulfate: Yellow powder. Melts at about 185°. Very soluble in water; freely soluble in methanol; slightly soluble in alcohol; practically insoluble in acetone and in ether. *NF category:* Chelating and/or complexing agent.

Oxytetracycline: Pale yellow to tan, odorless, crystalline powder. Is stable in air, but exposure to strong sunlight causes it to darken. It loses potency in solutions of pH below 2, and is rapidly destroyed by alkali hydroxide solutions. Freely soluble in 3 N hydrochloric acid and in alkaline solutions; sparingly soluble in alcohol; very slightly soluble in water.

Oxytetracycline Calcium: Yellow to light brown, crystalline powder. Insoluble in water.

Oxytetracycline Hydrochloride: Yellow, odorless, crystalline powder, having a bitter taste. Is hygroscopic. Decomposes at a temperature exceeding 180°, and exposure to strong sunlight or to temperatures exceeding 90° in moist air causes it to darken. Its potency is diminished in solutions having a pH below 2, and is rapidly destroyed by alkali hydroxide solutions. Freely soluble in water, but crystals of oxytetracycline base separate as a result of partial hydrolysis of the hydrochloride; sparingly soluble in alcohol and in methanol, and even less soluble in dehydrated alcohol; insoluble in chloroform and in ether.

Paclitaxel: White to off-white powder. Soluble in alcohol; insoluble in water.

Padimate O: A light yellow, mobile liquid having a faint, aromatic odor. Soluble in alcohol, in isopropyl alcohol, and in mineral oil; practically insoluble in water, in glycerin, and in propylene glycol.

Paliperidone: White to yellow powder. Sparingly soluble in 0.1 N hydrochloric acid and in methylene chloride; slightly soluble in *N,N*-dimethylformamide and in tetrahydrofuran; practically insoluble in water, in 0.1 N sodium hydroxide, and in hexane.

Palm Oil: White to yellowish, fatty solid to semisolid. Insoluble in water. *NF category:* Coating agent; emulsifying agent.

Hydrogenated Palm Oil: White to yellowish, fatty solid to semi-solid. Freely soluble in ether; very slightly soluble in alcohol; practically insoluble in water. *NF category:* Coating agent; lubricant; wet binder.

Palm Kernel Oil: White to yellowish, fatty solid. Insoluble in water. *NF category:* Coating agent; emulsifying agent; suppository base.

Palmitic Acid: Hard, white or faintly yellow, somewhat glossy crystalline solid, or white or yellowish-white powder. It has a slight characteristic odor and taste. Soluble in alcohol, in ether, and in chloroform; practically insoluble in water. *NF category:* Antifoaming agent; emulsifying agent; lubricant.

Palonosetron Hydrochloride: White to off-white, crystalline powder. Freely soluble in water; soluble in propylene glycol; slightly soluble in ethanol; very slightly soluble to slightly soluble in isopropyl alcohol.

Pamidronate Disodium: White, crystalline powder. Soluble in water and in 2 N sodium hydroxide; sparingly soluble in 0.1 N hydrochloric acid and in 0.1 N acetic acid; practically insoluble in organic solvents.

Pancreatin: Cream-colored, amorphous powder, having a faint, characteristic, but not offensive odor. It hydrolyzes fats to glycerol and fatty acids, changes protein into proteoses and derived substances, and converts starch into dextrins and sugars. Its greatest activities are in neutral or faintly alkaline media; more than traces of mineral acids or large amounts of alkali hydroxides make it inert. An excess of alkali carbonate also inhibits its action.

Pancrelipase: Cream-colored, amorphous powder, having a faint, characteristic, but not offensive odor. Pancrelipase hydrolyzes fats to glycerol and fatty acids, changes protein into proteoses and derived substances, and converts starch into dextrins and sugars. Its greatest activities are in neutral or faintly alkaline media; more than traces of mineral acids or large amounts of alkali hydroxides make it inert. An excess of alkali carbonate also inhibits its action.

Pancrelipase Capsules: The contents of Capsules conform to the *Description* under *Pancrelipase*, except that the odor may vary with the flavoring agent used.

Pancuronium Bromide: White, yellowish-white, or slightly pink, crystalline powder. Is hygroscopic. Freely soluble in water, in methylene chloride, and in alcohol.

Panthenol: White to creamy white, crystalline powder having a slight, characteristic odor. Freely soluble in water, in alcohol, and in propylene glycol; soluble in chloroform and in ether; slightly soluble in glycerin.

Pantoprazole Sodium: White to off-white powder. Freely soluble in water, in methanol, and in dehydrated alcohol; practically insoluble in hexane and in dichloromethane.

Papain: White to light tan, amorphous powder. Soluble in water, the solution being colorless to light yellow and more or less opalescent; practically insoluble in alcohol, in chloroform, and in ether.

Papaverine Hydrochloride: White crystals or white, crystalline powder. Is odorless, and has a slightly bitter taste. Is optically inactive. Its solutions are acid to litmus. Melts at about 220°, with decomposition. Soluble in water and in chloroform; slightly soluble in alcohol; practically insoluble in ether.

Parachlorophenol: White or pink crystals having a characteristic phenolic odor. When undiluted, it whitens and cauterizes the skin and mucous membranes. Melts at about 42°. Very soluble in alcohol, in glycerin, in chloroform, in ether, and in fixed and volatile oils; soluble in petrolatum; sparingly soluble in water and in liquid petrolatum.

Paraffin: Colorless or white, more or less translucent mass showing a crystalline structure. Is odorless and tasteless, and is slightly greasy to the touch. Freely soluble in chloroform, in ether, in volatile oils, and in most warm fixed oils; slightly soluble in dehydrated alcohol; insoluble in water and in alcohol. *NF category:* Stiffening agent; ointment base.

Synthetic Paraffin: Very hard, white, practically tasteless and odorless wax. Contains mostly long-chain, unbranched, saturated hydrocarbons, with a small amount of branched hydrocarbons. Is represented by the formula C_nH_{2n2}, in which *n* may range from 20 to about 100. The average molecular weight may range from 400 to 1400. Slightly soluble in aromatic and normal paraffinic solvents; very slightly soluble in aliphatic, oxygenated, and halogenated hydrocarbon solvents; insoluble in water. *NF category:* Stiffening agent.

Paraldehyde: Colorless, transparent liquid. Has a strong, characteristic but not unpleasant or pungent odor, and a disagreeable taste. Specific gravity is about 0.99. Soluble in water, but less soluble in boiling water. Miscible with alcohol, with chloroform, with ether, and with volatile oils.

Paricalcitol: White to almost white powder. Soluble in alcohol; insoluble in water.

Paromomycin Sulfate: Creamy white to light yellow powder. Is odorless or practically odorless, and is very

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hygroscopic. Very soluble in water; insoluble in alcohol, in chloroform, and in ether.

Paroxetine Hydrochloride: White to off-white solid. Soluble in methanol and in alcohol; slightly soluble in water.

Peanut Oil: Colorless or pale yellow, oily liquid with a bland taste. May have a characteristic, nutty odor. Very slightly soluble in alcohol. Miscible with ether, with chloroform, and with carbon disulfide. *Specific Gravity* ⟨841⟩: Between 0.912 and 0.920. *Refractive Index* ⟨831⟩: Between 1.462 and 1.464 at 40°. *NF category:* Solvent; vehicle (oleaginous).

Pectin: Coarse or fine powder, yellowish-white in color, almost odorless, and having a mucilaginous taste. Soluble in 20 parts of water, forming a viscous, opalescent, colloidal solution that flows readily and is acid to litmus; practically insoluble in alcohol or in diluted alcohol and in other organic solvents. Pectin dissolves in water more readily if first moistened with alcohol, glycerin, or simple syrup, or if first mixed with 3 or more parts of sucrose. *NF category:* Suspending and/or viscosity-increasing agent; emulsifying agent; film-forming agent.

Pemetrexed Disodium: White or almost white powder. Freely soluble in water; very slightly soluble in dehydrated alcohol; practically insoluble in methylene chloride.

Penbutolol Sulfate: White to off-white, crystalline powder. Melts at about 217°, with decomposition. Soluble in water and in methanol.

Penicillamine: White or practically white, crystalline powder, having a slight, characteristic odor. Freely soluble in water; slightly soluble in alcohol; insoluble in chloroform and in ether.

Penicillin G Benzathine: White, odorless, crystalline powder. Sparingly soluble in alcohol; very slightly soluble in water.

Penicillin G Potassium: Colorless or white crystals, or white, crystalline powder. Is odorless or practically so, and is moderately hygroscopic. Its solutions are dextrorotatory. Its solutions retain substantially full potency for several days at temperatures below 15°, but are rapidly inactivated by acids, by alkali hydroxides, by glycerin, and by oxidizing agents. Very soluble in water, in saline TS, and in dextrose solutions; sparingly soluble in alcohol.

Penicillin G Procaine: White crystals or white, very fine, microcrystalline powder. Is odorless or practically odorless, and is relatively stable in air. Its solutions are dextrorotatory. Is rapidly inactivated by acids, by alkali hydroxides, and by oxidizing agents. Soluble in alcohol and in chloroform; slightly soluble in water.

Penicillin G Sodium: Colorless or white crystals or white to slightly yellow, crystalline powder. Is odorless or practically odorless, and is moderately hygroscopic. Its solutions are dextrorotatory. Is relatively stable in air, but is inactivated by prolonged heating at about 100°, especially in the presence of moisture. Its solutions lose potency fairly rapidly at room temperature, but retain substantially full potency for several days at temperatures below 15°. Its solutions are rapidly inactivated by acids, by alkali hydroxides, by oxidizing agents, and by penicillinase.

Penicillin V: White, odorless, crystalline powder. Freely soluble in alcohol and in acetone; very slightly soluble in water; insoluble in fixed oils.

Penicillin V Benzathine: Practically white powder, having a characteristic odor. Sparingly soluble in chloroform; slightly soluble in alcohol and in ether; very slightly soluble in water.

Penicillin V Potassium: White, odorless, crystalline powder. Very soluble in water; slightly soluble in alcohol; insoluble in acetone.

Pentamidine Isethionate: White or almost white powder or colorless crystals, hygroscopic. Freely soluble in water; sparingly soluble in alcohol; practically insoluble in methylene chloride.

Pentazocine: White or very pale, tan-colored powder. Freely soluble in chloroform; soluble in alcohol, in acetone, and in ether; sparingly soluble in benzene and in ethyl acetate; practically insoluble in water.

Pentazocine Hydrochloride: White, crystalline powder. It exhibits polymorphism, one form melting at about 254° and the other at about 218°. Freely soluble in chloroform; soluble in alcohol; sparingly soluble in water; very slightly soluble in acetone and in ether; practically insoluble in benzene.

Pentetic Acid: White, odorless or almost odorless powder. Melts with foaming and degradation at 220°. *NF category:* Chelating and/or complexing agent; antimicrobial preservative; sequestering agent.

Pentobarbital: White to practically white, fine, practically odorless powder. May occur in a polymorphic form that melts at about 116°. This form gradually reverts to the more stable higher-melting form upon being heated at about 110°. Very soluble in alcohol, in methanol, in ether, in chloroform, and in acetone; soluble in benzene; very slightly soluble in water and in carbon tetrachloride.

Pentobarbital Sodium: White, crystalline granules or white powder. Is odorless or has a slight characteristic odor, and has a slightly bitter taste. Its solutions decompose on standing, heat accelerating the decomposition. Very soluble in water; freely soluble in alcohol; practically insoluble in ether.

Pentoxifylline: White to almost white crystalline powder. Freely soluble in chloroform and in methanol; soluble in water; sparingly soluble in alcohol; slightly soluble in ether.

Peppermint: Has an aromatic, characteristic odor and a pungent taste, and produces a cooling sensation in the mouth. *NF category:* Flavors and fragrance.

Peppermint Oil: Colorless or pale yellow liquid, having a strong, penetrating, characteristic odor and a pungent taste, followed by a sensation of cold when air is drawn into the mouth. *NF category:* Flavors and fragrance.

Peppermint Spirit: A clear, colorless liquid with a peppermint fragrance. Freely soluble in methanol and in diethyl ether; soluble in water. *NF category:* Flavors and fragrance.

Peppermint Water: *NF category:* Vehicle (flavored and/or sweetened).

Perflubron: Clear, colorless, practically odorless liquid.

Pergolide Mesylate: White to off-white powder. Sparingly soluble in methanol; slightly soluble in water, in dehydrated alcohol, and in chloroform; very slightly soluble in acetone; practically insoluble in ether.

Perindopril Erbumine: White or off-white, crystalline powder; freely soluble in water and in alcohol; sparingly soluble in methylene chloride.

Perphenazine: White to creamy white, odorless powder. Freely soluble in alcohol and in chloroform; soluble in acetone; practically insoluble in water.

Pertussis Immune Globulin: Transparent or slightly opalescent liquid, practically colorless, free from turbidity or particles, and practically odorless. May develop a slight, granular deposit during storage. Is standardized for agglutinating activity with the U.S. Standard Antipertussis Serum.

Petrolatum: Unctuous yellowish to light amber mass, having not more than a slight fluorescence even after being melted. Is transparent in thin layers. Is free or practically free from odor and taste. Freely soluble in benzene, in carbon disulfide, in chloroform, and in turpentine oil; soluble in ether, in solvent hexane, and in most fixed and volatile oils; practically insoluble in cold and hot alcohol and in cold dehydrated alcohol; insoluble in water. *NF category:* Ointment base; emollient.

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Hydrophilic Petrolatum: *NF category:* Ointment base.

White Petrolatum: White or faintly yellowish, unctuous mass, transparent in thin layers even after cooling to 0°. Freely soluble in benzene, in carbon disulfide, and in chloroform; soluble in ether, in solvent hexane, and in most fixed and volatile oils; slightly soluble in cold or hot alcohol, and in cold dehydrated alcohol; insoluble in water. *NF category:* Ointment base.

Phenazopyridine Hydrochloride: Light or dark red to dark violet, crystalline powder. Is odorless, or has a slight odor. Melts at about 235°, with decomposition. Slightly soluble in water, in alcohol, and in chloroform.

Phendimetrazine Tartrate: White, odorless, crystalline powder. Freely soluble in water; sparingly soluble in warm alcohol; insoluble in chloroform, in acetone, in ether, and in benzene. Phendimetrazine base is extracted by organic solvents from alkaline solution.

Phenelzine Sulfate: White to yellowish white powder, having a characteristic odor. Freely soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Pheniramine Maleate: White, crystalline powder having a faint amine-like odor. Soluble in water and in alcohol.

Phenmetrazine Hydrochloride: White to off-white, crystalline powder. Very soluble in water; freely soluble in alcohol and in chloroform.

Phenobarbital: White, odorless, glistening, small crystals, or white, crystalline powder, which may exhibit polymorphism. Is stable in air. Its saturated solution has a pH of about 5. Soluble in alcohol, in ether, and in solutions of fixed alkali hydroxides and carbonates; sparingly soluble in chloroform; very slightly soluble in water.

Phenobarbital Sodium: Flaky crystals, or white, crystalline granules, or white powder. Is odorless, has a bitter taste, and is hygroscopic. Its solutions are alkaline to phenolphthalein TS, and decompose on standing. Very soluble in water; soluble in alcohol; practically insoluble in ether and in chloroform.

Phenol: Colorless to light pink, interlaced or separate, needle-shaped crystals, or white to light pink, crystalline mass. Has a characteristic odor. Is liquefied by warming and by the addition of 10% of water. Boils at about 182°, and its vapor is flammable. Gradually darkens on exposure to light and air. Very soluble in alcohol, in glycerin, in chloroform, in ether, and in fixed and volatile oils; soluble in water; sparingly soluble in mineral oil. *NF category:* Antimicrobial preservative.

Liquefied Phenol: Colorless to pink liquid, which may develop a red tint upon exposure to air or light. Has a characteristic, somewhat aromatic odor. It whitens and cauterizes the skin and mucous membranes. Specific gravity is about 1.065. Miscible with alcohol, with ether, and with glycerin. A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

Camphorated Phenol Topical Gel: Clear, colorless, oily gel.

Phenolsulfonphthalein: A bright-red to dark-red, crystalline powder. Slightly soluble in alcohol; very slightly soluble in water.

Phenoxyethanol: A colorless, slightly viscous liquid. Slightly soluble in water, in peanut oil, and in olive oil. Miscible with acetone, with alcohol, and with glycerol. *NF category:* Antimicrobial preservative.

Phensuximide: White to off-white, crystalline powder. Is odorless, or has not more than a slight odor. Very soluble in chloroform; soluble in alcohol; slightly soluble in water.

Phentermine Hydrochloride: White, odorless, hygroscopic, crystalline powder. Soluble in water and in the lower alcohols; slightly soluble in chloroform; insoluble in ether.

Phentolamine Mesylate: White or off-white, odorless, crystalline powder. Its solutions are acid to litmus, having a pH of about 5, and slowly deteriorate. Melts at about 178°. Freely soluble in water and in alcohol; slightly soluble in chloroform.

Phenylalanine: White, odorless crystals, having a slightly bitter taste. Sparingly soluble in water; very slightly soluble in methanol, in alcohol, and in dilute mineral acids.

Phenylbenzimidazole Sulfonic Acid: White to ivory-colored, odorless powder. Soluble in alcohol; practically insoluble in oily solvents and in water. Its salts are freely soluble in water.

Phenylbutazone: White to off-white, odorless, crystalline powder. Freely soluble in acetone and in ether; soluble in alcohol; very slightly soluble in water.

Phenylephrine Bitartrate: White or almost white powder or colorless crystals. Freely soluble in water.

Phenylephrine Hydrochloride: White or practically white, odorless crystals, having a bitter taste. Freely soluble in water and in alcohol.

Phenylephrine Hydrochloride Nasal Solution: Clear, colorless or slightly yellow, odorless liquid. Is neutral or acid to litmus.

Phenylephrine Hydrochloride Ophthalmic Solution: Clear, colorless or slightly yellow liquid, depending on the concentration.

Phenylethyl Alcohol: Colorless liquid, having a rose-like odor and a sharp, burning taste. Very soluble in alcohol, in fixed oils, in glycerin, and in propylene glycol; sparingly soluble in water; slightly soluble in mineral oil. *NF category:* Antimicrobial preservative.

Phenylmercuric Acetate: White to creamy white, crystalline powder, or small white prisms or leaflets. Is odorless. Soluble in alcohol and in acetone; slightly soluble in water. *NF category:* Antimicrobial preservative.

Phenylmercuric Nitrate: White, crystalline powder. Is affected by light. Its saturated solution is acid to litmus. Slightly soluble in alcohol and in glycerin; very slightly soluble in water. It is more soluble in the presence of either nitric acid or alkali hydroxides. *NF category:* Antimicrobial preservative.

Phenylpropanolamine Bitartrate: White, crystalline powder.

Phenylpropanolamine Hydrochloride: White, crystalline powder, having a slight aromatic odor. Is affected by light. Freely soluble in water and in alcohol; insoluble in ether.

Phenyltoloxamine Citrate: White, crystalline powder. Very soluble in boiling water; slightly soluble in cold water and in alcohol; practically insoluble in cold acetone, in ethyl ether, and in toluene.

Phenytoin: White, odorless powder. Melts at about 295°. Soluble in hot alcohol; slightly soluble in cold alcohol, in chloroform, and in ether; practically insoluble in water.

Phenytoin Sodium: White, odorless powder. Is somewhat hygroscopic and on exposure to air gradually absorbs carbon dioxide. Freely soluble in water, the solution usually being somewhat turbid due to partial hydrolysis and absorption of carbon dioxide; soluble in alcohol; practically insoluble in ether and in chloroform.

Sodium Phosphate P 32 Solution: Clear, colorless solution. Upon standing, both the Solution and the glass container may darken as a result of the effects of the radiation.

Phosphoric Acid: Colorless, odorless liquid of syrupy consistency. Specific gravity is about 1.71. Miscible with water and with alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Diluted Phosphoric Acid: Clear, colorless, odorless liquid. Specific gravity is about 1.057. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Physostigmine: White, odorless, microcrystalline powder. Acquires a red tint when exposed to heat, light, air, or contact with traces of metals. Melts at a temperature not lower than 103°. Very soluble in chloroform and in dichloromethane;

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freely soluble in alcohol; soluble in benzene and in fixed oils; slightly soluble in water.

Physostigmine Salicylate: White, shining, odorless crystals or white powder. Acquires a red tint when exposed to heat, light, air, or contact with traces of metals for long periods. Melts at about 184°. Freely soluble in chloroform; soluble in alcohol; sparingly soluble in water; slightly soluble in ether.

Physostigmine Sulfate: White, odorless, microcrystalline powder. Is deliquescent in moist air and acquires a red tint when exposed to heat, light, air, or contact with traces of metals for long periods. Melts at about 143°. Freely soluble in water; very soluble in alcohol; very slightly soluble in ether.

Phytonadione: Clear, yellow to amber, very viscous, odorless or practically odorless liquid, having a specific gravity of about 0.967. Is stable in air, but decomposes on exposure to sunlight. Soluble in dehydrated alcohol, in benzene, in chloroform, in ether, and in vegetable oils; slightly soluble in alcohol; insoluble in water.

Pilocarpine: A viscous, oily liquid, or crystals melting at about 34°. Exceedingly hygroscopic. Soluble in water, in alcohol, and in chloroform; sparingly soluble in ether and in benzene; practically insoluble in petroleum ether.

Pilocarpine Hydrochloride: Colorless, translucent, odorless, faintly bitter crystals. Is hygroscopic and is affected by light. Its solutions are acid to litmus. Very soluble in water; freely soluble in alcohol; slightly soluble in chloroform; insoluble in ether.

Pilocarpine Nitrate: Shining, white crystals. Is stable in air but is affected by light. Its solutions are acid to litmus. Freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform and in ether.

Pimozide: White, crystalline powder. Freely soluble in chloroform; slightly soluble in ether and in alcohol; insoluble in water.

Pindolol: White to off-white, crystalline powder, having a faint odor. Slightly soluble in methanol; very slightly soluble in chloroform; practically insoluble in water.

Pioglitazone Hydrochloride: White crystals or crystalline powder. Soluble in dimethylformamide; slightly soluble in dehydrated alcohol; very slightly soluble in acetone and in acetonitrile; practically insoluble in water; insoluble in ether.

Piperacillin: White to off-white, crystalline powder. Very soluble in methanol; slightly soluble in isopropyl alcohol; very slightly soluble in ethyl acetate and practically insoluble to very slightly soluble in water.

Piperacillin Sodium: White to off-white solid. Freely soluble in water and in alcohol.

Piperazine: White to slightly off-white lumps or flakes, having an ammoniacal odor. Soluble in water and in alcohol; insoluble in ether.

Piperazine Adipate: White crystalline powder. Soluble in water; practically insoluble in alcohol.

Piperazine Citrate: White, crystalline powder, having not more than a slight odor. Its solution (1 in 10) has a pH of about 5. Soluble in water; insoluble in alcohol and in ether.

Piperazine Dihydrochloride: White crystalline powder. Soluble in water.

Piperazine Phosphate: White crystalline powder. Sparingly soluble in water; practically insoluble in alcohol.

Piroxicam: Off-white to light tan or light yellow, odorless powder. Forms a monohydrate that is yellow. Slightly soluble in alcohol and in aqueous alkaline solutions; very slightly soluble in water, in dilute acids, and in most organic solvents.

Plantago Seed: All varieties are practically odorless and have a bland, mucilaginous taste.

Plicamycin: Yellow, odorless, hygroscopic, crystalline powder. Freely soluble in ethyl acetate; slightly soluble in water and in methanol; very slightly soluble in alcohol.

Podophyllum: Has a slight odor and a disagreeably bitter and acrid taste.

Podophyllum Resin: Amorphous powder, varying in color from light brown to greenish yellow, turning darker when subjected to a temperature exceeding 25° or when exposed to light. Has a slight, peculiar, faintly bitter taste. Its alcohol solution is acid to moistened litmus paper. Soluble in alcohol with a slight opalescence; partially soluble in ether and in chloroform.

Polacrilin Potassium: White to off-white, free-flowing powder. Has a faint odor or is odorless. Insoluble in water and in most liquids. *NF category:* Disintegrant.

Polidocanol: White, waxy solid in appearance and texture at 15°–20°. Polidocanol is hygroscopic. Soluble in water and in alcohol; practically insoluble in light petroleum.

Poliovirus Vaccine Inactivated: Clear, reddish-tinged or yellowish liquid, that may have a slight odor because of the preservative.

Poloxalene: Colorless or pale yellow liquid. Soluble in water, in chloroform, and in ethylene dichloride.

Poloxamer: *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant.

Poloxamer 124: Colorless liquid, having a mild odor. When solidified, it melts at about 16°. Freely soluble in water, in alcohol, in isopropyl alcohol, in propylene glycol, and in xylene.

Poloxamer 188: White, prilled or cast solid. Is odorless, or has a very mild odor. Melts at about 52°. Freely soluble in water and in alcohol.

Poloxamer 237: White, prilled or cast solid. Is odorless, or has a very mild odor. Melts at about 49°. Freely soluble in water and in alcohol; sparingly soluble in isopropyl alcohol and in xylene.

Poloxamer 338: White, prilled or cast solid. Is odorless, or has a very mild odor. Melts at about 57°. Freely soluble in water and in alcohol; sparingly soluble in propylene glycol.

Poloxamer 407: White, prilled or cast solid. Is odorless, or has a very mild odor. Melts at about 56°. Freely soluble in water, in alcohol, and in isopropyl alcohol.

Polycarbophil: White to creamy white granules, having a characteristic, ester-like odor. Swells in water to a range of volumes, depending primarily on the pH. Insoluble in water, in dilute acids, in dilute alkalies, and in common organic solvents. *NF category:* Emulsifying agent; suspending and/or viscosity-increasing agent; wet binder.

Hydrogenated Polydecene: Clear, colorless, odorless, tasteless liquid. Very slightly soluble in water. *NF category:* Emollient; ointment base; solvent; vehicle (oleaginous).

Polydextrose: Off-white to light tan-colored solid. Very soluble in water; soluble in alcohol; slightly soluble in glycerin and in propylene glycol. *NF category:* Bulking agent; humectant; coating agent; suspending and/or viscosity-increasing agent; diluent.

Hydrogenated Polydextrose: Off-white to light tan-colored solid. Very soluble in water; soluble in alcohol; slightly soluble in glycerin and in propylene glycol. *NF category:* Bulking agent; coating agent; humectant; suspending and/or viscosity-increasing agent; wet binder.

Polyethylene Glycol: Polyethylene Glycol is usually designated by a number that corresponds approximately to its average molecular weight. As the average molecular weight increases, the water solubility, vapor pressure, hygroscopicity, and solubility in organic solvents decrease, while congealing temperature, specific gravity, flash point, and viscosity increase. Liquid grades occur as clear to slightly hazy, colorless or practically colorless, slightly hygroscopic, viscous liquids, having a slight, characteristic odor, and a specific gravity at 25° of about 1.12. Solid grades occur as practically odorless and tasteless, white, waxy, plastic material having a

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consistency similar to beeswax, or as creamy white flakes, beads, or powders. The accompanying table states the approximate congealing temperatures that are characteristic of commonly available grades. Liquid grades are miscible with water; solid grades are freely soluble in water; and all are soluble in acetone, in alcohol, in chloroform, in ethylene glycol monoethyl ether, in ethyl acetate, and in toluene; all are insoluble in ether and in hexane. *NF category:* Coating agent; plasticizer; solvent; suppository base; lubricant; film-forming agent; ointment base; diluent.

Nominal Molecular Weight Polyethylene Glycol	Approximate Congealing Temperature (°)
300	–11
400	6
600	20
900	34
1000	38
1450	44
4500	58
8000	60

Polyethylene Glycol 3350: White granular, powder, or flake. Approximate congealing temperature: 56°. Freely soluble in water; soluble in acetone, in alcohol, in chloroform, in ethylene glycol monoethyl ether, in ethyl acetate, and in toluene; insoluble in ether and in hexane. *NF category:* Coating agent; diluent; film-forming agent; lubricant; ointment base; plasticizer; solvent; suppository base.

Polyethylene Glycol Monomethyl Ether: Polyethylene Glycol Monomethyl Ether is usually designated by a number that corresponds approximately to its average molecular weight. As the average molecular weight increases, the water solubility, vapor pressure, hygroscopicity, and solubility in organic solvents decrease, while congealing temperature, specific gravity, flash point, and viscosity increase. Liquid grades occur as clear to slightly hazy, colorless or practically colorless, slightly hygroscopic, viscous liquids, having a slight, characteristic odor, and a specific gravity at 25° of about 1.09–1.10. Solid grades occur as practically odorless and tasteless, white, waxy, plastic material having a consistency similar to beeswax, or as creamy white flakes, beads, or powders. The accompanying table states the approximate congealing temperatures that are characteristic of commonly available grades. Liquid grades are miscible with water; solid grades are freely soluble in water; and all are soluble in acetone, in alcohol, in chloroform, in ethylene glycol monoethyl ether, in ethyl acetate, and in toluene; all are insoluble in ether and in hexane. *NF category:* Ointment base; solvent; plasticizer.

Nominal Molecular Weight Polyethylene Glycol Monomethyl Ether	Approximate Congealing Temperature (°)
350	–7
550	17
750	28
1000	35
2000	51
5000	59
8000	60
10000	61

Polyethylene Oxide: Polyethylene oxide resins are high molecular weight polymers having the common structure:

$$(-O-CH_2CH_2-)_n$$

in which *n*, the degree of polymerization, varies from about 2000 to over 100,000. Polyethylene oxide, being a polyether, strongly hydrogen, bonds with water. It is nonionic and undergoes salting-out effects associated with neutral molecules in solutions of high dielectric media. Salting-out effects manifest themselves in depressing the upper temperature limit of solubility, and in reducing the viscosity of both dilute and concentrated solutions of the polymers. All molecular weight grades are powdered or granular solids. They are soluble in water but, because of the high solution viscosities obtained (see *table*), solutions over 1% in water may be difficult to prepare. The water solubility, hygroscopicity, solubility in organic solvents, and melting point do not vary in the specified molecular weight range. At room temperature polyethylene oxide is miscible with water in all proportions. At concentrations of about 20% polymer in water, the solutions are nontacky, reversible, elastic gels. At higher concentrations, the solutions are tough, elastic materials with the water acting as a plasticizer. Polyethylene oxide is also freely soluble in acetonitrile, in ethylene dichloride, in trichloroethylene, and in methylene chloride. Heating may be required to obtain solutions in many other organic solvents. It is insoluble in aliphatic hydrocarbons, in ethylene glycol, in diethylene glycol, and in glycerol. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; coating agent; release-modifying agent.

Approximate Molecular Weight	Typical Solution Viscosity (cps), 25°	
	5% Solution	1% Solution
100,000	40	
200,000	100	
300,000	800	
400,000	3000	
600,000	6000	
900,000	15,000	
4,000,000		3500
5,000,000		5500

Polyethylene 50 Stearate: *NF category:* Emulsifying and/or solubilizing agent.

Polyglyceryl 3 Diisostearate: Viscous liquid. Soluble in alcohol, in methylene chloride, in mineral oil, and in vegetable oils; insoluble in water. *NF category:* Emulsifying agent; ointment base.

Polyglyceryl Dioleate: Viscous liquid. Soluble in methylene chloride, in mineral oil, and in vegetable oils; sparingly soluble in alcohol; insoluble in water. *NF category:* Emulsifying agent.

Polyisobutylene: Low molecular-weight grades are soft and gummy; high molecular-weight grades are tough and elastic. All grades are light in color, odorless, and tasteless. Soluble in diisobutylene, in toluene, and in chloroform; insoluble in water. *NF category:* Adhesive.

Polymyxin B Sulfate: White to buff-colored powder. Is odorless or has a faint odor. Freely soluble in water; slightly soluble in alcohol.

Polyoxyl Lauryl Ether: A material with 3–5 oxyethylene units per molecule is a colorless liquid. Soluble or dispersible in alcohol; practically insoluble in water and in hexane. A

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material with 9–23 oxyethylene units per molecule is a white, waxy mass. Soluble or dispersible in water; soluble in alcohol; practically insoluble in hexane. *NF category:* Emulsifying agent; wetting and/or solubilizing agent.

Polyoxyl Oleate: A slightly yellowish, viscous liquid. Dispersible in water and in oils. Soluble in alcohol and in isopropyl alcohol. Miscible with fatty oils and with waxes. Its refractive index is about 1.466.

Polyoxyl 10 Oleyl Ether: White, soft semisolid, or pale yellow liquid, having a bland odor. Soluble in water and in alcohol. Dispersible in mineral oil and in propylene glycol, with possible separation on standing. *NF category:* Emulsifying agent; lubricant; wetting and/or solubilizing agent.

Polyoxyl 15 Hydroxystearate: Yellowish to white waxy mass. Very soluble in water; soluble in alcohol and in 2-propanol; insoluble in mineral oil. It solidifies at 25°. *NF category:* Wetting and/or solubilizing agent; vehicle (oleaginous); lubricant; emulsifying agent.

Polyoxyl 20 Cetostearyl Ether: Cream-colored, waxy, unctuous mass, melting, when heated, to a clear brownish-yellow liquid. Soluble in water, in alcohol, and in acetone; insoluble in solvent hexane. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant.

Polyoxyl 35 Castor Oil: Yellow, oily liquid, having a faint, characteristic odor and a somewhat bitter taste. Very soluble in water, producing a practically odorless and colorless solution; soluble in alcohol and in ethyl acetate; insoluble in mineral oils. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant.

Polyoxyl 40 Hydrogenated Castor Oil: White to yellowish paste or pasty liquid, having a faint odor and a slight taste. Very soluble in water, producing a practically tasteless, odorless, and colorless solution; soluble in alcohol and in ethyl acetate; insoluble in mineral oils. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant.

Polyoxyl 40 Stearate: Waxy, white to light tan solid. Is odorless or has a faint, fat-like odor. Soluble in water, in alcohol, in ether, and in acetone; insoluble in mineral oil and in vegetable oils. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant.

Polyoxyl Stearate: White or slightly yellowish waxy mass. Soluble in alcohol and in isopropyl alcohol. Polyoxyl stearate corresponding to a product with 6–8 units of ethylene oxide per molecule is soluble in fatty oils and in waxes; practically insoluble in water. Polyoxyl stearate corresponding to a product with 20–100 units of ethylene oxide per molecule is soluble in water; practically insoluble in fatty oils and in waxes. *NF category:* Emulsifying agent; wetting and/or solubilizing agent.

Polyoxyl Stearyl Ether: A white to yellowish-white, waxy, unctuous mass, pellets, microbeads, or flakes. Polyoxyl Stearyl Ether with 2 oxyethylene units per molecule is soluble in alcohol, with heating, and in methylene chloride; practically insoluble in water. Polyoxyl Stearyl Ether with 10 oxyethylene units per molecule is soluble in water and in alcohol. Polyoxyl Stearyl Ether with 20 oxethylene units per molecule is soluble in water, in alcohol, and in methylene chloride. After melting, it solidifies at about 45°. *NF category:* Emulsifying agent; wetting and/or solubilizing agent.

Add the following:

▲**Polypropylene Glycol 11 Stearyl Ether:** Colorless or pale yellow, clear or slightly turbid liquid. Soluble in ethanol (96%), in mineral oils, and in 2-propanol; practically insoluble in water, in propylene glycol, and in glycerol. Relative density: about 0.94 at 25°. Refractive index: about 1.448 at 25°. *NF category:* Emollient; solvent; vehicle.▲ (NF 1-May-2020)

Polysorbate 20: Lemon to amber liquid having a faint characteristic odor. Soluble in water, in alcohol, in ethyl acetate, in methanol, and in dioxane; insoluble in mineral oil. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant; suspending and/or viscosity-increasing agent.

Polysorbate 40: Yellow liquid having a faint, characteristic odor. Soluble in water and in alcohol; insoluble in mineral oil and in vegetable oils. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant; suspending and/or viscosity-increasing agent.

Polysorbate 60: Lemon- to orange-colored, oily liquid or semi-gel having a faint, characteristic odor. Soluble in water, in ethyl acetate, and in toluene; insoluble in mineral oil and in vegetable oils. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Polysorbate 80: Lemon- to amber-colored, oily liquid having a faint, characteristic odor and a warm, somewhat bitter taste. Very soluble in water, producing an odorless and practically colorless solution; soluble in alcohol and in ethyl acetate; insoluble in mineral oil. *NF category:* Wetting and/or solubilizing agent; emulsifying agent; lubricant; suspending and/or viscosity-increasing agent.

Polyvinyl Acetate: White or off-white powder or colorless granules or beads. Freely soluble in ethyl acetate; soluble in alcohol, in acetone, and in chloroform; practically insoluble in water. It is hygroscopic and swells in water. *NF category:* Coating agent; desiccant; wet binder; film-forming agent.

Polyvinyl Acetate Dispersion: Opaque, white or off-white, slightly viscous liquid. Miscible with water and with ethanol. It is sensitive to spoilage by microbial contaminants. *NF category:* Coating agent; film-forming agent; release-modifying agent.

Polyvinyl Acetate Phthalate: Free-flowing white powder. May have a slight odor of acetic acid. Soluble in methanol and in alcohol; insoluble in water, in methylene chloride, and in chloroform. *NF category:* Coating agent; film-forming agent.

Polyvinyl Alcohol: White to cream-colored granules, or white to cream-colored powder. Is odorless. Freely soluble in water at room temperature. Solution may be effected more rapidly at somewhat higher temperatures. *NF category:* Suspending and/or viscosity-increasing agent; coating agent; film-forming agent; polymers for ophthalmic use.

Sulfurated Potash: Irregular, liver-brown pieces when freshly made, changing to a greenish yellow. Has an odor of hydrogen sulfide and a bitter, acrid, and alkaline taste, and decomposes on exposure to air. A solution (1 in 10) is light brown in color and is alkaline to litmus. Freely soluble in water, usually leaving a slight residue. Alcohol dissolves only the sulfides.

Potassium Acetate: Colorless, monoclinic crystals or white, crystalline powder having a saline and slightly alkaline taste. Is odorless, or has a faint acetous odor. Deliquesces on exposure to moist air. Very soluble in water; freely soluble in alcohol.

Potassium Alginate: White to yellow, fibrous or granular powder. Dissolves in water to form a viscous, colloidal solution; insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than 30% by weight; insoluble in chloroform, in ether, and in acids having a pH lower than about 3. *NF category:* Emulsifying agent; suspending and/or viscosity-increasing agent.

Potassium Benzoate: White, odorless, or practically odorless, granular or crystalline powder. Is stable in air. Freely soluble in water; soluble in 90% alcohol; sparingly soluble in alcohol. *NF category:* Antimicrobial preservative; lubricant.

Potassium Bicarbonate: Colorless, transparent, monoclinic prisms or as a white, granular powder. Is odorless,

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and is stable in air. Its solutions are neutral or alkaline to phenolphthalein TS. Freely soluble in water; practically insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Potassium Bitartrate: Colorless or slightly opaque crystals, or white, crystalline powder. A saturated solution is acid to litmus. Soluble in boiling water; slightly soluble in water; very slightly soluble in alcohol.

Potassium Bromide: White, crystalline powder or colorless, cubical crystals. Freely soluble in water and in glycerol; slightly soluble in alcohol.

Potassium Chloride: Colorless, elongated, prismatic, or cubical crystals, or white, granular powder. Is odorless, has a saline taste, and is stable in air. Its solutions are neutral to litmus. Freely soluble in water; insoluble in alcohol. *NF category:* Tonicity agent.

Potassium Citrate: Transparent crystals or white, granular powder. Is odorless, has a cooling, saline taste, and is deliquescent when exposed to moist air. Freely soluble in water; very slightly soluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); chelating and/or complexing agent.

Potassium Gluconate: White to yellowish-white, crystalline powder or granules. Is odorless, has a slightly bitter taste, and is stable in air. Its solutions are slightly alkaline to litmus. Freely soluble in water; practically insoluble in dehydrated alcohol, in ether, in benzene, and in chloroform.

Potassium Hydroxide: White or practically white, fused masses, or small pellets, or flakes, or sticks, or other forms. Is hard and brittle and shows a crystalline fracture. Exposed to air, it rapidly absorbs carbon dioxide and moisture, and deliquesces. Very soluble in boiling alcohol; freely soluble in water, in alcohol, and in glycerin. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Potassium Iodide: Hexahedral crystals, either transparent and colorless or somewhat opaque and white, or a white, granular powder. Is slightly hygroscopic. Its solutions are neutral or alkaline to litmus. Very soluble in water and even more soluble in boiling water; freely soluble in glycerin; soluble in alcohol.

Potassium Iodide Oral Solution: Clear, colorless, odorless liquid, having a characteristic, strongly salty taste. Is neutral or alkaline to litmus. Specific gravity is about 1.70.

Potassium Metabisulfite: White or colorless, free-flowing crystals, crystalline powder, or granules, usually having an odor of sulfur dioxide. Gradually oxidizes in air to the sulfate. Its solutions are acid to litmus. Soluble in water; insoluble in alcohol. *NF category:* Antioxidant; antimicrobial preservative.

Potassium Metaphosphate: White, odorless powder. Soluble in dilute solutions of sodium salts; insoluble in water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Potassium Nitrate: White, crystalline powder or colorless crystals. Very soluble in boiling water; freely soluble in water; soluble in glycerin; practically insoluble in alcohol.

Potassium Permanganate: Dark purple crystals, almost opaque by transmitted light and of a blue metallic luster by reflected light. Its color is sometimes modified by a dark bronze-like appearance. Is stable in air. Freely soluble in boiling water; soluble in water.

Dibasic Potassium Phosphate: Colorless or white, somewhat hygroscopic, granular powder. The pH of a solution (1 in 20) is about 8.5 to 9.6. Freely soluble in water; very slightly soluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Monobasic Potassium Phosphate: Colorless crystals or white, granular or crystalline powder. Is odorless, and is stable in air. The pH of a solution (1 in 100) is about 4.5. Freely soluble in water; practically insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Potassium Sodium Tartrate: Colorless crystals or white, crystalline powder, having a cooling, saline taste. As it effloresces slightly in warm, dry air, the crystals are often coated with a white powder. Freely soluble in water; practically insoluble in alcohol.

Potassium Sorbate: White crystals or powder, having a characteristic odor. Melts at about 270°, with decomposition. Freely soluble in water; soluble in alcohol. *NF category:* Antimicrobial preservative.

Povidone: White to slightly creamy white powder. Is hygroscopic. Freely soluble in water, in methanol, and in alcohol; slightly soluble in acetone; practically insoluble in ether. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; polymers for ophthalmic use.

Povidone-Iodine: Yellowish-brown to reddish-brown, amorphous powder, having a slight, characteristic odor. Its solution is acid to litmus. Soluble in water and in alcohol; practically insoluble in chloroform, in carbon tetrachloride, in ether, in solvent hexane, and in acetone.

Povidone-Iodine Topical Aerosol Solution: The liquid obtained from Povidone-Iodine Topical Aerosol Solution is transparent, having a reddish brown color.

Pralidoxime Chloride: White to pale-yellow, crystalline powder. Is odorless and is stable in air. Freely soluble in water.

Pramipexole Dihydrochloride: White to almost white crystalline powder. Freely soluble in water; soluble in methanol; slightly soluble in alcohol; practically insoluble in methylene chloride.

Pramoxine Hydrochloride: White to practically white, crystalline powder, having a numbing taste. May have a slight aromatic odor. The pH of a solution (1 in 100) is about 4.5. Freely soluble in water and in alcohol; soluble in chloroform; very slightly soluble in ether.

Prasugrel Hydrochloride: White to almost-white powder. Soluble in water; slightly soluble in acetonitrile; practically insoluble in ethyl acetate.

Pravastatin Sodium: White to yellowish white, hygroscopic powder. Freely soluble in water and in methanol; soluble in dehydrated alcohol; practically insoluble in acetonitrile and in chloroform.

Praziquantel: White or practically white, crystalline powder; odorless or having a faint characteristic odor. Freely soluble in alcohol and in chloroform; very slightly soluble in water.

Prazosin Hydrochloride: White to tan powder. Slightly soluble in water, in methanol, in dimethylformamide, and in dimethylacetamide; very slightly soluble in alcohol; practically insoluble in chloroform and in acetone.

Prednicarbate: White to almost white, crystalline powder. Freely soluble in acetone and in alcohol; sparingly soluble in propylene glycol; practically insoluble in water.

Prednisolone: White to practically white, odorless, crystalline powder. Melts at about 235°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Soluble in methanol and in dioxane; sparingly soluble in acetone and in alcohol; slightly soluble in chloroform; very slightly soluble in water.

Prednisolone Acetate: White to practically white, odorless, crystalline powder. Melts at about 235°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Slightly soluble in acetone, in alcohol, and in chloroform; practically insoluble in water.

Prednisolone Hemisuccinate: Fine, creamy white powder with friable lumps; practically odorless. Melts at about 205°, with decomposition. Freely soluble in alcohol; soluble in acetone; very slightly soluble in water.

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Prednisolone Sodium Phosphate: White or slightly yellow, friable granules or powder. Is odorless or has a slight odor. Is slightly hygroscopic. Freely soluble in water; soluble in methanol; slightly soluble in alcohol and in chloroform; very slightly soluble in acetone and in dioxane.

Prednisolone Sodium Succinate for Injection: Creamy white powder with friable lumps, having a slight odor.

Prednisolone Tebutate: White to slightly yellow, free-flowing powder, which may show some soft lumps. Is odorless or has not more than a moderate, characteristic odor. Is hygroscopic. Freely soluble in chloroform and in dioxane; soluble in acetone; sparingly soluble in alcohol and in methanol; very slightly soluble in water.

Prednisone: White to practically white, odorless, crystalline powder. Melts at about 230°, with some decomposition (see *Melting Range or Temperature* ⟨741⟩). Slightly soluble in alcohol, in chloroform, in dioxane, and in methanol; very slightly soluble in water.

Pregabalin: White to off-white, crystalline solid. Freely soluble in water and in both basic and acidic aqueous solutions.

Prilocaine: White or almost white powder or crystal aggregates. Very soluble in alcohol and in acetone; slightly soluble in water.

Prilocaine Hydrochloride: White, odorless, crystalline powder, having a bitter taste. Freely soluble in water and in alcohol; slightly soluble in chloroform; very slightly soluble in acetone; practically insoluble in ether.

Primaquine Phosphate: Orange-red, crystalline powder. Is odorless and has a bitter taste. Its solutions are acid to litmus. Melts at about 200°. Soluble in water; insoluble in chloroform and in ether.

Primidone: White, crystalline powder. Is odorless and has a slightly bitter taste. Slightly soluble in alcohol; very slightly soluble in water and in most organic solvents.

Probucol: White to off-white, crystalline powder. Freely soluble in chloroform and in *n*-propyl alcohol; soluble in alcohol and in solvent hexane; insoluble in water.

Probenecid: White or practically white, fine, crystalline powder. Is practically odorless. Soluble in dilute alkali, in chloroform, in alcohol, and in acetone; practically insoluble in water and in dilute acids.

Procainamide Hydrochloride: White to tan, crystalline powder. Is odorless. Its solution (1 in 10) has a pH between 5 and 6.5. Very soluble in water; soluble in alcohol; slightly soluble in chloroform; very slightly soluble in benzene and in ether.

Procainamide Hydrochloride Injection: Colorless, or having not more than a slight yellow color.

Procaine Hydrochloride: Small, white crystals or white, crystalline powder. Is odorless. Exhibits local anesthetic properties when placed on the tongue. Freely soluble in water; soluble in alcohol; slightly soluble in chloroform; practically insoluble in ether.

Procaine Hydrochloride Injection: Clear, colorless liquid.

Prochlorperazine: Clear, pale yellow, viscous liquid. Is sensitive to light. Freely soluble in alcohol, in chloroform, and in ether; very slightly soluble in water.

Prochlorperazine Edisylate: White to very light yellow, odorless, crystalline powder. Its solutions are acid to litmus. Freely soluble in water; very slightly soluble in alcohol; insoluble in ether and in chloroform.

Prochlorperazine Maleate: White or pale yellow, practically odorless, crystalline powder. Its saturated solution is acid to litmus. Slightly soluble in warm chloroform; practically insoluble in water and in alcohol.

Procyclidine Hydrochloride: White, crystalline powder, having a moderate, characteristic odor. Melts at about 225°, with decomposition. Soluble in water and in alcohol; insoluble in ether and in acetone.

Progesterone: White or creamy white, odorless, crystalline powder. Is stable in air. Soluble in alcohol, in acetone, and in dioxane; sparingly soluble in vegetable oils; practically insoluble in water.

Proguanil Hydrochloride: White, crystalline powder. Sparingly soluble in alcohol; slightly soluble in water; practically insoluble in methylene chloride.

Proline: White, odorless crystals, having a slightly sweet taste. Freely soluble in water and in absolute alcohol; insoluble in ether, in butanol, and in isopropanol.

Promazine Hydrochloride: White to slightly yellow, practically odorless, crystalline powder. It oxidizes upon prolonged exposure to air and acquires a blue or pink color. Freely soluble in water and in chloroform.

Promethazine Hydrochloride: White to faint yellow, practically odorless, crystalline powder. Slowly oxidizes, and acquires a blue color, on prolonged exposure to air. Freely soluble in water, in hot dehydrated alcohol, and in chloroform; practically insoluble in ether, in acetone, and in ethyl acetate.

Propafenone Hydrochloride: White powder. Soluble in methanol and in hot water; slightly soluble in alcohol and in chloroform; very slightly soluble in acetone; insoluble in diethyl ether and in toluene.

Propane: Colorless, flammable gas (boiling temperature is about –42°). One hundred volumes of water dissolves 6.5 volumes at 17.8° and 753 mm pressure; 100 volumes of anhydrous alcohol dissolves 790 volumes at 16.6° and 754 mm pressure; 100 volumes of ether dissolves 926 volumes at 16.6° and 757 mm pressure; 100 volumes of chloroform dissolves 1299 volumes at 21.6° and 757 mm pressure. Vapor pressure at 21° is about 10290 mm of mercury (108 psig). *NF category:* Propellant.

Propanediol: Clear, colorless, hygroscopic liquid. *Specific Gravity* ⟨841⟩, *Method I*: 1.040–1.065. Soluble in water, in alcohol, in methyl alcohol, in isopropyl alcohol, in butyl alcohol, in acetone, in propylene glycol, and miscible with many polar solvents. *NF category:* Humectant; solvent; wetting and/or solubilizing agent.

Propantheline Bromide: White or practically white crystals. Is odorless and has a bitter taste. Melts at about 160°, with decomposition. Very soluble in water, in alcohol, and in chloroform; practically insoluble in ether and in benzene.

Proparacaine Hydrochloride: White to off-white, or faintly buff-colored, odorless, crystalline powder. Its solutions are neutral to litmus. Soluble in water, in warm alcohol, and in methanol; insoluble in ether and in benzene.

Proparacaine Hydrochloride Ophthalmic Solution: Colorless or faint yellow solution.

Propionic Acid: Oily liquid having a slight pungent, rancid odor. Miscible with water and with alcohol and various other organic solvents. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative; antioxidant.

Propofol: Clear, colorless to slightly yellowish liquid. Very soluble in methanol and in ethanol; slightly soluble in cyclohexane and in isopropyl alcohol; very slightly soluble in water.

Propoxycaine Hydrochloride: White, odorless, crystalline solid, which discolors on prolonged exposure to light and air. The pH of a solution (1 in 50) is about 5.4. Freely soluble in water; soluble in alcohol; sparingly soluble in ether; practically insoluble in acetone and in chloroform.

Propoxyphene Hydrochloride: White, crystalline powder. Is odorless, and has a bitter taste. Freely soluble in water; soluble in alcohol, in chloroform, and in acetone; practically insoluble in benzene and in ether.

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Propoxyphene Napsylate: White powder, having essentially no odor, but having a bitter taste. Soluble in methanol, in alcohol, in chloroform, and in acetone; very slightly soluble in water.

Propranolol Hydrochloride: White to off-white, crystalline powder. Is odorless and has a bitter taste. Melts at about 164°. Soluble in water and in alcohol; slightly soluble in chloroform; practically insoluble in ether.

Propyl Gallate: White, crystalline powder having a very slight, characteristic odor. Freely soluble in alcohol; slightly soluble in water. *NF category:* Antioxidant.

Propylene Glycol: Clear, colorless, viscous liquid having a slight, characteristic taste. Is practically odorless. Absorbs moisture when exposed to moist air. Miscible with water, with acetone, and with chloroform. Soluble in ether and will dissolve many essential oils, but is immiscible with fixed oils. *NF category:* Humectant; plasticizer; solvent; antimicrobial preservative.

Propylene Glycol Alginate: White to yellowish fibrous or granular powder. Practically odorless and tasteless. Soluble in water, in solutions of dilute organic acids, and, depending on the degree of esterification, in hydroalcoholic mixture containing up to 60% by weight of alcohol to form stable, viscous colloidal solutions at a pH of 3. *NF category:* Suspending and/or viscosity-increasing agent; emulsifying agent.

Propylene Glycol Diacetate: Clear, colorless liquid having a mild, fruity odor. Soluble in water. *NF category:* Emulsifying agent; solvent.

Propylene Glycol Dicaprylate/Dicaprate: Clear, colorless or slightly yellow oily liquid at 20°. Soluble in fatty oils and in light petroleum; slightly soluble in dehydrated alcohol; practically insoluble in water. *NF category:* Emulsifying agent; vehicle.

Propylene Glycol Dilaurate: Clear, oily liquid at 20°. Colorless or slightly yellow. Very soluble in alcohol, in methanol, and in methylene chloride; practically insoluble in water. *NF category:* Emollient; emulsifying agent.

Propylene Glycol Monocaprylate: Clear, colorless, or slightly yellow, oily liquid at 20°. Very soluble in alcohol, in chloroform, and in methylene chloride; practically insoluble in water. *NF category:* Vehicle; emulsifying agent; diluent.

Propylene Glycol Monolaurate: Clear, oily liquid at 20°. Colorless or slightly yellow. Very soluble in alcohol, in methanol, and in methylene chloride; practically insoluble in water. *NF category:* Emollient; emulsifying agent.

Propylene Glycol Monostearate: White, wax-like solid or as white, wax-like beads or flakes. Has a slight, agreeable, fatty odor and taste. Soluble in organic solvents such as alcohol, mineral or fixed oils, benzene, ether, and acetone; insoluble in water, but may be dispersed in hot water with the aid of a small amount of soap or other suitable surface-active agent. *NF category:* Emulsifying agent.

Propylhexedrine: Clear, colorless liquid, having a characteristic, amine-like odor. Volatilizes slowly at room temperature. Absorbs carbon dioxide from the air, and its solutions are alkaline to litmus. Boils at about 205°. Very slightly soluble in water. Miscible with alcohol, with chloroform, and with ether.

Propyliodone: White or almost white, crystalline powder. Is odorless or has a faint odor. Soluble in acetone, in alcohol, and in ether; practically insoluble in water.

Propylparaben: Small, colorless crystals or white powder. Freely soluble in alcohol and in ether; slightly soluble in boiling water; very slightly soluble in water. *NF category:* Antimicrobial preservative.

Propylparaben Sodium: White powder. Is odorless and hygroscopic. Freely soluble in water; sparingly soluble in alcohol; insoluble in fixed oils. *NF category:* Antimicrobial preservative.

Propylthiouracil: White, powdery, crystalline substance. Is starch-like in appearance and to the touch, and has a bitter taste. Soluble in ammonium hydroxide and in alkali hydroxides; sparingly soluble in alcohol; slightly soluble in water, in chloroform, and in ether.

Protamine Sulfate Injection: Colorless solution, which may have the odor of a preservative.

Protamine Sulfate for Injection: White, odorless powder, having the characteristic appearance of solids dried from the frozen state.

Protein Hydrolysate Injection: Yellowish to reddish-amber, transparent liquid.

Protriptyline Hydrochloride: White to yellowish powder. Is odorless, or has not more than a slight odor. Melts at about 168°. Freely soluble in water, in alcohol, and in chloroform; practically insoluble in ether.

Pseudoephedrine Hydrochloride: Fine, white to off-white crystals or powder, having a faint characteristic odor. Very soluble in water; freely soluble in alcohol; slightly soluble in chloroform.

Pseudoephedrine Sulfate: White crystals or crystalline powder. Is odorless. Freely soluble in alcohol.

Pullulan: White powder. Freely soluble in water; practically insoluble in dehydrated alcohol. *NF category:* Coating agent; plasticizer; polymer membrane; sequestering agent; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent; bulking agent; diluent; disintegrant; wet binder; capsule shell; film-forming agent.

Pumice: Very light, hard, rough, porous, grayish masses or gritty, grayish powder. Is odorless and tasteless, and is stable in air. Practically insoluble in water; is not attacked by acids.

Pyrantel Pamoate: Yellow to tan solid. Soluble in dimethyl sulfoxide; slightly soluble in dimethylformamide; practically insoluble in water and in methanol.

Pyrazinamide: White to practically white, odorless or practically odorless, crystalline powder. Sparingly soluble in water; slightly soluble in alcohol, in ether, and in chloroform.

Pyrethrum Extract: Pale yellow liquid having a bland, flowery odor. Soluble in mineral oil and in most organic solvents; insoluble in water. *Pyrethrins I* denotes the group containing pyrethrin 1, cinerin 1, and jasmolin 1; *Pyrethrins II* denotes the group containing pyrethrin 2, cinerin 2, and jasmolin 2.

Pyridostigmine Bromide: White or practically white, crystalline powder, having an agreeable, characteristic odor. Is hygroscopic. Freely soluble in water, in alcohol, and in chloroform; slightly soluble in solvent hexane; practically insoluble in ether.

Pyridoxine Hydrochloride: White to practically white crystals or crystalline powder. Is stable in air, and is slowly affected by sunlight. Its solutions have a pH of about 3. Freely soluble in water; slightly soluble in alcohol; insoluble in ether.

Pyrilamine Maleate: White, crystalline powder, usually having a faint odor. Its solutions are acid to litmus. Very soluble in water; freely soluble in alcohol and in chloroform; slightly soluble in ether and in benzene.

Pyrimethamine: White, odorless, crystalline powder. Slightly soluble in acetone, in alcohol, and in chloroform; practically insoluble in water.

Pyrvinium Pamoate: Bright orange or orange-red to practically black, crystalline powder. Freely soluble in glacial acetic acid; slightly soluble in chloroform and in methoxyethanol; very slightly soluble in methanol; practically insoluble in water and in ether.

Pyrvinium Pamoate Oral Suspension: Dark red, opaque suspension of essentially very fine, amorphous particles or aggregates, usually less than 10 µm in size. Larger particles,

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some of which may be crystals, up to 100 µm in size also may be present.

Quazepam: Off-white to yellowish powder.

Quetiapine Fumarate: White to off-white crystalline powder. Soluble in 0.1 N hydrochloric acid; slightly soluble in water, in alcohol, and in methanol.

Quinapril Hydrochloride: White to off-white powder, with a pink cast at times. Freely soluble in aqueous solvents.

Quinidine Gluconate: White powder. Is odorless and has a very bitter taste. Freely soluble in water; slightly soluble in alcohol.

Quinidine Sulfate: Fine, needle-like, white crystals, frequently cohering in masses, or fine, white powder. Is odorless, and darkens on exposure to light. Its solutions are neutral or alkaline to litmus. Soluble in alcohol; sparingly soluble in chloroform; slightly soluble in water; insoluble in ether.

Quinine Sulfate: White, fine, needle-like crystals, usually lusterless, making a light and readily compressible mass. Is odorless. It darkens on exposure to light. Its saturated solution is neutral or alkaline to litmus. Freely soluble in alcohol at 80°, and in a mixture of 2 volumes of chloroform and 1 volume of dehydrated alcohol; sparingly soluble in water at 100°; slightly soluble in water, in alcohol, and in chloroform; very slightly soluble in ether.

Rabies Immune Globulin: Transparent or slightly opalescent liquid, practically colorless and practically odorless. May develop a slight, granular deposit during storage.

Rabies Vaccine: White to straw-colored, amorphous pellet, which may or may not become fragmented when shaken.

Racemethionine: Almost white, crystalline powder or small flakes. Sparingly soluble in water; very slightly soluble in alcohol. It dissolves in dilute acids and in dilute solutions of alkali hydroxides. It melts at about 270°. *NF category:* Antioxidant; pH modifier (acidifying agent/alkalizing agent/buffering agent); flavors and fragrance.

Racepinephrine: White to nearly white, crystalline, odorless powder, gradually darkening on exposure to light and air. With acids, it forms salts that are readily soluble in water, and the base may be recovered by the addition of ammonium hydroxide. Very slightly soluble in water and in alcohol; insoluble in ether, in chloroform, and in fixed and volatile oils.

Racepinephrine Hydrochloride: Fine, white, odorless powder. Darkens on exposure to light and air. Its solutions are acid to litmus. Melts at about 157°. Freely soluble in water; sparingly soluble in alcohol.

Raloxifene Hydrochloride: Almost white to pale yellow powder. Freely soluble in dimethylsulfoxide; slightly soluble to sparingly soluble in methanol; practically insoluble to very slightly soluble in water; practically insoluble in ether and in ethyl acetate.

Raltegravir Potassium: White to off-white powder. Soluble in water; slightly soluble in methanol; very slightly soluble in acetonitrile and in ethanol; insoluble in isopropyl alcohol.

Ramipril: White to almost white crystalline powder. Freely soluble in methanol; sparingly soluble in water.

Ranitidine Hydrochloride: White to pale yellow, crystalline, practically odorless powder. Is sensitive to light and moisture. Melts at about 140°, with decomposition. Very soluble in water; sparingly soluble in alcohol.

Fully Hydrogenated Rapeseed Oil: White, waxy solid. Insoluble in water and in alcohol. *NF category:* Coating agent; stiffening agent.

Superglycerinated Fully Hydrogenated Rapeseed Oil: White solid. Insoluble in water and in alcohol. *NF category:* Coating agent; emulsifying agent; stiffening agent.

Purified Rayon: White, lustrous or dull, fine, soft, filamentous fibers, appearing under the microscope as round, oval, or slightly flattened translucent rods, straight or crimped, striate and with serrate cross-sectional edges. Is practically odorless and practically tasteless. Very soluble in ammoniated cupric oxide TS and in dilute sulfuric acid (3 in 5); insoluble in ordinary solvents.

Repaglinide: White to off-white solid. Melts at about 132° to 136°. Soluble in methanol.

Reserpine: White or pale buff to slightly yellowish, odorless, crystalline powder. Darkens slowly on exposure to light, but more rapidly when in solution. Freely soluble in acetic acid and in chloroform; slightly soluble in benzene; very slightly soluble in alcohol and in ether; insoluble in water.

Resorcinol: White, or practically white, needle-shaped crystals or powder. Has a faint, characteristic odor and a sweetish, followed by a bitter, taste. Acquires a pink tint on exposure to light and air. Its solution (1 in 20) is neutral or acid to litmus. Freely soluble in water, in alcohol, in glycerin, and in ether; slightly soluble in chloroform.

Ribavirin: White, crystalline powder. Freely soluble in water; slightly soluble in dehydrated alcohol.

Riboflavin: Yellow to orange-yellow, crystalline powder having a slight odor. Melts at about 280°. Its saturated solution is neutral to litmus. When dry, it is not appreciably affected by diffused light, but when in solution, light induces quite rapid deterioration, especially in the presence of alkalies. Soluble in dilute solutions of alkalies; very slightly soluble in water, in alcohol, and in isotonic sodium chloride solution; insoluble in ether and in chloroform.

Riboflavin 5′-Phosphate Sodium: Fine, orange-yellow, crystalline powder, having a slight odor. Sparingly soluble in water. When dry, it is not affected by diffused light, but when in solution, light induces deterioration rapidly. Is hygroscopic.

Rifabutin: Amorphous red-violet powder. Soluble in chloroform and in methanol; sparingly soluble in alcohol; very slightly soluble in water.

Rifampin: Red-brown, crystalline powder. Freely soluble in chloroform; soluble in ethyl acetate and in methanol; very slightly soluble in water.

Riluzole: White to slightly yellow powder or crystalline powder. Freely soluble in acetonitrile, in alcohol, and in methylene chloride; slightly soluble in hexane; very slightly soluble in water.

Rimexolone: White to off-white powder. Freely soluble in chloroform; sparingly soluble in methanol.

Risedronate Sodium: White to off-white powder. Soluble in water and in aqueous solutions; insoluble in common organic solvents.

Risperidone: White or almost white powder. Soluble in methylene chloride; sparingly soluble in alcohol; practically insoluble in water.

Ritodrine Hydrochloride: White to nearly white, odorless or practically odorless, crystalline powder. Melts at about 200°. Freely soluble in water and in alcohol; soluble in *n*-propyl alcohol; practically insoluble in ether.

Ritonavir: White to light tan powder. Freely soluble in methanol and in methylene chloride; very slightly soluble in acetonitrile; practically insoluble in water.

Rivastigmine Tartrate: White to off-white powder. Very soluble in water and in methanol; very slightly soluble in ethyl acetate.

Rizatriptan Benzoate: White to almost white crystalline powder. Soluble in water; sparingly soluble in alcohol; slightly soluble in methylene chloride.

Rocuronium Bromide: Almost white or pale yellow. Slightly hygroscopic powder. Freely soluble in water and in dehydrated alcohol.

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Ropinirole Hydrochloride: Pale cream to yellow powder. Soluble in water.

Add the following:

▲Ropivacaine: White to off-white powder. Freely soluble in methylene chloride; soluble in ethanol; practically insoluble in water.▲ (USP 1-Dec-2019)

Ropivacaine Hydrochloride: White, crystalline powder. Soluble in water.

Rose Oil: Colorless or yellow liquid, having the characteristic odor and taste of rose. At 25° is a viscous liquid. Upon gradual cooling, changes to a translucent, crystalline mass, easily liquefied by warming. *NF category:* Flavors and fragrance.

Rose Water Ointment: *NF category:* Ointment base.

Stronger Rose Water: Practically colorless and clear, having the pleasant odor and taste of fresh rose blossoms. Is free from empyreuma, mustiness, and fungal growths. *NF category:* Flavors and fragrance.

Rosiglitazone Maleate: White to off-white solid. Sparingly soluble in alcohol; slightly soluble in methylene chloride; practically insoluble to very slightly soluble in water.

Add the following:

▲Rotigotine: White or light-brownish powder. Soluble in anhydrous alcohol; very slightly soluble in heptane; practically insoluble in water.▲ (USP 1-Dec-2019)

Roxarsone: Pale yellow, crystalline powder. Freely soluble in acetic acid, in acetone, in alkalies, in methanol, and in dehydrated alcohol; soluble in boiling water; sparingly soluble in dilute mineral acids; slightly soluble in cold water; insoluble in ether and in ethyl acetate. Puffs up and deflagrates on heating.

Rubella Virus Vaccine Live: Solid having the characteristic appearance of substances dried from the frozen state. Undergoes loss of potency on exposure to sunlight. The Vaccine is to be constituted with a suitable diluent just prior to use.

Rufinamide: White, crystalline neutral powder. Slightly soluble in tetrahydrofuran and in methanol; very slightly soluble in alcohol and in acetonitrile; practically insoluble in water.

Saccharin: White crystals or white, crystalline powder. Is odorless or has a faint, aromatic odor. In dilute solution, it is intensely sweet. Its solutions are acid to litmus. Soluble in boiling water; sparingly soluble in alcohol; slightly soluble in water, in chloroform, and in ether. Is readily dissolved by dilute solutions of ammonia, by solutions of alkali hydroxides, and by solutions of alkali carbonates with the evolution of carbon dioxide. *NF category:* Sweetening agent.

Saccharin Calcium: White crystals or white, crystalline powder. Is odorless, or has a faint, aromatic odor, and has an intensely sweet taste even in dilute solutions. Its dilute solution is about 300 times as sweet as sucrose. Freely soluble in water. *NF category:* Sweetening agent.

Saccharin Sodium: White crystals or white, crystalline powder. Is odorless, or has a faint, aromatic odor, and has an intensely sweet taste even in dilute solutions. Its dilute solution is about 300 times as sweet as sucrose. When in powdered form, it usually contains about one-third the theoretical amount of water of hydration as a result of efflorescence. Freely soluble in water; sparingly soluble in alcohol. *NF category:* Sweetening agent.

Saccharin Sodium Oral Solution: Clear, colorless, odorless liquid, having a sweet taste.

Safflower Oil: Light yellow oil. Thickens and becomes rancid on prolonged exposure to air. Insoluble in water. Miscible with ether and with chloroform. *NF category:* Vehicle (oleaginous); emollient; solvent.

Salicylamide: White, practically odorless, crystalline powder. Freely soluble in ether and in solutions of alkalies; soluble in alcohol and in propylene glycol; slightly soluble in water and in chloroform.

Salicylic Acid: White crystals, usually in fine needles, or fluffy, white, crystalline powder. Has a sweetish, followed by an acrid, taste and is stable in air. The synthetic form is white and odorless. When prepared from natural methyl salicylate, it may have a slightly yellow or pink tint, and a faint, mint-like odor. Freely soluble in alcohol and in ether; soluble in boiling water; sparingly soluble in chloroform; slightly soluble in water and in benzene.

Salmeterol Xinafoate: White to off-white powder. Soluble in methanol; slightly soluble in alcohol, in isopropanol, and in chloroform; practically insoluble in water (pH 8), and in saline solution (0.9% w/w).

Scopolamine Hydrobromide: Colorless or white crystals or white, granular powder. Melts at about 197°, with decomposition. Is odorless, and slightly efflorescent in dry air. Freely soluble in water; soluble in alcohol; slightly soluble in chloroform; insoluble in ether.

Secobarbital: White, amorphous or crystalline, odorless powder, having a slightly bitter taste. Its saturated solution has a pH of about 5.6. Freely soluble in alcohol, in ether, and in solutions of fixed alkali hydroxides and carbonates; soluble in chloroform; very slightly soluble in water.

Secobarbital Sodium: White powder. Is odorless, has a bitter taste, and is hygroscopic. Its solutions decompose on standing, heat accelerating the decomposition. Very soluble in water; soluble in alcohol; practically insoluble in ether.

Selegiline Hydrochloride: White, odorless, crystalline powder. Freely soluble in water, in chloroform, and in methanol.

Selenium Sulfide: Reddish-brown to bright orange powder, having not more than a faint odor. Practically insoluble in water and in organic solvents.

Sennosides: Brownish powder.

Serine: White, odorless crystals, having a sweet taste. Soluble in water; practically insoluble in absolute alcohol and in ether.

Sertraline Hydrochloride: White or off-white crystalline powder. Sparingly soluble or slightly soluble in absolute alcohol; slightly soluble in water and in isopropanol; and slightly or very slightly soluble in acetone.

Sesame Oil: Pale yellow, oily liquid. Is practically odorless, and has a bland taste. Slightly soluble in alcohol. Miscible with ether, with chloroform, with solvent hexane, and with carbon disulfide. *NF category:* Solvent, vehicle (oleaginous).

Sevoflurane: Clear, colorless, volatile, nonflammable liquid. Slightly soluble in water. Miscible with alcohol, with chloroform, and with ether.

Shellac: *Orange Shellac*—Thin, hard, brittle, transparent, pale lemon-yellow to brownish orange flakes, having little or no odor; *Bleached Shellac*—Opaque, amorphous cream to yellow granules or coarse powder, having little or no odor. Soluble (very slowly) in alcohol, 85% to 95% (w/w), in ether, 13% to 15%, in benzene, 10% to 20%, in petroleum ether, 2% to 6%; soluble in aqueous solutions of ethanolamines, alkalies, and borax; sparingly soluble in oil of turpentine; insoluble in water. *NF category:* Coating agent; film-forming agent; release-modifying agent.

Sibutramine Hydrochloride Monohydrate: White to cream crystalline powder. Slightly soluble in pH 5.2 water.

Sildenafil Citrate: White or almost white slightly hygroscopic crystalline powder. Slightly soluble in water and in methanol; practically insoluble in hexane.

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Dental-Type Silica: Fine, white, hygroscopic, odorless, amorphous powder, in which the diameter of the average particles ranges between 0.5 and 40 µm. Soluble in hot solutions of alkali hydroxides; insoluble in water, in alcohol, and in acid (except hydrofluoric acid). *NF category:* Glidant and/or anticaking agent; suspending and/or viscosity-increasing agent.

Hydrophobic Colloidal Silica: Light, fine, white or almost white, amorphous powder, not wettable by water. Dissolves slowly in hot solutions of alkali hydroxides. Practically insoluble in water and in mineral acids, except hydrofluoric acid. *NF category:* Glidant and/or anticaking agent; suspending and/or viscosity-increasing agent.

Purified Siliceous Earth: Very fine, white, light gray, or pale buff mixture of amorphous powder and lesser amounts of crystalline polymorphs, including quartz and cristobalite. Is gritty, readily absorbs moisture, and retains about four times its weight of water without becoming fluid. Insoluble in water, in acids, and in dilute solutions of the alkali hydroxides. *NF category:* Filtering aid; sorbent.

Silicon Dioxide: Fine, white, hygroscopic, odorless, amorphous powder, in which the diameter of the average particles ranges between 2 and 10 µm. Soluble in hot solutions of alkali hydroxides; insoluble in water, in alcohol, and in other organic solvents. *NF category:* Desiccant; suspending and/or viscosity-increasing agent.

Colloidal Silicon Dioxide: Light, white, nongritty powder of extremely fine particle size (about 15 nm). Soluble in hot solutions of alkali hydroxides; insoluble in water and in acid (except hydrofluoric). *NF category:* Glidant and/or anticaking agent; suspending and/or viscosity-increasing agent; disintegrant.

Silver Nitrate: Colorless or white crystals. The pH of its solutions is about 5.5. On exposure to light in the presence of organic matter, it becomes gray or grayish black. Very soluble in water and even more so in boiling water; freely soluble in boiling alcohol; sparingly soluble in alcohol; slightly soluble in ether.

Toughened Silver Nitrate: White, crystalline masses generally molded as pencils or cones. It breaks with a fibrous fracture. Its solutions are neutral to litmus. It becomes gray or grayish black upon exposure to light. Soluble in water to the extent of its nitrate content (there is always a residue of silver chloride); partially soluble in alcohol; slightly soluble in ether.

Simethicone: Translucent, gray, viscous fluid. The liquid phase is soluble in chloroform, in ether, and in benzene, but silicon dioxide remains as a residue in these solvents. Insoluble in water and in alcohol. *NF category:* Antifoaming agent; water-repelling agent; diluent.

Simvastatin: White to off-white powder. Freely soluble in chloroform, in methanol, and in alcohol; sparingly soluble in propylene glycol; very slightly soluble in hexane; practically insoluble in water.

Sitagliptin Phosphate: White or almost white powder. Soluble in water; very slightly soluble in anhydrous ethanol; practically insoluble in heptane.

Smallpox Vaccine: Liquid Vaccine is a turbid, whitish to greenish suspension, which may have a slight odor due to the antimicrobial agent. Dried Vaccine is a yellow to grayish pellet, which may or may not become fragmented when shaken.

Soda Lime: White or grayish-white granules. May have a color if an indicator has been added. *NF category:* Sorbent, carbon dioxide.

Sodium Acetate: Colorless, transparent crystals, or white, granular crystalline powder, or white flakes. Is odorless or has a faint acetous odor, and has a slightly bitter, saline taste. Is efflorescent in warm, dry air. Very soluble in water; soluble in alcohol. *NF category:* Flavors and fragrance; pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative; transfer ligand.

Sodium Alginate: Practically odorless and tasteless, coarse or fine powder, yellowish white in color. Soluble in water, forming a viscous, colloidal solution; insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than about 30% by weight, in chloroform, in ether, and in acids when the pH of the resulting solution becomes lower than about 3. *NF category:* Suspending and/or viscosity-increasing agent; film-forming agent; release-modifying agent; disintegrant; wet binder.

Sodium Ascorbate: White or very faintly yellow crystals or crystalline powder. Is odorless or practically odorless. Is relatively stable in air. On exposure to light it gradually darkens. Freely soluble in water; very slightly soluble in alcohol; insoluble in chloroform and in ether. *NF category:* Antioxidant.

Sodium Benzoate: White, odorless or practically odorless, granular or crystalline powder. Is stable in air. Freely soluble in water; soluble in 90% alcohol; sparingly soluble in alcohol. *NF category:* Antimicrobial preservative; lubricant.

Sodium Bicarbonate: White, crystalline powder. Is stable in dry air, but slowly decomposes in moist air. Its solutions, when freshly prepared with cold water, without shaking, are alkaline to litmus. The alkalinity increases as the solutions stand, as they are agitated, or as they are heated. Soluble in water; insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Sodium Bisulfite: White, crystalline powder. Freely soluble in cold water and in hot water; sparingly soluble in alcohol. *NF category:* Antioxidant.

Sodium Borate: Colorless, transparent crystals or white, crystalline powder. Is odorless. Its solutions are alkaline to phenolphthalein TS. As it effloresces in warm, dry air, the crystals are often coated with white powder. Freely soluble in boiling water and in glycerin; soluble in water; insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative; emulsifying agent.

Sodium Bromide: White, crystalline powder or colorless, cubical crystals. Freely soluble in water; soluble in alcohol.

Sodium Butyrate: Clear, colorless, hygroscopic powder. Soluble in water and in methanol. Melting range is about 250° to 253°.

Sodium Caprylate: A white, crystalline powder. Very soluble or freely soluble in water; freely soluble in acetic acid; sparingly soluble in alcohol; practically insoluble in acetone.

Sodium Carbonate: Colorless crystals, or white, crystalline powder or granules. Is stable in air under ordinary conditions. When exposed to dry air above 50°, the hydrous salt effloresces and, at 100°, becomes anhydrous. Very soluble in boiling water; freely soluble in water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Sodium Cetostearyl Sulfate: A white or pale yellow, amorphous or crystalline powder. Soluble in hot water giving an opalescent solution; slightly soluble in alcohol; practically insoluble in cold water. *NF category:* Emulsifying agent.

Sodium Chloride: Colorless, cubic crystals or white crystalline powder. Has a saline taste. Freely soluble in water; soluble in glycerin; slightly soluble in alcohol. *NF category:* Tonicity agent; diluent.

Sodium Chloride Inhalation Solution: Clear, colorless solution.

Bacteriostatic Sodium Chloride Injection: Clear, colorless solution, odorless or having the odor of the bacteriostatic substance. *NF category:* Vehicle (sterile).

Sodium Chloride Irrigation: Clear, colorless solution.

Sodium Citrate: Colorless crystals, or white, crystalline powder. Hydrous form very soluble in boiling water; freely soluble in water; insoluble in alcohol. *NF category:* pH modifier

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(acidifying agent/alkalizing agent/buffering agent); sequestering agent; transfer ligand.

Sodium Citrate and Citric Acid Oral Solution: Clear solution having the color of any added preservative or flavoring agents.

Sodium Dehydroacetate: White or practically white, odorless powder, having a slight characteristic taste. Freely soluble in water, in propylene glycol, and in glycerin. *NF category:* Antimicrobial preservative.

Sodium Fluoride: White, odorless powder. Soluble in water; insoluble in alcohol.

Sodium Formaldehyde Sulfoxylate: White crystals or hard, white masses, having the characteristic odor of garlic. Freely soluble in water; slightly soluble in alcohol, in ether, in chloroform, and in benzene. *NF category:* Antioxidant.

Sodium Hydroxide: White, or practically white, fused masses, in small pellets, in flakes, or sticks, and in other forms. Is hard and brittle and shows a crystalline fracture. Exposed to the air, it rapidly absorbs carbon dioxide and moisture. Freely soluble in water and in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Sodium Hypochlorite Solution: Clear, pale greenish-yellow liquid, having the odor of chlorine. Is affected by light.

Sodium Iodide: Colorless, odorless crystals, or white, crystalline powder. Is deliquescent in moist air, and develops a brown tint upon decomposition. Very soluble in water; freely soluble in alcohol and in glycerin.

Sodium Lactate Solution: Clear, colorless or practically colorless, slightly viscous liquid, odorless or having a slight, not unpleasant odor. Miscible with water. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); antimicrobial preservative; humectant; flavors and fragrance.

Sodium Lauryl Sulfate: Small, white or light yellow crystals having a slight, characteristic odor. Freely soluble in water, forming an opalescent solution. *NF category:* Emulsifying agent; lubricant; wetting and/or solubilizing agent.

Sodium Metabisulfite: White crystals or white to yellowish, crystalline powder, having the odor of sulfur dioxide. Freely soluble in water and in glycerin; slightly soluble in alcohol. *NF category:* Antioxidant; antimicrobial preservative.

Sodium Monofluorophosphate: White to slightly gray, odorless powder. Freely soluble in water.

Sodium Nitrite: White to slightly yellow, granular powder, or white or practically white, opaque, fused masses or sticks. Has a mild, saline taste and is deliquescent in air. Its solutions are alkaline to litmus. Freely soluble in water; sparingly soluble in alcohol.

Sodium Nitrite Injection: Clear, colorless liquid.

Sodium Nitroprusside: Reddish-brown, practically odorless, crystals or powder. Freely soluble in water; slightly soluble in alcohol; very slightly soluble in chloroform; insoluble in benzene.

Dibasic Sodium Phosphate (*dried*): White powder that readily absorbs moisture. Freely soluble in water; insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); chelating and/or complexing agent.

Dibasic Sodium Phosphate (*heptahydrate*): Colorless or white, granular or caked salt. Effloresces in warm, dry air. Its solutions are alkaline to phenolphthalein TS, a 0.1 M solution having a pH of about 9. Freely soluble in water; very slightly soluble in alcohol. *NF category:* Buffering agent.

Monobasic Sodium Phosphate: Colorless crystals or white, crystalline powder. Is odorless and is slightly deliquescent. Its solutions are acid to litmus and effervesce with sodium carbonate. Freely soluble in water; practically insoluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); chelating and/or complexing agent.

Tribasic Sodium Phosphate: The formula for a crystalline material is approximately $4(Na_3PO_4 \cdot 12H_2O)NaOH$. It occurs as white, odorless crystals or granules or as a crystalline powder. Freely soluble in water; insoluble in alcohol. The pH of a 1 in 100 solution is between 11.5 and 12.0.

Sodium Picosulfate: White or almost white crystalline powder. Freely soluble in water; slightly soluble in alcohol.

Sodium Polystyrene Sulfonate: Golden brown, fine powder. Is odorless and has a characteristic taste. Insoluble in water.

Sodium Propionate: Colorless, transparent crystals or granular, crystalline powder. Is odorless, or has a faint acetic-butyric odor and is deliquescent in moist air. Very soluble in water; soluble in alcohol. *NF category:* Antimicrobial preservative.

Sodium Salicylate: Amorphous or microcrystalline powder or scales. Is colorless, or has not more than a faint, pink tinge. Is odorless, or has a faint, characteristic odor, and is affected by light. A freshly made solution (1 in 10) is neutral or acid to litmus. Very soluble in boiling water and in boiling alcohol; freely (and slowly) soluble in water and in glycerin; slowly soluble in alcohol.

Sodium Starch Glycolate: White, tasteless, odorless, relatively free-flowing powder; available in several different viscosity grades. A 2% (w/v) dispersion in cold water settles, on standing, in the form of a highly hydrated layer. *NF category:* Disintegrant.

Sodium Stearate: Fine, white powder, soapy to the touch, usually having a slight, tallow-like odor. Is affected by light. Its solutions are alkaline to phenolphthalein TS. Slowly soluble in cold water and in cold alcohol; readily soluble in hot water and in hot alcohol. *NF category:* Emulsifying agent; glidant and/or anticaking agent; stiffening agent; lubricant.

Sodium Stearyl Fumarate: Fine, white powder. Slightly soluble in methanol; practically insoluble in water. *NF category:* Lubricant.

Sodium Succinate: White crystals or white powder. Freely soluble in water. *NF category:* Flavors and fragrance; pH modifier.

Sodium Sulfate: Large, colorless, odorless, transparent crystals, or a granular powder. Effloresces rapidly in air, liquefies in its water of hydration at about 33°, and loses all of its water of hydration at about 100°. Freely soluble in water; soluble in glycerin; insoluble in alcohol.

Sodium Sulfite: Colorless crystals. Freely soluble in water; very slightly soluble in alcohol. *NF category:* Antioxidant; antimicrobial preservative.

Sodium Tartrate: Transparent, colorless, odorless crystals. Freely soluble in water; insoluble in alcohol. *NF category:* Sequestering agent; transfer ligand.

Sodium Thiosulfate: Large, colorless crystals or coarse, crystalline powder. Is deliquescent in moist air and effloresces in dry air at temperatures exceeding 33°. Its solutions are neutral or faintly alkaline to litmus. Very soluble in water; insoluble in alcohol. *NF category:* Antioxidant.

Solifenacin Succinate: White to pale, yellowish-white crystal or crystalline powder. Freely soluble in water and in methanol.

Sorbic Acid: Free-flowing, white, crystalline powder, having a characteristic odor. Soluble in alcohol and in ether; slightly soluble in water. *NF category:* Antimicrobial preservative.

Sorbitan Monolaurate: Yellow to amber-colored, oily liquid, having a bland, characteristic odor. Soluble in mineral oil; slightly soluble in cottonseed oil and in ethyl acetate; insoluble in water. *NF category:* Emulsifying agent; lubricant;

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suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitan Monooleate: Viscous, yellow to amber-colored, oily liquid, having a bland, characteristic odor. Insoluble in water and in propylene glycol. Miscible with mineral and vegetable oils. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitan Monopalmitate: Cream-colored, waxy solid having a faint fatty odor. Soluble in warm absolute alcohol; soluble, with haze, in warm peanut oil and in warm mineral oil; insoluble in water. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitan Monostearate: Cream-colored to tan, hard, waxy solid, having a bland odor and taste. Soluble, with haze, above 50° in mineral oil and in ethyl acetate; insoluble in cold water and in acetone. Dispersible in warm water. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitan Sesquioleate: Viscous, yellow to amber-colored, oily liquid. Soluble in alcohol, in isopropyl alcohol, in cottonseed oil, and in mineral oil; insoluble in water and in propylene glycol. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitan Trioleate: Yellow to amber-colored, oily liquid. Soluble in methyl alcohol, in alcohol, in isopropyl alcohol, in corn oil, in cottonseed oil, and in mineral oil; insoluble in water, in ethylene glycol, and in propylene glycol. *NF category:* Emulsifying agent; lubricant; suspending and/or viscosity-increasing agent; wetting and/or solubilizing agent.

Sorbitol: D-Sorbitol occurs as white granules, powder, or crystalline masses. Is odorless, and has a sweet taste with a cold sensation. Very soluble in water; sparingly soluble in alcohol; and practically insoluble in ethyl ether. Is hygroscopic. *NF category:* Humectant; sweetening agent; diluent; plasticizer.

Sorbitol Solution: Clear, colorless, syrupy liquid. Is odorless and has a sweet taste. It sometimes separates into crystalline masses. Miscible with water, with alcohol, with glycerin, and with propylene glycol. Is neutral to litmus. *NF category:* Sweetening agent; vehicle (flavored and/or sweetened).

Sorbitol Sorbitan Solution: A clear, colorless to pale yellow, syrupy liquid. Is odorless and has a sweet taste. Insoluble in mineral oil and in vegetable oil. Miscible with water, with alcohol, with glycerin, and with propylene glycol. *NF category:* Humectant; plasticizer.

Sotalol Hydrochloride: White to off-white powder. Freely soluble in water; soluble in alcohol; very slightly soluble in chloroform.

Soybean Oil: Clear, pale yellow, oily liquid having a characteristic odor and taste. Insoluble in water. Miscible with ether and with chloroform. *Specific Gravity* ⟨841⟩: between 0.916 and 0.922. *Refractive Index* ⟨831⟩: between 1.465 and 1.475. *NF category:* Vehicle (oleaginous); solvent.

Hydrogenated Soybean Oil: A white mass or powder that melts to a clear, pale yellow liquid when heated. Freely soluble in methylene chloride, in hexane after heating, and in toluene; very slightly soluble in alcohol; practically insoluble in water. *NF category:* Emollient.

Spectinomycin Hydrochloride: White to pale-buff crystalline powder. Freely soluble in water; practically insoluble in alcohol, in chloroform, and in ether.

Spironolactone: Light cream-colored to light tan, crystalline powder. Has a faint to mild mercaptan-like odor; is stable in air. Freely soluble in benzene and in chloroform; soluble in ethyl acetate and in alcohol; slightly soluble in methanol and in fixed oils; practically insoluble in water.

Squalane: Colorless, practically odorless transparent oil. Slightly soluble in acetone; very slightly soluble in absolute alcohol; insoluble in water. Miscible with ether and with chloroform. *NF category:* Ointment base; vehicle (oleaginous).

Stannous Chloride: White, crystalline powder or colorless crystals, efflorescent in air. Freely soluble in water (the solution becomes cloudy after standing or on dilution) and in alcohol. Dissolves in dilute hydrochloric acid. *NF category:* Emulsifying agent; antioxidant; reducing agent.

Stannous Fluoride: White, crystalline powder, having a bitter, salty taste. Melts at about 213°. Freely soluble in water; practically insoluble in alcohol, in ether, and in chloroform. *NF category:* Reducing agent.

Stanozolol: Odorless, crystalline powder, occurring in two forms: as needles, melting at about 155°, and as prisms, melting at about 235°. Soluble in dimethylformamide; sparingly soluble in alcohol and in chloroform; slightly soluble in ethyl acetate and in acetone; very slightly soluble in benzene; insoluble in water.

Add the following:

▲**Star Anise Oil:** Clear, colorless to light yellow in the liquid state having a powerful and characteristic licorice–like scent. Congeals between 15° and 19°. Miscible with alcohol. *NF category:* Flavors and fragrance.▲ (NF 1-Dec-2019)

Corn Starch: Irregular, angular, white masses (which may be bleached) or fine powder. Is odorless, and has a slight, characteristic taste. Insoluble in cold water and in alcohol. *NF category:* Diluent; disintegrant; wet binder; suspending and/or viscosity-increasing agent.

Hydroxypropyl Corn Starch: White or slightly yellowish powder. Practically insoluble in cold water and in alcohol. *NF category:* Diluent; disintegrant; wet binder; emulsifying agent; suspending and/or viscosity-increasing agent.

Pregelatinized Hydroxypropyl Corn Starch: White or slightly yellowish powder. It swells in water and produces a clear or translucent, viscous, colloidal mixture. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder; emulsifying agent.

Pea Starch: White or almost white, very fine powder. Practically insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder.

Hydroxypropyl Pea Starch: White or slightly yellowish powder. Practically insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder; emulsifying agent.

Pregelatinized Hydroxypropyl Pea Starch: White or slightly yellowish powder. It swells in water and produces a clear or translucent, viscous, colloidal mixture. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; diluent; disintegrant.

Potato Starch: Irregular, angular, white masses (which may be bleached) or fine powder. Is odorless, and has a slight, characteristic taste. Insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder.

Hydroxypropyl Potato Starch: White or slightly yellowish powder. Practically insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder; emulsifying agent.

Pregelatinized Hydroxypropyl Potato Starch: White or slightly yellowish powder. It swells in water and produces a clear or translucent, viscous, colloidal mixture. *NF category:* Suspending and/or viscosity-increasing agent; wet binder; diluent; disintegrant.

Pregelatinized Starch: Moderately coarse to fine, white to off-white powder. Is odorless and has a slight, characteristic

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taste. Slightly soluble to soluble in cold water; insoluble in alcohol. *NF category:* Wet binder; diluent; disintegrant; release-modifying agent.

Pregelatinized Modified Starch: Moderately coarse to fine, white to off-white powder. Is odorless and has a slight, characteristic taste. Soluble to slightly soluble in cold water; insoluble in alcohol. *NF category:* Coating agent; diluent; disintegrant; wet binder; release-modifying agent.

Tapioca Starch: Irregular, angular, white to pale yellow masses (which may be bleached) or fine powder. Insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder.

Wheat Starch: Irregular, angular, white masses (which may be bleached) or fine powder. Is odorless and has a slight, characteristic taste. Insoluble in cold water and in alcohol. *NF category:* Suspending and/or viscosity-increasing agent; diluent; disintegrant; wet binder.

Hydrogenated Starch Hydrolysate: Concentrated, aqueous solution or spray-dried or dried powder. Very soluble in water; insoluble in alcohol. *NF category:* Sweetening agent; humectant; diluent; wet binder.

Stavudine: White to off-white, crystalline powder. Soluble in water, in dimethylacetamide, and in dimethyl sulfoxide; sparingly soluble in methanol, in alcohol, and in acetonitrile; slightly soluble in dichloromethane; insoluble in hexane.

Stearic Acid: Hard, white or faintly yellowish, somewhat glossy and crystalline solid, or white or yellowish-white powder. Its odor and taste are slight, suggesting tallow. Freely soluble in chloroform and in ether; soluble in alcohol; practically insoluble in water. *NF category:* Emulsifying agent; lubricant.

Purified Stearic Acid: Hard, white or faintly yellowish, somewhat glossy and crystalline solid, or white or yellowish-white powder. Its odor and taste are slight, suggesting tallow. Freely soluble in chloroform and in ether; soluble in alcohol; practically insoluble in water. *NF category:* Lubricant.

Stearoyl Polyoxylglycerides: Pale yellow, waxy solids. Dispersible in warm water and in warm paraffin. Freely soluble in methylene chloride; soluble in warm methanol. *NF category:* Ointment base; solvent; emulsifying agent; wetting and/or solubilizing agent.

Stearyl Alcohol: Unctuous, white flakes or granules. Has a faint, characteristic odor and a bland, mild taste. Soluble in alcohol and in ether; insoluble in water. *NF category:* Stiffening agent.

Storax: Semiliquid, grayish to grayish-brown, sticky, opaque mass depositing on standing a heavy dark brown layer (Levant Storax); or semisolid, sometimes a solid mass, softened by gently warming (American Storax). Is transparent in thin layers, has a characteristic odor and taste, and is more dense than water. Soluble, usually incompletely, in an equal weight of warm alcohol, in acetone, in carbon disulfide, and in ether, some insoluble residue usually remaining; insoluble in water.

Streptomycin Sulfate: White or practically white powder. Is odorless or has not more than a faint odor. Is hygroscopic, but is stable in air and on exposure to light. Its solutions are acid to practically neutral to litmus. Freely soluble in water; very slightly soluble in alcohol; practically insoluble in chloroform.

Streptomycin Sulfate Injection: Clear, colorless to yellow, viscous liquid. Is odorless or has a slight odor.

Strontium Chloride: Colorless, odorless crystals or white granules. Effloresces in air; deliquesces in moist air. Very soluble in water; soluble in alcohol.

Succinic Acid: White, odorless crystals. Freely soluble in boiling water; soluble in water, in alcohol, and in glycerin. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Succinylcholine Chloride: White, odorless, crystalline powder. Its solutions have a pH of about 4. The dihydrate form melts at about 160°; the anhydrous form melts at about 190°, and is hygroscopic. Freely soluble in water; slightly soluble in alcohol and in chloroform; practically insoluble in ether.

Add the following:

▲**Sucralfate:** White or almost-white amorphous powder. Soluble in a dilute solution of mineral acids or alkali hydroxides; practically insoluble in water, in alcohol, and in methylene chloride.▲ (USP 1-Dec-2019)

Sucralose: White to off-white, crystalline powder. Freely soluble in water, in methanol, and in alcohol; slightly soluble in ethyl acetate. *NF category:* Sweetening agent.

Sucrose: White, crystalline powder or lustrous, dry, colorless or white crystals. Very soluble in water; slightly soluble in alcohol; practically insoluble in dehydrated alcohol. *NF category:* Coating agent; sweetening agent; diluent; suspending and/or viscosity-increasing agent; wet binder.

Sucrose Palmitate: White or almost white, unctuous powder. Sparingly soluble in ethanol (96%); very slightly soluble in water. *NF category:* Suspending and/or viscosity-increasing agent; emulsifying agent.

Sucrose Octaacetate: White, practically odorless powder, having an intensely bitter taste. Is hygroscopic. Very soluble in methanol and in chloroform; soluble in alcohol and in ether; very slightly soluble in water. *NF category:* Alcohol denaturant.

Sucrose Stearate: White or almost white, unctuous powder. Sparingly soluble in ethanol (96%); very slightly soluble in water. *NF category:* Emulsifying agent; lubricant.

Sufentanil Citrate: White powder. Freely soluble in methanol; soluble in water; sparingly soluble in acetone, in alcohol, and in chloroform. Melts between 133° and 140°.

Compressible Sugar: Practically white, crystalline, odorless powder, having a sweet taste. Is stable in air. The sucrose portion of Compressible Sugar is very soluble in water. *NF category:* Sweetening agent; diluent.

Confectioner's Sugar: Fine, white, odorless powder, having a sweet taste. Is stable in air. The sucrose portion of Confectioner's Sugar is soluble in cold water. Confectioner's Sugar is freely soluble in boiling water. *NF category:* Sweetening agent; diluent; coating agent.

Sugar Spheres: Hard, brittle, free-flowing, spherical masses ranging generally in size from 10- to 60-mesh. Usually white, but may be colored. Solubility in water varies according to the sugar-to-starch ratio. *NF category:* Vehicle (solid carrier); diluent.

Sulbactam Sodium: White to off-white, crystalline powder. Freely soluble in water and in dilute acid; sparingly soluble in acetone, in ethyl acetate, and in chloroform.

Sulconazole Nitrate: White to off-white, crystalline powder. Melts at about 130°, with decomposition. Freely soluble in pyridine; sparingly soluble in methanol; slightly soluble in alcohol, in chloroform, in acetone, and in methylene chloride; very slightly soluble in water, in toluene, and in dioxane.

Sulfabenzamide: Fine, white, practically odorless powder. Soluble in alcohol, in acetone, and in sodium hydroxide TS; insoluble in water and in ether.

Sulfacetamide: White, crystalline powder, odorless and having a characteristic sour taste. Its aqueous solutions are sensitive to light, and are unstable when acidic or strongly alkaline. Freely soluble in dilute mineral acids and in solutions of potassium and sodium hydroxides; soluble in alcohol; slightly soluble in water and in ether; very slightly soluble in chloroform; practically insoluble in benzene.

Sulfacetamide Sodium: White, crystalline powder. Is odorless and has a bitter taste. Freely soluble in water;

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sparingly soluble in alcohol; practically insoluble in chloroform and in ether.

Sulfadiazine: White or slightly yellow powder. Is odorless or nearly odorless and is stable in air, but slowly darkens on exposure to light. Freely soluble in dilute mineral acids, in solutions of potassium and sodium hydroxides, and in ammonia TS; sparingly soluble in alcohol and in acetone; slightly soluble in human serum at 37°; practically insoluble in water.

Silver Sulfadiazine: White to creamy-white, crystalline powder, odorless to having a slight odor. Is stable in air, but turns yellow on exposure to light. Freely soluble in 30% ammonium solution; slightly soluble in acetone; practically insoluble in alcohol, in chloroform, and in ether. Decomposes in moderately strong mineral acids.

Sulfadiazine Sodium: White powder. On prolonged exposure to humid air it absorbs carbon dioxide with the liberation of sulfadiazine and becomes incompletely soluble in water. Its solutions are alkaline to phenolphthalein. Is affected by light. Freely soluble in water; slightly soluble in alcohol.

Sulfadimethoxine: Practically white, crystalline powder. Soluble in 2 N sodium hydroxide; sparingly soluble in 2 N hydrochloric acid; slightly soluble in alcohol, in ether, in chloroform, and in hexane; practically insoluble in water.

Sulfamethazine: White to yellowish-white powder, which may darken on exposure to light. Has a slightly bitter taste and is practically odorless. Soluble in acetone; slightly soluble in alcohol; very slightly soluble in water and in ether.

Sulfamethizole: White crystals or powder, having a slightly bitter taste. Is practically odorless, and has no odor of hydrogen sulfide. Freely soluble in solutions of ammonium, potassium, and sodium hydroxides; soluble in dilute mineral acids and in acetone; sparingly soluble in alcohol; very slightly soluble in water, in chloroform, and in ether; practically insoluble in benzene.

Sulfamethoxazole: White to off-white, practically odorless, crystalline powder. Freely soluble in acetone and in dilute solutions of sodium hydroxide; sparingly soluble in alcohol; practically insoluble in water, in ether, and in chloroform.

Sulfapyridine: White or faintly yellowish-white crystals, granules, or powder. Is odorless or practically odorless, and is stable in air, but slowly darkens on exposure to light. Freely soluble in dilute mineral acids and in solutions of potassium and sodium hydroxides; sparingly soluble in acetone; slightly soluble in alcohol; very slightly soluble in water.

Sulfasalazine: Bright yellow or brownish-yellow, odorless, fine powder. Melts at about 255°, with decomposition. Soluble in aqueous solutions of alkali hydroxides; very slightly soluble in alcohol; practically insoluble in water, in ether, in chloroform, and in benzene.

Sulfathiazole: Fine, white or faintly yellowish-white, practically odorless powder. Soluble in acetone, in dilute mineral acids, in solutions of alkali hydroxides, and in 6 N ammonium hydroxide; slightly soluble in alcohol; very slightly soluble in water.

Sulfinpyrazone: White to off-white powder. Soluble in alcohol and in acetone; sparingly soluble in dilute alkali; practically insoluble in water and in solvent hexane.

Sulfisoxazole: White to slightly yellowish, odorless, crystalline powder. Soluble in boiling alcohol and in 3 N hydrochloric acid; very slightly soluble in water.

Sulfisoxazole Acetyl: White or slightly yellow, crystalline powder. Sparingly soluble in chloroform; slightly soluble in alcohol; practically insoluble in water.

Precipitated Sulfur: Very fine, pale yellow, amorphous or microcrystalline powder. Is odorless and tasteless. Very soluble in carbon disulfide; slightly soluble in olive oil; very slightly soluble in alcohol; practically insoluble in water.

Sublimed Sulfur: Fine, yellow, crystalline powder, having a faint odor and taste. Sparingly soluble in olive oil; practically insoluble in water and in alcohol.

Sulfur Dioxide: Colorless, nonflammable gas, possessing a strong, suffocating odor characteristic of burning sulfur. Under pressure, it condenses readily to a colorless liquid that boils at −10° and has a density of approximately 1.5. At 20° and at standard pressure, approximately 36 volumes dissolve in 1 volume of water and approximately 114 volumes dissolve in 1 volume of alcohol. Soluble also in ether and in chloroform. *NF category:* Antioxidant; antimicrobial preservative.

Sulfuric Acid: Clear, colorless, oily liquid. Miscible with water and with alcohol with the generation of much heat. Is very caustic and corrosive. Specific gravity is about 1.84. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent).

Sulindac: Yellow, crystalline powder, which is odorless or practically so. Slightly soluble in methanol, in alcohol, in acetone, and in chloroform; very slightly soluble in isopropanol and in ethyl acetate; practically insoluble in hexane and in water.

Sulisobenzone: Light tan powder, with a melting point of about 145°. Freely soluble in methanol, in alcohol, and in water; sparingly soluble in ethyl acetate.

Sumatriptan: White to pale yellow powder. Very slightly soluble in water.

Sumatriptan Succinate: White or almost white powder. Freely soluble in water; sparingly soluble in methanol; practically insoluble in methylene chloride.

Sunflower Oil: Clear, light yellow liquid. It is miscible with petroleum at a boiling point of 40°–60°. Practically insoluble in water and in alcohol. *Refractive Index* ⟨831⟩: 1.472–1.474 at 25°. *Specific Gravity* ⟨841⟩: 0.914–0.924 at 20°. *NF category*: Coating agent; emollient; solvent; diluent; vehicle (oleaginous).

Suprofen: White to off-white powder, odorless to having a slight odor. Sparingly soluble in water.

Syrup: *NF category:* Sweetening agent; wet binder; vehicle.

Tacrine Hydrochloride: White powder. Freely soluble in water, in 0.1 N hydrochloric acid, in pH 4.0 acetate buffer, in phosphate buffer (pH between 7.0 and 7.4), in methanol, in dimethylsulfoxide, in alcohol, and in propylene glycol; sparingly soluble in linoleic acid and in polyethylene glycol 400.

Tacrolimus: White crystals or white crystalline powder. Very soluble in methanol; freely soluble in *N,N*-dimethylformamide, and in alcohol; practically insoluble in water.

Tadalafil: White or almost white powder. Freely soluble in dimethyl sulfoxide; slightly soluble in methylene chloride; practically insoluble in water.

Tagatose: White or almost white crystals, having a sweet taste. Very soluble in water; very slightly soluble in alcohol. *NF category:* Sweetening agent; humectant.

Talc: Very fine, white or grayish-white, crystalline powder. Is unctuous, adheres readily to the skin, and is free from grittiness. *NF category:* Glidant and/or anticaking agent; lubricant; diluent.

Tamoxifen Citrate: White, fine, crystalline powder. Soluble in methanol; very slightly soluble in water, in acetone, in chloroform, and in alcohol. Melts at about 142°, with decomposition.

Tamsulosin Hydrochloride: White or almost white crystalline powder. Melts with decomposition at approximately 230°. Freely soluble in formic acid; sparingly soluble in methanol; slightly soluble in water and in dehydrated alcohol; practically insoluble in ether.

Tannic Acid: Amorphous powder, glistening scales, or spongy masses, varying in color from yellowish-white to light

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brown. Is odorless or has a faint, characteristic odor, and has a strongly astringent taste. Very soluble in water, in acetone, and in alcohol; freely soluble in diluted alcohol; slightly soluble in dehydrated alcohol; practically insoluble in benzene, in chloroform, in ether, and in solvent hexane; 1 g dissolves in about 1 mL of warm glycerin.

Tartaric Acid: Colorless or translucent crystals or white, fine to granular, crystalline powder. Is odorless, has an acid taste, and is stable in air. Very soluble in water; freely soluble in alcohol. *NF category:* pH modifier (acidifying agent/alkalizing agent/buffering agent); flavors and fragrance; sequestering agent.

Taurine: White crystals or crystalline powder. Soluble in water.

Tazobactam: White to pale yellow, nonhygroscopic, crystalline powder. Soluble in dimethylformamide; slightly soluble in water, in methanol, in acetone, and in alcohol; very slightly soluble in ethyl acetate, in ethyl ether, and in chloroform; insoluble in hexane.

Technetium Tc 99m Aggregated Albumin Injection: Milky suspension, from which particles settle upon standing.

Technetium Tc 99m Pentetate Injection: Clear, colorless solution.

Sodium Pertechnetate Tc 99m Injection: Clear, colorless solution.

Technetium Tc 99m (Pyro- and trimeta-) Phosphates Injection: Clear solution.

Technetium Tc 99m Sulfur Colloid Injection: Colloidal dispersion. Slightly opalescent, colorless to light tan liquid.

Telmisartan: White or slightly yellowish, crystalline powder. Sparingly soluble in methylene chloride; slightly soluble in methanol; practically insoluble in water. It dissolves in 1 M sodium hydroxide.

Temazepam: White or nearly white, crystalline powder. Sparingly soluble in alcohol; very slightly soluble in water. Melts between 157° and 163°, within a 3° range.

Temozolomide: White to light pink/light tan powder. Soluble in dimethyl sulfoxide; sparingly soluble in water; practically insoluble in toluene.

Teniposide: White to off-white, crystalline powder. Very soluble in acetone and in dimethylformamide; slightly soluble in methanol; insoluble in water and in ether.

Terazosin Hydrochloride: White to pale yellow, crystalline powder. Freely soluble in isotonic saline solution; soluble in methanol and in water; slightly soluble in alcohol and in 0.1 N hydrochloric acid; very slightly soluble in chloroform; practically insoluble in acetone and in hexanes.

Terbinafine Hydrochloride: White or off-white powder. Freely soluble in dehydrated alcohol and in methanol; slightly soluble in acetone; very slightly or slightly soluble in water.

Terbutaline Sulfate: White to gray-white, crystalline powder. Is odorless or has a faint odor of acetic acid. Soluble in water and in 0.1 N hydrochloric acid; slightly soluble in methanol; insoluble in chloroform.

Terconazole: White to off-white powder. Freely soluble in methylene chloride; soluble in acetone; sparingly soluble in alcohol; practically insoluble in water. It exhibits polymorphism.

Terpin Hydrate: Colorless, lustrous crystals or white powder. Has a slight odor, and effloresces in dry air. A hot solution (1 in 100) is neutral to litmus. When dried in vacuum at 60° for 2 hours, it melts at about 103°. Very soluble in boiling alcohol; soluble in alcohol; sparingly soluble in boiling water; slightly soluble in water, in chloroform, and in ether.

Testolactone: White to off-white, practically odorless, crystalline powder. Melts at about 218°. Soluble in alcohol and in chloroform; slightly soluble in water and in benzyl alcohol; insoluble in ether and in solvent hexane.

Testosterone: White or slightly creamy white crystals or crystalline powder. Is odorless, and is stable in air. Freely soluble in dehydrated alcohol and in chloroform; soluble in dioxane and in vegetable oils; slightly soluble in ether; practically insoluble in water.

Testosterone Cypionate: White or creamy white, crystalline powder. Is odorless or has a slight odor, and is stable in air. Freely soluble in alcohol, in chloroform, in dioxane, and in ether; soluble in vegetable oils; insoluble in water.

Testosterone Enanthate: White or creamy white, crystalline powder. Is odorless or has a faint odor characteristic of heptanoic acid. Very soluble in ether; soluble in vegetable oils; insoluble in water.

Testosterone Propionate: White or creamy white crystals or crystalline powder. Is odorless and is stable in air. Freely soluble in alcohol, in dioxane, in ether, and in other organic solvents; soluble in vegetable oils; insoluble in water.

Tetanus Immune Globulin: Transparent or slightly opalescent liquid, practically colorless and practically odorless. May develop a slight granular deposit during storage.

Tetanus Toxoid: Clear, colorless to brownish-yellow, or slightly turbid liquid, free from evident clumps or particles, having a characteristic odor or an odor of formaldehyde.

Tetanus Toxoid Adsorbed: Turbid, white, slightly gray, or slightly pink suspension, free from evident clumps after shaking.

Tetanus and Diphtheria Toxoids Adsorbed for Adult Use: Turbid, white, slightly gray, or cream-colored suspension, free from evident clumps after shaking.

Tetracaine: White or light yellow, waxy solid. Soluble in alcohol, in ether, in benzene, and in chloroform; very slightly soluble in water.

Tetracaine Hydrochloride: Fine, white, crystalline, odorless powder. Has a slightly bitter taste followed by a sense of numbness. Its solutions are neutral to litmus. Melts at about 148°, or may occur in either of two other polymorphic modifications that melt at about 134° and 139°, respectively. Mixtures of the forms may melt within the range of 134° to 147°. Is hygroscopic. Very soluble in water; soluble in alcohol; insoluble in ether and in benzene.

Tetracycline: Yellow, odorless, crystalline powder. Is stable in air, but exposure to strong sunlight causes it to darken. It loses potency in solutions of pH below 2, and is rapidly destroyed by alkali hydroxide solutions. Freely soluble in dilute acid and in alkali hydroxide solutions; sparingly soluble in alcohol; very slightly soluble in water; practically insoluble in chloroform and in ether.

Tetracycline Hydrochloride: Yellow, odorless, crystalline powder. Is moderately hygroscopic. Is stable in air, but exposure to strong sunlight in moist air causes it to darken. It loses potency in solution at a pH below 2, and is rapidly destroyed by alkali hydroxide solutions. Soluble in water and in solutions of alkali hydroxides and carbonates; slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Tetrahydrozoline Hydrochloride: White, odorless solid. Melts at about 256°, with decomposition. Freely soluble in water and in alcohol; very slightly soluble in chloroform; practically insoluble in ether.

Thalidomide: White to off-white powder. Very soluble in dimethyl sulfoxide; sparingly soluble in ethanol and in water.

Theophylline: White, odorless, crystalline powder, having a bitter taste. Is stable in air. Freely soluble in solutions of alkali hydroxides and in ammonia; sparingly soluble in alcohol, in chloroform, and in ether; slightly soluble in water, but more soluble in hot water.

Theophylline Sodium Glycinate: White, crystalline powder having a slight ammoniacal odor and a bitter taste.

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Freely soluble in water; very slightly soluble in alcohol; practically insoluble in chloroform.

Thiabendazole: White to practically white, odorless or practically odorless powder. Slightly soluble in acetone and in alcohol; very slightly soluble in chloroform and in ether; practically insoluble in water.

Thiacetarsamide: White to yellowish, crystalline powder. Soluble in warm dehydrated alcohol and in warm methanol; sparingly soluble in cold dehydrated alcohol, in cold methanol, and in cold water; more soluble in water above 90°; insoluble in warm isopropyl alcohol. pK_a is 4.

Thiamine Hydrochloride: White crystals or crystalline powder, usually having a slight, characteristic odor. When exposed to air, the anhydrous product rapidly absorbs about 4% of water. Melts at about 248°, with some decomposition. Freely soluble in water; soluble in glycerin; slightly soluble in alcohol; insoluble in ether and in benzene.

Thiamine Mononitrate: White crystals or crystalline powder, usually having a slight, characteristic odor. Sparingly soluble in water; slightly soluble in alcohol; very slightly soluble in chloroform.

Thiethylperazine Maleate: Yellowish, granular powder. Is odorless or has not more than a slight odor. Melts at about 183°, with decomposition. Slightly soluble in methanol; practically insoluble in water and in chloroform.

Thimerosal: Light cream-colored, crystalline powder, having a slight characteristic odor. Is affected by light. The pH of a solution (1 in 100) is about 6.7. Freely soluble in water; soluble in alcohol; practically insoluble in ether. *NF category:* Antimicrobial preservative.

Thimerosal Topical Solution: Clear liquid, having a slight characteristic odor. Is affected by light.

Thimerosal Tincture: Transparent, mobile liquid, having the characteristic odor of alcohol and acetone. Is affected by light.

Thioguanine: Pale yellow, odorless or practically odorless, crystalline powder. Freely soluble in dilute solutions of alkali hydroxides; insoluble in water, in alcohol, and in chloroform.

Thiopental Sodium: White to off-white, crystalline powder, or yellowish-white to pale greenish-yellow, hygroscopic powder. May have a disagreeable odor. Its solutions are alkaline to litmus. Its solutions decompose on standing, and on boiling precipitation occurs. Soluble in water and in alcohol; insoluble in benzene, in absolute ether, and in solvent hexane.

Thiopental Sodium for Injection: White to off-white, crystalline powder, or yellowish-white to pale greenish-yellow, hygroscopic powder. May have a disagreeable odor. Its solutions are alkaline to litmus. Its solutions decompose on standing, and on boiling precipitation occurs.

Thioridazine: White to slightly yellow, crystalline or micronized powder, odorless or having a faint odor. Very soluble in chloroform; freely soluble in dehydrated alcohol and in ether; practically insoluble in water.

Thioridazine Hydrochloride: White to slightly yellow, granular powder, having a faint odor and a very bitter taste. Freely soluble in water, in methanol, and in chloroform; insoluble in ether.

Thiostrepton: White to off-white, crystalline solid. Soluble in glacial acetic acid, in chloroform, in dimethylformamide, in dimethyl sulfoxide, in dioxane, and in pyridine; practically insoluble in water, in the lower alcohols, in nonpolar organic solvents, and in dilute aqueous acids or alkali.

Thiotepa: Fine, white, crystalline flakes, having a faint odor. Freely soluble in water, in alcohol, in chloroform, and in ether.

Thiotepa for Injection: White powder.

Thiothixene: White to tan, practically odorless crystals. Is affected by light. Very soluble in chloroform; slightly soluble in methanol and in acetone; practically insoluble in water.

Thiothixene Hydrochloride: White, or practically white, crystalline powder, having a slight odor. Is affected by light. Soluble in water; slightly soluble in chloroform; practically insoluble in benzene, in acetone, and in ether.

Threonine: White, odorless crystals, having a slightly sweet taste. Freely soluble in water; insoluble in absolute alcohol, in ether, and in chloroform.

Thrombin: White to grayish, amorphous substance dried from the frozen state.

Thymol: Colorless, often large, crystals, or white, crystalline powder, having an aromatic, thyme-like odor and a pungent taste. Is affected by light. Its alcohol solution is neutral to litmus. Freely soluble in alcohol, in chloroform, in ether, and in olive oil; soluble in glacial acetic acid and in fixed and volatile oils; very slightly soluble in water. *NF category:* Antimicrobial preservative; flavors and fragrance; antioxidant.

Thyroid: Yellowish to buff-colored, amorphous powder, having a slight, characteristic, meat-like odor and a saline taste.

Tiagabine Hydrochloride: White to off-white powder. Freely soluble in methanol and in alcohol; soluble in isopropanol; very slightly soluble in chloroform; sparingly soluble in water; practically insoluble in *n*-heptane.

Tiamulin: A sticky, translucent yellowish mass, slightly hygroscopic. Very soluble in dichloromethane; freely soluble in dehydrated alcohol; practically insoluble in water.

Ticarcillin Disodium: White to pale yellow powder, or white to pale yellow solid. Freely soluble in water.

Ticlopidine: White or almost white crystalline powder. Sparingly soluble in water and in alcohol; very slightly soluble in ethyl acetate.

Tigecycline: Orange powder. Freely soluble in water; soluble in 1-octanol; slightly soluble in methanol, in ethanol, and in isopropyl alcohol.

Tiletamine Hydrochloride: White to off-white, crystalline powder. Freely soluble in water and in 0.1 N hydrochloric acid; soluble in methanol; slightly soluble in chloroform; practically insoluble in ether.

Tilmicosin: White to off-white, amorphous solid. Slightly soluble in water and in *n*-hexane.

Timolol Maleate: White to practically white, odorless or practically odorless powder. Soluble in water, in alcohol, and in methanol; sparingly soluble in chloroform and in propylene glycol; insoluble in ether and in cyclohexane.

Tinidazole: Almost white or pale yellow, crystalline powder. Soluble in acetone and in methylene chloride; sparingly soluble in methanol; practically insoluble in water.

Titanium Dioxide: White, odorless, tasteless powder. Its 1 in 10 suspension in water is neutral to litmus. Insoluble in water, in hydrochloric acid, in nitric acid, and in 2 N sulfuric acid. Dissolves in hydrofluoric acid and in hot sulfuric acid. Is rendered soluble by fusion with potassium bisulfate or with alkali carbonates or hydroxides. *NF category:* Coating agent.

Tizanidine Hydrochloride: Almost white to slightly yellow, crystalline powder. Slightly soluble in water and in methanol.

Tobramycin: White to off-white, hygroscopic powder. Freely soluble in water; very slightly soluble in alcohol; practically insoluble in chloroform and in ether.

Tobramycin Sulfate Injection: Clear, colorless solution.

Tocainide Hydrochloride: Fine, white, odorless powder. Freely soluble in water and in alcohol; practically insoluble in chloroform and in ether.

Tocopherol: Clear, colorless to yellow, yellowish-brown, or greenish-yellow, viscous oil. Is odorless. Soluble in oils, in

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fats, in acetone, in alcohol, in chloroform, in ether, and in alcohol; insoluble in water. *NF category:* Antioxidant.

Tocopherols Excipient: Brownish-red to red, clear, viscous oil, having a mild, characteristic odor and taste. May show a slight separation of waxlike constituents in microcrystalline form. Oxidizes and darkens slowly in air and on exposure to light, particularly in alkaline media. Soluble in alcohol; insoluble in water. Miscible with acetone, with chloroform, with ether, and with vegetable oils. *NF category:* Antioxidant.

Tolazamide: White to off-white, crystalline powder, odorless or having a slight odor. Melts with decomposition in the approximate range of 161° to 173°. Freely soluble in chloroform; soluble in acetone; slightly soluble in alcohol; very slightly soluble in water.

Tolazoline Hydrochloride: White to off-white, crystalline powder. Its solutions are slightly acid to litmus. Freely soluble in water and in alcohol.

Tolbutamide: White, or practically white, crystalline powder. Is slightly bitter and practically odorless. Soluble in alcohol and in chloroform; practically insoluble in water.

Tolbutamide Sodium: White to off-white, practically odorless, crystalline powder, having a slightly bitter taste. Freely soluble in water; soluble in alcohol and in chloroform; very slightly soluble in ether.

Tolcapone: Yellow, fine powder or fine powder with lumps. Freely soluble in acetone and in tetrahydrofuran; soluble in methanol and in ethyl acetate; sparingly soluble in chloroform and in dichloromethane; insoluble in water and in *n*-hexane.

Tolmetin Sodium: Light yellow to light orange, crystalline powder. Freely soluble in water and in methanol; slightly soluble in alcohol; very slightly soluble in chloroform.

Tolnaftate: White to creamy white, fine powder, having a slight odor. Freely soluble in acetone and in chloroform; sparingly soluble in ether; slightly soluble in alcohol; practically insoluble in water.

Tolterodine Tartrate: White or almost white, crystalline powder. Sparingly soluble in water; slightly soluble in anhydrous ethanol; practically insoluble in heptane.

Tolu Balsam: Brown or yellowish-brown, plastic solid, transparent in thin layers and brittle when old, dried, or exposed to cold temperatures. Has a pleasant, aromatic odor resembling that of vanilla, and a mild, aromatic taste. Soluble in alcohol, in chloroform, and in ether, sometimes with slight residue or turbidity; practically insoluble in water and in solvent hexane. *NF category:* Flavors and perfumes.

Topiramate: White to off-white powder. Freely soluble in dichloromethane.

Torsemide: White to off-white, crystalline powder. Slightly soluble in 0.1 N sodium hydroxide, in 0.1 N hydrochloric acid, in alcohol, and in methanol; very slightly soluble in acetone and in chloroform; practically insoluble in water and in ether.

Tragacanth: Is odorless, and has an insipid, mucilaginous taste. *NF category:* Suspending and/or viscosity-increasing agent.

Tramadol Hydrochloride: White, crystalline powder. Freely soluble in water and in methanol; very slightly soluble in acetone.

Trandalopril: White or almost white powder. Freely soluble in methylene chloride; sparingly soluble in absolute alcohol; practically insoluble in water.

Tranexamic Acid: White, crystalline powder. Freely soluble in water and in glacial acetic acid; practically insoluble in acetone and in alcohol.

Tranylcypromine Sulfate: White or almost white crystalline powder. Freely soluble in water; very slightly soluble in alcohol and in ether; practically insoluble in chloroform.

Travoprost: Clear, colorless, viscous oil. Insoluble in water.

Trazodone Hydrochloride: White to off-white, crystalline powder. Sparingly soluble in chloroform and in water. Melts between 231° and 234° when the melting point determination is carried out in an evacuated capillary tube; otherwise melts with decomposition over a broad range below 230°.

Trehalose: White, odorless, nonhygroscopic crystalline powder. Soluble in water, solubility increases with temperature; practically insoluble in dehydrated alcohol. Trehalose is typically used in the dihydrate form. *NF category:* Bulking agent; sweetening agent; diluent; disintegrant; wet binder; vehicle.

Tretinoin: Yellow to light-orange, crystalline powder. Slightly soluble in alcohol, in chloroform, and in methanol; insoluble in water.

Triacetin: Colorless, somewhat oily liquid having a slight, fatty odor and a bitter taste. Soluble in water; slightly soluble in carbon disulfide. Miscible with alcohol, with ether, and with chloroform. *NF category:* Plasticizer; humectant; solvent.

Triamcinolone: White or practically white, odorless, crystalline powder. Slightly soluble in alcohol and in methanol; very slightly soluble in water, in chloroform, and in ether.

Triamcinolone Acetonide: White to cream-colored, crystalline powder, having not more than a slight odor. Sparingly soluble in dehydrated alcohol, in chloroform, and in methanol; practically insoluble in water.

Triamcinolone Diacetate: Fine, white to off-white, crystalline powder, having not more than a slight odor. Soluble in chloroform; sparingly soluble in alcohol and in methanol; slightly soluble in ether; practically insoluble in water.

Triamcinolone Hexacetonide: White to cream-colored powder. Soluble in chloroform; slightly soluble in methanol; practically insoluble in water.

Triamterene: Yellow, odorless, crystalline powder. Soluble in formic acid; sparingly soluble in methoxyethanol; very slightly soluble in acetic acid, in alcohol, and in dilute mineral acids; practically insoluble in water, in benzene, in chloroform, in ether, and in dilute alkali hydroxides.

Triazolam: White to off-white, practically odorless, crystalline powder. Soluble in chloroform; slightly soluble in alcohol; practically insoluble in ether and in water.

Tributyl Citrate: Clear, practically colorless, oily liquid. Freely soluble in alcohol, in isopropyl alcohol, in acetone, and in toluene; insoluble in water. *NF category:* Plasticizer.

Trichlorfon: White crystalline powder. Very soluble in methylene chloride; freely soluble in acetone, in alcohol, in benzene, in chloroform, in ether, and in water; very slightly soluble in hexane and in pentane. Decomposed by alkali. Melts at about 78° with decomposition.

Trichlormethiazide: White or practically white, crystalline powder. Is odorless, or has a slight characteristic odor. Melts at about 274°, with decomposition. Freely soluble in acetone; soluble in methanol; sparingly soluble in alcohol; very slightly soluble in water, in ether, and in chloroform.

Trichloromonofluoromethane: Clear, colorless gas, having a faint, ethereal odor. Its vapor pressure at 25° is about 796 mm of mercury (1 psig). *NF category:* Propellant.

Triclocarban: White powder. Freely soluble in ethanol, in acetone, and in polyethylene glycol; slightly soluble in acetonitrile and in propylene glycol; insoluble in water.

Triclosan: Fine, whitish, crystalline powder. Melts at about 57°. Soluble in methanol, in alcohol, and in acetone; slightly soluble in hexane; practically insoluble in water.

Trientine Hydrochloride: White to pale yellow, crystalline powder. Melts at about 117°. Freely soluble in water; soluble in methanol; slightly soluble in alcohol; insoluble in chloroform and in ether.

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Triethyl Citrate: Practically colorless, oily liquid. Soluble in water. Miscible with alcohol and with ether. *NF category:* Plasticizer; solvent.

Trifluoperazine Hydrochloride: White to pale yellow, crystalline powder. Is practically odorless, and has a bitter taste. Melts at about 242°, with decomposition. Freely soluble in water; soluble in alcohol; sparingly soluble in chloroform; insoluble in ether and in benzene.

Triflupromazine: Viscous, light amber-colored, oily liquid, which crystallizes on prolonged standing into large, irregular crystals. Practically insoluble in water.

Triflupromazine Hydrochloride: White to pale tan, crystalline powder, having a slight, characteristic odor. Melts between 170° and 178°. Soluble in water, in alcohol, and in acetone; insoluble in ether.

Trifluridine: Odorless, white powder appearing under the microscope as rodlike crystals; melts at 175°, with sublimation.

Change to read:

Medium-Chain Triglycerides: Colorless or slightly yellowish, oily liquid. Practically insoluble in water. Miscible with alcohol, with methylene chloride, with hexane, and with fatty oils. ▲The refractive index is 1.440–1.452 at 20° (see *Refractive Index* ⟨831⟩)▲ (NF 1-May-2020). *NF category:* Emulsifying agent; solvent; suspending and/or viscosity-increasing agent.

Trihexyphenidyl Hydrochloride: White or slightly off-white, crystalline powder, having not more than a very faint odor. Melts at about 250°. Soluble in alcohol and in chloroform; slightly soluble in water.

Trimeprazine Tartrate: White to off-white, odorless, crystalline powder. Freely soluble in water and in chloroform; soluble in alcohol; very slightly soluble in ether and in benzene.

Trimethobenzamide Hydrochloride: White, crystalline powder having a slight phenolic odor. Soluble in water and in warm alcohol; insoluble in ether and in benzene.

Trimethoprim: White to cream-colored, odorless crystals, or crystalline powder. Soluble in benzyl alcohol; sparingly soluble in chloroform and in methanol; slightly soluble in alcohol and in acetone; very slightly soluble in water; practically insoluble in ether and in carbon tetrachloride.

Trimethoprim Sulfate: White to off-white, crystalline powder. Soluble in water, in alcohol, in dilute mineral acids, and in fixed alkalies.

Trimipramine Maleate: White to almost white crystalline powder. Slightly soluble in water and in alcohol.

Trioxsalen: White to off-white or grayish, odorless, crystalline solid. Melts at about 230°. Sparingly soluble in chloroform; slightly soluble in alcohol; practically insoluble in water.

Tripelennamine Hydrochloride: White, crystalline powder. Slowly darkens on exposure to light. Its solutions are practically neutral to litmus. Freely soluble in water, in alcohol, and in chloroform; slightly soluble in acetone; insoluble in benzene, in ether, and in ethyl acetate.

Triprolidine Hydrochloride: White, crystalline powder, having no more than a slight, but unpleasant, odor. Its solutions are alkaline to litmus, and it melts at about 115°. Soluble in water, in alcohol, and in chloroform; insoluble in ether.

Trolamine: Colorless to pale yellow, viscous, hygroscopic liquid having a slight, ammoniacal odor. Soluble in chloroform. Miscible with water and with alcohol. *NF category:* Emulsifying agent; pH modifier (acidifying agent/alkalizing agent/buffering agent).

Troleandomycin: White, odorless, crystalline powder. Freely soluble in alcohol; soluble in chloroform; slightly soluble in ether and in water.

Tromethamine: White, crystalline powder, having a slight, characteristic odor. Freely soluble in water.

Tropicamide: White or practically white, crystalline powder, odorless or having not more than a slight odor. Freely soluble in chloroform and in solutions of strong acids; slightly soluble in water.

Trospium Chloride: Colorless or white to slightly yellow crystalline powder. Very soluble in water; freely soluble in methanol.

Crystallized Trypsin: White to yellowish white, odorless, crystalline or amorphous powder.

Tryptophan: White to slightly yellowish-white crystals or crystalline powder, having a slightly bitter taste. Soluble in hot alcohol and in dilute hydrochloric acid.

Tuberculin: Old Tuberculin is a clear, brownish liquid, which is readily miscible with water and has a characteristic odor. Purified Protein Derivative (PPD) of Tuberculin is a very slightly opalescent, colorless solution. Old Tuberculin and PPD concentrates contain 50% of glycerin for use with various application devices. Old Tuberculin and PPD are also dried on the tines of multiple-puncture devices.

Tubocurarine Chloride: White or yellowish-white to grayish-white, crystalline powder. Melts at about 270°, with decomposition. Soluble in water; sparingly soluble in alcohol.

Tylosin: White to buff-colored powder. Freely soluble in methanol; soluble in alcohol, in amyl acetate, in chloroform, and in dilute mineral acids; slightly soluble in water.

Tylosin Tartrate: Almost white or slightly yellow, hygroscopic powder. Freely soluble in water and in dichloromethane; slightly soluble in alcohol. It dissolves in dilute solutions of mineral acids.

Tyloxapol: Viscous, amber liquid, having a slight, aromatic odor. May exhibit a slight turbidity. Slowly but freely miscible with water. Soluble in glacial acetic acid, in benzene, in toluene, in carbon tetrachloride, in chloroform, and in carbon disulfide. *NF category:* Wetting and/or solubilizing agent.

Tyrosine: White, odorless, tasteless crystals or crystalline powder. Very slightly soluble in water; insoluble in alcohol and in ether.

Ubidecarenone: Yellow to orange, crystalline powder. Melts at about 48°. Soluble in ether; very slightly soluble in dehydrated alcohol; practically insoluble in water.

Undecylenic Acid: Clear, colorless to pale yellow liquid having a characteristic odor. Practically insoluble in water. Miscible with alcohol, with chloroform, with ether, with benzene, and with fixed and volatile oils.

Urea: Colorless to white, prismatic crystals, or white, crystalline powder, or small white pellets. Is practically odorless, but may gradually develop a slight odor of ammonia upon long standing. Its solutions are neutral to litmus. Freely soluble in water and in boiling alcohol; practically insoluble in chloroform and in ether.

Urea C 13: See *Urea*.

Ursodiol: White or almost white, crystalline powder. Freely soluble in alcohol and in glacial acetic acid; sparingly soluble in chloroform; slightly soluble in ether; practically insoluble in water.

Vaccinia Immune Globulin: Transparent or slightly opalescent liquid. Is practically colorless and practically odorless. May develop a slight, granular deposit during storage.

Valacyclovir Hydrochloride: White to off-white powder. Soluble in water; insoluble in dichloromethane.

Powdered Valerian Extract: Brown, hygroscopic, powdery or easily pulverizable mass. Soluble in water to form a slightly cloudy solution; sparingly soluble in 70 percent alcohol; practically insoluble in alcohol.

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Valganciclovir Hydrochloride: White to off-white powder. Very slightly soluble in alcohol; practically insoluble in 2-propanol, in hexane, in acetone, and in ethyl acetate.

Valine: White, odorless, tasteless crystals. Soluble in water; practically insoluble in ether, in alcohol, and in acetone.

Valproic Acid: Colorless to pale yellow, slightly viscous, clear liquid, having a characteristic odor. Refractive index: about 1.423 at 20°. Freely soluble in 1 N sodium hydroxide, in methanol, in alcohol, in acetone, in chloroform, in benzene, in ether, and in *n*-heptane; slightly soluble in water and in 0.1 N hydrochloric acid.

Valrubicin: Orange to orange-red, crystalline powder. Soluble in methylene chloride, in dehydrated alcohol, in methanol, and in acetone; very slightly soluble in water, in hexane, and in petroleum ether.

Valsartan: White or almost white, hygroscopic powder. Freely soluble in anhydrous ethanol; sparingly soluble in methylene chloride; practically insoluble in water.

Vancomycin Hydrochloride: White, almost white, or tan to brown, free-flowing powder, odorless, and having a bitter taste. Freely soluble in water; insoluble in ether and in chloroform.

Vanillin: Fine, white to slightly yellow crystals, usually needle-like, having an odor and taste suggestive of vanilla. Is affected by light. Its solutions are acid to litmus. Freely soluble in alcohol, in chloroform, in ether, and in solutions of the fixed alkali hydroxides; soluble in glycerin and in hot water; slightly soluble in water. *NF category:* Flavors and fragrance.

Vardenafil Hydrochloride: White or slightly brown or yellow powder. Freely soluble in anhydrous alcohol; slightly soluble in water; practically insoluble in heptane.

Vasopressin Injection: Clear, colorless or practically colorless liquid, having a faint, characteristic odor.

Vecuronium Bromide: White or creamy white crystals, or a crystalline powder. Sparingly soluble in alcohol; slightly soluble in water and in acetone.

Hydrogenated Vegetable Oil: Type I Hydrogenated Vegetable Oil—Fine, white powder, beads, or small flakes. Type II Hydrogenated Vegetable Oil—Plastic (semi-solid) or flakes having a softer consistency than Type I. Soluble in hot isopropyl alcohol, in hexane, and in chloroform; insoluble in water. *NF category:* Hydrogenated Vegetable Oil—Wet binder; Type I Hydrogenated Vegetable Oil—Lubricant; Type II Hydrogenated Vegetable Oil—Ointment base.

Venlafaxine Hydrochloride: Off-white to white crystalline powder. Soluble in methanol and in water.

Verapamil Hydrochloride: White or practically white, crystalline powder. Is practically odorless and has a bitter taste. Freely soluble in chloroform; soluble in water; sparingly soluble in alcohol; practically insoluble in ether.

Vidarabine: White to off-white powder. Slightly soluble in dimethylformamide; very slightly soluble in water.

Vigabatrin: White or almost white powder. Freely soluble in water; slightly soluble in methanol; very slightly soluble in alcohol and in chloroform; practically insoluble in methylene chloride; insoluble in hexane and in toluene.

Vinblastine Sulfate: White or slightly yellow, odorless, amorphous or crystalline powder. Is hygroscopic. Freely soluble in water.

Vincristine Sulfate: White to slightly yellow, odorless, amorphous or crystalline powder. Is hygroscopic. Freely soluble in water; soluble in methanol; slightly soluble in alcohol.

Vincristine Sulfate for Injection: Yellowish-white solid, having the characteristic appearance of products prepared by freeze-drying.

Vinorelbine Tartrate: White to yellow or light brown, amorphous powder. Freely soluble in water.

Vitamin A: In liquid form, a light-yellow to red oil that may solidify upon refrigeration. In solid form, has the appearance of any diluent that has been added. May be practically odorless or may have a mild fishy odor, but has no rancid odor or taste. Is unstable to air and light. In liquid form, very soluble in chloroform and in ether; soluble in absolute alcohol and in vegetable oils; insoluble in water and in glycerin. In solid form, may be dispersible in water.

Vitamin E: Practically odorless and tasteless. The alpha tocopherols and alpha tocopheryl acetates occur as clear, yellow, or greenish yellow, viscous oils. *d*-Alpha tocopheryl acetate may solidify in the cold. Alpha tocopheryl acid succinate occurs as a white powder; the *d*-isomer melts at about 75°, and the *dl*-form melts at about 70°. The alpha tocopherols are unstable to air and light, particularly when in alkaline media. The esters are stable to air and light, but are unstable to alkali; the acid succinate is also unstable when held molten. Alpha tocopheryl acid succinate is very soluble in chloroform; soluble in alcohol, in ether, in acetone, and in vegetable oils; slightly soluble in alkaline solutions; insoluble in water. The other forms of Vitamin E are insoluble in water; soluble in alcohol; miscible with ether, with acetone, with vegetable oils, and with chloroform. *NF category:* Antioxidant; plasticizer.

Vitamin E Preparation: The liquid forms are clear, yellow to brownish red, viscous oils. The solid forms are white to tan-white granular powders. The liquid forms are soluble in alcohol; insoluble in water. Miscible with ether, with acetone, with vegetable oils, and with chloroform. The solid forms disperse in water to give cloudy suspensions.

Voriconazole: White to almost white powder. Freely soluble in acetone and in methylene chloride; very slightly soluble in water.

Warfarin Sodium: White, odorless, amorphous or crystalline powder, having a slightly bitter taste. Is discolored by light. Very soluble in water; freely soluble in alcohol; very slightly soluble in chloroform and in ether.

Water for Injection: Clear, colorless, odorless liquid. *NF category:* Solvent; pharmaceutical water.

Bacteriostatic Water for Injection: Clear, colorless liquid, odorless or having the odor of the antimicrobial substance. *NF category:* Vehicle (sterile); pharmaceutical water.

Sterile Water for Inhalation: Clear, colorless solution.

Sterile Water for Injection: Clear, colorless, odorless liquid. *NF category:* Solvent; pharmaceutical water.

Sterile Water for Irrigation: Clear, colorless, odorless liquid. *NF category:* Solvent; pharmaceutical water.

Purified Water: Clear, colorless, odorless liquid. *NF category:* Solvent; pharmaceutical water.

Carnauba Wax: Light brown to pale yellow, moderately coarse powder or flakes, possessing a characteristic bland odor, and free from rancidity. Specific gravity is about 0.99. Freely soluble in warm benzene; soluble in warm chloroform and in warm toluene; slightly soluble in boiling alcohol; insoluble in water. *NF category:* Coating agent.

Emulsifying Wax: Creamy white, wax-like solid, having a mild, characteristic odor. Freely soluble in ether, in chloroform, in most hydrocarbon solvents, and in aerosol propellants; soluble in alcohol; insoluble in water. *NF category:* Emulsifying agent; wetting and/or solubilizing agent; stiffening agent.

Microcrystalline Wax: White or cream-colored, odorless, waxy solid. Soluble in chloroform, in ether, in volatile oils, and in most warm fixed oils; sparingly soluble in dehydrated alcohol; insoluble in water. *NF category:* Coating agent; stiffening agent.

White Wax: Yellowish-white solid, somewhat translucent in thin layers. Has a faint, characteristic odor, and is free from rancidity. Specific gravity is about 0.95. Sparingly soluble in cold alcohol; insoluble in water. Boiling alcohol dissolves the

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cerotic acid and a portion of the myricin, which are constituents of White Wax. Completely soluble in chloroform, in ether, and in fixed and volatile oils. Partly soluble in cold benzene and in cold carbon disulfide; completely soluble in these liquids at about 30°. *NF category:* Stiffening agent.

Yellow Wax: Solid varying in color from yellow to grayish brown. Has an agreeable, honey-like odor. Is somewhat brittle when cold, and presents a dull, granular, noncrystalline fracture when broken. It becomes pliable from the heat of the hand. Specific gravity is about 0.95. Sparingly soluble in cold alcohol; insoluble in water. Boiling alcohol dissolves the cerotic acid and a portion of the myricin, that are constituents of Yellow Wax. Soluble in chloroform, in ether, in fixed oils, and in volatile oils; sparingly soluble in cold benzene and in cold carbon disulfide; soluble in these liquids at about 30°. *NF category:* Stiffening agent.

Wheat Bran: Light tan powder having a characteristic aroma. Practically insoluble in cold water and in alcohol. Available in a variety of particle sizes depending upon the degree of milling to which it is subjected. Color and flavor development variable, depending on the extent to which it is heat-stabilized.

Xanthan Gum: Cream-colored powder. Its solutions in water are neutral to litmus. Soluble in hot or cold water. *NF category:* Suspending and/or viscosity-increasing agent; film-forming agent; polymers for ophthalmic use; release-modifying agent.

Xenon Xe 127: Clear, colorless gas.

Xenon Xe 133 Injection: Clear, colorless solution.

Xylazine: Colorless to white crystals. Sparingly soluble in dilute acid, in acetone, and in chloroform; insoluble in dilute alkali.

Xylazine Hydrochloride: Colorless to white crystals. Sparingly soluble in dilute acid, in acetone, and in methanol; insoluble in dilute alkali.

Xylitol: White crystals or crystalline powder. It has a sweet taste and produces a cooling sensation in the mouth. One g dissolves in about 0.65 mL of water. Sparingly soluble in alcohol. Crystalline xylitol has a melting range between 92° and 96°. *NF category:* Coating agent; emollient; humectant; sweetening agent; diluent.

Xylometazoline Hydrochloride: White to off-white, odorless, crystalline powder. Melts above 300°, with decomposition. Freely soluble in alcohol; soluble in water; sparingly soluble in chloroform; practically insoluble in benzene and in ether.

Xylose: Colorless needles or white, crystalline powder. Is odorless, and has a slightly sweet taste. Very soluble in water; slightly soluble in alcohol.

Yellow Fever Vaccine: Slightly dull, light-orange colored, flaky or crustlike, desiccated mass.

Yohimbine Hydrochloride: White to yellow powder. Melts at about 295°, with decomposition. Soluble in boiling water; slightly soluble in water and in alcohol.

Yttrium Chloride: Colorless, deliquescent crystals. Soluble in water and in alcohol.

Zalcitabine: White to off-white, crystalline powder. Soluble in water and in methanol; sparingly soluble in alcohol, in acetonitrile, in chloroform, and in methylene chloride; slightly soluble in cyclohexane.

Zaleplon: White to off-white powder. Sparingly soluble in alcohol; slightly soluble in propylene glycol; practically insoluble in water.

Zanamivir: White, crystalline powder. Sparingly soluble in water; insoluble in acetone, in acetonitrile, in alcohol, and in isopropyl alcohol.

Zein: White to yellow powder. Soluble in aqueous alcohols, in glycols, in ethylene glycol ethyl ether, in furfuryl alcohol, in tetrahydrofurfuryl alcohol, in aqueous alkaline solutions of pH 11.5 or greater, and in acetone-water mixtures between the limits of 60% and 80% of acetone by volume; insoluble in water, in acetone, and in all anhydrous alcohols except methanol. *NF category:* Coating agent; wet binder.

Zidovudine: White to yellowish powder. Melts at about 124°. Exhibits polymorphism. Soluble in alcohol; sparingly soluble in water.

Zileuton: White to off-white powder.

Zinc Acetate: White crystals or granules, having a slight acetous odor and an astringent taste. Is slightly efflorescent. Freely soluble in water and in boiling alcohol; slightly soluble in alcohol. *NF category:* Emollient.

Zinc Chloride: White or practically white, odorless, crystalline powder, or white or practically white crystalline granules. May also be in porcelain-like masses or molded into cylinders. Is very deliquescent. A solution (1 in 10) is acid to litmus. Very soluble in water; freely soluble in alcohol and in glycerin. Its solution in water or in alcohol is usually slightly turbid, but the turbidity disappears when a small quantity of hydrochloric acid is added.

Zinc Gluconate: White or practically white powder or granules. Soluble in water; very slightly soluble in alcohol.

Zinc Oxide: Very fine, odorless, amorphous, white or yellowish white powder, free from gritty particles. It gradually absorbs carbon dioxide from air. Soluble in dilute acids; insoluble in water and in alcohol. *NF category:* Antimicrobial preservative; coating agent.

Zinc Stearate: Fine, white, bulky powder, free from grittiness. Has a faint, characteristic odor. Is neutral to moistened litmus paper. Insoluble in water, in alcohol, and in ether. *NF category:* Lubricant.

Zinc Sulfate: Colorless, transparent prisms, or small needles. May occur as a white, granular, crystalline powder. Is odorless and is efflorescent in dry air. Its solutions are acid to litmus. Very soluble in water (heptahydrate); freely soluble in water (monohydrate) and in glycerin (heptahydrate); practically insoluble in alcohol (monohydrate); insoluble in alcohol (heptahydrate).

Zinc Undecylenate: Fine, white powder. Practically insoluble in water and in alcohol.

Ziprasidone Hydrochloride (anhydrous): White to slightly pink powder. Soluble in anhydrous formic acid; insoluble in *n*-hexane.

Ziprasidone Hydrochloride (monohydrate): White to slightly pink powder. Slightly soluble in methanol and in methylene chloride; practically insoluble in water.

Zolazepam Hydrochloride: White to off-white, crystalline powder. Freely soluble in water and in 0.1 N hydrochloric acid; soluble in methanol; slightly soluble in chloroform; practically insoluble in ether.

Zolmitriptan: White to off-white, crystalline powder. Freely soluble to soluble in methanol; soluble in water; sparingly soluble in dichloromethane; practically insoluble in toluene.

Zolpidem Tartrate: White to off-white powder, hygroscopic. Sparingly soluble in methanol; slightly soluble in water; practically insoluble in methylene chloride.

Zonisamide: White to off-white powder. Freely soluble in dimethylformamide; soluble in methanol.

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SOLUBILITIES

Approximate Solubilities of USP and NF Articles

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Acenocoumarol	67,000		280	130	1800	
Acetaminophen		20	10			1 N sodium hydroxide, 15
Acetohexamide			230	210		
Acetylcysteine	5		4			
Acetyldigitoxin	6100		62.5	12	>10,000	
Ammonium Alum	7	0.5				
Aluminum Chloride	0.9		4			
Aluminum Sulfate	1					
Amantadine Hydrochloride	2.5		5.1	18		polyethylene glycol 400, 70
Amaranth	15					
Aminocaproic Acid	3					methanol, 450
Aminohippuric Acid	45		50			3 N hydrochloric acid, 5
Aminosalicylate Sodium	2					
Ammonium Carbonate	4					
Amodiaquine Hydrochloride	25		78	>10,000	>10,000	benzene, 10,000
Anethole[1]			2			
Anileridine	>10,000		2	1		
Anileridine Hydrochloride	5		80	>10,000	>10,000	
Antimony Potassium Tartrate	12	3				glycerin, 15
Apomorphine Hydrochloride	50		50			water at 80°, 20
Apraclonidine Hydrochloride	34		74	>10,000		methanol, 13; ethyl acetate, >10,000; hexanes, >10,000
Ascorbic Acid	3		40			
Ascorbyl Palmitate	>1000		125	>1000	>1000	
Aspirin	300		5	17	10 to 15	
Atropine	460		2	1	25	water at 80°, 90
Atropine Sulfate	0.5	2.5	5			glycerin, 2.5
Bendroflumethiazide			23		200	
Benoxinate Hydrochloride	0.8		2.6	2.5	>10,000	
Benzalkonium Chloride (anhydrous)					100	benzene, 6
Gamma Benzene Hexachloride				3.5	40	dehydrated alcohol, 20
Benzethonium Chloride	<1		<1	<1	6000	
Benzocaine	2500		5	2	4	almond oil or olive oil, 30–50
Benzoic Acid	300		3	5	3	
Benzonatate	<1		<1	<1	<1	
Betadex	54					
Betamethasone	5300		65	325		warm alcohol, 15; methanol, 3
Betamethasone Acetate	2000		9	16		
Betamethasone Sodium Phosphate	2		470	>10,000	>10,000	
Betamethasone Valerate	10,000		16	<10	400	
Bisacodyl	>10,000		210	2.5	275	

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Boric Acid	18	4	18			boiling alcohol, 6; glycerin, 4
Bromodiphenhydramine Hydrochloride	<1		2	2	3500	isopropyl alcohol, 31
Brompheniramine Maleate	5		15	15		
Busulfan						acetone, 45
Butabarbital Sodium	2		7	7000	10,000	
Butamben	7000					
Butylated Hydroxyanisole			4.0	2.0	1.2	
Butylated Hydroxytoluene			4.0	1.1	1.1	
Caffeine (hydrous)	50		75	6	600	
Calcium Chloride	0.7	0.2	4			boiling alcohol, 2
Calcium Gluconate	30 (slowly)	5				
Calcium Hydroxide	630	1300				
Calcium Lactate	20					
Calcium Pantothenate	3					
Calcium Sulfate	375	485				
Camphor	800		1	0.5	1	
Carbinoxamine Maleate	<1		1.5	1.5	8300	
Carisoprodol	2083		2.5	2.3		acetone, 2.5
Cephaloridine	5		1000	10,000	10,000	
Cetylpyridinium Chloride	4.5		2.5	4.5		
Chloral Betaine	1		4	>10,000	>10,000	0.1 N hydrochloric acid, 1; 0.1 N sodium hydroxide, 1
Chloral Hydrate	0.25		1.3	2	1.5	
Chlorambucil						acetone, 2
Chloramphenicol	400					
Chlordiazepoxide	>10,000		50	6250	130	
Chlorobutanol	125		1			glycerin, 10
Chlorocresol	260		0.4			
Chloroprocaine Hydrochloride	20		100			
Chlorpheniramine Maleate	4		10	10		
Chlorpromazine			3	2	3	benzene, 2
Chlorpromazine Hydrochloride	1		1.5	1.5		
Chlortetracycline Hydrochloride	75		560			
Cholesterol			100 (slowly)			dehydrated alcohol, 50
Citric Acid	0.5		2		30	
Clindamycin Palmitate Hydrochloride			3			ethyl acetate, 9
Clindamycin Phosphate	2.5		>1000	>1000	>1000	
Clioquinol	>100,000		3500	120	4500	
Cocaine	600		7	1	3.5	olive oil, 12; liquid petrolatum, 80–100
Cocaine Hydrochloride	0.5		3.5	15		
Codeine	120		2	0.5	50	
Codeine Phosphate	2.5		325			boiling alcohol, 125; water at 80°, 0.5
Codeine Sulfate	30		1300			water at 80°, 6.5
Colchicine	25				220	

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Cortisone Acetate			350	4		acetone, 75; dioxane, 30
Cupric Sulfate	3	0.5	500			glycerin, 3
Cyanocobalamin	80					
Cyclizine Hydrochloride	115		115	75		
Cyproheptadine Hydrochloride	275		35	26		methanol, 1.5
Dehydrocholic Acid			100	35	2200 (15°)	acetic acid at 15°, 135; acetone at 15°, 130; benzene at 15°, 960; ethyl acetate at 15°, 135
Demeclocycline			200			methanol, 40
Demeclocycline Hydrochloride	60					methanol, 50
Denatonium Benzoate	20		2.4	2.9	5000	
Desipramine Hydrochloride	12		14	3.5	>10,000	
Dexamethasone Sodium Phosphate	2					
Dexbrompheniramine Maleate	1.2		2.5	2	3000	
Dexchlorpheniramine Maleate	1.1		2	1.7	2500	
Dextroamphetamine Sulfate	10		800			
Dextromethorphan Hydrobromide	65					
Dextrose	1		100			
Diazepam	333		16	2	39	
Dibucaine	4600		0.7	0.5	1.4	
Dicyclomine Hydrochloride	13		5	2	770	glacial acetic acid, 2
Diethylpropion Hydrochloride	0.5		3	3		
Digitoxin			150	40		
Dihydroergotamine Mesylate	125		90	175	2600	
Dimercaprol	20					
Dimethisterone			3	0.7		
Diphenhydramine Hydrochloride	1		2	2		acetone, 50
Disulfiram	>5000		30		15	
Docusate Calcium	3300		>1	>1	>1	
Docusate Sodium	70 (slowly)					
Doxapram Hydrochloride	50					
Doxylamine Succinate	1		2	2	370	
Droperidol	10,000		140	4	500	
Dyclonine Hydrochloride	60		24	2.3	>10,000	hexane, >10,000
Dydrogesterone	>10,000		40	2	200	
Echothiophate Iodide	1					dehydrated alcohol, 25; methanol, 3
Edrophonium Chloride	0.5		5			
Enalaprilat	200		>1000			dimethylformamide, 40; methanol, 68
Ephedrine	20		0.2			
Ephedrine Hydrochloride	3		14			
Ephedrine Sulfate	1.3		90			
Epinephrine Bitartrate	3					
Ergotamine Tartrate	500		500			
Erythromycin	1000					

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Erythromycin Estolate			20	10		acetone, 15
Estradiol			28	435	150	
Estradiol Cypionate	>10,000		40	7	2800	
Estrone			250 (15°)	110 (15°)		boiling alcohol, 50; boiling chloroform, 80; acetone at 50°, 50; boiling acetone, 33; boiling benzene, 145
Estropipate	>2000		>2000	>2000	>2000	warm alcohol, 500
Ethacrynic Acid			1.6	6	3.5	
Ether[1]	12					
Ethyl Vanillin	100 (50°)		2			
Ferrous Gluconate	5					
Ferrous Sulfate	1.5	0.5				
Flumethasone Pivalate	>10,000		89	350	2800	
Fluocinolone Acetonide	>1000		45	25	350	
Fluorometholone	>10,000		200	2200	>10,000	
Fluphenazine Enanthate			<1	<1	2	
Fluphenazine Hydrochloride	1.4		6.7			
Flurandrenolide			72	10		methanol, 25
Flurazepam Hydrochloride	2		4	90	5000	methanol, 3; isopropanol, 69; benzene, 2500; petroleum ether, 5000
Fluroxene	220					
Fructose			15			methanol, 14
Gentian Violet			10			glycerin, 15
Glyceryl Monostearate				10.0	100.0	methanol, 100; isopropyl alcohol, 33
Glycine	4		1254			water at 50°, 2.6; at 75°, 1.9; at 100°, 1.5
Glycopyrrolate	4.2		30	260	35,000	
Guaifenesin	60–70					
Halazone	>1000		140	>1000	>2000	
Haloperidol	>10,000		60	15	200	
Heparin Sodium	20					
Histamine Phosphate	4					
Homatropine Hydrobromide	6		40	420		
Hydralazine Hydrochloride	25		500			
Hydrocortisone			40			acetone, 80
Hydrocortisone Acetate			230	200		
Hydrocortisone Sodium Phosphate	1.5					
Hydroflumethiazide	>5000		39	>5000	2500	
Hydromorphone Hydrochloride	3					
Hydroquinone	17		4	51	16.5	
Hydroxocobalamin	50		100	10,000	10,000	
Hydroxyzine Hydrochloride	1		4.5	13	>1000	
Hydroxyzine Pamoate	>1000		700	>1000	>1000	dimethylformamide, 10; 10 N sodium hydroxide, 3.5
Hyoscyamine Hydrobromide			2.5	1.7	2300	
Hyoscyamine Sulfate	0.5		5			

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Ichthammol	10					
Indigotindisulfonate Sodium	100					
Indomethacin			50	30	40	
Iodine	3000		13			carbon disulfide, 4; glycerin, 80
Ipodate Sodium	<1		2			dimethylacetamide, 2; dimethylformamide, 3.5; dimethylsulfoxide, 3.5
Isoniazid	8		50			
Isopropamide Iodide	50		10	5		
Isopropyl Alcohol	<1		<1	<1	<1	
Isoproterenol Hydrochloride	3		50			
Isoproterenol Sulfate	4		>2000	>2000	>2000	
Isoxsuprine Hydrochloride	500		100	>10,000	>10,000	0.1 N hydrochloric acid, 2500; 0.1 N sodium hydroxide, 100
Ketamine Hydrochloride	4		14	60	>10,000	methanol, 6; absolute alcohol, 60
Lactose	5 (slowly)	2.6				
Levorphanol Tartrate	50		120			
Levothyroxine Sodium	700		300			
Lisinopril	10		>10,000	>10,000	>10,000	methanol, 70
Magnesium Hydroxide	>10,000		>10,000	>10,000	>10,000	
Magnesium Sulfate	0.8	0.5				glycerin, 1 (slowly)
Mannitol	5.5					
Menadione			60			benzene, 10; vegetable oils, 50
Mesoridazine Besylate	1		11	3	6300	
Methacholine Chloride	1.2		1.7	2.1		
Methacrylic Acid Copolymer						water in methanol (≥3 in 100), 10; water in alcohol (≥3 in 100), 10; water in isopropyl alcohol (≥3 in 100), 10; water in acetone (≥3 in 100), 10
Methacycline Hydrochloride	100		300	>1000	>1000	0.1 N sodium hydroxide, 25
Methdilazine Hydrochloride	2		2	6	>10,000	0.1 N hydrochloric acid, 1; 0.1 N sodium hydroxide, 1
Methenamine	1.5		12.5	10	320	
Methenamine Mandelate			10	20	350	
Methimazole	5		5	4.5	125	
Methocarbamol	40 (20°)					
Methotrimeprazine	10		10	2		methanol, 10
Methoxyflurane	500		<1	<1	<1	
Methsuximide	350		3	<1	2	
Methyclothiazide	>10,000		92.5	>10,000	2700	
Methylbenzethonium Chloride	0.8		0.9	>10,000	0.7	
Methylene Blue	25		65			
Methylergonovine Maleate	100		175	1900	8400	
Methylparaben	400		3		10	water at 80°, 50
Methylprednisolone	10,000		100	800	800	
Methylprednisolone Acetate	1500		400	250	1500	
Methylprednisolone Sodium Succinate	1.5		12	>10,000	>10,000	

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Methysergide Maleate	200		165	3400	>10,000	
Miconazole	>100,000		9.5	2	15	isopropyl alcohol, 4; propylene glycol, 9; methanol, 5.3
Miconazole Nitrate	6250		312	525	50,000	isopropyl alcohol, 1408; propylene glycol, 119; methanol, 75
Morphine Sulfate	16		570			alcohol at 60°, 240; water at 80°, 1
Nalidixic Acid	>1000		910	29	>1000	
Neomycin Sulfate	1					
Niacin	60					
Niacinamide	1.5	10	5.5			
Nifedipine	>10,000					acetone, 10
Nitrofurazone	4200		590			propylene glycol, 350
Nitromersol	>2000		>2000	>2000	>2000	
Norepinephrine Bitartrate	2.5		300			
Norethindrone Acetate	>10,000		10	<1	18	dioxane, 2
Nortriptyline Hydrochloride	90		30	20		methanol, 10
Oxandrolone	5200		57	<5	860	acetone, 69
Oxazepam	>10,000		220	270	2200	
Oxtriphylline	1					
Oxymetazoline Hydrochloride	6.7		3.6	862		
Oxymetholone	>10,000		40	5	82	dioxane, 14
Oxymorphone Hydrochloride	4		100	>1000	>1000	methanol, 25
Oxytetracycline	4150			>10,000	6250	absolute alcohol, 66
Oxytetracycline Calcium	>1000		>1000	>1000	>1000	0.1 N sodium hydroxide, 15
Papaverine Hydrochloride	30		120			
Paraldehyde[1]	10	17				
Paramethasone Acetate				50		methanol, 40
Paromomycin Sulfate	<1		>10,000	>10,000	>10,000	
Pectin	20					
Penicillin G Benzathine	5000		65			
Sterile Penicillin G Procaine	250		30	60		
Sterile Penicillin G Sodium	40					
Penicillin V Benzathine	3200		330	42	910	acetone, 37
Penicillin V Potassium			150			
Pentazocine	>1000		11	2	42	
Pentazocine Hydrochloride	30		20	4	>10,000	
Pentobarbital	>2000		4.5	4.0	10	
Pentolinium Tartrate	0.5		475	>1000	>2000	
Perphenazine			7			acetone, 13
Phenazopyridine Hydrochloride	<10	20	59	331	>5000	cold water, 300; glycerin, 100
Phenindamine Tartrate	40		350			
Phenmetrazine Hydrochloride	0.4		2	2		
Phenobarbital	1000		10			
Phenol	15					mineral oil, 70
Phentolamine Mesylate	1		4	700		

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Phenylethyl Alcohol	60		<1	<1	<1	alcohol solution (1 in 2), 2; diethylphthalate, <1; benzyl benzoate, <1
Phenylmercuric Acetate	180		225	6.8	200	
Phenylmercuric Nitrate	600					
Phenylpropanolamine Hydrochloride	1.1		7.4	4100		
Physostigmine Salicylate	75		16	6	250	
Physostigmine Sulfate	4		0.4		1200	
Pilocarpine Hydrochloride	0.3		3	360		
Pilocarpine Nitrate	4		75			
Piminodine Esylate	>1000		6	2	>1000	
Pimozide	>10,000		1000	10	1000	acetone, 100; methanol, 1000; 0.1 N hydrochloride acid, >1000
Polyethylene Glycol 1540	1			3		absolute alcohol, 100
Polyethylene Glycol 4000	4		2.5	2		
Potash, Sulfurated	2					
Potassium Acetate	0.5	0.2	3			
Potassium Alum	7	0.3				
Potassium Benzoate	2		75			alcohol solution (9 in 10), 50
Potassium Chloride	2.8	2				
Potassium Citrate	1					glycerin, 2.5
Potassium Gluconate	3					
Potassium Hydroxide	1		3			glycerin, 2.5
Potassium Iodide	0.7	0.5	22			glycerin, 2
Potassium Permanganate	15	3.5				
Potassium Sodium Tartrate	1					
Potassium Sorbate	4.5		35	>1000	>1000	
Pramoxine Hydrochloride				35		
Prednisolone			30	180		acetone, 50
Prednisolone Acetate			120			
Prednisolone Hemisuccinate	4170		6.3	1064	248	
Prednisolone Sodium Phosphate	4					methanol, 13
Prednisone			150	200		
Prilocaine Hydrochloride	3.5		4.2	175		
Primaquine Phosphate	15					
Primidone	2000		200			
Procaine Hydrochloride	1		15			
Prochlorperazine Edisylate	2		1500			
Prochlorperazine Maleate			1200			
Procyclidine Hydrochloride	35		9	6	11,000	
Promazine Hydrochloride	3					
Propoxycaine Hydrochloride	2		10	>10,000	80	
Propoxyphene Napsylate	10,000		15	10		
Propylhexedrine	>500		0.4	0.2	0.1	
Propylparaben	2500	400	1.5		3	

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Protriptyline Hydrochloride	2		3.5	2.5	>10,000	
Pseudoephedrine Hydrochloride	0.5		3.6	91	7000	
Pyrazinamide	67			135	1000	absolute alcohol, 175; methanol, 72
Pyridoxine Hydrochloride	5		115			
Pyrilamine Maleate	0.5		3	2		absolute alcohol, 15
Pyrimethamine			200	125		
Pyrrocaine Hydrochloride	1.5		12	8		
Quinidine Sulfate	100		10	15		
Quinine Sulfate	500		120			
Reserpine			1800	6		
Resorcinol	1		1			
Rotoxamine Tartrate	10		100	>10,000	>10,000	
Saccharin	290	25	31			
Saccharin Calcium	2.6		4.7			
Saccharin Sodium	1.5		50			
Salicylic Acid	460	15	3	45	3	benzene, 135
Scopolamine Hydrobromide	1.5		20			
Secobarbital						0.5 N sodium hydroxide, 8.5
Selenium Sulfide				161	1667	
Sennosides	35		2100	3700	6100	
Silver Nitrate	0.4	0.1	30			boiling alcohol, 6.5
Simethicone[2]	>10,000			10^2	10^2	benzene, 10^2; absolute alcohol, >10,000
Sodium Acetate	0.8	0.6	19			
Sodium Ascorbate	1.3					
Sodium Benzoate	2		75			90 percent alcohol, 50
Sodium Bicarbonate	12					
Sodium Bisulfite	4					
Sodium Borate	16	1				glycerin, 1
Sodium Carbonate	3	1.8				
Sodium Chloride	2.8	2.7				glycerin, 10
Sodium Citrate (hydrous)	1.5	0.6				
Sodium Fluoride	25					
Sodium Formaldehyde Sulfoxylate	3.4		510	175	180	
Sodium Hydroxide	1					
Sodium Iodide	0.6		2			glycerin, 1
Sodium Lauryl Sulfate	10					
Sodium Nitrite	1.5					
Sodium Phosphate, Dried	8					
Sodium Propionate	1	0.65	24	>10,000	>10,000	
Sodium Thiosulfate	0.5					
Sorbic Acid	1000		10	15	30	absolute alcohol, 8; methanol, 8; propylene glycol, 19
Sorbitol	0.45					
Stanozolol	>1000		41	74	370	

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	Water	Boiling Water	Alcohol	Chloroform	Ether	Other
Stearic Acid			20	2	3	
Stibophen	1		>10,000	>10,000	10,000	
Succinylcholine Chloride	1		350			
Sucrose	0.5	0.2	170			
Sucrose Octaacetate	1100		11			acetone, 0.3; benzene, 0.6; toluene, 0.5
Sulconazole Nitrate	3333		100	333		pyridine, 10; methanol, 71; acetone, 130; methylene chloride, 286; toluene, 2000; dioxane, 2000
Sulfacetamide Sodium	2.5					
Sulfadiazine	13,000					human serum at 37°, 620
Sulfadiazine Sodium	2					
Sulfadimethoxine			200			2 N hydrochloric acid, 50
Sulfaethidole	>3000		75	1300	1700	methanol, 51; acetone, 13; benzene, 2277
Sulfamethizole	2000		38	1900	1900	acetone, 13
Sulfamethoxazole	3400		50	1000	1000	carbon disulfide, 2 (slowly and usually incompletely)
Sulfapyridine	3500		440			acetone, 65
Sulfasalazine	>10,000		2900	>10,000	>10,000	methanol, 1500
Sulfisoxazole	7700					boiling alcohol, 10
Sulfisoxazole Acetyl			176	35	1064	methanol, 203
Precipitated Sulfur						carbon disulfide, 2 (slowly and usually incompletely); olive oil, 100
Tartaric Acid	0.8	0.5	3		250	methanol, 1.7
Terpin Hydrate	200	35	13	140	140	boiling alcohol, 3
Testolactone	4050					
Testosterone				2	100	absolute alcohol, 6
Tetracaine	>1000		5	2	2	
Tetracycline	2500		50			
Tetracycline Hydrochloride	10		100			
Tetrahydrozoline Hydrochloride	3.5		7.5	>1000	>1000	
Theophylline Sodium Glycinate	6					
Thiamine Hydrochloride	1		170			
Thiamine Mononitrate	44					
Thiethylperazine Maleate	1700		530	>10,000	>10,000	
Thimerosal	1		12			
Thioguanine			7700			
Thiotepa	13		8.3	1.9	4.1	
Thiothixene	>10,000			2	120	absolute alcohol, 110
Thiothixene Hydrochloride	8			280	>10,000	absolute alcohol, 270
Thymol	1000		1	1	1.5	olive oil, 2
Tolazoline Hydrochloride	<1		2	3	>10,000	
Triamcinolone Diacetate			13	80		methanol, 40
Triamterene						formic acid, 30; 2-methoxyethanol, 85
Triazolam	>10,000		1000	25	>10,000	0.1 N hydrochloric acid, 600

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Approximate Solubilities of USP and NF Articles *(continued)*

	Name and Volume, in mL, of Solvent					
Solute (1 g)	**Water**	**Boiling Water**	**Alcohol**	**Chloroform**	**Ether**	**Other**
Trichlormethiazide	1100		48	5000	1400	dioxane, 9.1; dimethylformamide, 4.35
Trichloroethylene	>10,000					
Triethylenemelamine	2.5		13	3.6		methanol, 8; acetone, 9.5; benzene, 18
Trifluoperazine Hydrochloride	3.5		11	100		
Triflupromazine Hydrochloride	<1		<1	1.7		
Trimeprazine Tartrate	2		20	5	1800	
Trimethobenzamide Hydrochloride	2		59	67	720	
Trioxsalen			1150	84		methylenedichloride, 43; 4-methyl-2-pentanone, 100
Tripelennamine Hydrochloride	1		6	6		acetone, 350
Triprolidine Hydrochloride	2.1		1.8	1	2000	
Tromethamine	1.8		45.5	>10,000		
Tubocurarine Chloride	20		45			
Urea	1.5		10			boiling alcohol, 1
Vanillin	100					glycerin, 20; water at 80°, 20
Xylometazoline Hydrochloride	35					
Zinc Acetate	2.5		30			
Zinc Chloride	0.5		1.5			glycerin, 2
Zinc Sulfate	0.6					glycerin, 2.5

[1] Solubility data for compounds that ordinarily are liquids at 25° are expressed in terms of the ratio of the *volume* of solute to the *volume* of solvent; i.e., 1 mL dissolved in mL of solvent.
[2] Liquid phase only; silicon dioxide remains as a residue in these solvents.

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ATOMIC WEIGHTS

Standard Atomic Weights of the Elements, Recommended by the Commission on Atomic Weights and Isotopic Abundances of the International Union of Pure and Applied Chemistry

This table can be found at http://www.sbcs.qmul.ac.uk/iupac/AtWt/.

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Half-Lives of Selected Radionuclides

This table can be found at http://www.nist.gov/pml/data/halflife-html.cfm.

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ALCOHOLOMETRIC TABLE

Based on data appearing in the National Bureau of Standards Bulletin, vol. 9, pp. 424–425 (publication of the National Institute of Standards and Technology).

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Percentage of C_2H_5OH		Specific gravity in air		Percentage of C_2H_5OH		Specific gravity in air	
By volume at 15.56°C	By weight	At 25°/25°	At 15.56°/15.56°	By weight	By volume at 15.56°C	At 25°/25°	At 15.56°/15.56°
0	0.00	1.0000	1.0000	0	0.00	1.0000	1.0000
1	0.80	0.9985	0.9985	1	1.26	0.9981	0.9981
2	1.59	0.9970	0.9970	2	2.51	0.9963	0.9963
3	2.39	0.9956	0.9956	3	3.76	0.9945	0.9945
4	3.19	0.9941	0.9942	4	5.00	0.9927	0.9928
5	4.00	0.9927	0.9928	5	6.24	0.9911	0.9912
6	4.80	0.9914	0.9915	6	7.48	0.9894	0.9896
7	5.61	0.9901	0.9902	7	8.71	0.9879	0.9881
8	6.42	0.9888	0.9890	8	9.94	0.9863	0.9867
9	7.23	0.9875	0.9878	9	11.17	0.9848	0.9852
10	8.05	0.9862	0.9866	10	12.39	0.9833	0.9839
11	8.86	0.9850	0.9854	11	13.61	0.9818	0.9825
12	9.68	0.9838	0.9843	12	14.83	0.9804	0.9812
13	10.50	0.9826	0.9832	13	16.05	0.9789	0.9799
14	11.32	0.9814	0.9821	14	17.26	0.9776	0.9787
15	12.14	0.9802	0.9810	15	18.47	0.9762	0.9774
16	12.96	0.9790	0.9800	16	19.68	0.9748	0.9763
17	13.79	0.9778	0.9789	17	20.88	0.9734	0.9751
18	14.61	0.9767	0.9779	18	22.08	0.9720	0.9738
19	15.44	0.9756	0.9769	19	23.28	0.9706	0.9726
20	16.27	0.9744	0.9759	20	24.47	0.9692	0.9714
21	17.10	0.9733	0.9749	21	25.66	0.9677	0.9701
22	17.93	0.9721	0.9739	22	26.85	0.9663	0.9688
23	18.77	0.9710	0.9729	23	28.03	0.9648	0.9675
24	19.60	0.9698	0.9719	24	29.21	0.9633	0.9662
25	20.44	0.9685	0.9708	25	30.39	0.9617	0.9648
26	21.29	0.9673	0.9697	26	31.56	0.9601	0.9635
27	22.13	0.9661	0.9687	27	32.72	0.9585	0.9620
28	22.97	0.9648	0.9676	28	33.88	0.9568	0.9605
29	23.82	0.9635	0.9664	29	35.03	0.9551	0.9590
30	24.67	0.9622	0.9653	30	36.18	0.9534	0.9574
31	25.52	0.9609	0.9641	31	37.32	0.9516	0.9558
32	26.38	0.9595	0.9629	32	38.46	0.9498	0.9541
33	27.24	0.9581	0.9617	33	39.59	0.9480	0.9524
34	28.10	0.9567	0.9604	34	40.72	0.9461	0.9506
35	28.97	0.9552	0.9590	35	41.83	0.9442	0.9488
36	29.84	0.9537	0.9576	36	42.94	0.9422	0.9470
37	30.72	0.9521	0.9562	37	44.05	0.9402	0.9451

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(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Percentage of C_2H_5OH		Specific gravity in air		Percentage of C_2H_5OH		Specific gravity in air	
By volume at 15.56°C	By weight	At 25°/25°	At 15.56°/15.56°	By weight	By volume at 15.56°C	At 25°/25°	At 15.56°/15.56°
38	31.60	0.9506	0.9548	38	45.15	0.9382	0.9432
39	32.48	0.9489	0.9533	39	46.24	0.9362	0.9412
40	33.36	0.9473	0.9517	40	47.33	0.9341	0.9392
41	34.25	0.9456	0.9501	41	48.41	0.9320	0.9372
42	35.15	0.9439	0.9485	42	49.48	0.9299	0.9352
43	36.05	0.9421	0.9469	43	50.55	0.9278	0.9331
44	36.96	0.9403	0.9452	44	51.61	0.9256	0.9310
45	37.87	0.9385	0.9434	45	52.66	0.9235	0.9289
46	38.78	0.9366	0.9417	46	53.71	0.9213	0.9268
47	39.70	0.9348	0.9399	47	54.75	0.9191	0.9246
48	40.62	0.9328	0.9380	48	55.78	0.9169	0.9225
49	41.55	0.9309	0.9361	49	56.81	0.9147	0.9203
50	42.49	0.9289	0.9342	50	57.83	0.9124	0.9181
51	43.43	0.9269	0.9322	51	58.84	0.9102	0.9159
52	44.37	0.9248	0.9302	52	59.85	0.9079	0.9137
53	45.33	0.9228	0.9282	53	60.85	0.9056	0.9114
54	46.28	0.9207	0.9262	54	61.85	0.9033	0.9092
55	47.25	0.9185	0.9241	55	62.84	0.9010	0.9069
56	48.21	0.9164	0.9220	56	63.82	0.8987	0.9046
57	49.19	0.9142	0.9199	57	64.80	0.8964	0.9024
58	50.17	0.9120	0.9177	58	65.77	0.8941	0.9001
59	51.15	0.9098	0.9155	59	66.73	0.8918	0.8978
60	52.15	0.9076	0.9133	60	67.79	0.8895	0.8955
61	53.15	0.9053	0.9111	61	68.64	0.8871	0.8932
62	54.15	0.9030	0.9088	62	69.59	0.8848	0.8909
63	55.17	0.9006	0.9065	63	70.52	0.8824	0.8886
64	56.18	0.8983	0.9042	64	71.46	0.8801	0.8862
65	57.21	0.8959	0.9019	65	72.38	0.8777	0.8839
66	58.24	0.8936	0.8995	66	73.30	0.8753	0.8815
67	59.28	0.8911	0.8972	67	74.21	0.8729	0.8792
68	60.33	0.8887	0.8948	68	75.12	0.8706	0.8768
69	61.38	0.8862	0.8923	69	76.02	0.8682	0.8745
70	62.44	0.8837	0.8899	70	76.91	0.8658	0.8721
71	63.51	0.8812	0.8874	71	77.79	0.8634	0.8697
72	64.59	0.8787	0.8848	72	78.67	0.8609	0.8673
73	65.67	0.8761	0.8823	73	79.54	0.8585	0.8649
74	66.77	0.8735	0.8797	74	80.41	0.8561	0.8625
75	67.87	0.8709	0.8771	75	81.27	0.8537	0.8601
76	68.98	0.8682	0.8745	76	82.12	0.8512	0.8576
77	70.10	0.8655	0.8718	77	82.97	0.8488	0.8552
78	71.23	0.8628	0.8691	78	83.81	0.8463	0.8528
79	72.38	0.8600	0.8664	79	84.64	0.8439	0.8503
80	73.53	0.8572	0.8636	80	85.46	0.8414	0.8479

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(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Percentage of C_2H_5OH		Specific gravity in air		Percentage of C_2H_5OH		Specific gravity in air	
By volume at 15.56°C	By weight	At 25°/25°	At 15.56°/15.56°	By weight	By volume at 15.56°C	At 25°/25°	At 15.56°/15.56°
81	74.69	0.8544	0.8608	81	86.28	0.8389	0.8454
82	75.86	0.8516	0.8580	82	87.08	0.8364	0.8429
83	77.04	0.8487	0.8551	83	87.89	0.8339	0.8404
84	78.23	0.8458	0.8522	84	88.68	0.8314	0.8379
85	79.44	0.8428	0.8493	85	89.46	0.8288	0.8354
86	80.66	0.8397	0.8462	86	90.24	0.8263	0.8328
87	81.90	0.8367	0.8432	87	91.01	0.8237	0.8303
88	83.14	0.8335	0.8401	88	91.77	0.8211	0.8276
89	84.41	0.8303	0.8369	89	92.52	0.8184	0.8250
90	85.69	0.8271	0.8336	90	93.25	0.8158	0.8224
91	86.99	0.8237	0.8303	91	93.98	0.8131	0.8197
92	88.31	0.8202	0.8268	92	94.70	0.8104	0.8170
93	89.65	0.8167	0.8233	93	95.41	0.8076	0.8142
94	91.03	0.8130	0.8196	94	96.10	0.8048	0.8114
95	92.42	0.8092	0.8158	95	96.79	0.8020	0.8086
96	93.85	0.8053	0.8118	96	97.46	0.7992	0.8057
97	95.32	0.8011	0.8077	97	98.12	0.7962	0.8028
98	96.82	0.7968	0.8033	98	98.76	0.7932	0.7998
99	98.38	0.7921	0.7986	99	99.39	0.7902	0.7967
100	100.00	0.7871	0.7936	100	100.00	0.7871	0.7936

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INTRINSIC VISCOSITY TABLE

This table is based on data appearing in Table 3 of ASTM Test Method D 1795 (publication of the American Society for Testing and Materials).

Intrinsic Viscosity, [η]c, at Different Values of Relative Viscosity, η_{rel}[A,B]

	[η]c									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.941	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762
4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017

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Intrinsic Viscosity, [η]c, at Different Values of Relative Viscosity, η_{rel}[A,B] *(continued)*

	[η]c									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.521	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915
8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064

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Intrinsic Viscosity, [η]c, at Different Values of Relative Viscosity, η_{rel}[A,B] *(continued)*

	[η]c									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66

[A] Swedish Method CCA 27:57, Karin Wilson, *Svensk Papperstidning,* Vol. 60, 1957, pp. 513 to 521.
[B] Derived from the equation: $\eta_{rel} - 1 = \eta_{sp} = [\eta]ce^{k'[\eta]c}$, where $k' = 0.30$.

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Chapter Charts

CHART GUIDE—USP GENERAL CHAPTERS[1]

Official Articles

- Chemical Medicines Drug Substances—Universal Tests: See *Chart 1a*
- Chemical Medicines Drug Substances—Specific Tests: See *Chart 1b*
- Biologics Drug Substances: See *Chart 2*
- Excipients—Universal Tests: See *Chart 3a*
- Excipients—Specific Tests: See *Chart 3b*
- Chemical Medicines Drug Products—Universal Tests: See *Chart 4a*
- Chemical Medicines Drug Products—Specific Tests: See *Chart 4b*
- Biologics Drug Products: See *Chart 5*
- Vaccines: See *Chart 6*
- Blood and Blood Products: See *Chart 7*
- Cell, Gene, and Tissue Based Products: See *Chart 8*
- Dietary Supplement Ingredients: See *Chart 11a*
- Dietary Supplement Ingredients and Dosage Forms: See *Chart 11b*
- Dietary Supplement Products: See *Chart 12*
- Compounding—Substance/Preparation/Practice: See *Chart 13*
- Medical Devices
  - ⟨691⟩ *Cotton*
  - ⟨861⟩ *Sutures—Diameter*
  - ⟨871⟩ *Sutures—Needle Attachment*

Generally Applicable

- Basic Elements
  - ⟨1010⟩ *Analytical Data—Interpretation and Treatment*
  - ⟨1029⟩ *Good Documentation Guidelines*
  - ⟨1039⟩ *Chemometrics*
  - ⟨1058⟩ *Analytical Instrument Qualification*
  - ⟨1097⟩ *Bulk Powder Sampling Procedures*
  - ⟨1151⟩ *Pharmaceutical Dosage Forms*
  - ⟨1210⟩ *Statistical Tools for Procedure Validation*
  - ⟨1224⟩ *Transfer of Analytical Procedures*
  - ⟨1225⟩ *Validation of Compendial Procedures*
  - ⟨1226⟩ *Verification of Compendial Procedures*
- Drug Product Distribution: See *Chart 9*
- Microbiology—Nonsterile Products: See *Chart 10a*
- Microbiology—Sterile Products: See *Chart 10b*

Chart 1a. Chemical Medicines Drug Substances—Universal Tests

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨7⟩ *Labeling*	◆					
⟨11⟩ *USP Reference Standards*			◆			
⟨81⟩ *Antibiotics—Microbial Assays*			◆			
⟨181⟩ *Identification Organic Nitrogenous Bases*		◆				
⟨191⟩ *Identification Tests—General*		◆				
⟨193⟩ *Identification Tests—Tetracyclines*		◆				
⟨197⟩ *Spectrophotometric Identification Test*		◆				
⟨201⟩ *Thin-Layer Chromatographic Identification Test*		◆				

[1] This table and the *Charts 1–13* that follow are intended as a guide to the chapters in this publication. They may not be all-inclusive, and they are not intended to describe expectations for articles or limit the application of tests to any article in the *USP–NF*.

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Chart 1a. Chemical Medicines Drug Substances—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*		◆				
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*		◆				
⟨206⟩ *Aluminum*					◆	
⟨211⟩ *Arsenic*					◆	
⟨221⟩ *Chloride and Sulfate*					◆	
⟨223⟩ *Dimethylaniline*				◆		
⟨232⟩ *Elemental Impurities—Limits*					◆	
⟨233⟩ *Elemental Impurities—Procedures*					◆	
⟨241⟩ *Iron*					◆	
⟨251⟩ *Lead*					◆	
⟨261⟩ *Mercury*					◆	
⟨281⟩ *Residue on Ignition*					◆	
⟨291⟩ *Selenium*					◆	
⟨351⟩ *Assay for Steroids*			◆			
⟨391⟩ *Epinephrine Assay*			◆			
⟨401⟩ *Fats and Fixed Oils*		◆				
⟨425⟩ *Iodometric Assay—Antibiotics*			◆			
⟨451⟩ *Nitrate Titration*			◆			
⟨461⟩ *Nitrogen Determination*				◆		
⟨466⟩ *Ordinary Impurities*				◆		
⟨467⟩ *Residual Solvents*						◆
⟨471⟩ *Oxygen Flask Combustion*					◆	
⟨511⟩ *Single-Steroid Assay*			◆			
⟨541⟩ *Titrimetry*		◆	◆			
⟨621⟩ *Chromatography*		◆	◆	◆		◆
⟨630⟩ *Visual Comparison*				◆	◆	
⟨659⟩ *Packaging and Storage Requirements*	◆					
⟨730⟩ *Plasma Spectrochemistry*					◆	
⟨731⟩ *Loss on Drying*		◆				◆
⟨733⟩ *Loss on Ignition*					◆	
⟨735⟩ *X-Ray Fluorescence Spectrometry*		◆	◆		◆	
⟨736⟩ *Mass Spectrometry*		◆	◆			
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*		◆				
⟨781⟩ *Optical Rotation*		◆		◆		
⟨801⟩ *Polarography*			◆	◆		
⟨852⟩ *Atomic Absorption Spectroscopy*		◆	◆	◆		
⟨853⟩ *Fluorescence Spectroscopy*		◆	◆	◆		
⟨854⟩ *Mid-Infrared Spectroscopy*		◆	◆	◆		
⟨855⟩ *Nephelometry and Turbidimetry*		◆		◆		
⟨856⟩ *Near-Infrared Spectroscopy*		◆	◆	◆		
⟨857⟩ *Ultraviolet-Visible Spectroscopy*		◆	◆	◆		

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Chart 1a. Chemical Medicines Drug Substances—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨941⟩ *Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD)*		◆			◆	
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*		◆				
⟨1086⟩ *Impurities in Drug Substances and Drug Products*				◆		
⟨1120⟩ *Raman Spectroscopy*		◆	◆			
⟨1223.1⟩ *Validation of Alternative Methods to Antibiotic Microbial Assays*			◆			
⟨1467⟩ *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures*						◆
⟨1730⟩ *Plasma Spectrochemistry—Theory and Practice*		◆	◆			
⟨1735⟩ *X-Ray Fluorescence Spectrometry—Theory and Practice*		◆	◆			
⟨1736⟩ *Applications of Mass Spectrometry*		◆	◆			
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*		◆				
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*		◆				
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*		◆	◆	◆		

Chart 1b. Chemical Medicines Drug Substances—Specific Tests

Chapter	Physicochemical Characterization	Equipment	Water Content
⟨31⟩ *Volumetric Apparatus*		◆	
⟨41⟩ *Balances*		◆	
⟨268⟩ *Porosity by Nitrogen Adsorption–Desorption*	◆		
⟨301⟩ *Acid-Neutralizing Capacity*	◆		
⟨429⟩ *Light Diffraction Measurement of Particle Size*	◆		
⟨541⟩ *Titrimetry*			◆
⟨616⟩ *Bulk Density and Tapped Density*	◆		
⟨631⟩ *Color and Achromicity*	◆		
⟨641⟩ *Completeness of Solution*	◆		
⟨651⟩ *Congealing Temperature*	◆		
⟨695⟩ *Crystallinity*	◆		
⟨699⟩ *Density of Solids*	◆		
⟨721⟩ *Distilling Range*	◆		
⟨731⟩ *Loss on Drying*	◆		◆
⟨735⟩ *X-Ray Fluorescence Spectrometry*	◆		
⟨741⟩ *Melting Range or Temperature*	◆		
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*	◆		

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Chart 1b. Chemical Medicines Drug Substances—Specific Tests *(continued)*

Chapter	Physicochemical Characterization	Equipment	Water Content
⟨776⟩ *Optical Microscopy*	◆		
⟨781⟩ *Optical Rotation*	◆		
⟨782⟩ *Vibrational Circular Dichroism Spectroscopy*	◆		
⟨785⟩ *Osmolality and Osmolarity*	◆		
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*	◆		
⟨791⟩ *pH*	◆		
⟨811⟩ *Powder Fineness*	◆		
⟨831⟩ *Refractive Index*	◆		
⟨841⟩ *Specific Gravity*	◆		
⟨846⟩ *Specific Surface Area*	◆		
⟨856⟩ *Near-Infrared Spectroscopy*	◆		
⟨881⟩ *Tensile Strength*	◆		
⟨891⟩ *Thermal Analysis*			◆
⟨911⟩ *Viscosity—Capillary Methods*	◆		
⟨912⟩ *Viscosity—Rotational Methods*	◆		
⟨913⟩ *Viscosity—Rolling Ball Method*	◆		
⟨921⟩ *Water Determination*			◆
⟨941⟩ *Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD)*	◆		
⟨1051⟩ *Cleaning Glass Apparatus*		◆	
⟨1120⟩ *Raman Spectroscopy*	◆		
⟨1251⟩ *Weighing on an Analytical Balance*		◆	
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*	◆		
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*	◆		
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*	◆		
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*	◆		
⟨1730⟩ *Plasma Spectrochemistry—Theory and Practice*	◆		
⟨1735⟩ *X-Ray Fluorescence Spectrometry—Theory and Practice*	◆		
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*	◆		
⟨1782⟩ *Vibrational Circular Dichroism Spectroscopy—Theory and Practice*	◆		
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*	◆		
⟨1911⟩ *Rheometry*	◆		

Chart 2. Biologics Drug Substances

Chapter	Description	Identification	Safety	Assay	Physicochemical	Equipment	Impurities		Characterization
							Process Related	Product Related	
⟨7⟩ *Labeling*	◆								
⟨11⟩ *USP Reference Standards*		◆	◆	◆	◆		◆	◆	
⟨31⟩ *Volumetric Apparatus*						◆			
⟨41⟩ *Balances*						◆			

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Chart 2. Biologics Drug Substances *(continued)*

Chapter	Description	Identification	Safety	Assay	Physicochemical	Equipment	Impurities		Characterization
							Process Related	Product Related	
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*			◆						
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*			◆						
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*			◆						
⟨63⟩ *Mycoplasma Tests*			◆				◆		
⟨71⟩ *Sterility Tests*			◆						
⟨81⟩ *Antibiotics—Microbial Assays*							◆		
⟨85⟩ *Bacterial Endotoxins Test*			◆						
⟨87⟩ *Biological Reactivity Tests, In Vitro*			◆						
⟨88⟩ *Biological Reactivity Tests, In Vivo*			◆						
⟨111⟩ *Design and Analysis of Biological Assays*				◆			◆	◆	◆
⟨121⟩ *Insulin Assays*		◆		◆					
⟨121.1⟩ *Physicochemical Analytical Procedures for Insulins*		◆			◆		◆	◆	◆
⟨126⟩ *Somatropin Bioidentity Tests*		◆							
⟨129⟩ *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*					◆			◆	◆
⟨191⟩ *Identification Tests—General*		◆							
⟨197⟩ *Spectrophotometric Identification Tests*		◆							
⟨208⟩ *Anti-factor Xa and Anti-factor IIA Assays for Unfractionated and Low Molecular Weight Heparins*				◆					
⟨209⟩ *Low Molecular Weight Heparin Molecular Weight Determinations*		◆							
⟨210⟩ *Monosaccharide Analysis*					◆			◆	◆
⟨212⟩ *Oligosaccharide Analysis*					◆			◆	◆
⟨232⟩ *Elemental Impurities—Limits*							◆		
⟨233⟩ *Elemental Impurities—Procedures*							◆		
⟨467⟩ *Residual Solvents*							◆		
⟨503⟩ *Acetic Acid in Peptides*							◆		
⟨503.1⟩ *Trifluoroacetic Acid (TFA) in Peptides*							◆		
⟨507⟩ *Protein Determination Procedures*					◆				◆
⟨509⟩ *Residual DNA Testing*							◆		◆
⟨541⟩ *Titrimetry*				◆			◆		
⟨621⟩ *Chromatography*		◆		◆	◆		◆	◆	◆
⟨630⟩ *Visual Comparison*							◆	◆	
⟨631⟩ *Color and Achromicity*	◆								
⟨695⟩ *Crystallinity*	◆								
⟨730⟩ *Plasma Spectrochemistry*							◆		
⟨731⟩ *Loss on Drying*							◆		
⟨736⟩ *Mass Spectrometry*		◆		◆	◆		◆	◆	◆
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*		◆		◆	◆		◆	◆	◆

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Chart 2. Biologics Drug Substances *(continued)*

Chapter	Description	Identification	Safety	Assay	Physicochemical	Equipment	Impurities		Characterization
							Process Related	Product Related	
⟨781⟩ *Optical Rotation*				◆					
⟨782⟩ *Vibrational Circular Dichroism Spectroscopy*				◆					
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*					◆				
⟨831⟩ *Refractive Index*					◆				
⟨852⟩ *Atomic Absorption Spectroscopy*		◆		◆	◆		◆		◆
⟨853⟩ *Fluorescence Spectroscopy*		◆		◆	◆		◆		◆
⟨854⟩ *Mid-Infrared Spectroscopy*		◆		◆	◆		◆		◆
⟨855⟩ *Nephelometry and Turbidimetry*				◆	◆		◆		◆
⟨856⟩ *Near-Infrared Spectroscopy*		◆		◆	◆		◆		◆
⟨857⟩ *Ultraviolet-Visible Spectroscopy*		◆		◆	◆		◆		◆
⟨921⟩ *Water Determination*							◆		
⟨1025⟩ *Pancreatin*	◆	◆	◆	◆	◆		◆	◆	◆
⟨1030⟩ *Biological Assay Chapters—Overview and Glossary*		◆		◆					◆
⟨1032⟩ *Design and Development of Biological Assays*		◆		◆					◆
⟨1033⟩ *Biological Assay Validation*		◆		◆					◆
⟨1034⟩ *Analysis of Biological Assays*		◆		◆					◆
⟨1041⟩ *Biologics*	◆								
⟨1044⟩ *Cryopreservation of Cells*				◆					◆
⟨1048⟩ *Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products*									◆
⟨1050.1⟩ *Design, Evaluation, and Characterization of Viral Clearance Procedures*			◆						
⟨1052⟩ *Biotechnology-Derived Articles—Amino Acid Analysis*		◆		◆	◆		◆		◆
⟨1053⟩ *Capillary Electrophoresis*		◆		◆	◆		◆		◆
⟨1054⟩ *Biotechnology-Derived Articles—Isoelectric Focusing*		◆		◆	◆		◆		◆
⟨1055⟩ *Biotechnology-Derived Articles—Peptide Mapping*		◆		◆	◆		◆		◆
⟨1056⟩ *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis*		◆		◆	◆		◆		◆
⟨1057⟩ *Biotechnology-Derived Articles—Total Protein Assay*		◆		◆	◆		◆		◆
⟨1065⟩ *Ion Chromatography*					◆				
⟨1084⟩ *Glycoprotein and Glycan Analysis—General Considerations*							◆		◆
⟨1085⟩ *Guidelines on Endotoxins Test*			◆						
⟨1102⟩ *Immunological Test Methods—General Considerations*		◆		◆			◆	◆	◆
⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)*		◆		◆			◆	◆	◆
⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis*		◆					◆	◆	◆
⟨1105⟩ *Immunological Test Methods—Surface Plasmon Resonance*				◆					◆

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Chart 2. Biologics Drug Substances *(continued)*

Chapter	Description	Identification	Safety	Assay	Physicochemical	Equipment	Impurities		Characterization
							Process Related	Product Related	
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*			◆						
⟨1121⟩ *Nomenclature*	◆								
⟨1125⟩ *Nucleic Acid-Based Techniques—General*		◆							◆
⟨1126⟩ *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*		◆							◆
⟨1127⟩ *Nucleic Acid-Based Techniques—Amplification*		◆							◆
⟨1128⟩ *Nucleic Acid-Based Techniques—Microarray*									◆
⟨1129⟩ *Nucleic Acid-Based Techniques—Genotyping*									◆
⟨1130⟩ *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*			◆				◆		◆
⟨1132⟩ *Residual Host Cell Protein Measurement in Biopharmaceuticals*			◆				◆		
⟨1180⟩ *Human Plasma*			◆						
⟨1181⟩ *Scanning Electron Microscopy*									◆
⟨1211⟩ *Sterility Assurance*			◆						
⟨1228⟩ *Depyrogenation*			◆						
⟨1228.1⟩ *Dry Heat Depyrogenation*			◆						
⟨1228.3⟩ *Depyrogenation by Filtration*			◆						
⟨1228.4⟩ *Depyrogenation by Rinsing*			◆			◆			
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*			◆						
⟨1229⟩ *Sterilization of Compendial Articles*			◆						
⟨1229.4⟩ *Sterilizing Filtration of Liquids*			◆						
⟨1229.14⟩ *Sterilization Cycle Development*			◆						
⟨1229.15⟩ *Sterilizing Filtration of Gases*			◆			◆			
⟨1229.16⟩ *Prion Sterilization*			◆			◆	◆		
⟨1251⟩ *Weighing on an Analytical Balance*						◆			
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*					◆				◆
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*					◆				◆
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*					◆				◆
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*					◆				◆
⟨1736⟩ *Applications of Mass Spectrometry*		◆	◆						
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*		◆		◆	◆		◆	◆	◆
⟨1782⟩ *Vibrational Circular Dichroism Spectroscopy—Theory and Practice*				◆					
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*		◆		◆	◆		◆		◆

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Chart 2. Biologics Drug Substances *(continued)*

Chapter	Description	Identification	Safety	Assay	Physicochemical	Equipment	Impurities		Characterization
							Process Related	Product Related	
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*		◆		◆	◆		◆		◆
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*		◆		◆	◆		◆		◆
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*		◆		◆	◆		◆		◆
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*		◆		◆	◆		◆		◆

Chart 3a. Excipients—Universal Tests

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨7⟩ *Labeling*	◆					
⟨11⟩ *USP Reference Standards*		◆	◆	◆		
⟨181⟩ *Identification—Organic Nitrogenous Bases*		◆				
⟨191⟩ *Identification Tests—General*		◆				
⟨197⟩ *Spectrophotometric Identification Tests*		◆				
⟨201⟩ *Thin-Layer Chromatographic Identification Test*		◆				
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*		◆				
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*		◆				
⟨206⟩ *Aluminum*					◆	
⟨211⟩ *Arsenic*					◆	
⟨221⟩ *Chloride and Sulfate*					◆	
⟨226⟩ *4-Epianhydrotetracycline*				◆		
⟨228⟩ *Ethylene Oxide and Dioxane*						◆
⟨232⟩ *Elemental Impurities—Limits*					◆	
⟨233⟩ *Elemental Impurities—Procedures*					◆	
⟨241⟩ *Iron*					◆	
⟨251⟩ *Lead*					◆	
⟨261⟩ *Mercury*					◆	
⟨281⟩ *Residue on Ignition*					◆	
⟨291⟩ *Selenium*					◆	
⟨311⟩ *Alginates Assay*			◆			
⟨345⟩ *Assay for Citric Acid/Citrate and Phosphate*			◆			
⟨401⟩ *Fats and Fixed Oils*		◆				
⟨425⟩ *Iodometric Assay—Antibiotics*			◆			
⟨431⟩ *Methoxy Determination*			◆			
⟨461⟩ *Nitrogen Determination*				◆		

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Chart 3a. Excipients—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨466⟩ *Ordinary Impurities*				◆		
⟨467⟩ *Residual Solvents*						◆
⟨469⟩ *Ethylene Glycol, Diethylene Glycol, and Triethylene Glycol in Ethoxylated Substances*				◆		
⟨471⟩ *Oxygen Flask Combustion*					◆	
⟨541⟩ *Titrimetry*			◆			
⟨621⟩ *Chromatography*		◆	◆	◆		◆
⟨630⟩ *Visual Comparison*				◆	◆	◆
⟨730⟩ *Plasma Spectrochemistry*					◆	
⟨731⟩ *Loss on Drying*		◆				◆
⟨733⟩ *Loss on Ignition*					◆	
⟨735⟩ *X-Ray Fluorescence Spectrometry*		◆			◆	
⟨736⟩ *Mass Spectrometry*		◆				
⟨781⟩ *Optical Rotation*		◆		◆		
⟨801⟩ *Polarography*			◆	◆		
⟨852⟩ *Atomic Absorption Spectroscopy*		◆	◆	◆		
⟨853⟩ *Fluorescence Spectroscopy*		◆	◆	◆		
⟨854⟩ *Mid-Infrared Spectroscopy*		◆	◆	◆		
⟨855⟩ *Nephelometry and Turbidimetry*			◆	◆		
⟨856⟩ *Near-Infrared Spectroscopy*		◆	◆	◆		
⟨857⟩ *Ultraviolet-Visible Spectroscopy*		◆	◆	◆		
⟨941⟩ *Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD)*		◆				
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*		◆				
⟨1086⟩ *Impurities in Drug Substances and Drug Products*				◆		
⟨1091⟩ *Labeling of Inactive Ingredients*	◆					
⟨1467⟩ *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures*						◆
⟨1730⟩ *Plasma Spectrochemistry—Theory and Practice*		◆	◆			
⟨1735⟩ *X-Ray Fluorescence Spectrometry—Theory and Practice*		◆	◆			
⟨1736⟩ *Applications of Mass Spectrometry*		◆				
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*		◆	◆	◆		

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Chart 3a. Excipients—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*		◆				
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*		◆	◆	◆		

Chart 3b. Excipients—Specific Tests

Chapter	Physicochemical Characterization	Equipment	Pharmaceutical Water	Functionality/Safety/GMPs
⟨31⟩ *Volumetric Apparatus*		◆		
⟨41⟩ *Balances*		◆		
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*			◆	◆
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*			◆	◆
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests For Specified Microorganisms*				◆
⟨85⟩ *Bacterial Endotoxins Test*				◆
⟨268⟩ *Porosity by Nitrogen Adsorption–Desorption*	◆			
⟨301⟩ *Acid-Neutralizing Capacity*				◆
⟨429⟩ *Light Diffraction Measurement of Particle Size*	◆			
⟨541⟩ *Titrimetry*			◆	
⟨616⟩ *Bulk Density and Tapped Density*	◆			
⟨631⟩ *Color and Achromicity*	◆			
⟨641⟩ *Completeness of Solution*	◆			
⟨643⟩ *Total Organic Carbon*			◆	
⟨645⟩ *Water Conductivity*			◆	
⟨651⟩ *Congealing Temperature*	◆			
⟨695⟩ *Crystallinity*	◆			
⟨699⟩ *Density of Solids*	◆			
⟨721⟩ *Distilling Range*	◆			
⟨731⟩ *Loss on Drying*	◆			
⟨735⟩ *X-Ray Fluorescence Spectrometry*	◆			
⟨741⟩ *Melting Range or Temperature*	◆			
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*	◆			
⟨776⟩ *Optical Microscopy*	◆			
⟨781⟩ *Optical Rotation*	◆			
⟨782⟩ *Vibrational Circular Dichroism Spectroscopy*	◆			

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Chart 3b. Excipients—Specific Tests *(continued)*

Chapter	Physicochemical Characterization	Equipment	Pharmaceutical Water	Functionality/Safety/GMPs
⟨785⟩ *Osmolality and Osmolarity*	◆			
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*	◆			
⟨791⟩ *pH*	◆		◆	
⟨811⟩ *Powder Fineness*	◆			
⟨831⟩ *Refractive Index*	◆			
⟨841⟩ *Specific Gravity*	◆			
⟨846⟩ *Specific Surface Area*	◆			
⟨881⟩ *Tensile Strength*	◆			
⟨891⟩ *Thermal Analysis*			◆	
⟨911⟩ *Viscosity—Capillary Methods*	◆			
⟨912⟩ *Viscosity—Rotational Methods*	◆			
⟨913⟩ *Viscosity—Rolling Ball Method*	◆			
⟨914⟩ *Viscosity—Pressure Driven Methods*	◆			
⟨921⟩ *Water Determination*			◆	
⟨941⟩ *Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD)*	◆			
⟨1051⟩ *Cleaning Glass Apparatus*		◆		
⟨1059⟩ *Excipient Performance*				◆
⟨1063⟩ *Shear Cell Methodology for Powder Flow Testing*	◆	◆		
⟨1074⟩ *Excipient Biological Safety Evaluation Guidelines*				◆
⟨1078⟩ *Good Manufacturing Practices for Bulk Pharmaceutical Excipients*				◆
⟨1080⟩ *Bulk Pharmaceutical Excipients—Certificate of Analysis*				◆
⟨1085⟩ *Guidelines on Endotoxins Test*				◆
⟨1097⟩ *Bulk Powder Sampling Procedures*				◆
⟨1174⟩ *Powder Flow*	◆			◆
⟨1195⟩ *Significant Change Guide for Bulk Pharmaceutical Excipients*				◆
⟨1197⟩ *Good Distribution Practices for Bulk Pharmaceutical Excipients*				◆
⟨1230⟩ *Water for Hemodialysis Applications*			◆	
⟨1231⟩ *Water for Pharmaceutical Purposes*			◆	
⟨1251⟩ *Weighing on an Analytical Balance*		◆		

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Chart 3b. Excipients—Specific Tests *(continued)*

Chapter	Physicochemical Characterization	Equipment	Pharmaceutical Water	Functionality/Safety/GMPs
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*	◆			
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*	◆			
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*	◆			
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*	◆			
⟨1644⟩ *Theory and Practice of Electrical Conductivity Measurements of Solutions*			◆	
⟨1730⟩ *Plasma Spectrochemistry—Theory and Practice*	◆			
⟨1735⟩ *X-Ray Fluorescence Spectrometry—Theory and Practice*	◆			
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*	◆			
⟨1782⟩ *Vibrational Circular Dichroism Spectroscopy—Theory and Practice*	◆			
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*	◆			
⟨1911⟩ *Rheometry*	◆			

Chart 4a. Chemical Medicines Drug Products—Universal Tests

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨1⟩ *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests*	◆	◆	◆	◆	◆	◆
⟨2⟩ *Oral Drug Products—Product Quality Tests*	◆	◆	◆	◆	◆	◆
⟨3⟩ *Topical and Transdermal Drug Products—Product Quality Tests*	◆	◆	◆	◆	◆	◆
⟨4⟩ *Mucosal Drug Products—Product Quality Tests*	◆	◆	◆	◆	◆	◆
⟨5⟩ *Inhalation and Nasal Drug Products—General Information and Product Quality Checks*	◆	◆	◆	◆	◆	◆
⟨7⟩ *Labeling*	◆					
⟨81⟩ *Antibiotics—Microbial Assays*			◆			
⟨191⟩ *Identification Tests—General*		◆				
⟨197⟩ *Spectrophotometric Identification Tests*		◆				
⟨201⟩ *Thin-Layer Chromatographic Identification Test*		◆				
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*		◆				

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Chart 4a. Chemical Medicines Drug Products—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*		◆				
⟨227⟩ *4-Aminophenol in Acetaminophen-Containing Drug Products*				◆		
⟨232⟩ *Elemental Impurities—Limits*					◆	
⟨233⟩ *Elemental Impurities—Procedures*					◆	
⟨281⟩ *Residue on Ignition*					◆	
⟨341⟩ *Antimicrobial Agents—Content*			◆			
⟨351⟩ *Assay for Steroids*			◆			
⟨391⟩ *Epinephrine Assay*			◆			
⟨413⟩ *Impurities Testing in Medical Gases*				◆		
⟨415⟩ *Medical Gases Assay*			◆			
⟨451⟩ *Nitrate Titration*			◆			
⟨461⟩ *Nitrogen Determination*				◆		
⟨466⟩ *Ordinary Impurities*				◆		
⟨467⟩ *Residual Solvents*						◆
⟨501⟩ *Salts of Organic Nitrogenous Bases*		◆	◆			
⟨541⟩ *Titrimetry*			◆			
⟨611⟩ *Alcohol Determination*			◆			
⟨621⟩ *Chromatography*		◆	◆	◆		◆
⟨730⟩ *Plasma Spectrochemistry*					◆	
⟨733⟩ *Loss on Ignition*					◆	
⟨735⟩ *X-Ray Fluorescence Spectrometry*		◆			◆	
⟨736⟩ *Mass Spectrometry*		◆				
⟨781⟩ *Optical Rotation*				◆		
⟨801⟩ *Polarography*			◆	◆		
⟨821⟩ *Radioactivity*		◆	◆	◆	◆	◆
⟨823⟩ *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses*		◆	◆	◆	◆	◆
⟨852⟩ *Atomic Absorption Spectroscopy*		◆	◆	◆		
⟨853⟩ *Fluorescence Spectroscopy*		◆	◆	◆		
⟨854⟩ *Mid-Infrared Spectroscopy*		◆	◆	◆		
⟨855⟩ *Nephelometry and Turbidimetry*			◆	◆		
⟨856⟩ *Near-Infrared Spectroscopy*		◆	◆	◆		
⟨857⟩ *Ultraviolet-Visible Spectroscopy*		◆	◆	◆		
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*		◆				
⟨1065⟩ *Ion Chromatography*			◆			

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Chart 4a. Chemical Medicines Drug Products—Universal Tests *(continued)*

Chapter	Description	Identification	Assay	Impurities		
				Organic	Inorganic	Residual Solvents
⟨1086⟩ *Impurities in Drug Substances and Drug Products*				◆		
⟨1121⟩ *Nomenclature*	◆					
⟨1223.1⟩ *Validation of Alternative Methods to Antibiotic Microbial Assays*			◆			
⟨1467⟩ *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures*						◆
⟨1730⟩ *Plasma Spectrochemistry—Theory and Practice*		◆	◆			
⟨1735⟩ *X-Ray Fluorescence Spectrometry—Theory and Practice*		◆	◆			
⟨1736⟩ *Applications of Mass Spectrometry*		◆				
⟨1821⟩ *Radioactivity—Theory and Practice*	◆					
⟨1823⟩ *Positron Emission Tomography Drugs—Information*	◆					
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*		◆	◆	◆		
⟨1853⟩ *Fluorescence Spectroscopy —Theory and Practice*		◆	◆	◆		
⟨1854⟩ *Mid-Infrared Spectroscopy —Theory and Practice*		◆	◆	◆		
⟨1856⟩ *Near-Infrared Spectroscopy —Theory and Practice*		◆	◆	◆		
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*		◆	◆	◆		

Chart 4b. Chemical Medicines Drug Products—Specific Tests

Chapter	Equipment	Water Content	Performance Tests						
			Mucosal		Parenteral	Oral	Topical	Inhalational	Mucosal (7 membrane surfaces)
			Ophthalmic[1]	Nasal					
⟨31⟩ *Volumetric Apparatus*	◆								
⟨41⟩ *Balances*	◆								
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*				◆		◆	◆	◆	
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*				◆		◆	◆	◆	
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*				◆		◆	◆	◆	
⟨71⟩ *Sterility Tests*					◆				
⟨85⟩ *Bacterial Endotoxins Test*					◆				
⟨87⟩ *Biological Reactivity Tests, In Vitro*					◆				
⟨88⟩ *Biological Reactivity Tests, In Vivo*					◆				
⟨151⟩ *Pyrogen Test*					◆				

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Chart 4b. Chemical Medicines Drug Products—Specific Tests *(continued)*

Chapter	Equipment	Water Content	Performance Tests						
			Mucosal		Parenteral	Oral	Topical	Inhalational	Mucosal (7 membrane surfaces)
			Ophthalmic[1]	Nasal					
⟨301⟩ *Acid-Neutralizing Capacity*						◆			
⟨381⟩ *Elastomeric Closures for Injections*					◆				
⟨541⟩ *Titrimetry*		◆							
⟨601⟩ *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests*				◆				◆	
⟨602⟩ *Propellants*								◆	
⟨603⟩ *Topical Aerosols*							◆		
⟨604⟩ *Leak Rate*				◆			◆	◆	
⟨697⟩ *Container Content for Injections*					◆				
⟨698⟩ *Deliverable Volume*						◆			
⟨701⟩ *Disintegration*						◆			
⟨705⟩ *Quality Attributes of Tablets Labeled as Having a Functional Score*						◆			
⟨711⟩ *Dissolution*						◆			
⟨724⟩ *Drug Release*						◆	◆		
⟨729⟩ *Globule Size Distribution in Lipid Injectable Emulsions*					◆				
⟨731⟩ *Loss on Drying*		◆							
⟨755⟩ *Minimum Fill*			◆			◆	◆		◆
⟨771⟩ *Ophthalmic Products—Quality Tests*			◆						
⟨785⟩ *Osmolality and Osmolarity*			◆						
⟨787⟩ *Subvisible Particulate Matter in Therapeutic Protein Injections*					◆				
⟨788⟩ *Particulate Matter in Injections*					◆				
⟨789⟩ *Particulate Matter in Ophthalmic Solutions*			◆						
⟨790⟩ *Visible Particulates in Injections*					◆				
⟨791⟩ *pH*			◆	◆	◆		◆	◆	
⟨891⟩ *Thermal Analysis*		◆							
⟨905⟩ *Uniformity of Dosage Units*			◆	◆	◆	◆	◆	◆	◆
⟨921⟩ *Water Determination*		◆							
⟨1004⟩ *Mucosal Drug Products—Performance Tests*									◆
⟨1005⟩ *Acoustic Emission*						◆			
⟨1051⟩ *Cleaning Glass Apparatus*	◆								
⟨1085⟩ *Guidelines on Endotoxins Test*			◆		◆				

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Chart 4b. Chemical Medicines Drug Products—Specific Tests *(continued)*

Chapter	Equipment	Water Content	Performance Tests						
			Mucosal		Parenteral	Oral	Topical	Inhalational	Mucosal (7 membrane surfaces)
			Ophthalmic[1]	Nasal					
⟨1087⟩ *Apparent Intrinsic Dissolution—Dissolution Testing Procedures for Rotating Disk and Stationary Disk*						◆			
⟨1088⟩ *In Vitro and In Vivo Evaluation of Dosage Forms*						◆			
⟨1090⟩ *Assessment of Solid Oral Drug Product Performance and Interchangeability, Bioavailability, Bioequivalence, and Dissolution*						◆			
⟨1092⟩ *The Dissolution Procedure: Development and Validation*						◆			
⟨1094⟩ *Capsules—Dissolution Testing and Related Quality Attributes*						◆			
⟨1099⟩ *Limit on Number of Large Deviations when Assessing Content Uniformity in Large Samples*			◆	◆	◆	◆	◆		◆
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*					◆				
⟨1211⟩ *Sterility Assurance*					◆				
⟨1216⟩ *Tablet Friability*						◆			
⟨1217⟩ *Tablet Breaking Force*						◆			
⟨1228⟩ *Depyrogenation*					◆				
⟨1228.1⟩ *Dry Heat Depyrogenation*					◆				
⟨1228.3⟩ *Depyrogenation by Filtration*					◆				
⟨1228.4⟩ *Depyrogenation by Rinsing*	◆				◆				
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*					◆				
⟨1229⟩ *Sterilization of Compendial Articles*					◆				
⟨1229.2⟩ *Moist Heat Sterilization of Aqueous Liquids*					◆				
⟨1229.3⟩ *Monitoring of Bioburden*					◆				
⟨1229.4⟩ *Sterilizing Filtration of Liquids*					◆				
⟨1229.13⟩ *Sterilization-in-Place*	◆								
⟨1229.14⟩ *Sterilization Cycle Development*					◆				
⟨1229.15⟩ *Sterilizing Filtration of Gases*	◆				◆				
⟨1251⟩ *Weighing on an Analytical Balance*	◆								
⟨1601⟩ *Products for Nebulization—Characterization Tests*								◆	
⟨1602⟩ *Spacers and Valved Holding Chambers Used With Inhalation Aerosols—Characterization Tests*								◆	

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Chart 4b. Chemical Medicines Drug Products—Specific Tests *(continued)*

Chapter	Equipment	Water Content	Performance Tests						
			Mucosal		Parenteral	Oral	Topical	Inhalational	Mucosal (7 membrane surfaces)
			Ophthalmic[1]	Nasal					
⟨1724⟩ *Semisolid Drug Products—Performance Tests*							◆		
⟨1771⟩ *Ophthalmic Products—Performance Tests*			◆						
⟨1788⟩ *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*					◆				
⟨1787⟩ *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections*					◆				

[1] See *Microbiology (Chart 10)*.

Chart 5. Biologics Drug Products

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1⟩ *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests*				◆						
⟨7⟩ *Labeling*				◆						
⟨11⟩ *USP Reference Standards*	◆					◆	◆	◆	◆	◆
⟨31⟩ *Volumetric Apparatus*			◆							
⟨41⟩ *Balances*			◆							
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*						◆			◆	
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*						◆			◆	
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*						◆			◆	
⟨63⟩ *Mycoplasma Tests*						◆			◆	
⟨71⟩ *Sterility Tests*						◆				
⟨85⟩ *Bacterial Endotoxins Test*						◆				
⟨87⟩ *Biological Reactivity Tests, In Vitro*						◆				
⟨88⟩ *Biological Reactivity Tests, In Vivo*						◆				
⟨89⟩ *Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing*	◆	◆			◆	◆	◆		◆	
⟨89.1⟩ *Collagenase I*	◆	◆			◆	◆	◆		◆	
⟨89.2⟩ *Collagenase II*	◆	◆			◆	◆	◆		◆	
⟨90⟩ *Fetal Bovine Serum—Quality Attributes and Functionality Tests*									◆	
⟨111⟩ *Design and Analysis of Biological Assays*		◆					◆		◆	◆
⟨121⟩ *Insulin Assays*	◆						◆			

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Chart 5. Biologics Drug Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨121.1⟩ *Physicochemical Analytical Procedures for Insulins*	◆	◆						◆	◆	◆
⟨123⟩ *Glucagon Bioidentity Tests*	◆									
⟨124⟩ *Erythropoietin Bioassays*		◆					◆			
⟨126⟩ *Somatropin Bioidentity Tests*	◆									
⟨129⟩ *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*		◆						◆		◆
⟨130⟩ *Protein A Quality Attributes*									◆	
⟨151⟩ *Pyrogen Test*						◆				
⟨191⟩ *Identification Tests—General*	◆									
⟨197⟩ *Spectrophotometric Identification Tests*	◆									
⟨208⟩ *Anti-factor Xa and Anti-factor IIa Assays for Unfractionated and Low Molecular Weight Heparins*		◆					◆			
⟨209⟩ *Low Molecular Weight Heparin Molecular Weight Determinations*		◆								
⟨212⟩ *Oligosaccharide Analysis*		◆						◆		◆
⟨232⟩ *Elemental Impurities —Limits*									◆	
⟨233⟩ *Elemental Impurities —Procedures*									◆	
⟨341⟩ *Antimicrobial Agents —Content*						◆				
⟨467⟩ *Residual Solvents*									◆	
⟨503⟩ *Acetic Acid in Peptides*									◆	
⟨507⟩ *Protein Determination Procedures*		◆						◆		
⟨509⟩ *Residual DNA Testing*		◆							◆	
⟨541⟩ *Titrimetry*								◆		◆
⟨621⟩ *Chromatography*	◆	◆					◆	◆	◆	◆
⟨630⟩ *Visual Comparison*									◆	◆
⟨631⟩ *Color and Achromicity*					◆					
⟨660⟩ *Containers—Glass*				◆						
⟨661⟩ *Plastic Packaging Systems and Their Materials of Construction*				◆						
⟨661.1⟩ *Plastic Materials of Construction*				◆						
⟨661.2⟩ *Plastic Packaging Systems for Pharmaceutical Use*				◆						
⟨671⟩ *Containers—Performance Testing*				◆						
⟨695⟩ *Crystallinity*					◆					

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Chart 5. Biologics Drug Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨697⟩ *Container Content for Injections*				◆						
⟨730⟩ *Plasma Spectrochemistry*									◆	
⟨731⟩ *Loss on Drying*									◆	
⟨736⟩ *Mass Spectrometry*	◆	◆					◆	◆	◆	◆
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆	◆	◆	◆
⟨781⟩ *Optical Rotation*							◆			
⟨782⟩ *Vibrational Circular Dichroism Spectroscopy*							◆			
⟨785⟩ *Osmolality and Osmolarity*								◆		
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*								◆		
⟨787⟩ *Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨788⟩ *Particulate Matter in Injections*				◆						
⟨791⟩ *pH*					◆			◆		
⟨821⟩ *Radioactivity*	◆	◆	◆			◆	◆		◆	◆
⟨831⟩ *Refractive Index*								◆		
⟨852⟩ *Atomic Absorption Spectroscopy*	◆	◆					◆	◆	◆	
⟨853⟩ *Fluorescence Spectroscopy*	◆	◆					◆	◆	◆	
⟨854⟩ *Mid-Infrared Spectroscopy*	◆	◆					◆	◆	◆	
⟨855⟩ *Nephelometry and Turbidimetry*		◆					◆	◆	◆	
⟨857⟩ *Ultraviolet-Visible Spectroscopy*	◆	◆					◆	◆	◆	
⟨921⟩ *Water Determination*									◆	
⟨1024⟩ *Bovine Serum*									◆	
⟨1025⟩ *Pancreatin*	◆	◆			◆	◆	◆	◆	◆	◆
⟨1030⟩ *Biological Assay Chapters—Overview and Glossary*	◆	◆					◆			
⟨1032⟩ *Design and Development of Biological Assays*	◆	◆					◆			
⟨1033⟩ *Biological Assay Validation*	◆	◆					◆			
⟨1034⟩ *Analysis of Biological Assays*	◆	◆					◆			
⟨1041⟩ *Biologics*					◆					
⟨1044⟩ *Cryopreservation of Cells*		◆					◆			
⟨1050⟩ *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*						◆				

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Chart 5. Biologics Drug Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1050.1⟩ *Design, Evaluation, and Characterization of Viral Clearance Procedures*						◆				
⟨1052⟩ *Biotechnology Derived Articles—Amino Acid Analysis*	◆	◆					◆	◆	◆	
⟨1053⟩ *Capillary Electrophoresis*	◆	◆					◆	◆	◆	
⟨1054⟩ *Biotechnology Derived Articles—Isoelectric Focusing*	◆	◆					◆	◆	◆	
⟨1055⟩ *Biotechnology Derived Articles—Peptide Mapping*	◆	◆					◆	◆	◆	
⟨1056⟩ *Biotechnology Derived Articles—Polyacrylamide Gel Electrophoresis*	◆	◆					◆	◆	◆	
⟨1057⟩ *Biotechnology Derived Articles—Total Protein Assay*	◆	◆					◆	◆	◆	
⟨1065⟩ *Ion Chromatography*										◆
⟨1084⟩ *Glycoprotein and Glycan Analysis—General Considerations*		◆						◆	◆	
⟨1085⟩ *Guidelines on Endotoxins Test*						◆				
⟨1102⟩ *Immunological Test Methods—General Considerations*	◆	◆					◆		◆	◆
⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)*	◆	◆					◆		◆	◆
⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis*	◆	◆							◆	◆
⟨1105⟩ *Immunological Test Methods—Surface Plasmon Resonance*		◆					◆			
⟨1106⟩ *Immunogenicity Assays—Design and Validation of Immunoassays to Detect Anti-Drug Antibodies*						◆				
⟨1106.1⟩ *Immunogenicity Assays—Design and Validation of Assays to Detect Anti-Drug Neutralizing Antibody*						◆				
⟨1111⟩ *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use*						◆				
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*						◆			◆	
⟨1121⟩ *Nomenclature*					◆					
⟨1125⟩ *Nucleic Acid-Based Techniques—General*							◆		◆	

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Chart 5. Biologics Drug Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1126⟩ *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*							◆		◆	
⟨1127⟩ *Nucleic Acid-Based Techniques—Amplification*							◆		◆	
⟨1128⟩ *Nucleic Acid-Based Techniques—Microarray*									◆	
⟨1129⟩ *Nucleic Acid-Based Techniques—Genotyping*							◆		◆	
⟨1130⟩ *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*						◆			◆	
⟨1132⟩ *Residual Host Cell Protein Measurement in Biopharmaceuticals*						◆			◆	
⟨1181⟩ *Scanning Electron Microscopy*		◆								
⟨1211⟩ *Sterility Assurance*						◆				
⟨1228⟩ *Depyrogenation*						◆				
⟨1228.1⟩ *Dry Heat Depyrogenation*						◆				
⟨1228.3⟩ *Depyrogenation by Filtration*						◆				
⟨1228.4⟩ *Depyrogenation by Rinsing*			◆			◆				
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*						◆				
⟨1229⟩ *Sterilization of Compendial Articles*						◆				
⟨1229.4⟩ *Sterilizing Filtration of Liquids*						◆				
⟨1229.13⟩ *Sterilization-in-Place*			◆							
⟨1229.14⟩ *Sterilization Cycle Development*						◆				
⟨1229.15⟩ *Sterilizing Filtration of Gases*			◆			◆				
⟨1229.16⟩ *Prion Sterilization*			◆			◆			◆	
⟨1251⟩ *Weighing on an Analytical Balance*			◆							
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*		◆						◆		
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*		◆						◆		
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*		◆						◆		

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Chart 5. Biologics Drug Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*		◆						◆		
⟨1660⟩ *Evaluation of the Inner Surface Durability of Glass Containers*				◆						
⟨1661⟩ *Evaluation of Plastic Packaging Systems and Their Materials of Construction with Respect to Their User Safety Impact*				◆						
⟨1663⟩ *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1664⟩ *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1736⟩ *Applications of Mass Spectrometry*	◆	◆					◆	◆	◆	◆
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆	◆	◆	◆
⟨1782⟩ *Vibrational Circular Dichroism Spectroscopy—Theory and Practice*							◆			
⟨1787⟩ *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨1788⟩ *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*				◆						
⟨1821⟩ *Radioactivity—Theory and Practice*					◆					
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*	◆	◆					◆			
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	

Chart 6. Vaccines

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1⟩ *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests*				◆						

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Chart 6. Vaccines *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨11⟩ *USP Reference Standards*						◆				
⟨31⟩ *Volumetric Apparatus*			◆							
⟨41⟩ *Balances*			◆							
⟨63⟩ *Mycoplasma Tests*						◆				
⟨71⟩ *Sterility Tests*						◆				
⟨81⟩ *Antibiotics—Microbial Assays*									◆	
⟨85⟩ *Bacterial Endotoxins Test*						◆				
⟨88⟩ *Biological Reactivity Tests, In Vivo*						◆				
⟨90⟩ *Fetal Bovine Serum—Quality Attributes and Functionality Tests*						◆			◆	
⟨111⟩ *Design and Analysis of Biological Assays*		◆					◆		◆	◆
⟨151⟩ *Pyrogen Test*						◆				
⟨198⟩ *Nuclear Magnetic Resonance Spectroscopy Identity Testing of Bacterial Polysaccharides Used in Vaccine Manufacture*	◆									
⟨341⟩ *Antimicrobial Agents—Content*						◆				
⟨507⟩ *Protein Determination Procedures*		◆						◆		
⟨509⟩ *Residual DNA Testing*									◆	
⟨621⟩ *Chromatography*	◆	◆					◆	◆	◆	◆
⟨660⟩ *Containers—Glass*				◆						
⟨661⟩ *Plastic Packaging Systems and Their Materials of Construction*				◆						
⟨661.1⟩ *Plastic Materials of Construction*				◆						
⟨661.2⟩ *Plastic Packaging Systems for Pharmaceutical Use*				◆						
⟨671⟩ *Containers—Performance Testing*				◆						
⟨695⟩ *Crystallinity*										
⟨697⟩ *Container Content for Injections*				◆						
⟨731⟩ *Loss on Drying*									◆	
⟨736⟩ *Mass Spectrometry*	◆	◆					◆	◆	◆	◆
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆	◆	◆	◆
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*								◆		
⟨787⟩ *Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨788⟩ *Particulate Matter in Injections*				◆						

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Chart 6. Vaccines *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨790⟩ *Visible Particulates in Injections*				◆						
⟨791⟩ *pH*					◆			◆		
⟨852⟩ *Atomic Absorption Spectroscopy*							◆	◆		
⟨853⟩ *Fluorescence Spectroscopy*							◆	◆		
⟨854⟩ *Mid-Infrared Spectroscopy*							◆	◆		
⟨855⟩ *Nephelometry and Turbidimetry*							◆	◆		
⟨856⟩ *Near-Infrared Spectroscopy*							◆	◆		
⟨857⟩ *Ultraviolet-Visible Spectroscopy*							◆	◆		
⟨921⟩ *Water Determination*									◆	
⟨1024⟩ *Bovine Serum*						◆			◆	
⟨1030⟩ *Biological Assay Chapters—Overview and Glossary*	◆	◆					◆			
⟨1032⟩ *Design and Development of Biological Assays*	◆	◆					◆			
⟨1033⟩ *Biological Assay Validation*	◆	◆					◆			
⟨1034⟩ *Analysis of Biological Assays*	◆	◆					◆			
⟨1041⟩ *Biologics*					◆					
⟨1044⟩ *Cryopreservation of Cells*		◆					◆			
⟨1052⟩ *Biotechnology Derived Articles—Amino Acid Analysis*	◆	◆					◆	◆	◆	
⟨1053⟩ *Capillary Electrophoresis*	◆	◆					◆	◆	◆	
⟨1054⟩ *Biotechnology Derived Articles—Isoelectric Focusing*	◆	◆					◆	◆	◆	
⟨1055⟩ *Biotechnology Derived Articles—Peptide Mapping*	◆	◆					◆	◆	◆	
⟨1056⟩ *Biotechnology Derived Articles—Polyacrylamide Gel Electrophoresis*	◆	◆					◆	◆	◆	
⟨1057⟩ *Biotechnology Derived Articles—Total Protein Assay*	◆	◆					◆	◆	◆	
⟨1065⟩ *Ion Chromatography*								◆		
⟨1071⟩ *Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach*						◆				
⟨1084⟩ *Glycoprotein and Glycan Analysis—General Considerations*		◆						◆	◆	
⟨1085⟩ *Guidelines on Endotoxins Test*						◆				
⟨1102⟩ *Immunological Test Methods—General Considerations*	◆	◆							◆	◆

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Chart 6. Vaccines *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)*	◆	◆							◆	◆
⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis*	◆	◆							◆	◆
⟨1105⟩ *Immunological Test Methods—Surface Plasmon Resonance*		◆					◆			
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*						◆				
⟨1121⟩ *Nomenclature*						◆				
⟨1126⟩ *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*	◆	◆							◆	
⟨1127⟩ *Nucleic Acid-Based Techniques—Amplification*	◆	◆							◆	
⟨1128⟩ *Nucleic Acid-Based Techniques—Microarray*	◆									
⟨1129⟩ *Nucleic Acid-Based Techniques—Genotyping*	◆									
⟨1130⟩ *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*									◆	
⟨1211⟩ *Sterility Assurance*						◆				
⟨1228⟩ *Depyrogenation*						◆				
⟨1228.1⟩ *Dry Heat Depyrogenation*						◆				
⟨1228.3⟩ *Depyrogenation by Filtration*						◆				
⟨1228.4⟩ *Depyrogenation by Rinsing*			◆			◆				
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*						◆				
⟨1229⟩ *Sterilization of Compendial Articles*						◆				
⟨1229.4⟩ *Sterilizing Filtration of Liquids*						◆				
⟨1229.13⟩ *Steriliza-tion-in-Place*			◆							
⟨1229.14⟩ *Sterilization Cycle Development*						◆				
⟨1229.15⟩ *Sterilizing Filtration of Gases*			◆			◆				
⟨1229.16⟩ *Prion Sterilization*			◆			◆			◆	
⟨1234⟩ *Vaccines for Human Use—Polysaccharide and Glycoconjugate Vaccines*	◆	◆				◆	◆	◆	◆	◆
⟨1235⟩ *Vaccines for Human Use—General Considerations*	◆	◆					◆			◆
⟨1237⟩ *Virology Test Methods*						◆	◆		◆	◆
⟨1238⟩ *Vaccines for Human Use—Bacterial Vaccines*	◆	◆					◆	◆	◆	◆

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Chart 6. Vaccines *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physico-chemical	Impurities	
									Process Related	Product Related
⟨1251⟩ *Weighing on an Analytical Balance*			◆							
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*		◆						◆		
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*		◆						◆		
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*		◆						◆		
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*		◆						◆		
⟨1660⟩ *Evaluation of the Inner Surface Durability of Glass Containers*				◆						
⟨1661⟩ *Evaluation of Plastic Packaging Systems and Their Materials of Construction with Respect to Their User Safety Impact*				◆						
⟨1663⟩ *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1664⟩ *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1736⟩ *Applications of Mass Spectrometry*	◆	◆					◆	◆	◆	◆
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆		◆	◆
⟨1787⟩ *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨1788⟩ *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*				◆						
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*							◆	◆		
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*							◆	◆		
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*							◆	◆		
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*							◆	◆		

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Chart 6. Vaccines *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*							◆	◆		

Chart 7. Blood and Blood Products

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1⟩ *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests*				◆						
⟨11⟩ *USP Reference Standards*	◆					◆	◆	◆	◆	◆
⟨31⟩ *Volumetric Apparatus*			◆							
⟨41⟩ *Balances*			◆							
⟨51⟩ *Antimicrobial Effectiveness Testing*						◆				
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*						◆				
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*						◆				
⟨71⟩ *Sterility Tests*						◆				
⟨85⟩ *Bacterial Endotoxins Test*						◆				
⟨87⟩ *Biological Reactivity Tests, In Vitro*						◆				
⟨88⟩ *Biological Reactivity Tests, In Vivo*						◆				
⟨111⟩ *Design and Analysis of Biological Assays*		◆					◆		◆	◆
⟨151⟩ *Pyrogen Test*						◆				
⟨191⟩ *Identification Tests—General*	◆									
⟨197⟩ *Spectrophotometric Identification Tests*	◆									
⟨208⟩ *Anti-factor Xa and Anti-factor IIa Assays for Unfractionated and Low Molecular Weight Heparins*							◆			
⟨209⟩ *Low Molecular Weight Heparin Molecular Weight Determinations*	◆									
⟨341⟩ *Antimicrobial Agents—Content*						◆				
⟨507⟩ *Protein Determination Procedures*		◆						◆		
⟨509⟩ *Residual DNA Testing*									◆	
⟨621⟩ *Chromatography*	◆	◆					◆	◆	◆	◆
⟨631⟩ *Color and Achromicity*					◆					
⟨660⟩ *Containers—Glass*				◆						
⟨661⟩ *Plastic Packaging Systems and Their Materials of Construction*				◆						

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Chart 7. Blood and Blood Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨661.1⟩ *Plastic Materials of Construction*				◆						
⟨661.2⟩ *Plastic Packaging Systems for Pharmaceutical Use*				◆						
⟨671⟩ *Containers—Performance Testing*				◆						
⟨695⟩ *Crystallinity*					◆					
⟨697⟩ *Container Content for Injections*				◆						
⟨731⟩ *Loss on Drying*									◆	
⟨736⟩ *Mass Spectrometry*	◆	◆					◆	◆	◆	◆
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆	◆	◆	◆
⟨785⟩ *Osmolality and Osmolarity*								◆		
⟨787⟩ *Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨788⟩ *Particulate Matter in Injections*				◆						
⟨790⟩ *Visible Particulates in Injections*				◆						
⟨791⟩ *pH*					◆			◆		
⟨852⟩ *Atomic Absorption Spectroscopy*	◆	◆					◆	◆	◆	
⟨853⟩ *Fluorescence Spectroscopy*	◆	◆					◆	◆	◆	
⟨854⟩ *Mid-Infrared Spectroscopy*	◆	◆					◆	◆	◆	
⟨855⟩ *Nephelometry and Turbidimetry*		◆					◆	◆	◆	
⟨856⟩ *Near-Infrared Spectroscopy*	◆	◆					◆	◆	◆	
⟨857⟩ *Ultraviolet-Visible Spectroscopy*	◆	◆					◆	◆	◆	
⟨921⟩ *Water Determination*									◆	
⟨1030⟩ *Biological Assay Chapters—Overview and Glossary*	◆	◆					◆			
⟨1032⟩ *Design and Development of Biological Assays*	◆	◆					◆			
⟨1033⟩ *Biological Assay Validation*	◆	◆					◆			
⟨1034⟩ *Analysis of Biological Assays*	◆	◆					◆			
⟨1041⟩ *Biologics*					◆					
⟨1044⟩ *Cryopreservation of Cells*		◆								
⟨1052⟩ *Biotechnology Derived Articles—Amino Acid Analysis*	◆	◆					◆	◆	◆	
⟨1053⟩ *Capillary Electrophoresis*	◆	◆					◆	◆	◆	
⟨1054⟩ *Biotechnology Derived Articles—Isoelectric Focusing*	◆	◆					◆	◆	◆	

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Chart 7. Blood and Blood Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1055⟩ *Biotechnology Derived Articles—Peptide Mapping*	◆	◆					◆	◆	◆	
⟨1056⟩ *Biotechnology Derived Articles—Polyacrylamide Gel Electrophoresis*	◆	◆					◆	◆	◆	
⟨1057⟩ *Biotechnology Derived Articles—Total Protein Assay*	◆	◆					◆	◆	◆	
⟨1065⟩ *Ion Chromatography*	◆									
⟨1085⟩ *Guidelines on Endotoxins Test*						◆				
⟨1102⟩ *Immunological Test Methods—General Considerations*	◆	◆							◆	◆
⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)*	◆	◆							◆	◆
⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis*	◆	◆							◆	◆
⟨1105⟩ *Immunological Test Methods—Surface Plasmon Resonance*		◆					◆			
⟨1106⟩ *Immunogenicity Assays—Design and Validation of Immunoassays to Detect Anti-Drug Antibodies*						◆				
⟨1106.1⟩ *Immunogenicity Assays—Design and Validation of Assays to Detect Anti-Drug Neutralizing Antibody*						◆				
⟨1111⟩ *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use*						◆				
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*						◆				
⟨1116⟩ *Microbiological Control and Monitoring of Aseptic Processing Environments*						◆				
⟨1121⟩ *Nomenclature*					◆					
⟨1125⟩ *Nucleic Acid-Based Techniques—General*		◆								
⟨1130⟩ *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*		◆								
⟨1180⟩ *Human Plasma*						◆				
⟨1211⟩ *Sterility Assurance*						◆				
⟨1228⟩ *Depyrogenation*						◆				
⟨1228.1⟩ *Dry Heat Depyrogenation*						◆				
⟨1228.3⟩ *Depyrogenation by Filtration*						◆				

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Chart 7. Blood and Blood Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1228.4⟩ *Depyrogenation by Rinsing*			◆			◆				
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*						◆				
⟨1229⟩ *Sterilization of Compendial Articles*						◆				
⟨1229.4⟩ *Sterilizing Filtration of Liquids*						◆				
⟨1229.5⟩ *Biological Indicators for Sterilization*						◆				
⟨1229.13⟩ *Sterilization-in-Place*			◆							
⟨1229.14⟩ *Sterilization Cycle Development*						◆				
⟨1229.15⟩ *Sterilizing Filtration of Gases*			◆			◆				
⟨1237⟩ *Virology Test Methods*						◆				
⟨1240⟩ *Virus Testing of Human Plasma for Further Manufacture*						◆				
⟨1251⟩ *Weighing on an Analytical Balance*			◆							
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*		◆						◆		
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*		◆						◆		
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*		◆						◆		
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*		◆						◆		
⟨1660⟩ *Evaluation of the Inner Surface Durability of Glass Containers*				◆						
⟨1661⟩ *Evaluation of Plastic Packaging Systems and Their Materials of Construction with Respect to Their User Safety Impact*				◆						
⟨1663⟩ *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1664⟩ *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*				◆						
⟨1736⟩ *Applications of Mass Spectrometry*	◆	◆					◆	◆	◆	◆

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Chart 7. Blood and Blood Products *(continued)*

Chapter	Identification	Characterization	Equipment	Misc. Tests	Description	Safety	Assay	Physicochemical	Impurities	
									Process Related	Product Related
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*	◆	◆					◆	◆	◆	◆
⟨1787⟩ *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections*				◆						
⟨1788⟩ *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*				◆						
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*	◆	◆					◆	◆	◆	

Chart 8. Cell, Gene, and Tissue Based Products

Chapter	Universal Tests		Specific Tests[1, 2]					
	Identification	Assay	Biocompatibility	Microbial/Sterility Issues	Production Issues	Product Issues	Equipment	Characterization
⟨1⟩ *Injections and Implanted Drug Products (Parenterals)—Product Quality Tests*					◆			
⟨11⟩ *USP Reference Standards*	◆							
⟨31⟩ *Volumetric Apparatus*							◆	
⟨41⟩ *Balances*							◆	
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*				◆				
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*				◆				
⟨63⟩ *Mycoplasma Tests*				◆				
⟨71⟩ *Sterility Tests*				◆				
⟨85⟩ *Bacterial Endotoxins Test*				◆				
⟨87⟩ *Biological Reactivity Tests, In Vitro*			◆					
⟨88⟩ *Biological Reactivity Tests, In Vivo*			◆					
⟨90⟩ *Fetal Bovine Serum—Quality Attributes and Functionality Tests*					◆			
⟨92⟩ *Growth Factors and Cytokines Used in Cell Therapy Manufacturing*					◆			
⟨111⟩ *Design and Analysis of Biological Assays*								◆

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Chart 8. Cell, Gene, and Tissue Based Products *(continued)*

	Universal Tests		Specific Tests[1,2]					
Chapter	Identification	Assay	Biocompatibility	Microbial/Sterility Issues	Production Issues	Product Issues	Equipment	Characterization
⟨151⟩ *Pyrogen Test*				◆				
⟨161⟩ *Medical Devices—Bacterial Endotoxin and Pyrogen Tests*				◆				
⟨381⟩ *Elastomeric Closures for Injections*						◆		
⟨507⟩ *Protein Determination Procedures*								◆
⟨621⟩ *Chromatography*		◆						◆
⟨785⟩ *Osmolality and Osmolarity*								◆
⟨787⟩ *Subvisible Particulate Matter in Therapeutic Protein Injections*								◆
⟨788⟩ *Particulate Matter in Injections*								◆
⟨791⟩ *pH*								◆
⟨797⟩ *Pharmaceutical Compounding—Sterile Preparations*					◆			
⟨905⟩ *Uniformity of Dosage Units*								◆
⟨911⟩ *Viscosity—Capillary Methods*								◆
⟨912⟩ *Viscosity—Rotational Methods*								◆
⟨913⟩ *Viscosity—Rolling Ball Method*								◆
⟨1024⟩ *Bovine Serum*					◆			
⟨1027⟩ *Flow Cytometry*								◆
⟨1030⟩ *Biological Assay Chapters—Overview and Glossary*	◆	◆						◆
⟨1031⟩ *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants*			◆					
⟨1032⟩ *Design and Development of Biological Assays*	◆	◆						◆
⟨1033⟩ *Biological Assay Validation*	◆	◆						◆
⟨1034⟩ *Analysis of Biological Assays*	◆	◆						◆
⟨1041⟩ *Biologics*					◆			
⟨1043⟩ *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products*					◆			
⟨1044⟩ *Cryopreservation of Cells*	◆	◆			◆			◆
⟨1046⟩ *Cell-based Advanced Therapies and Tissue-Based Products*					◆	◆		◆
⟨1047⟩ *Gene Therapy Products*					◆			
⟨1048⟩ *Quality of Biotechnology Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products*								◆

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Chart 8. Cell, Gene, and Tissue Based Products *(continued)*

	Universal Tests		Specific Tests[1, 2]					
Chapter	Identification	Assay	Biocompatibility	Microbial/Sterility Issues	Production Issues	Product Issues	Equipment	Characterization
⟨1049⟩ *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products*								◆
⟨1050⟩ *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*				◆				
⟨1051⟩ *Cleaning Glass Apparatus*							◆	
⟨1052⟩ *Biotechnology Derived Articles—Amino Acid Analysis*								◆
⟨1053⟩ *Capillary Electrophoresis*								◆
⟨1054⟩ *Biotechnology Derived Articles—Isoelectric Focusing*								◆
⟨1055⟩ *Biotechnology Derived Articles—Peptide Mapping*								◆
⟨1056⟩ *Biotechnology Derived Articles—Polyacrylamide Gel Electrophoresis*								◆
⟨1057⟩ *Biotechnology Derived Articles—Total Protein Assay*								◆
⟨1071⟩ *Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach*				◆				
⟨1074⟩ *Excipient Biological Safety Evaluation Guidelines*					◆			
⟨1084⟩ *Glycoprotein and Glycan Analysis—General Considerations*								◆
⟨1085⟩ *Guidelines on Endotoxins Test*				◆				
⟨1086⟩ *Impurities in Drug Substances and Drug Products*						◆		
⟨1102⟩ *Immunological Test Methods—General Considerations*	◆	◆						◆
⟨1103⟩ *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)*	◆	◆						◆
⟨1104⟩ *Immunological Test Methods—Immunoblot Analysis*	◆							◆
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*				◆				
⟨1116⟩ *Microbiological Control and Monitoring of Aseptic Processing Environments*				◆				
⟨1121⟩ *Nomenclature*						◆		
⟨1126⟩ *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing*					◆			◆
⟨1127⟩ *Nucleic Acid-Based Techniques—Amplification*					◆			◆
⟨1128⟩ *Nucleic Acid-Based Techniques—Microarray*								◆
⟨1129⟩ *Nucleic Acid-Based Techniques—Genotyping*								◆

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Chart 8. Cell, Gene, and Tissue Based Products *(continued)*

	Universal Tests		Specific Tests[1, 2]					
Chapter	Identification	Assay	Biocompatibility	Microbial/Sterility Issues	Production Issues	Product Issues	Equipment	Characterization
⟨1130⟩ *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*								◆
⟨1151⟩ *Pharmaceutical Dosage Forms*						◆		
⟨1184⟩ *Sensitization Testing*			◆					
⟨1208⟩ *Sterility Testing—Validation of Isolator Systems*				◆				
⟨1211⟩ *Sterility Assurance*				◆				
⟨1227⟩ *Validation of Microbial Recovery from Pharmacopeial Articles*				◆				
⟨1228⟩ *Depyrogenation*				◆				
⟨1228.1⟩ *Dry Heat Depyrogenation*				◆				
⟨1228.3⟩ *Depyrogenation by Filtration*				◆				
⟨1228.4⟩ *Depyrogenation by Rinsing*				◆			◆	
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*				◆				
⟨1229⟩ *Sterilization of Compendial Articles*				◆				
⟨1229.3⟩ *Monitoring of Bioburden*				◆				
⟨1229.4⟩ *Sterilizing Filtration of Liquids*				◆				
⟨1229.13⟩ *Sterilization-in-Place*							◆	
⟨1229.14⟩ *Sterilization Cycle Development*				◆				
⟨1229.15⟩ *Sterilizing Filtration of Gases*				◆			◆	
⟨1229.16⟩ *Prion Sterilization*					◆		◆	
⟨1237⟩ *Virology Test Methods*					◆			◆
⟨1251⟩ *Weighing on an Analytical Balance*							◆	
⟨1285⟩ *Preparation of Biological Specimens for Histologic and Immunohistochemical Analysis*	◆				◆			◆
⟨1285.1⟩ *Hematoxylin and Eosin Staining of Sectioned Tissue for Microscopic Examination*	◆				◆			◆
⟨1787⟩ *Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections*								◆
⟨1788⟩ *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*								◆

[1] For Functionality test, see ⟨881⟩ *Tensile Strength*.
[2] For Water test, see ⟨1231⟩ *Water for Pharmaceutical Purposes*.

Chart 9. Drug Product Distribution

Chapter	Manufacturer	Shipper	Wholesaler	Sample Distribution	Repackager	Pharmacy	Practitioner
⟨7⟩ *Labeling*	◆						

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Chart 9. Drug Product Distribution *(continued)*

Chapter	Manufacturer	Shipper	Wholesaler	Sample Distribution	Repackager	Pharmacy	Practitioner
⟨17⟩ *Prescription Container Labeling*						◆	
⟨87⟩ *Biological Reactivity Tests, In Vitro*	◆				◆	◆	
⟨88⟩ *Biological Reactivity Tests, In Vivo*	◆				◆	◆	
⟨381⟩ *Elastomeric Closures for Injections*	◆					◆	
⟨659⟩ *Packaging and Storage Requirements*	◆	◆	◆	◆	◆	◆	◆
⟨660⟩ *Containers—Glass*	◆				◆	◆	
⟨661⟩ *Plastic Packaging Systems and Their Materials of Construction*	◆				◆	◆	
⟨661.1⟩ *Plastic Materials of Construction*	◆				◆	◆	
⟨661.2⟩ *Plastic Packaging Systems for Pharmaceutical Use*	◆				◆	◆	
⟨670⟩ *Auxiliary Packaging Components*	◆						
⟨671⟩ *Containers—Performance Testing*	◆				◆	◆	
⟨698⟩ *Deliverable Volume*	◆				◆		
⟨735⟩ *X-Ray Fluorescence Spectrometry*	◆				◆		
⟨755⟩ *Minimum Fill*	◆				◆		
⟨1066⟩ *Physical Environments That Promote Safe Medication Use*					◆	◆	◆
⟨1079⟩ *Good Storage and Distribution Practices for Drug Products*	◆	◆	◆	◆	◆	◆	
⟨1079.1⟩ *Storage and Transportation of Investigational Drug Products*	◆						
⟨1118⟩ *Monitoring Devices—Time, Temperature, and Humidity*	◆	◆	◆	◆	◆	◆	
⟨1151⟩ *Pharmaceutical Dosage Forms*	◆						
⟨1152⟩ *Animal Drugs for Use in Animal Feeds*	◆						◆
⟨1178⟩ *Good Repackaging Practices*					◆		
⟨1191⟩ *Stability Considerations in Dispensing Practice*	◆					◆	◆
⟨1265⟩ *Written Prescription Drug Information—Guidelines*						◆	
⟨1660⟩ *Evaluation of the Inner Surface Durability of Glass Containers*	◆				◆	◆	
⟨1661⟩ *Evaluation of Plastic Packaging Systems and Their Materials of Construction with Respect to Their User Safety Impact*	◆				◆	◆	
⟨1663⟩ *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems*	◆						

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Chart 9. Drug Product Distribution *(continued)*

Chapter	Manufacturer	Shipper	Wholesaler	Sample Distribution	Repackager	Pharmacy	Practitioner
⟨1664⟩ *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*	◆						

Chart 10a. Microbiology—Nonsterile Products

Chapter	Microbial Enumeration	Absence of Objectionable Microorganisms
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*		◆
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*	◆	
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*		◆
⟨63⟩ *Mycoplasma Tests*		◆
⟨610⟩ *Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products*	◆	◆
⟨1111⟩ *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use*	◆	◆
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*		◆
⟨1115⟩ *Bioburden Control of Nonsterile Drug Substances and Products*		◆
⟨2021⟩ *Microbial Enumeration Tests—Nutritional and Dietary Supplements*	◆	
⟨2022⟩ *Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements*		◆
⟨2023⟩ *Microbiological Attributes for Nonsterile Nutritional and Dietary Supplements*	◆	

Chart 10b. Microbiology—Sterile Products[1]

Chapter	Sterility Tests	Endotoxins/Pyrogens	Mycoplasma	Aseptic Processing	Filtration	Assembly[2]		Terminal Sterilization						
						Assembly	Other	Terminal Sterilization	Moist Heat	Dry Heat	Radiation	EtO	Liquid Phase	Vapor Phase
⟨55⟩ *Biological Indicators—Resistance Performance Tests*									◆	◆	◆	◆		
⟨63⟩ *Mycoplasma Tests*			◆											
⟨71⟩ *Sterility Tests*	◆													
⟨151⟩ *Pyrogen Tests*							◆							
⟨1071⟩ *Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach*	◆													
⟨1072⟩ *Disinfectants and Antiseptics*							◆							
⟨1085⟩ *Guidelines on Endotoxins Test*		◆												

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Chart 10b. Microbiology—Sterile Products[1] *(continued)*

Chapter	Sterility Tests	Endotoxins/Pyrogens	Mycoplasma	Aseptic Processing	Filtration	Assembly[2]		Terminal Sterilization						
						Assembly	Other	Terminal Sterilization	Moist Heat	Dry Heat	Radiation	EtO	Liquid Phase	Vapor Phase
⟨1112⟩ *Application of Water Activity Determination to Nonsterile Pharmaceutical Products*							◆							
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*	◆		◆	◆										
⟨1116⟩ *Microbiological Control and Monitoring of Aseptic Processing Environments*				◆		◆	◆							
⟨1117⟩ *Microbiological Best Laboratory Practices*							◆							
⟨1207⟩ *Package Integrity Evaluation—Sterile Products*						◆								
⟨1207.1⟩ *Package Integrity Testing in the Product Life Cycle—Test Method Selection and Validation*						◆								
⟨1207.2⟩ *Package Integrity Leak Test Technologies*						◆								
⟨1207.3⟩ *Package Seal Quality Test Technologies*						◆								
⟨1208⟩ *Sterility Testing—Validation of Isolator Systems*	◆			◆										
⟨1211⟩ *Sterility Assurance*				◆	◆				◆	◆	◆	◆		

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Chart 10b. Microbiology—Sterile Products[1] *(continued)*

Chapter	Sterility Tests	Endotoxins/Pyrogens	Mycoplasma	Aseptic Processing	Filtration	Assembly[2]		Terminal Sterilization						
						Assembly	Other	Terminal Sterilization	Moist Heat	Dry Heat	Radiation	EtO	Liquid Phase	Vapor Phase
⟨1222⟩ *Terminally Sterilized Pharmaceutical Products—Parametric Release*								◆						
⟨1223⟩ *Validation of Alternative Microbiological Methods*							◆							
⟨1228⟩ *Depyrogenation*				◆	◆	◆		◆	◆	◆	◆	◆		
⟨1228.1⟩ *Dry Heat Depyrogenation*				◆		◆		◆		◆				
⟨1228.3⟩ *Depyrogenation by Filtration*				◆	◆						◆			
⟨1228.4⟩ *Depyrogenation by Rinsing*				◆	◆			◆						
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*				◆	◆	◆		◆		◆				
⟨1229⟩ *Sterilization of Compendial Articles*				◆	◆	◆		◆	◆	◆	◆	◆		
⟨1229.1⟩ *Steam Sterilization by Direct Contact*									◆					
⟨1229.2⟩ *Moist Heat Sterilization of Aqueous Liquids*									◆					
⟨1229.3⟩ *Monitoring of Bioburden*				◆	◆				◆		◆			
⟨1229.4⟩ *Sterilizing Filtration of Liquids*				◆	◆									
⟨1229.5⟩ *Biological Indicators for Sterilization*									◆	◆	◆	◆		
⟨1229.6⟩ *Liquid-Phase Sterilization*						◆							◆	
⟨1229.7⟩ *Gaseous Sterilization*												◆		

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Chart 10b. Microbiology—Sterile Products[1] *(continued)*

Chapter	Sterility Tests	Endotoxins/Pyrogens	Mycoplasma	Aseptic Processing	Filtration	Assembly[2]		Terminal Sterilization						
						Assembly	Other	Terminal Sterilization	Moist Heat	Dry Heat	Radiation	EtO	Liquid Phase	Vapor Phase
⟨1229.8⟩ *Dry Heat Sterilization*										◆				
⟨1229.9⟩ *Physicochemical Integrators and Indicators for Sterilization*									◆			◆		
⟨1229.10⟩ *Radiation Sterilization*											◆			
⟨1229.11⟩ *Vapor Phase Sterilization*						◆								◆
⟨1229.12⟩ *New Sterilization Methods*				◆				◆						
⟨1229.13⟩ *Steriliza-tion-in-Place*								◆	◆	◆		◆	◆	◆
⟨1229.14⟩ *Sterilization Cycle Development*				◆	◆			◆	◆	◆	◆	◆	◆	◆
⟨1229.15⟩ *Sterilizing Filtration of Gases*						◆								

[1] For endotoxins limits, see ⟨85⟩ *Bacterial Endotoxins Test.*
[2] For BFS, FFS, and SFS, see ⟨1116⟩ *Microbiological Control and Monitoring of Aseptic Processing Environments.*

Chart 11a. Dietary Supplement Ingredients

Chapter	Botanicals						Nonbotanicals[1,2]
	Description	Identification	Content	Safety/Purity	Physicochemical Characterization	Other	Vitamin Assays[3]
⟨7⟩ *Labeling*	◆						
⟨91⟩ *Calcium Pantothenate Assay*							◆
⟨115⟩ *Dexpanthenol Assay*							◆
⟨171⟩ *Vitamin B_{12} Activity Assay*							◆
⟨197⟩ *Spectrophotometric Identification Tests*		◆					
⟨201⟩ *Thin-Layer Chromatographic Identification Test*		◆					
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*		◆					
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*		◆					
⟨211⟩ *Arsenic*				◆			
⟨233⟩ *Elemental Impurities—Procedures*				◆			
⟨251⟩ *Lead*				◆			
⟨261⟩ *Mercury*				◆			

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Chart 11a. Dietary Supplement Ingredients *(continued)*

	Botanicals						Nonbotanicals[1, 2]
Chapter	Description	Identification	Content	Safety/ Purity	Physicochemical Characterization	Other	Vitamin Assays[3]
⟨281⟩ *Residue on Ignition*				◆			
⟨401⟩ *Fats and Fixed Oils*					◆		
⟨411⟩ *Folic Acid Assay*							◆
⟨441⟩ *Niacin or Niacinamide Assay*							◆
⟨451⟩ *Nitrate Titration*				◆			
⟨461⟩ *Nitrogen Determination*				◆			
⟨467⟩ *Residual Solvents*				◆			
⟨481⟩ *Riboflavin Assay*							◆
⟨531⟩ *Thiamine Assay*							◆
⟨541⟩ *Titrimetry*			◆				
⟨551⟩ *Vitamin E Assay*							◆
⟨561⟩ *Articles of Botanical Origin*	◆	◆	◆	◆	◆		
⟨563⟩ *Identification of Articles of Botanical Origin*	◆	◆					
⟨565⟩ *Botanical Extracts*	◆			◆			
⟨571⟩ *Vitamin A Assay*							◆
⟨581⟩ *Vitamin D Assay*							◆
⟨591⟩ *Zinc Determination*							◆
⟨611⟩ *Alcohol Determination*				◆			
⟨621⟩ *Chromatography*		◆	◆		◆		
⟨630⟩ *Visual Comparison*				◆			
⟨730⟩ *Plasma Spectrochemistry*				◆			
⟨731⟩ *Loss on Drying*				◆			
⟨733⟩ *Loss on Ignition*				◆			
⟨735⟩ *X-Ray Fluorescence Spectrometry*		◆		◆	◆		◆
⟨736⟩ *Mass Spectrometry*					◆		
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*					◆		
⟨776⟩ *Optical Microscopy*	◆	◆					
⟨791⟩ *pH*					◆		
⟨852⟩ *Atomic Absorption Spectroscopy*			◆		◆		
⟨853⟩ *Fluorescence Spectroscopy*			◆		◆		
⟨854⟩ *Mid-Infrared Spectroscopy*			◆		◆		
⟨855⟩ *Nephelometry and Turbidimetry*					◆		
⟨856⟩ *Near-Infrared Spectroscopy*			◆		◆		
⟨857⟩ *Ultraviolet-Visible Spectroscopy*			◆		◆		
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*		◆					
⟨1065⟩ *Ion Chromatography*					◆		
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*				◆			
⟨1181⟩ *Scanning Electron Microscopy*	◆	◆					
⟨1225⟩ *Validation of Compendial Procedures*						◆	
⟨1226⟩ *Verification of Compendial Procedures*						◆	

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Chart 11a. Dietary Supplement Ingredients *(continued)*

	Botanicals						Nonbotanicals[1,2]
Chapter	Description	Identification	Content	Safety/ Purity	Physicochemical Characterization	Other	Vitamin Assays[3]
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*					◆		
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*					◆		
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*					◆		
⟨1430.5⟩ *Analytical Methodologies Based onScattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*					◆		
⟨1467⟩ *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures*				◆			
⟨1736⟩ *Applications of Mass Spectrometry*					◆		
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*					◆		
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*			◆		◆		
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*			◆		◆		
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*			◆		◆		
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*			◆		◆		
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*			◆		◆		
⟨2021⟩ *Microbial Enumeration Tests—Nutritional and Dietary Supplements*				◆			
⟨2022⟩ *Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements*				◆			
⟨2023⟩ *Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements*				◆			
⟨2030⟩ *Supplemental Information for Articles of Botanical Origin*						◆	
⟨2232⟩ *Elemental Contaminants in Dietary Supplements*				◆			
⟨2250⟩ *Detection of Irradiated Dietary Supplements*				◆			

[1] For complex see *Biologics Drug Substances (Chart 2)*.
[2] For noncomplex Minerals, Amino Acids, and Metabolites, see *Chemical Medicines Drug Substances (Chart 1a and 1b)*.
[3] See also *Chemical Medicines Drug Substances—Universal Tests (Chart 1a)*.

Chart 11b. Probiotics. Dietary Supplements Ingredients and Dosage Forms

	Probiotic Ingredients				Probiotic Dosage Forms		
Chapter	Description/Labeling	Identification	Content/Enumeration	Safety/ Purity	Description/Labeling	Performance	Safety/ Purity
⟨64⟩ *Probiotic Tests*	◆	◆	◆	◆	◆	◆	◆
⟨2040⟩ *Disintegration and Dissolution of Dietary Supplements*						◆	
⟨2091⟩ *Weight Variation of Dietary Supplements*						◆	

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Chart 11b. Probiotics. Dietary Supplements Ingredients and Dosage Forms *(continued)*

Chapter	Probiotic Ingredients				Probiotic Dosage Forms		
	Description/Labeling	Identification	Content/Enumeration	Safety/Purity	Description/Labeling	Performance	Safety/Purity
⟨2021⟩ *Microbiological Enumeration Tests—Nutritional and Dietary Supplements*				◆			◆
⟨2022⟩ *Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements*				◆			◆

Chart 12. Dietary Supplement Products

Chapter	Universal Tests						Specific Tests		
				Impurities					
	Description	Identification	Assay/Content	Organic	Inorganic	Residual Solvents	Equipment	Performance Tests	Safety/Purity
⟨7⟩ *Labeling*	◆								
⟨31⟩ *Volumetric Apparatus*							◆		
⟨41⟩ *Balances*							◆		
⟨91⟩ *Calcium Pantothenate Assay*			◆						
⟨171⟩ *Vitamin B_{12} Assay*			◆						
⟨191⟩ *Identification Tests—General*		◆							
⟨197⟩ *Spectrophotometric Identification Tests*		◆							
⟨201⟩ *Thin-Layer Chromatographic Identification Test*		◆							
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*		◆							
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*		◆							
⟨211⟩ *Arsenic*									◆
⟨251⟩ *Lead*									◆
⟨261⟩ *Mercury*									◆
⟨281⟩ *Residue on Ignition*					◆				
⟨411⟩ *Folic Acid Assay*			◆						
⟨441⟩ *Niacin or Niacinamide Assay*			◆						
⟨451⟩ *Nitrate Titration*									◆
⟨466⟩ *Ordinary Impurities*				◆					
⟨467⟩ *Residual Solvents*						◆			
⟨531⟩ *Thiamine Assay*			◆						
⟨541⟩ *Titrimetry*			◆						
⟨551⟩ *Vitamin E Assay*			◆						
⟨561⟩ *Articles of Botanical Origin*			◆						◆
⟨563⟩ *Identification of Articles of Botanical Origin*		◆							
⟨565⟩ *Botanical Extracts*									◆
⟨571⟩ *Vitamin A Assay*			◆						
⟨581⟩ *Vitamin D Assay*			◆						
⟨621⟩ *Chromatography*		◆	◆	◆		◆			

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Chart 12. Dietary Supplement Products *(continued)*

Chapter	Universal Tests						Specific Tests		
				Impurities					
	Description	Identification	Assay/ Content	Organic	Inorganic	Residual Solvents	Equipment	Performance Tests	Safety/ Purity
⟨630⟩ *Visual Comparison*				◆	◆				
⟨730⟩ *Plasma Spectrochemistry*			◆		◆				◆
⟨733⟩ *Loss on Ignition*					◆				
⟨735⟩ *X-Ray Fluorescence Spectrometry*		◆	◆		◆				◆
⟨736⟩ *Mass Spectrometry*		◆							
⟨776⟩ *Optical Microscopy*		◆							
⟨781⟩ *Optical Rotation*				◆					
⟨782⟩ *Vibrational Circular Dichroism Spectroscopy*				◆					
⟨801⟩ *Polarography*				◆					
⟨852⟩ *Atomic Absorption Spectroscopy*		◆	◆	◆					
⟨853⟩ *Fluorescence Spectroscopy*		◆	◆	◆					
⟨854⟩ *Mid-Infrared Spectroscopy*		◆	◆	◆					
⟨855⟩ *Nephelometry and Turbidimetry*			◆	◆					
⟨856⟩ *Near-Infrared Spectroscopy*		◆	◆	◆					
⟨857⟩ *Ultraviolet-Visible Spectroscopy*		◆	◆	◆					
⟨1051⟩ *Cleaning Glass Apparatus*							◆		
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*		◆							
⟨1065⟩ *Ion Chromatography*		◆							
⟨1086⟩ *Impurities in Drug Substances and Drug Products*				◆					
⟨1094⟩ *Capsules—Dissolution Testing and Related Quality Attributes*								◆	
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*									◆
⟨1151⟩ *Pharmaceutical Dosage Forms*	◆								
⟨1216⟩ *Tablet Friability*								◆	
⟨1251⟩ *Weighing on an Analytical Balance*							◆		
⟨1467⟩ *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures*						◆			
⟨1736⟩ *Applications of Mass Spectrometry*		◆							
⟨1782⟩ *Vibrational Circular Dichroism Spectroscopy—Theory and Practice*				◆					
⟨1852⟩ *Atomic Absorption Spectroscopy—Theory and Practice*		◆	◆	◆					

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Chart 12. Dietary Supplement Products *(continued)*

Chapter	Universal Tests						Specific Tests		
				Impurities					
	Description	Identification	Assay/ Content	Organic	Inorganic	Residual Solvents	Equipment	Performance Tests	Safety/ Purity
⟨1853⟩ *Fluorescence Spectroscopy—Theory and Practice*		◆	◆	◆					
⟨1854⟩ *Mid-Infrared Spectroscopy—Theory and Practice*		◆	◆	◆					
⟨1856⟩ *Near-Infrared Spectroscopy—Theory and Practice*		◆	◆	◆					
⟨1857⟩ *Ultraviolet-Visible Spectroscopy—Theory and Practice*		◆	◆	◆					
⟨2021⟩ *Microbiological Enumeration Tests—Nutritional and Dietary Supplements*									◆
⟨2022⟩ *Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements*									◆
⟨2023⟩ *Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements*									◆
⟨2030⟩ *Supplemental Information for Articles of Botanical Origin*									◆
⟨2040⟩ *Disintegration and Dissolution of Dietary Supplements*								◆	
⟨2091⟩ *Weight Variation of Dietary Supplements*								◆	
⟨2232⟩ *Elemental Contaminants in Dietary Supplements*					◆				◆
⟨2250⟩ *Detection of Irradiated Dietary Supplements*					◆				
⟨2750⟩ *Manufacturing Practices for Dietary Supplements*							◆	◆	◆

Chart 13. Compounding—Substance/Preparation/Practice

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨7⟩ *Labeling*		◆								
⟨11⟩ *USP Reference Standards*			◆	◆		◆	◆		◆	◆
⟨31⟩ *Volumetric Apparatus*								◆		
⟨41⟩ *Balances*								◆		
⟨60⟩ *Microbiological Examination of Nonsterile Products: Tests for Burkholderia Cepacia Complex (BCC)*							◆			
⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*							◆			

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*							◆			
⟨71⟩ *Sterility Tests*							◆			
⟨81⟩ *Antibiotics—Microbial Assays*				◆					◆	
⟨85⟩ *Bacterial Endotoxins Test*							◆			
⟨87⟩ *Biological Reactivity Tests, In Vitro*							◆			
⟨88⟩ *Biological Reactivity Tests, In Vivo*							◆			
⟨111⟩ *Design and Analysis of Biological Assays*									◆	◆
⟨191⟩ *Identification Tests—General*			◆							
⟨197⟩ *Spectrophotometric Identification Tests*			◆					◆		
⟨201⟩ *Thin-Layer Chromatographic Identification Test*			◆							
⟨202⟩ *Identification of Fixed Oils by Thin-Layer Chromatography*			◆							
⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*			◆							
⟨541⟩ *Titrimetry*				◆					◆	
⟨621⟩ *Chromatography*			◆	◆		◆		◆	◆	◆
⟨630⟩ *Visual Comparison*									◆	
⟨659⟩ *Packaging and Storage Requirements*					◆					

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨660⟩ *Containers—Glass*					◆					
⟨661⟩ *Plastic Packaging Systems and Their Materials of Construction*					◆					
⟨661.1⟩ *Plastic Materials of Construction*					◆					
⟨661.2⟩ *Plastic Packaging Systems for Pharmaceutical Use*					◆					
⟨671⟩ *Containers—Performance Testing*					◆					
⟨730⟩ *Plasma Spectrochemistry*								◆	◆	
⟨731⟩ *Loss on Drying*									◆	
⟨736⟩ *Mass Spectrometry*			◆	◆		◆		◆	◆	◆
⟨761⟩ *Nuclear Magnetic Resonance Spectroscopy*			◆	◆		◆		◆	◆	◆
⟨781⟩ *Optical Rotation*				◆						
⟨786⟩ *Particle Size Distribution Estimation by Analytical Sieving*						◆				
⟨795⟩ *Pharmaceutical Compounding—Nonsterile Preparations*	◆	◆			◆		◆	◆		
⟨797⟩ *Pharmaceutical Compounding—Sterile Preparations*	◆	◆			◆		◆	◆		
⟨800⟩ *Hazardous Drugs—Handling in Healthcare Settings*	◆				◆		◆	◆		
⟨823⟩ *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses*	◆									
⟨831⟩ *Refractive Index*						◆				

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨852⟩ *Atomic Absorption Spectroscopy*			◆	◆		◆		◆	◆	
⟨853⟩ *Fluorescence Spectroscopy*			◆	◆		◆		◆	◆	
⟨854⟩ *Mid-Infrared Spectroscopy*			◆	◆		◆		◆	◆	
⟨855⟩ *Nephelometry and Turbidimetry*				◆		◆		◆	◆	
⟨856⟩ *Near-Infrared Spectroscopy*			◆	◆		◆		◆	◆	
⟨857⟩ *Ultraviolet-Visible Spectroscopy*			◆	◆		◆		◆	◆	
⟨921⟩ *Water Determination*									◆	
⟨1031⟩ *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants*							◆			
⟨1052⟩ *Biotechnology-Derived Articles—Amino Acid Analysis*								◆	◆	
⟨1053⟩ *Capillary Electrophoresis*								◆	◆	
⟨1054⟩ *Biotechnology-Derived Articles—Isoelectric Focusing*								◆	◆	
⟨1055⟩ *Biotechnology-Derived Articles—Peptide Mapping*								◆	◆	
⟨1056⟩ *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis*								◆	◆	
⟨1057⟩ *Biotechnology-Derived Articles—Total Protein Assay*								◆	◆	

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*			◆							
⟨1065⟩ *Ion Chromatography*			◆							
⟨1066⟩ *Physical Environments That Promote Safe Medication Use*							◆			
⟨1085⟩ *Guidelines on Endotoxins Test*							◆			
⟨1113⟩ *Microbial Characterization, Identification, and Strain Typing*							◆			
⟨1121⟩ *Nomenclature*		◆								
⟨1151⟩ *Pharmaceutical Dosage Forms*	◆									
⟨1152⟩ *Animal Drugs for Use in Animal Feeds*	◆									
⟨1160⟩ *Pharmaceutical Calculations in Pharmacy Practice*	◆						◆			
⟨1163⟩ *Quality Assurance in Pharmaceutical Compounding*	◆	◆		◆	◆		◆	◆		
⟨1168⟩ *Compounding for Phase I Investigational Studies*	◆	◆			◆		◆	◆		
⟨1176⟩ *Prescription Balances and Volumetric Apparatus Used in Compounding*	◆	◆					◆	◆		
⟨1191⟩ *Stability Considerations in Dispensing Practice*					◆					
⟨1228⟩ *Depyrogenation*							◆			

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨1228.1⟩ *Dry Heat Depyrogenation*							◆			
⟨1228.3⟩ *Depyrogenation by Filtration*							◆			
⟨1228.4⟩ *Depyrogenation by Rinsing*							◆	◆		
⟨1228.5⟩ *Endotoxin Indicators for Depyrogenation*							◆			
⟨1229⟩ *Sterilization of Compendial Articles*							◆			
⟨1229.2⟩ *Moist Heat Sterilization of Aqueous Liquids*							◆			
⟨1229.3⟩ *Monitoring of Bioburden*							◆			
⟨1229.4⟩ *Sterilizing Filtration of Liquids*							◆			
⟨1229.6⟩ *Liquid-Phase Sterilization*							◆			
⟨1229.7⟩ *Gaseous Sterilization*							◆			
⟨1229.8⟩ *Dry Heat Sterilization*							◆			
⟨1229.10⟩ *Radiation Sterilization*							◆			
⟨1229.11⟩ *Vapor Phase Sterilization*							◆			
⟨1229.12⟩ *New Sterilization Methods*							◆			
⟨1229.13⟩ *Sterilization-in-Place*							◆			
⟨1229.14⟩ *Sterilization Cycle Development*							◆			
⟨1229.15⟩ *Sterilizing Filtration of Gases*					◆		◆	◆		
⟨1231⟩ *Water for Pharmaceutical Purposes*	◆									
⟨1251⟩ *Weighing on an Analytical Balance*								◆		

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨1265⟩ *Written Prescription Drug Information—Guidelines*	◆									
⟨1430.1⟩ *Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering*						◆				
⟨1430.2⟩ *Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size*						◆				
⟨1430.4⟩ *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)*						◆				
⟨1430.5⟩ *Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering*						◆				
⟨1660⟩ *Evaluation of the Inner Surface Durability of Glass Containers*					◆					
⟨1661⟩ *Evaluation of Plastic Packaging Systems and their Materials of Construction with Respect to Their User Safety Impact*					◆					
⟨1663⟩ *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems*					◆					

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Chart 13. Compounding—Substance/Preparation/Practice *(continued)*

Chapter	Global	Description	Identification	Assay	Packaging	Physicochemical Characterization	Safety	Equipment	Impurities	
									Process Related	Product Related
⟨1664⟩ *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*					◆					
⟨1736⟩ *Applications of Mass Spectrometry*			◆	◆		◆		◆	◆	◆
⟨1761⟩ *Applications of Nuclear Magnetic Resonance Spectroscopy*			◆	◆		◆		◆	◆	◆
⟨1852⟩ *Atomic Absorption Spectroscopy —Theory and Practice*			◆	◆		◆		◆	◆	
⟨1853⟩ *Fluorescence Spectroscopy —Theory and Practice*			◆	◆		◆		◆	◆	
⟨1854⟩ *Mid-Infrared Spectroscopy —Theory and Practice*			◆	◆		◆		◆	◆	
⟨1856⟩ *Near-Infrared Spectroscopy —Theory and Practice*			◆	◆		◆		◆	◆	
⟨1857⟩ *Ultraviolet-Visible Spectroscopy —Theory and Practice*			◆	◆		◆		◆	◆	

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General Chapters

General Tests And Assays

General Requirements for Tests and Assays

⟨1⟩ INJECTIONS AND IMPLANTED DRUG PRODUCTS (PARENTERALS)—PRODUCT QUALITY TESTS

INTRODUCTION
PRODUCT QUALITY TESTS COMMON TO PARENTERAL DOSAGE FORMS
- Universal Tests
- Specific Tests

PRODUCT QUALITY TESTS FOR SPECIFIC PARENTERAL DOSAGE FORMS
- Solutions
- Sterile Powders for Solutions
- Suspensions
- Liposomes
- Sterile Powders for Suspensions
- Emulsions
- Implants
- Drug-Eluting Stents

INTRODUCTION

Parenteral drug products include both injections and implanted drug products that are injected through the skin or other external boundary tissue, or implanted within the body to allow the direct administration of the active drug substance(s) into blood vessels, organs, tissues, or lesions. Injections may exist as either immediate- or extended-release dosage forms. Implanted parenteral drug products are long-acting dosage forms that provide continuous release of the active drug substance(s), often for periods of months to years. For systemic delivery, they may be placed subcutaneously; for local delivery, they may be placed in a specific region of the body. Routes of administration for parenteral drug products include intravenous, intraventricular, intra-arterial, intra-articular, subcutaneous, intramuscular, intrathecal, intracisternal, and intraocular.

Parenteral dosage forms include solutions, suspensions, emulsions, sterile powders for solutions and suspensions (including liposomes), implants (including microparticles), and products that consist of both a drug and a device such as drug-eluting stents. The definitions and descriptions of these dosage forms, and brief information about their composition and manufacturing processes, are found in *Pharmaceutical Dosage Forms* ⟨1151⟩. [NOTE—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.]

This chapter is divided into three main sections: (1) universal product quality tests that are applicable to parenteral dosage forms; (2) specific product quality tests, which are tests that should be considered in addition to *Universal Tests*; and (3) product quality tests for specific dosage forms, which list applicable tests (universal and specific) for the specific dosage form.

This chapter applies, in whole or in part, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*).

The pharmacopeial definitions for sterile preparations for parenteral use may not apply to some biologics because of their special nature and licensing requirements (see *Biologics* ⟨1041⟩). However, some biological finished drug products containing "Injection" in the monograph title must meet the requirements of ⟨1⟩ or indicated chapter subparts, where it is specified in the monograph.

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PRODUCT QUALITY TESTS COMMON TO PARENTERAL DOSAGE FORMS

Universal Tests

Universal tests are listed below and are applicable to parenteral dosage forms.

IDENTIFICATION

Identification tests are discussed in *General Notices, 5.40 Identification* should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structure that are likely to be present..

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. In cases where the use of a nonspecific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay.

IMPURITIES

Tests for *Impurities* are discussed in *General Notices, 5.60 Impurities and Foreign Substances*. All articles should be tested to ensure that they meet the requirements.

FOREIGN AND PARTICULATE MATTER

Articles intended for parenteral administration should be prepared in a manner designed to exclude particulate matter as defined in *Subvisible Particulate Matter in Therapeutic Protein Injections* ⟨787⟩, *Particulate Matter in Injections* ⟨788⟩, or *Particulate Matter in Ophthalmic Solutions* ⟨789⟩, as well as excluding other foreign matter as appropriate for the dosage form. Each final container of all parenteral preparations should be inspected to the extent possible for the presence of observable foreign and particulate matter (hereafter termed visible particulates) in its contents. The inspection process should be designed and qualified to ensure that every lot of all parenteral preparations is essentially free from visible particulates, as defined in *Visible Particulates in Injections* ⟨790⟩. Qualification of the inspection process should be performed with reference to particulates in the visible range and those particulates that might emanate from the manufacturing or filling process. Every container in which the contents show evidence of visible particulates must be rejected. The inspection for visible particulates may take place during examination for other defects such as cracked or defective containers or seals, or when characterizing the appearance of a lyophilized product.

When the nature of the contents or the container–closure system permits only limited inspection of the total contents, the 100% inspection of a lot should be supplemented with the inspection of constituted (e.g., dried) or withdrawn (e.g., from a dark amber container) contents of a sample of containers from the lot.

Large-volume injections for single-dose infusion, small-volume injections, and pharmacy bulk packages (PBPs) are subject to the light obscuration or microscopic procedures and limits for subvisible particulate matter set forth in ⟨788⟩, unless otherwise specified in the chapter or in the individual monograph. An article packaged as both a large-volume and a small-volume injection meets the requirements set forth for small-volume injections where the container is labeled as containing 100 mL or less. It meets the requirements set forth for large-volume injections for single-dose infusion where the container is labeled as containing more than 100 mL.

STERILITY

The sterility of all drug products intended for parenteral administration should be confirmed by the use of methods described in *Sterility Tests* ⟨71⟩ or by an approved alternative method.

BACTERIAL ENDOTOXINS

All articles intended for parenteral administration should be prepared in a manner designed to limit bacterial endotoxins as defined in *Bacterial Endotoxins Test* ⟨85⟩ or *Pyrogen Test* ⟨151⟩.

CONTAINER CONTENT

Container contents should be determined when appropriate (see *Container Content for Injections* ⟨697⟩).

PACKAGING SYSTEMS

The packaging system should not interact physically or chemically with the preparation to alter its strength, quality, or purity beyond the official or established requirements. The packaging system should meet the requirements in *Elastomeric Closures for Injections* ⟨381⟩, *Packaging and Storage Requirements* ⟨659⟩, *Containers—Glass* ⟨660⟩, *Plastic Packaging Systems and their Materials of Construction* ⟨661⟩, *Plastic Materials of Construction* ⟨661.1⟩, and *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩. Further information regarding packaging systems testing may be found in *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1663⟩ and *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1664⟩.

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CONTAINER–CLOSURE INTEGRITY

The packaging system should be closed or sealed in such a manner as to prevent contamination or loss of contents. Validation of container integrity must demonstrate no penetration of microbial contamination or gain or loss of any chemical or physical parameter deemed necessary to protect the product (see *Package Integrity Evaluation—Sterile Products* ⟨1207⟩, *Package Integrity Testing in the Product Life Cycle—Test Method Selection and Validation* ⟨1207.1⟩, *Package Integrity Leak Test Technologies* ⟨1207.2⟩, and *Package Seal Quality Test Technologies* ⟨1207.3⟩).

LABELING

All articles intended for parenteral administration should meet the labeling requirements defined in *Labeling* ⟨7⟩.

Change to read:

Specific Tests

In addition to the *Universal Tests* listed above, the following specific tests may be necessary depending on the dosage form.

UNIFORMITY OF DOSAGE UNITS

This test is applicable for parenteral drug products and dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the active substance in the dosage form (see *Uniformity of Dosage Units* ⟨905⟩).

VEHICLES AND ADDED SUBSTANCES

There are other vehicles, both aqueous and nonaqueous, beyond those that are discussed below. All vehicles should be suitable for their intended use and not impact drug product quality.

Aqueous vehicles: Aqueous vehicles must meet the requirements of ⟨151⟩ or ⟨85⟩, whichever is specified in the monograph. Water for injection is generally used as the vehicle. Sodium chloride or dextrose may be added to render the resulting solution isotonic, and sodium chloride injection or Ringer's injection may be used in whole or in part instead of water for injection.

Nonaqueous vehicles: Fixed oils are classified under *Nonaqueous vehicles*. Fixed oils used as vehicles are of vegetable origin and are odorless. They meet the requirements in the test for *Solid Paraffin* in the *Mineral Oil* monograph with the cooling bath maintained at 10°.

Nonaqueous vehicles should also meet the requirements of the following tests:

- *Fats and Fixed Oils* ⟨401⟩, *Saponification Value*: Between 185 and 200
- *Fats and Fixed Oils* ⟨401⟩, *Iodine Value*: Between 79 and 141
- *Fats and Fixed Oils* ⟨401⟩, *Unsaponifiable Matter*: NMT 1.5%
- *Fats and Fixed Oils* ⟨401⟩, *Acid Value*: NMT 0.2
- *Fats and Fixed Oils* ⟨401⟩, *Peroxide Value*: NMT 5.0
- *Water Determination* ⟨921⟩, *Method Ic*: NMT 0.1%
- *Limit of Copper, Iron, Lead, and Nickel*: [NOTE—The test for nickel is not required if the oil has not been subjected to hydrogenation, or a nickel catalyst has not been used in processing.] Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Trace Metals* or *Elemental Impurities—Procedures* ⟨233⟩. Meet the requirements in *Elemental Impurities—Limits* ⟨232⟩.

Synthetic mono- or diglycerides of fatty acids may be used provided they are liquid and remain clear when cooled to 10° and have a *Iodine Value* of NMT 140.

Added substances: Suitable substances may be added to preparations in order to increase stability or usefulness unless they are proscribed in the monograph. No coloring agent may be added to a preparation solely for the purpose of coloring the finished preparation (see *General Notices, 5.20 Added Substances* and *Antimicrobial Effectiveness Testing* ⟨51⟩).

Observe special care in the choice and use of added substances in preparations with volumes that exceed 5 mL. The following limits prevail unless otherwise directed:

- Mercury and cationic surface-active agents: NMT 0.01%
- Chlorobutanol, cresol, phenol, and similar substances: NMT 0.5%
- Sulfur dioxide or an equivalent amount of sulfite, bisulfite, or metabisulfite of potassium or sodium: NMT 0.2%

ANTIMICROBIAL PRESERVATIVES

Antimicrobial agents must be added to preparations intended for injection that are packaged in multiple-dose containers unless one of the following conditions prevails: (1) there are different directions in the individual monograph; (2) the substance contains a radionuclide with a physical half-life of less than 24 h; or (3) the active ingredients are themselves antimicrobial. Substances must meet the requirements of ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩.

WATER CONTENT

The water content of freeze-dried (lyophilized) products should be determined when appropriate (see ⟨921⟩).

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ALUMINUM CONTENT

See ▲*Labeling* ⟨7⟩, *Aluminum in Large-Volume Injections (LVIs), Small-Volume Injections (SVIs), and Pharmacy Bulk Packages (PBPs) Used in Parenteral Nutrition (PN) Therapy*▲ (CN 1-May-2019) for information related to specific labeling requirements associated with aluminum content.

COMPLETENESS AND CLARITY OF SOLUTIONS

The following tests are performed to demonstrate suitability of constituted solutions prepared before administration. Constitute the solution as directed in the labeling supplied by the manufacturer:

- The solid dissolves completely, leaving no undissolved matter.
- The constituted solution is not significantly less clear than an equal volume of the diluent or of purified water contained in a similar vessel and examined similarly. Protein solutions may exhibit an inherent opalescence.

The constituted solution is free from particulate matter that can be observed on visual inspection (see ⟨790⟩).

PRODUCT QUALITY TESTS FOR SPECIFIC PARENTERAL DOSAGE FORMS

Product quality tests for the specific dosage forms are listed below. Specific chapter(s) referenced for the test can be found in the *Universal Tests* and *Specific Tests* sections.

Solutions

A solution is a clear, homogeneous liquid dosage form that contains one or more chemical substances (e.g., drug substances or excipients) dissolved in a solvent (aqueous or nonaqueous) or a mixture of mutually miscible solvents. Solutions intended for parenteral administration (e.g., by injection or for irrigation) must be sterile and biocompatible with the intended administration site. This includes consideration of factors such as tonicity, pH, pyrogenicity, extraneous particulate matter, and physicochemical compatibility, among others.

Unless otherwise justified, the following tests are required for solutions for injection:

- *Universal Tests*
- *Specific Tests*
  - *Antimicrobial Preservatives*

Sterile Powders for Solutions

Sterile powders for solutions (also referred to as sterile powders for injection) consist of drug substances and other components as dry-formulation ingredients to ensure the chemical and physical stability of the presentation within a final-use container. Companion sterile diluent or diluent compartments may be provided to facilitate constitution to the desired final volume.

The sterile article for injection may be presented in several forms: lyophilized powder intended for final solution, powdered solids intended for final solution, or dry solids that form viscous liquids upon constitution.

The description should include a section that deals with ease of dispersion and reconstitution. The dosage form is a homogeneous solid that is readily constituted to the final form with the specified diluent, and dispersion is completed with gentle agitation.

Unless otherwise justified, the following tests apply to sterile powders for injection:

- *Universal Tests*

The following applies to constituted solutions:

- Chapter ⟨905⟩: To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms that contain a single dose or a part of a dose of drug substance in each unit. For liquid dosage forms analysts should conduct the assay on an amount of well-mixed constituted material that is removed from an individual container under conditions of normal use, should express the results as delivered dose, and should calculate the acceptance value.

The following applies to dry cake:

- *Loss on Drying* ⟨731⟩: The procedure set forth in this chapter determines the amount of volatile matter of any kind that is driven off under the conditions specified.
- Chapter ⟨921⟩: Water or solvent content may have important effects on reconstitution and stability. For articles that require water or solvent content control, analysts should perform one of the methods described in ⟨921⟩ or a suitable replacement.
- *Appearance*: Analysts should assess the level of and the unit variation for the following parameters:
  - Color of Dry Cake: Varies within target parameters
  - Texture and Homogeneity of Dry Cake: Varies within target parameters
  - Presence of Foreign Material: All units with visible foreign material must be rejected

Suspensions

Parenteral suspensions are liquid dosage forms that contain solid particles in a state of uniform dispersion. Suspensions for parenteral administration must be sterile and compatible with the administration site. Consideration should be given to pH and

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pyrogenicity, and appropriate limits should be identified. Physical stability evaluations of parenteral suspension preparations should include evaluations to confirm that the particle size range of suspended matter does not change with time and to confirm that the solids in the preparation can be readily resuspended to yield a uniform preparation.

The following tests are required for suspensions for injection unless otherwise justified:

- *Universal Tests*
- *Specific Tests*
  - *Uniformity of Dosage Units*
  - *Antimicrobial Preservatives*

Liposomes

Liposomes are unique drug products with unique properties that can be either solutions or suspensions. Liposomes are aqueous dispersions of amphiphilic lipids and have low water solubility. They are organized as a bilayer sheet that encloses an internal aqueous compartment and are known as lipid bilayer vesicles. Liposomes can have a single lipid bilayer (unilamellar vesicle) or can have an onion-like multilayered structure (multilamellar vesicle). The amphiphilic lipids comprise a hydrated head group at the water interface of the bilayer attached to a hydrophobic group that forms the interior of the bilayer by association with the hydrophobic group of lipids from the opposite leaflet of the bilayer. The physical properties of the liposome and its bilayer can vary widely and depend on lipid composition, aqueous composition, and temperature relative to the acyl components' phase transition points. Because of the central aqueous compartment, a simple test for the presence of liposomes in a lipid dispersion is to determine the presence of an entrapped aqueous phase.

A liposome drug product consists of the drug substance, liposome components, and other inactive but critical ingredients such as an aqueous dispersion unless the contents are a lyophilized product.

Unless otherwise justified, the following tests are required for liposomes:

- *Universal Tests*
- *Specific Tests*
  - *Globule Size Distribution in Lipid Injectable Emulsions* ⟨729⟩

Sterile Powders for Suspensions

Sterile powders for suspensions consist of drug substances and other components as dry-formulation ingredients to ensure the chemical and physical stability of the presentation within a final-use container. Companion sterile diluent or diluent compartments may be provided to facilitate constitution to the desired final volume.

The sterile article for injection may be presented in several forms: lyophilized powder intended for final suspension, powdered solids intended for final suspension, and microparticles that retain their integrity and are delivered as a sterile suspension. The description should include a section that deals with ease of dispersion and reconstitution. The dosage form is a homogeneous solid that is readily constituted to the final form with the specified diluent, and dispersion is completed with gentle agitation.

Unless otherwise justified, the following tests apply to sterile powders for injection:

- *Universal Tests*

Microparticles: Some microparticles are provided as a sterile powder to be reconstituted as a suspension before injection. The majority of microparticle preparations are for reconstitution as a suspension for injection. For quality test requirements, please refer to *Implants*.

Emulsions

Emulsions for parenteral dosage forms are liquid preparations of drug substances dissolved or dispersed in a suitable emulsion medium. Oil-in-water or water-in-oil emulsions typically entrap the drug substance.

Emulsions typically are white, turbid, homogeneous liquid dosage forms that contain one or more chemical substances (e.g., drug substances and excipients) dissolved in a solvent (aqueous or nonaqueous) or mixture of mutually miscible solvents. Emulsions intended for intravenous administration must be sterile and must be compatible with the intended administration site.

Unless otherwise justified, the following tests are required for emulsions for injection:

- *Universal Tests*
- *Specific Tests*
  - Chapter ⟨729⟩

Implants

Implants for extended release consist of a matrix of drug substance and polymeric excipient that may or may not have an outer rate-controlling membrane. The polymeric excipient must be biocompatible but may or may not be bioresorbable. Some implants are made from medical-grade metal with an osmotic pump inside that effects the extended release of the drug substance. Implants must be sterile and usually are formed in the shape of a cylinder, although other shapes are used. Solvents used to dissolve the formulation can lead to sterilization, and thus the internal sterility test method should demonstrate that the sample preparation does not lead to sterilization of the test sample.

Cylindrically shaped implants for systemic delivery usually are provided in an inserter for subcutaneous or local administration such as local ocular delivery. Implants also can be surgically implanted for local delivery, e.g., ocular delivery.

Unless otherwise justified, the following tests are required for implants:

- *Universal Tests*

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- *Specific Tests*
  - *Uniformity of Dosage Units*

In situ Gels

Sterile in situ gels are liquid preparations that are intended for injection into specific therapeutic targets. Typically they consist of polymers in organic solvents, and upon injection the solvents migrate away from the site, leaving a gelled mass. The preparations may be injected as-is, upon reconstitution, from in situ formation, or from chemically initiated catalysis that results in the final form.

Unless otherwise justified, the following tests are required for in situ gels:

- *Universal Tests*
- *Specific Tests*
  - *Antimicrobial Preservatives*

Microparticles

Injectable, resorbable microparticles for extended release generally range from 20 to 100 µm in diameter. They consist of drug substances embedded within a biocompatible, bioresorbable polymeric excipient, e.g., polyester excipients. Microparticles are provided as a sterile powder in a vial or syringe.

Just before intramuscular or subcutaneous administration, the microparticle powder should be suspended in an aqueous injection vehicle (diluent). The injection vehicle usually consists of water for injection, surfactant, and a viscosity enhancer, and the vehicle may contain a compound that adjusts osmolality, e.g., a sugar with or without a compound that controls pH, e.g., an acid. The injection vehicle must be sterile and must be tested according to requirements for solutions that are intended for parenteral administration.

Unless otherwise justified, the following tests are required for microparticles for injection:

- *Universal Tests*
- *Specific Tests*
  - *Uniformity of Dosage Units*
  - *Water Content*

Drug-Eluting Stents

Drug-eluting stents are tiny metal or polymer scaffolds used to keep arteries open following a medical intervention; the drug substance is incorporated into or onto the stent platform. Drug-eluting stents typically have two components of testing: (1) functional tests that generally are American Society for Testing and Materials (ASTM) International methods that fall outside the scope of this chapter and (2) analytical tests.

Unless otherwise justified, the following tests are required for drug-eluting stents:

- *Universal Tests*
- *Specific Tests*
  - *Uniformity of Dosage Units*. The content of the active substance in the dosage form is applicable for drug-eluting stents packaged in single-unit containers. The test can be performed by either content uniformity or weight variation (see ⟨905⟩). With appropriate justification, the number of stents needed for this test may be fewer than the recommended number of stents in ⟨905⟩.
  - ⟨88⟩ *Biological ReactivityTests, In Vivo*

⟨2⟩ ORAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

INTRODUCTION

Oral delivery is the most common route of administration for drug products. All oral drug products lead to systemic and/or local action in the oral cavity and/or gastrointestinal tract. Oral drug products fall primarily into two main categories: solids and liquids. Solid oral drug products include but are not restricted to capsules, tablets, granules, and powders. Similarly, liquid oral drug products include but are not restricted to solutions, suspensions, and emulsions. The definitions and descriptions of these dosage forms and brief information about their composition and manufacturing process are found in *Pharmaceutical Dosage Forms* ⟨1151⟩. [NOTE—All references to chapters above ⟨1000⟩ are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.]

This chapter focuses on the product quality tests that are generally necessary for oral drug products for a single or combination of small molecules of active ingredients. Biologics in solid dosage forms are not considered. In this chapter, the terms "drug substance" and "active ingredient" are used interchangeably. The contents of this chapter do not necessarily apply to drug products that are intended for use other than by oral administration. For example, the chapter does not address oromucosal dosage forms. Some of the tests indicated in this chapter may be performed on an in-process basis or omitted as routine tests based on process validation. However, the product must meet USP compendial requirements when sampled and tested, once the product is on the market.

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This chapter provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in part or whole, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*). This chapter includes the quality tests for the specific route of administration. The quality tests listed can be used as appropriate by manufacturers toward the development of new drug product monographs for submission to USP. If a validated performance test procedure is available for the specific drug product, it is identified in a general chapter below ⟨1000⟩. In cases where a validated procedure cannot be recommended, but if the information is available for a product quality and/or product performance test, it is described in an informational chapter above ⟨1000⟩.

Drug Product Quality Tests and Performance Tests

Monograph tests, analytical procedures, and acceptance criteria for testing oral drug products are divided into two categories: 1) those that assess general product quality attributes, and 2) those that assess product performance, which is a specific quality attribute typically linked to bioavailability and bioequivalence studies (see *Assessment of Solid Oral Drug Product Performance and Interchangeability, Bioavailability, Bioequivalence, and Dissolution* ⟨1090⟩). Drug product quality tests are intended to assess attributes such as identification, strength (assay), impurities , dose content uniformity, pH, minimum fill, alcohol content, volatile content, and microbial content. Drug product performance tests are designed to assess in vitro drug release from dosage forms (e.g., *Dissolution* ⟨711⟩ and *Drug Release* ⟨724⟩). For liquid oral drug products in solution, performance is considered optimal, and a monograph performance test is not included.

Each of these attributes is important for a primary understanding of the quality and performance of a drug product. Thus, they form the basis for the monograph. A compendial product should meet all drug product quality tests and drug product performance tests contained in its monograph.

[NOTE—Dissolution tests, specifically dissolution profile similarity between higher strengths and lower strengths of a given manufacturer's product and dissolution profile similarity between the generic product and the reference product, are used for granting biowaivers. See ⟨1090⟩.]

Change to read:

PRODUCT QUALITY TESTS FOR ORAL DRUG PRODUCTS

Drug product quality tests for oral drug products fall into two categories: 1) universal tests that are applicable to all oral drug products and should be included in the monograph, and 2) specific tests that should be considered for inclusion for specific types of oral products.

Universal Tests for Oral Drug Products

Product quality attributes for oral dosage forms are important to ensure that commercialized products meet minimum quality requirements. Universal tests should be applied to all oral dosage forms and include *Description*, *Identification*, *Strength* (*Assay*), and *Impurities* (organic, inorganic, and residual solvents).

DESCRIPTION

Description is general in nature and is not a standard in itself. It communicates the appearance of an article that complies with monograph standards.

IDENTIFICATION

The identification test is defined in *General Notices, 5.40 Identification*. It is included in a monograph as an aid to confirm that the article contains the labeled drug substance by providing a positive identification of the drug substance or substances in a drug product.

One method of confirming the identity is to compare the retention time of the sample with that obtained for the standard injections in a chromatographic assay procedure. Other methods often used to orthogonally confirm the identity of the active ingredient are: *Thin-Layer Chromatographic Identification Test* ⟨201⟩, ▲*Spectroscopic Identification Tests* ⟨197⟩▲ (CN 1-May-2020), *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩, ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), and *Raman Spectroscopy* ⟨1120⟩, among others. The analytical procedure must be able to distinguish the active ingredient from all excipients that are present or from potential degradation products likely to be present. Care must also be taken to ensure that the chromatographic system separates the article from other closely related drug substances, impurities, and additives. Infrared and ultraviolet absorption also can be used for identification (see ⟨197⟩), if the procedure has been demonstrated to be selective for the drug substance via an appropriate validation or verification study. The results of the identification test must be compared to the results obtained from a similarly prepared, suitable Reference Standard.

ASSAY

The assay is a specific and stability-indicating test to determine the potency (content) of the drug product. When a nonspecific assay (e.g., titration) is justified, other supporting analytical procedures should ensure that any interfering species can be detected. In general the *a priori* acceptance of ±10% variation in limits of a quality attribute (e.g., assay) from the target label claim (100%) in most cases is intended to account for manufacturing variability and shelf-life stability and is primarily based on the notion that such variation in a quality attribute is less likely to have any noticeable adverse impact on the desired clinical

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outcome. Acceptance criteria of 95.0%–105.0% are used with justification (e.g., for drug products with narrow therapeutic index). Activity assays and absolute content assays also are acceptable when justified.

IMPURITIES

Process impurities, synthetic by-products, and other inorganic and organic impurities may be present in the drug substance and in the excipients used in the manufacture of the drug product. These impurities are limited by drug substance and excipient monographs. During product manufacture and over the shelf life of the product, degradation products can form. These can be a result of degradation of the drug substance or from interactions between the drug substance and excipient(s), among other factors. The procedures and acceptance criteria should specifically limit toxic materials. See specific requirements in the *General Notices 5.60, Impurities and Foreign Substances*. [NOTE—For additional information, see *Impurities in Drug Substances and Drug Products* ⟨1086⟩.]

Specific Tests for Tablets

In addition to the *Universal Tests for Oral Drug Products* described above, the following specific tests for tablets should be considered, depending upon the nature of the drug substance and formulation.

VOLATILE CONTENT

The test and the specific method depend on the nature of the article. Special consideration should be given to dosage forms for which water content has been shown to be a potential quality attribute and to products where solvent is used in the manufacture of the drug product.

When the presence of moisture or other volatile material may become critical, analysts must determine the amount of unbound volatile solvents or volatile matter of any kind that is driven off by *Loss on Drying* ⟨731⟩ or another suitable technique (e.g., water activity). For substances that appear to contain water as the only volatile constituent, the procedure given in *Water Determination* ⟨921⟩ may be appropriate. For drug products, analysts also should consult *Residual Solvents* ⟨467⟩.

DISINTEGRATION

Disintegration is an essential attribute of oral solids, except for those intended to be chewed before being swallowed and for delayed- or extended-release products. This test measures the time it takes for the dosage unit to disintegrate in an aqueous medium and is described in detail in *Disintegration* ⟨701⟩. Certain dosage forms (e.g., effervescent tablets and others) may require special procedures and identity tests for bicarbonate when dissolved in water. The disintegration test for some of the dosage forms in this chapter is included for completeness. For detailed procedures, refer to ⟨701⟩. The disintegration test, if included, is used only as a quality control test and not as a product performance test and should conform with the specifications in the monograph. Only when disintegration has been correlated with dissolution of a dosage form can a disintegration test be used as a product performance test [International Council for Harmonisation (ICH) Guidance Q6A, available at www.ich.org]. In all other instances, a dissolution test should be considered as a product performance test.

TABLET FRIABILITY

The test procedure is applicable to most compressed, uncoated tablets. Friability determines the ability of tablets to withstand mechanical stresses and their resistance to chipping and surface abrasion. [NOTE—For additional information, see *Tablet Friability* ⟨1216⟩.]

TABLET BREAKING FORCE

Tablet breaking force measures the mechanical integrity of tablets, which is the force required to cause them to fail (i.e., break) in a specific plane. [NOTE—For additional information, see *Tablet Breaking Force* ⟨1217⟩.]

UNIFORMITY OF DOSAGE UNITS

Uniformity of dosage units must be demonstrated by either content uniformity or weight variation. Content uniformity is based on the assay of the individual content of drug substance(s) in a number of dosage units to determine whether the individual contents are sufficiently close to label claim. Weight variation can be used as an alternative to estimate content uniformity under certain conditions (see *Uniformity of Dosage Units* ⟨905⟩).

Specific Tests for Uncoated Tablets

Uncoated tablets include single-layer tablets that result from a single compression of particles and multilayer tablets that consist of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used generally are not specifically intended to modify the release of the active substance in the digestive fluids. Uncoated tablets include but are not limited to: effervescent tablets, buccal tablets, sublingual tablets, chewable tablets, orally disintegrating tablets, tablets for oral solution, and tablets for oral suspension. For uncoated tablets, disintegration should be tested as directed in ⟨701⟩.

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BUCCAL, SUBLINGUAL, AND ORALLY DISINTEGRATING TABLETS

These dosage forms are discussed in *Mucosal Drug Products—Product Quality Tests* ⟨4⟩. They are listed here for informational purposes and completeness.

CHEWABLE TABLETS

Chewable tablets (intact) should undergo dissolution and disintegration testing, as a product performance test (if cited in the monograph), because they might be swallowed without proper chewing by a patient. In general, the dissolution and disintegration test conditions for chewable tablets should be the same as for nonchewable tablets of the same active ingredient or moiety.

TABLETS FOR ORAL SOLUTION AND TABLETS FOR ORAL SUSPENSION

These are tablets intended to be dissolved or dispersed in water before administration, giving a homogeneous solution or dispersion. They are listed here for informational purposes and completeness.

Specific Tests for Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble excipients, sugars, plasticizers, polyols, waxes, coloring matter authorized by the competent authority, and sometimes flavoring substances and active substances. Tablets coated by sugar or film include but are not limited to: plain coated tablets, extended-release tablets, and delayed-release tablets. A disintegration test, when applicable, should be performed as directed in ⟨701⟩.

There are no additional specific quality tests for extended-release tablets and delayed-release tablets. Universal quality tests should be applied to these products.

Specific Tests for Capsules

In addition to the *Universal Tests for Oral Drug Products* described above, the specific tests included below should be considered, depending on the nature of the drug substance and formulation. Modified release capsules include delayed-release capsules and extended-release capsules.

Product quality tests that are considered specific to the type of capsule include those for volatile content (see ⟨731⟩ and ⟨921⟩). One-piece capsules typically are used to deliver a drug substance as a solution or suspension. Two-piece capsules consist of two telescoping cap-and-body pieces that are used to deliver solid material as powder, granules, or small tablets.

UNIFORMITY OF DOSAGE UNITS

Uniformity of dosage units must be demonstrated by either content uniformity or weight variation. Content uniformity is based on the assay of the individual content of drug substance(s) in a number of dosage units to determine whether the individual contents are sufficiently close to label claim. Weight variation can be used as an alternative to estimate content uniformity under certain conditions (see ⟨905⟩).

DISINTEGRATION

For capsules, proceed as directed in ⟨701⟩, if a disintegration test is required. Disintegration for modified release capsules may require special procedures.

There are no additional specific quality tests for modified release capsules. Universal quality tests should be applied to these products.

Specific Tests for Granules

In addition to the *Universal Tests for Oral Drug Products* described above, the specific tests included below should be considered, depending on the nature of the drug substance and formulation.

Granules are solid dosage forms that are composed of agglomerations of smaller particles. Granules include but are not limited to: effervescent granules, coated granules, extended-release granules, and delayed-release granules.

Tests that are considered specific to the type of granules include volatile content (see ⟨731⟩ and ⟨921⟩). For disintegration for effervescent granules, refer to ⟨701⟩. On the basis of the nature of the article and scientific criteria, additional tests may apply, including powder fineness and content uniformity (see ⟨905⟩) if granules are packaged in single-unit containers.

Specific Tests for Powders

Oral powders should indicate: "For Oral Use Only". Tests that are considered specific to the type of powders include: *Minimum Fill* ⟨755⟩ for products packaged in multiple-dose containers, content uniformity (see ⟨905⟩) for products packaged in single-unit containers, and volatile content (see ⟨731⟩ and ⟨921⟩).

On the basis of the nature of the article and scientific criteria, additional tests may apply, including pH in an aqueous solution, powder fineness, microbial limits, and others.

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Specific Tests for Liquids

The recommended product quality tests for a liquid drug product include the *Universal Tests for Oral Drug Products* described above and the specific tests included below. Most of the quality tests for liquids require the evaluation of single-dose products to estimate the quality attribute. Specific directions to perform the quality tests (including preservative content) for either single-dose or multiple-dose products are provided in the monograph or the general chapter. For example, weight variation may be used when adequacy of mix for the active substance(s) and excipients in the blend is well controlled to ensure their uniform distribution, as in solutions.

DELIVERABLE VOLUME

When the liquid formulation is packaged in a multiple-dose container, compliance with *Deliverable Volume* ⟨698⟩ is required.

ALCOHOL DETERMINATION

If the liquid formulation contains a quantity of alcohol, *Alcohol Determination* ⟨611⟩ should be included. The limits may be an absolute concentration, in percentage, or relative to a labeled content.

pH

Liquid oral products typically are aqueous formulations that are susceptible to pH changes from exposure to atmospheric carbon dioxide (CO_2). The uptake of atmospheric carbon dioxide (CO_2) and consequent pH change of oral liquid products is only relevant to aqueous-based products. The pH of an oral liquid formulation can affect flavor and stability. The pH range as outlined in *pH* ⟨791⟩ is indicated in the monograph.

MICROBIAL CONTENT

The presence of certain microorganisms in nonsterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Some liquid oral products can be subject to extreme microbiological control, and others require none. The needed microbial specification for a given liquid oral product depends on its formulation and use and is indicated in the monograph.

[NOTE—For additional information, see *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* ⟨1111⟩.]

ANTIOXIDANT

Release testing should be performed. Shelf-life testing may be unnecessary where justified by development and stability data (ICH Guidance Q6A).

EXTRACTABLES

Where development and stability data show no significant evidence of extractables, elimination of this test may be proposed. Where data demonstrate the need and acceptance criteria for oral solutions—rubber stopper, cap liner, plastic bottle—data should be collected as early in the development process as possible (ICH Guidance Q6A).

TYPES OF LIQUID DOSAGE FORMS

Specific quality tests for these dosage forms are provided in their respective monographs.

Solutions, powders, and granules for solution: Tests of "for Solution" formulations are conducted on a well-mixed solution of the drug product constituted as described in the labeling.

Emulsions, suspensions, and powders and granules for suspension: Tests of "for Suspension" formulations are conducted on a well-mixed suspension of the drug product constituted as described in the labeling. Product quality tests for suspensions should include a test of suspendability.

Powders and granules for solutions: After dissolution or suspension, they comply with monograph requirements for the final dosage form. Volatile content (see ⟨731⟩ and ⟨921⟩) may be an additional quality test for powders and granules for reconstitution.

Specific Tests for Miscellaneous Oral Dosage Forms

LYOPHILIZED ORAL PRODUCTS

Water Determination ⟨921⟩, *Method I, Method Ia*: Lyophilized oral products comply with the test. The limits are approved as indicated in the specific monograph.

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⟨3⟩ TOPICAL AND TRANSDERMAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

INTRODUCTION

Topically applied drug products fall into two general categories: those applied to achieve local action and those applied to achieve systemic effects after absorption through the skin into the blood circulation. Local action can occur at or on the surface of the application site (e.g., stratum corneum); in the underlying tissues (e.g., epidermis and/or dermis); and in subcutaneous tissues (e.g., muscle or joint). Topically applied drug products include, but are not limited to, creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, solutions, and transdermal delivery systems (TDS). The definitions and descriptions of these dosage forms, as well as brief information on their composition and/or manufacturing processes, can be found in *Pharmaceutical Dosage Forms* ⟨1151⟩.

Procedures and acceptable criteria for testing topically applied drug products can be divided into those that assess general product quality attributes and those that assess product performance. The product quality attributes include the following: description, identification, assay (strength), impurities, physicochemical properties, uniformity of dosage units, water content, pH, apparent viscosity, microbial limits, antimicrobial preservative content, antioxidant content, sterility (if applicable), and other tests that may be product specific. Product performance testing assesses drug release and other attributes that affect drug release from the finished dosage form.

This chapter provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in whole or in part, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*) and includes the quality tests for the specific route of administration. The quality tests listed can be used as appropriate by manufacturers toward the development of new drug product monographs for submission to *USP–NF*.

TDS release their active ingredients by different mechanisms. They can be passive or active. This chapter covers only the tests related to passive TDS.

Change to read:

PRODUCT QUALITY TESTS FOR TOPICAL AND TRANSDERMAL DRUG PRODUCTS

▲Additional Procedure for Products Packaged in Containers with a Non-Metered Pump

For some tests (e.g., viscosity, assay, etc.), with the exception of the uniformity in containers test, samples need to be collected from the pumped-out product. In these instances, the samples should be collected as follows:

1. Remove cap from container.
2. Fully depress and release the actuator, disposing of any material dispensed, and allowing the pump actuator to return to the initial position after each actuation. Repeat the sequence, as indicated in the patient instructions, until a full amount of material is dispensed.
3. Collect the next material dispensed as the sample. Generally, collect NMT the amount needed to perform a single analysis (for the assay test, typically NMT 2 actuations).▲ (USP 1-Dec-2019)

Universal Tests

Universal tests [see International Council for Harmonisation (ICH) guidance *Q6A—Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances*, available at www.ich.org] are listed as follows and are applicable to all topically applied drug products.

DESCRIPTION

A qualitative description of the drug product should be provided. The acceptance criteria should include the final acceptable appearance of the finished dosage form and packaging. A visual examination should identify changes in color, adhesive migration (i.e., cold flow; see *Cold Flow Test*) for TDS, separations, crystallization, and others that are specific to the drug product. The description should specify the content or the label claim of the article. For TDS, a visual examination should also be done to assess potential use issues with the product. The examination should include an assessment of the difficulty of removing the TDS from the pouch (e.g., due to adhesive migration adhering the system to the pouch); inability to remove the TDS from the pouch without damage to the system; and adhesive residue remaining on the pouch after removal of the TDS. This is not a compendial test but is part of the manufacturer's specification for the drug product.

IDENTIFICATION

Identification tests are discussed in *General Notices, 5.40 Identification*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structures that are likely to be present. Identification tests should be specific for the drug substance(s) (e.g., infrared spectroscopy). Near-infrared (NIR) or Raman spectrophotometric methods also could be acceptable for the identification of the drug product (for additional

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information, see ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020) and *Raman Spectroscopy* ⟨1120⟩). Identification solely by a single chromatographic retention time is not specific.

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. This assay requirement can be satisfied for topical products containing antibiotics by a standard microbiological method (see *Antibiotics—Microbial Assays* ⟨81⟩). In cases when the use of a nonspecific assay (e.g., *Titrimetry* ⟨541⟩) is justified, other supporting analytical procedures should be used to achieve overall specificity.

IMPURITIES

Process impurities, synthetic byproducts, impurities associated with the adhesive (e.g., residual monomers), residual solvents (see *Residual Solvents* ⟨467⟩), and other inorganic and organic impurities may be present in the drug substance and in the excipients used in the manufacture of the drug product and should be assessed and controlled. Impurities arising from the degradation of the drug substance and those arising during the manufacturing process of the drug product also should be assessed and controlled.

Specific Tests

In addition to the *Universal Tests* listed previously, the following *Specific Tests* should be considered on a case-by-case basis.

UNIFORMITY OF DOSAGE UNITS

This test is applicable for TDS and for topical dosage forms intended for systemic delivery, or where tight control of the dose is necessary to limit local irritation or undesired systemic exposure, packaged in single-unit containers, such as packets (see *Uniformity of Dosage Units* ⟨905⟩). The uniformity of dosage units specification is not intended to apply to solutions, suspensions, emulsions, ointments, or gels in single-unit containers intended for local action following external, cutaneous administration.

WATER CONTENT

A test for water content should be included when appropriate (see *Water Determination* ⟨921⟩). This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

MICROBIAL LIMITS

Microbial examination of nonsterile drug products is performed according to the methods given in *Microbial Enumeration Tests* ⟨61⟩ and *Tests for Specified Microorganisms* ⟨62⟩, unless the formulation itself is demonstrated to have antimicrobial properties. Acceptance criteria for nonsterile pharmaceutical products based on total aerobic microbial count and total combined yeasts and molds count are given in *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* ⟨1111⟩.

ANTIMICROBIAL PRESERVATIVE CONTENT

Acceptance criteria for antimicrobial preservative content in multi-unit products should be established. They should be based on the levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life (see *Antimicrobial Effectiveness Testing* ⟨51⟩).

ANTIOXIDANT CONTENT

If antioxidants are present in the drug product, tests of their content should be established unless oxidative degradation can be detected by another test method such as impurity testing. Acceptance criteria for antioxidant content should be established. They should be based on the levels of antioxidant necessary to maintain the product's stability at all stages throughout the product's proposed usage and shelf life.

STERILITY

Depending on the use of the dosage form (e.g., products that will be applied to open wounds or burned areas), sterility of the product should be demonstrated as appropriate (see *Sterility Tests* ⟨71⟩).

pH

When applicable, topically applied drug products should be tested for pH. Because some topically applied drug products contain very limited quantities of water or aqueous phase, pH measurements may not always be warranted. This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product.

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PARTICLE SIZE

When the finished product contains a suspended solid drug substance, the product should be examined for particle size. The particle size of the active drug substance(s) in topically applied drug products is usually established and controlled at the formulation development stage. However, topically applied drug products should be examined for evidence of particle alteration (i.e., drug polymorphic form, appearance of particles, size, shape, morphology, agglomeration, or aggregation) of the drug substance that may occur during the course of product processing and storage. These types of tests are generally formulation dependent. Therefore, such tests are not included in compendial drug product monographs but are part of the manufacturer's specification for the drug product.

CRYSTAL FORMATION

When the drug substance is dissolved in the finished product, the product should be microscopically examined for evidence of crystal formation of the active drug substance. This test is generally formulation dependent. Therefore, it is not included in the compendial drug product monograph but is part of the manufacturer's specification for the drug product. It is recommended that the potential for the drug product to form crystals of drug substance be examined during product development. ▲▲ (USP 1-Dec-2019)

Add the following:

▲IN VITRO DRUG RELEASE TEST

For TDS, the in vitro drug release test is required as a batch-to-batch quality control. See *Drug Release* ⟨724⟩ for apparatus and test conditions. The development and validation report of the in vitro drug release test needs to include sufficient detail and data to facilitate the assessment of whether the method is adequate as a quality control test for batch release and stability testing.

For semisolid dosage forms, in vitro drug release testing is currently not required for batch release. See *Semisolid Drug Products—Performance Tests* ⟨1724⟩ for apparatus, test conditions, and test applicability. ▲ (USP 1-Dec-2019)

SPECIFIC TESTS FOR OPHTHALMIC DRUG PRODUCTS

See *Ophthalmic Products—Quality Tests* ⟨771⟩.

Add the following:

▲SPECIFIC TESTS FOR TOPICAL AEROSOLS

See *Topical Aerosols* ⟨603⟩. ▲ (USP 1-Dec-2019)

Change to read:

SPECIFIC TESTS FOR TOPICALLY APPLIED SEMISOLID DRUG PRODUCTS

Minimum Fill

Single- and multiple-unit containers must meet minimum fill requirements as established by testing described in *Minimum Fill* ⟨755⟩. For single-unit containers where the test for ⟨905⟩ is applied, the test for ⟨755⟩ is not required.

Apparent Viscosity

Viscosity is a measure of a formulation's resistance to flow and is an assessment of a rheological property of a semisolid dosage form. The term "apparent viscosity" applies to non-Newtonian fluids, which comprise the majority of semisolid pharmaceutical dosage forms.

Measurement procedures should be developed as outlined in *Viscosity—Capillary Methods* ⟨911⟩, *Viscosity—Rotational Methods* ⟨912⟩, and *Viscosity—Rolling Ball Method* ⟨913⟩. For semisolids that show thixotropy and/or irreversible changes in viscosity after shearing, specific attention should be given to sample preparation procedures to minimize variability in the measurement of apparent viscosity caused by variable shear histories (e.g., mixing speed and temperature, filling operation, and sample handling). Furthermore, for some products it may be warranted to have apparent viscosity specifications at more than one stage of the manufacturing process or with more than one set of test conditions (e.g., bulk in-process stage, final packaged product, high and low shear rates, and different temperatures).

Apart from single-point viscosity measurements, more advanced rheological techniques (flow, oscillatory, creep, and stress relaxation testing) can be applied to develop a mechanistic understanding of a formulation and its structure. These techniques may be useful for product development using the principles of quality by design or for comparative physicochemical characterization of the test and reference formulations in the case of a biowaiver argument in a generic drug application. However, these techniques are not generally suitable for routine quality testing. Common parameters derived from rheological

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testing of semisolid pharmaceutical dosage forms that may be useful for characterization and comparison are the storage modulus, loss modulus, relaxation modulus, compliance, thixotropic index, and yield stress.

Acceptance criteria are product specific and defined to ensure that the apparent viscosity of each batch of semisolid drug product is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life.

Uniformity in Containers

Topically applied semisolid drug products may show physical separation during manufacturing processes and during their shelf life. To ensure the integrity of the drug product, it is essential to evaluate the uniformity of the finished product. This test applies only to multiple-unit containers, such as tubes and jars. This test does not apply to more fluid topical drug products in multiple-unit containers, such as emulsions, lotions, two-phase gels, or topical suspensions, in which the labeling directs the user to mix the product (e.g., shake well) before use.

PRODUCTS PACKAGED IN TUBES

Visual uniformity: Carefully remove or cut off the bottom tube seal and make a vertical cut from the bottom to the top of the tube. Carefully cut the tube around the upper rim, open the two flaps, and lay the flaps open to expose the product.

Inspect the product visually for the presence of phase separation, change in physical appearance and texture (e.g., color change, crystallization, lumping), and other properties described in the product specification for *Description*. If there is no significant phase separation or change in physical appearance and texture, and if the product meets the *Description* criteria, the product passes the test. If the product exhibits significant phase separation or change in physical appearance or texture, the product fails the test.

Uniformity of active ingredient(s): The following procedures can be modified depending on the sensitivity of the quantitative procedure used to determine the amount of the drug substance(s) present in the formulation.

For multiple-unit tubes that contain 5 g or more of product

STAGE 1:

1. Using a single tube, after performing the test for *Visual uniformity*, remove an appropriate amount of the product from the top (i.e., cap end), middle, and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s) and should not exceed the maximum dose recommended by the product labeling for a single application.
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the single tube using the *Stage 1* acceptance criteria outlined in number *3*.
3. *Stage 1* acceptance criteria are met if:
   - None of the three results are outside of the product assay range, and
   - The maximum difference in the amount of active ingredient(s) determined within the tube is NMT 10.0%. For example, if the three measurements within the tube are 97.0%, 95.2%, and 99.7%, the maximum difference would be 4.5% (i.e., 99.7% – 95.2% = 4.5%).
4. Proceed to *Stage 2* testing if *Stage 1* acceptance criteria are not met and none of the test results are outside the product assay range by more than 5.0% (e.g., if the product assay range is 90.0%–120%, the range will be 85.0%–125.0%), and the maximum difference in the amount of active ingredient(s) measured within the tube is NMT 10.0%. ▲An example of a product that fails to meet *Stage 1* criteria:▲ (USP 1-Dec-2019) if the highest and lowest assay values were 106.0% and 94.7% of label claim, then the difference would be 106.0% – 94.7% = ▲11.3%.▲ (USP 1-Dec-2019)
5. Proceed to *Stage 3* testing if *Stage 1* acceptance criteria are not met, *Stage 2* acceptance criteria cannot be met, NMT one of the three test results is outside of the product assay range by more than ±5.0%, ▲▲ (USP 1-Dec-2019) and the maximum difference of the amount of active ingredient(s) measured within the tube is NMT 15.0%.

STAGE 2:

1. Test an additional 2 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 3 samples each from 3 tubes.
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 3 tubes using the *Stage 2* acceptance criteria.
3. *Stage 2* acceptance criteria are met if:
   - The *Visual uniformity* test is met for all tubes;
   - None of the 9 results (i.e., 3 each from 3 tubes) are outside of the product assay range by NMT 5.0%; and
   - The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the samples tested, is NMT 10.0%.
4. Proceed to *Stage 3* testing if NMT one of the 9 test results is outside of the product assay range by ±5.0% ▲▲ (USP 1-Dec-2019) and the maximum difference of the amount of active ingredient(s) measured within each tube is NMT 15.0%.

STAGE 3:

1. If *Stage 2* has been completed, test an additional 7 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 3 samples each from 10 tubes. If *Stage 2* was skipped, test an additional 9 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 3 samples each from 10 tubes.
2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3* acceptance criteria as outlined in number *3*.

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3. *Stage 3* acceptance criteria are met if:
   - The *Visual uniformity* test is met for all tubes;
   - ▲NMT 1 of the 30 test results is outside of the product assay range by ±5.0%;▲ (USP 1-Dec-2019) and
   - The maximum difference of the amount of active ingredient(s) measured within each tube, for each of the 10 tubes tested, is NMT 15.0%.

For multiple-unit tubes that contain less than 5 g of product

STAGE 1:

1. Using a single tube, after performing the test for *Visual uniformity*, remove an appropriate amount of product from the top (i.e., cap end) and bottom (i.e., seal end) portions of the tube. The sample size should be sufficient for at least one quantitative determination of the active ingredient(s) and should not exceed the maximum dose recommended by the product labeling for a single application.
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the tube using the *Stage 1* acceptance criteria outlined in number *3*.
3. *Stage 1* acceptance criteria are met if:
   - Neither result is outside of the product assay range; and
   - The difference between the amount of active ingredient(s) determined for the 2 samples within the tube tested is NMT 10.0%. For example, if the two measurements within a tube were 95.2% and 89.7%, the difference would be 5.5%.
4. Proceed to *Stage 2* testing if *Stage 1* acceptance criteria are not met and neither of the test results are outside the product assay range by more than ±5.0% (e.g., if the product assay range is 90.0%–120.0%, the range will be 85.0%–125.0%), and the difference between the amounts of active ingredient(s) measured within the tube is NMT 10.0%.
5. Proceed to *Stage 3* testing if *Stage 1* acceptance criteria are not met, *Stage 2* acceptance criteria cannot be met, NMT one of the test results is outside of the product assay range by more than ±5.0%, ▲ (USP 1-Dec-2019) and the difference between the amounts of active ingredient(s) measured within the tube is NMT 15.0%.

STAGE 2:

1. Test an additional 2 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 2 samples each from 3 tubes.
2. Determine the amount of the active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 3 tubes using the *Stage 2* acceptance criteria as outlined in number *3*.
3. *Stage 2* acceptance criteria are met if:
   - The *Visual uniformity* test is met for all tubes;
   - None of the 6 test results (i.e., 2 each from 3 tubes) are outside of the product assay range by ±5.0%; and
   - The difference between the amount of active ingredient(s) determined for the 2 samples within each tube, for each of the 3 tubes tested is NMT 10.0%.
4. Proceed to *Stage 3* testing if NMT one of the 6 test results is outside of the product assay range by ±5.0%, ▲ (USP 1-Dec-2019) and the difference between the amounts of active ingredient(s) measured within each tube is NMT 15.0%.

STAGE 3:

1. If *Stage 2* has been completed, test an additional 7 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 2 samples each from 10 tubes. If *Stage 2* was skipped, test an additional 9 tubes for *Visual uniformity* and *Uniformity of active ingredient(s)* for a total of 2 samples each from 10 tubes.
2. Determine the amount of active ingredient(s) in each portion of the product using any appropriate validated quantitative procedure, and evaluate the test results from the 10 tubes using the *Stage 3* acceptance criteria as outlined in number *3*.
3. *Stage 3* acceptance criteria are met if:
   - The *Visual uniformity* test is met for all tubes;
   - 19 of 20 test results are within ±5.0% of the product assay range; ▲ (USP 1-Dec-2019) and
   - The difference between the amount of active ingredient(s) determined for the 2 samples within each tube, for each of the 10 tubes tested, is NMT 15.0%.

PRODUCTS PACKAGED IN CONTAINERS OTHER THAN TUBES

For semisolid products packaged in a container other than a tube when the sampling method presented previously cannot be used, other sampling methods are acceptable, such as this method described for a jar:

1. Select a suitable syringe of sufficient length to extend to the bottom of the container.
2. Remove and set aside the syringe plunger, and cut off the bottom of the syringe barrel. Sampling should take place from a location to the left or right of the mid-line of the jar surface to preserve an undisturbed region on the other side for any additional investigation (see *Figure 1*).

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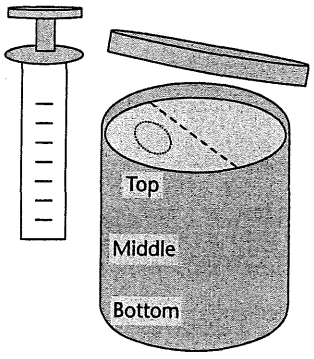


Figure 1. Sampling from a jar container.

3. Slowly push the syringe barrel into the container until it reaches the bottom. Then twist the syringe barrel containing the sample core, and remove the syringe from the container.
4. Insert the syringe plunger into the barrel, and carefully extrude the sample core onto a clean surface in three equal portions to represent the top, middle, and bottom portions of the container.
5. Remove an appropriate sample representative of the top, middle, and bottom portions of the container samples, and test according to the instructions outlined in *Products Packaged in Tubes*.

▲Delivered-Dose Uniformity in Metered Dose Containers

The test for delivered-dose uniformity is required for drug products contained either in metered-dose containers or in premetered unit presentations. The test for delivered-dose uniformity includes dose uniformity over the entire unit shelf life. Select 1 container. Using separate collection vessels, quantitatively collect delivered doses representing the initial, middle, and the final dose from the container. Appropriately discard doses not collected for testing. Determine the drug substance content of each of the 3 collected samples using an appropriate and validated quantitative procedure or the procedure indicated in the individual monograph. A dose in this test is defined as the minimum recommended number of actuations specified in the product labeling or in the instructions for use but NMT 2 actuations. The target-delivered dose is specified by the product label claim, unless otherwise specified in the individual monograph.

In-Process Testing

PUMP FUNCTIONALITY TEST FOR METERED AND NON-METERED DOSE CONTAINER CLOSURES

This test is conducted during product development to establish that the airless pumps are performing as intended. This test is product dependent. Therefore, it is part of the manufacturer's controls for the drug product and it is not included in compendial drug product monographs.

During the filling process for semisolid or liquid products packaged in a non-metered dose container closure requiring an actuator to dispense the product (for example, an airless pump container), in-process testing to verify pump functionality should be examined. Example tests include pumps to prime and total amount dispensed. ▲ (USP 1-Dec-2019)

Change to read:

SPECIFIC TESTS FOR TDS

TDS are formulated with an adhesive layer to ensure intimate contact with the skin and allow the delivery of the desired dose of the drug. Adhesives in TDS must permit easy removal of the release liner before use, adhere properly to human skin upon application, maintain adhesion to the skin during the prescribed period of use, and permit easy removal of the TDS at the end of use without leaving a residue or causing damage to the skin or other undesirable effect(s). Additionally, adhesives must be able to maintain the performance of the TDS throughout the shelf life of the drug product.

Testing of the physical properties of the TDS generally include peel adhesion, release liner peel, tack, cold flow, shear, and crystal formation (see *Crystal Formation*). The peel adhesion, release liner peel, and tack tests measure the adhesion properties of the TDS. Each of these tests measures the force required to separate the TDS from another surface. The cold flow and shear tests measure the cohesive properties of the TDS. These latter tests measure the resistance to flow of the adhesive matrix.

Acceptance criteria are product specific and defined to ensure that adhesion of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life.

In addition to physical testing, this section also discusses the *Leak Test* applicable to form-fill-seal-type (reservoir or pouched) TDS.

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Peel Adhesion Test

This test measures the force required to remove (peel away) a TDS attached to a standard substrate surface (e.g., polished stainless steel). The TDS is applied to the substrate using specified techniques for application and is conditioned at a specified temperature and time. Then the TDS is peeled away from the substrate with an instrument that allows for control of the peel angle (e.g., 90° or 180°) and peel rate (e.g., 300 mm/min), and the ▲▲ (USP 1-Dec-2019) force ▲profile▲ (USP 1-Dec-2019) is recorded. This procedure is repeated using a minimum of 5 independent samples ▲yielding results from a suitable method (see *Development of Peel Adhesion and Static Shear Test Methods*). The in vitro adhesive properties of the drug product should thus be characterized with the specifications limits for the specified tests in accordance with the results obtained on clinical batches for which satisfactory in vivo adhesive properties under product use have been demonstrated and used to support their justification of the drug product specification. Release and shelf life limits should be the same, unless justified by reference to clinical batches.

The geometry of a TDS (e.g., round or rectangular shaped) and its design (e.g., TDS with different surfaces such as an inner part and with a peripheral adhesive ring) should be considered in the method.

The substrate surface should be cleaned regularly and checked for scratches, as this may influence test results.

Development of Peel Adhesion and Static Shear Test Methods

During method development, suitable methods (including test panel surface) need to be identified. For peel adhesion testing, the method should allow the TDS to be removed entirely and cleanly, leaving no visually noticeable matrix residue on the substrate surface (i.e., cohesive failure).

In contrast, for static shear testing, cohesive failure (i.e., adhesive is left on the TDS and on the substrate plate) should occur.

Suitable peel adhesion and static shear test methods do not result in undesired adhesive failures. Undesired adhesive failure occurs when the TDS delaminates at an interface (e.g., between a membrane and an adhesive layer, or between two different adhesive layers of a bilayer product). During peel adhesion testing, transfer of adhesive to the test panel (i.e., cohesive failure) is an additional undesired adhesive failure mode. ▲ (USP 1-Dec-2019)

Release Liner Peel Test

This test measures the force required to separate the release liner from the adhesive layer of the TDS. The test is performed with a finished product sample. The test sample is conditioned using specific procedures (temperature and time). Then, the release liner is pulled away from the TDS with an instrument that allows for control of the peel angle (e.g., 90° or 180°) and peel rate (300 mm/min), and the ▲force profile▲ (USP 1-Dec-2019) is recorded. This procedure is repeated using a minimum of 5 independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

Tack Test

▲A few tack test methods▲ (USP 1-Dec-2019) have been developed ▲and the current predominantly used method is▲ (USP 1-Dec-2019) the *Probe Tack Method*. ▲▲ (USP 1-Dec-2019) It is up to the TDS manufacturer to decide which tack test is most appropriate for each drug product.

PROBE TACK METHOD

This test measures the force required to separate the tip of the test probe from the adhesive layer of the TDS. This test uses an instrument designed to create a bond between the tip of the stainless steel test probe (of defined geometry) and the TDS using a controlled force (light pressure) and specified test conditions (i.e., rate, contact time, contact pressure, and temperature). Then while controlling the rate of probe removal, the test measures the ▲▲ (USP 1-Dec-2019) force required to separate the probe tip from the TDS and the maximum force required to break the bond (tack). This procedure is repeated using a minimum of 5 ▲individual▲ (USP 1-Dec-2019) samples. The product fails the test if the mean test result ▲▲ (USP 1-Dec-2019) is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.

▲The probe head surface should be cleaned regularly and checked for scratches, as this may influence the test results.▲ (USP 1-Dec-2019)

Cold Flow Test

Cold flow is the migration of the adhesive matrix beyond the edge of the TDS backing, and through the slit in the release liner, which may occur during the course of product processing and storage. Cold flow is an inherent property of TDS due to the use of pressure-sensitive adhesives that flow when force is applied (i.e., if the adhesive matrix did not flow, the TDS would not stick). The magnitude of the cold flow is generally dependent on the product formulation, ▲packaging design,▲ (USP 1-Dec-2019) storage conditions, and storage time. Cold flow should be ▲assessed using a combination of quantitative and qualitative methods. No single quantitative method has been identified to work universally for all TDS.▲ (USP 1-Dec-2019) The TDS manufacturer ▲should▲ (USP 1-Dec-2019) determine the most suitable cold flow test, ▲or tests, as cold flow may manifest differently for different products. Several different cold flow tests have been developed. Examples include:

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- Linear measurement of the radial cold flow using microscopy
- Measuring the distance of migrated adhesive matrix at predefined and evenly spaced positions of a TDS
- Measuring cold flow by applying a reference plate in the size of the TDS plus the acceptable cold flow
- Swabbing and stripping the migrated part of the matrix and determining it gravimetrically or by assay of the drug substance
- Die cutting and punching out the original size of the TDS and determining the amount of migrated matrix on the outside
- Overall area determining methods of cold flow using image analysis tools ▲(USP 1-Dec-2019)

Acceptance criteria are product specific and defined to ensure that the cold flow of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple batches over the product's shelf life.

Shear Test

The shear test measures the cohesive strength of a TDS. It can be measured under static (see *Static Shear Test*) or dynamic conditions. Shear testing may not be feasible for all TDS because the presence of multiple layers of adhesive in the system, the presence of a membrane or scrim, or the use of an emulsion adhesive system may result in the inability to achieve cohesive failure. TDS that are constructed with a peripheral adhesive ring or form-fill-seal TDS may not be suitable for this test. ▲▲(USP 1-Dec-2019) The TDS manufacturer ▲should▲ (USP 1-Dec-2019) decide if a shear test is appropriate, and if so, which shear test is most appropriate for each drug product. Acceptance criteria are product specific and defined to ensure that the shear of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications and statistical assessment of multiple product batches over the product's shelf life.

Static Shear Test

For the static shear test, the time required to remove a standard area of the TDS from the substrate (i.e., stainless steel test panel) under a standard load (e.g., 250 g) is measured. The TDS is applied to a test panel, and the sample is subjected to a shearing force by means of a given weight suspended from the TDS. The test apparatus holds the test panels at 0°– 2° from vertical to ensure that the TDS will not experience peeling action when the weight is attached. Dwell time, weight used, test panel type, mode of failure, and sample size should be noted; the time taken for the TDS sample to detach from the test panel is reported. This procedure is repeated using a minimum of 5 independent samples yielding ▲results from a suitable method (see *Development of Peel Adhesion and Static Shear Test Methods*).▲ (USP 1-Dec-2019) The product fails the test if the mean shear force (i.e., arithmetic mean or geometric mean as determined by the manufacturer) is outside the acceptable range determined during product development and based on statistical assessment of multiple product batches over the product's shelf life.
▲▲(USP 1-Dec-2019)

Leak Test

This test is applicable only for form-fill-seal-type (reservoir or pouched) TDS. Form-fill-seal TDS must be manufactured with zero tolerance for leaks because of their potential for dose dumping if leaking occurs.

In-process control methods to examine TDS for leaks or potential leaks are needed and require considerable development on the part of TDS manufacturers.

IN-PROCESS TESTING

During the manufacturing process, the presence of leakage (or potential for leakage) due to TDS perforation, cuts, and faulty seals resulting from failures such as air bubbles, gel splash, or misalignment of a TDS backing and release liner layers must be examined. Unless automated process analytical technology is implemented, in-process testing to identify these defects should be performed using the following test procedures.

Visual inspection:

- A specified number of TDS, defined on the basis of batch size, should be examined randomly
- Each sampled TDS should be thoroughly visually inspected for leakage
- The product fails if any of the TDS examined are detected with a leak

Seal integrity: TDS seals should be stress tested to ensure that the application of pressure does not force seals to open, thereby leading to leakage.

- A specified number of TDS, defined on the basis of batch size, should be randomly examined.
- Each sampled TDS should be thoroughly visually inspected for leakage.
- Each sampled TDS is placed on a hard, flat surface and overlaid with a weight so that it is subjected to 13.6 kg. The weight should be left in place for 2 min. Upon removal of the weight, the TDS should be visually inspected for leakage.
- The product fails if the number of TDS detected with a leak is greater than the acceptable limit established by the manufacturer.

Packaged product testing: TDS may leak after they have been individually placed in the primary packaging material as a result of the packaging operation itself or by a user opening the packaging. Therefore, TDS should be tested for leakage after they have been manufactured and packaged in their primary packaging material.

- A specified number of TDS, defined on the basis of batch size, should be randomly examined after they have been placed in their primary packaging material.

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- The sampled TDS should be removed from their packaging and thoroughly visually inspected for leakage.
- Each sampled TDS should then be uniformly wiped with a solvent-moistened swab. Both the backing side and the release liner side of the TDS should be wiped. The inside surface of the pouch should also be wiped. The swab(s) is then extracted and assayed for the drug.
- The product fails if the total amount of drug from the TDS, and the corresponding pouch, exceed the acceptable limit established by the manufacturer.

⟨4⟩ MUCOSAL DRUG PRODUCTS—PRODUCT QUALITY TESTS

INTRODUCTION

The mucosal route of drug administration is subdivided into seven membrane surfaces for the purposes of taxonomic distinction of dosage forms by route of administration. These membrane surfaces are characterized as otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal, and rectal. This grouping does not include the pulmonary mucosal route addressed in *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩. A drug product is administered to any of these seven mucosal surfaces to effect either local action or systemic absorption. Local action is to the area proximate to application. Where local action is intended, systemic absorption is not typically desired and is unnecessary for therapeutic effect. In some cases, however, the mucosal delivery of a drug for systemic absorption is used because it avoids first-pass metabolism, it provides more rapid systemic delivery, or it provides an alternative when oral delivery (to the gastrointestinal tract) is not possible due to a disease state. A large number of the dosage forms listed in *Pharmaceutical Dosage Forms* ⟨1151⟩ can be delivered by way of the various membrane surfaces in the mucosal category. [NOTE—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.]

Analytical procedures and acceptance criteria for testing drug products are divided into two categories: those that assess general product quality attributes and those that assess product performance. Drug product quality tests assess attributes such as identification, assay (strength), dose content uniformity, and impurities and are usually part of the compendial monograph. Product performance tests include the dissolution test for a solid oral dosage form, *Dissolution* ⟨711⟩, and the drug release test, *Drug Release* ⟨724⟩. Taken together, quality and performance tests ensure the identity, strength, quality, and purity of a mucosal drug product.

This chapter provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in part or in its entirety, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*). The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the *USP*.

PRODUCT QUALITY TESTS FOR MUCOSAL DRUG PRODUCTS

This chapter provides product quality tests that are generally necessary, tests that apply to specific products, and tests that apply to one or more of the specific mucosal routes. Quality tests listed under a specific mucosal route in this chapter represent expectations for any dosage form administered by that specific route.

Generally Necessary Tests

Product quality attributes for mucosal dosage forms should reflect acceptable requirements for marketed products. The following tests should be generally applied to all dosage forms intended for mucosal delivery. Tests that are generally necessary for any article include: *Definition*, *Identification*, *Assay*, and *Impurities* (organic, inorganic, and residual solvents). *Uniformity of Dosage Units* ⟨905⟩ is typically included in a *USP* product monograph.

DEFINITION

The definition section (see *General Notices, 4.10 Monographs*) in a *USP* monograph describes the drug product and specifies the range of acceptable assayed content of the drug substance(s) present in the dosage form. For certain products, the definition includes any relevant additional information, such as the presence or absence of other components, excipients, or adjuvants, and cautionary statements on toxicity and stability. Appearance information is used in a regulatory submission to aid in product identification. Because the size, shape, color, and other attributes are attributes of individual marketed products, a qualitative description is typically not required as part of a *USP* monograph (see ⟨1151⟩).

IDENTIFICATION

Identification is included in a monograph as an aid in verifying the identity of the article (see *General Notices, 5.40 Identification.*)

ASSAY

The assay is used to determine the strength (content) of the drug product. Typically, the assay is specific and stability-indicating. When a nonspecific assay is justified, other supporting analytical procedures should ensure that any

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interfering species will be detected and can be limited. Assay results are often reported as a percentage of the label claim, with acceptance criteria that are typically in the range from 90.0% to 110.0%. For some antibiotic products, the range may be wider. The width of these limits is intended to allow for manufacturing variability, including changes in stability, as well as analytical variation. The narrower acceptance range of 95.0%–105.0% is used less often and with justification.

IMPURITIES

Process impurities include those arising from starting materials, synthetic byproducts, and other inorganic and organic impurities that may be present in the drug substance and in the excipients used in the manufacture of the drug product. These impurities are limited, as specified within the drug substance and excipient monographs. Impurities in the drug product may also result from degradation of the drug substance or excipients, from interactions between the drug substance and an excipient, or from interactions between the drug substance and the packaging components. The procedures and acceptance criteria should specifically limit toxic degradation products as well as degradation products that compromise the quality of the article if they exceed certain levels. A more complete discussion of impurities is provided in *Impurities in Drug Substances and Drug Products* ⟨1086⟩[1] and in ICH Q3B Impurities in New Drug Products.[1]

UNIFORMITY OF DOSAGE UNITS

Chapter ⟨905⟩ is used to ensure the consistency of drug substance content in dosage units within a narrow range around the label claim. The test is applied only to dosage forms containing a single dose or a part of a dose of the drug substance in each unit. Uniformity of dosage units may be demonstrated by one of two methods: content uniformity or weight variation. Content uniformity is based on the assays of a number of individual dosage units. Weight variation can be used to estimate content uniformity under certain conditions.

Dosage Forms by Specific Mucosal Route and Product-Specific Tests

In addition to the generally necessary product quality tests already discussed, the dosage form may require specific quality tests that are common across routes of administration. *Injections and Implanted Drug Products* ⟨1⟩ provides testing requirements common to injectable and implantable products. *Oral Drug Products—Product Quality Tests* ⟨2⟩ provides testing requirements for tablets and lozenges. *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩ provides testing requirements common to semisolids (creams, ointments, and gels). Chapter ⟨5⟩ presents testing requirements for sprays and aerosols. Where a dosage form has no specific test given in this chapter, no additional test is required unless included in the individual monograph specification.

OTIC ROUTE

The otic route is characterized by administration of a preparation into, or by way of, the ear. Demonstration of sterility (see *Sterility Tests* ⟨71⟩) is not always required for products delivered to the ear. Typically, sterility is required where the product is administered to the inner ear or where the eardrum is damaged. Where sterility is not required, the quantitative enumeration of mesophilic bacteria and fungi that grow under anaerobic conditions, *Microbial Enumeration Tests* ⟨61⟩, or the determination of the absence or limited occurrence of specified organisms, *Tests for Specified Microorganisms* ⟨62⟩, may be required.

If an antimicrobial preservative is used, *Antimicrobial Effectiveness Testing* ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩ may be required.

Dosage forms given by the otic route include liquids, solutions, and suspensions.

OPHTHALMIC ROUTE

The ophthalmic route is to the eye. In addition to the generally necessary tests, the following specific tests for ophthalmic drug products should be considered (see *Table 1*). For products that are injected or implanted into the eye, see ⟨1⟩. Some of the important product quality tests for products administered by the ophthalmic route are listed below. See *Ophthalmic Products—Quality Tests* ⟨771⟩ for details and other product quality information.

- Particulate and Foreign Matter
- Sterility
- Particle Size and Particle Size Distribution
- Antimicrobial Preservative

Table 1. Drug Products Administered by the Ophthalmic Route, with Product-Specific Tests

Ophthalmic Route	
Dosage Form	**Product-Specific Tests**
Gels	*Minimum Fill* ⟨755⟩
	⟨3⟩

[1] ICH Q3B (R2) Impurities in New Drug Products, 2006, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed 28 May 2015.

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Table 1. Drug Products Administered by the Ophthalmic Route, with Product-Specific Tests *(continued)*

Ophthalmic Route	
Dosage Form	**Product-Specific Tests**
Emulsions	*Osmolality and Osmolarity* ⟨785⟩
	pH ⟨791⟩
	Surface tension
	Viscosity—Capillary Methods ⟨911⟩
	Viscosity—Rotational Methods ⟨912⟩
	Zeta potential
Inserts	*Bacterial Endotoxins Test* ⟨85⟩
Ointments	⟨755⟩
	⟨3⟩
Solutions	*Particulate Matter in Ophthalmic Solutions* ⟨789⟩
	⟨791⟩
	⟨911⟩
	⟨912⟩
	Viscosity—Rolling Ball Method ⟨913⟩
	⟨785⟩
Strips	Currently no specific tests (additional specific monograph requirements may apply)
Suspensions	⟨791⟩
	⟨785⟩
	Particle size and particle size distribution
	⟨911⟩
	⟨912⟩
	⟨913⟩

NASAL ROUTE

The nasal route is administration to the nose, or by way of the nose, for local or systemic effect (see *Table 2*).

Table 2. Drug Products Administered by the Nasal Route, with Product-Specific Tests

Nasal Route	
Dosage Form	**Product-Specific Tests**
Aerosols	⟨5⟩
Gels (Jelly)	⟨755⟩
	⟨3⟩
Ointments	⟨755⟩
	⟨3⟩
Sprays	⟨5⟩
Solutions	⟨5⟩

OROPHARYNGEAL ROUTE

The oropharyngeal route is into the oral cavity and/or pharyngeal region. The oropharyngeal route is subclassified by the specific intra-oral surfaces, such as buccal or sublingual. Buccal and sublingual administrations are typically intended to promote systemic absorption by permeation through the respective mucosa. However, in this context, oral administration may mean topical application for local action (see *Table 3*). Product quality tests for products administered to oropharyngeal surfaces often conform to those for oral administration to the gastrointestinal tract (see ⟨2⟩).

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Table 3. Drug Products Administered by the Oropharyngeal Route, with Product-Specific Tests

Oropharyngeal Route	
Dosage Form	**Product-Specific Tests**
Buccal patches	See ⟨3⟩ for testing requirements common to patches.
Films	Currently no specific tests (additional specific monograph requirements may apply)
Gels	⟨755⟩
	⟨3⟩
Gums	Currently no specific tests (additional specific monograph requirements may apply)
Lozenges	Currently no specific tests (additional specific monograph requirements may apply)
Ointments	⟨3⟩
	⟨755⟩
Solutions (Rinses)	Currently no specific tests (additional specific monograph requirements may apply)
Sprays	⟨5⟩
Tablets	⟨2⟩

URETHRAL ROUTE

The urethral route is into the urethra, typically for local action, but systemic distribution is also possible. Chapters ⟨61⟩ and ⟨62⟩ may apply. Drug products in this category include urethral inserts.

VAGINAL ROUTE

The vaginal route is into the vagina, typically for local action, but systemic distribution is also possible. Chapters ⟨61⟩ and ⟨62⟩ may apply (see *Table 4*).

Relative foam density: Determine relative foam density by weighing a mass of foam (*m*) and a mass of the same volume of water (*e*) in a flat-bottom dish. Relative foam density = *m*/*e*.

Volume of foam expansion: Estimate the volume of foam expansion at 25° using a graduated buret and a foam-generating container equipped with a dose-actuating device and fitted to the buret.

Table 4. Drug Products Administered by the Vaginal Route, with Product-Specific Tests

Vaginal Route	
Dosage Form	**Product-Specific Tests**
Creams	⟨755⟩
	⟨3⟩
Foams	⟨755⟩
	Physical appearance (of the foam and of the collapsed foam) *Relative foam density* *Volume of foam expansion*
Gels	⟨755⟩
	⟨3⟩
Inserts	Currently no specific tests (additional specific monograph requirements may apply)

RECTAL ROUTE

The rectal route is into the rectum. Rectally administered products may produce local effect(s) or delivery to the systemic circulation.

Softening time of lipophilic suppositories: The test is intended to determine, under defined conditions, the time that elapses until a suppository maintained in water at 37 ± 0.5° softens to the extent that it no longer offers resistance when a defined weight is applied (see *Table 5*).

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Table 5. Drug Products Administered by the Rectal Route, with Product-Specific Tests

Rectal Route	
Dosage Form	**Product-Specific Tests**
Foams	⟨755⟩
	Physical appearance (of the foam and of the collapsed foam)
	Relative foam density
	Volume of foam expansion
Ointments	⟨755⟩
	⟨3⟩
Suppositories	Softening time of lipophilic suppositories
Solutions	Currently no specific tests (additional specific monograph requirements may apply)
Suspensions	Currently no specific tests (additional specific monograph requirements may apply)

⟨5⟩ INHALATION AND NASAL DRUG PRODUCTS—GENERAL INFORMATION AND PRODUCT QUALITY TESTS

Add the following:

▲

1. INTRODUCTION
2. GENERAL QUALITY TESTS FOR INHALATION DRUG PRODUCTS
3. GENERAL QUALITY TESTS FOR NASAL DRUG PRODUCTS
4. DESCRIPTION OF PRODUCT QUALITY TESTS

▲ 2S (USP41)

Change to read:

1. INTRODUCTION

Inhalation drug products deliver ▲drugs▲ 2S (USP41) into the lungs by oral inhalation and include inhalation aerosols, inhalation powders, inhalation sprays, inhalation solutions, inhalation suspensions, solutions for inhalation, and drugs for inhalation solutions dosage forms. Nasal drug products deliver drugs into the nasal cavity and include nasal sprays, nasal solutions, nasal aerosols, and nasal powder dosage forms. This chapter does not address nasal products in gel and ointment form. See *Table 1* for established names and definitions of these dosage forms. Definitions of these drug product dosage forms, brief information about their manufacture, and a glossary of dosage form names can be found in *Pharmaceutical Dosage Forms* ⟨1151⟩.

[NOTE—All references to chapters above 1000 are for informational purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.▲ ▲ 2S (USP41)]

Table 1. Established Names and Definitions

Established Name	Definition
Inhalation Aerosol	A drug product for oral inhalation that is packaged under pressure, ▲aerosolizes,▲ 2S (USP41) and delivers a specified amount of ▲▲ 2S (USP41) active ingredient(s) upon activation of an ▲accurate metering▲ 2S (USP41) valve system ▲in association with an actuator mechanism.▲ 2S (USP41) Inhalation aerosol ▲drug products▲ 2S (USP41) are more commonly known as metered-dose inhalers or MDIs.
Inhalation Powder	Drug powder for oral inhalation with the use of a device that aerosolizes and delivers an accurately metered amount of ▲▲ 2S (USP41) active ingredient(s). Inhalation powder ▲drug products▲ 2S (USP41) are more commonly known as dry powder inhalers or DPIs.
Inhalation Spray	A nonpressurized ▲▲ 2S (USP41) liquid, ▲sterile (if aqueous-based)▲ 2S (USP41) drug dosage form for oral inhalation that is packaged in a container▲–closure system▲ 2S (USP41) that upon activation ▲aerosolizes and▲ 2S (USP41) delivers ▲in fine droplets of formulation an accurately metered amount of active ingredient(s).▲ 2S (USP41)
Inhalation Solution	A ▲sterile▲ 2S (USP41) drug solution for oral inhalation with the use of a nebulization system.
Inhalation Suspension	A ▲sterile▲ 2S (USP41) drug suspension for oral inhalation with the use of a nebulization system.

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Table 1. Established Names and Definitions *(continued)*

Established Name	Definition
Solution for Inhalation	A ▲sterile▲ 2S (USP41) drug solution for oral inhalation that must be diluted before it is administered with the use of a nebulization system.
[Drug] for Inhalation Solution	A ▲sterile▲ 2S (USP41) drug powder that, upon the addition of a suitable vehicle, yields a ▲sterile▲ 2S (USP41) solution conforming in all respects to an inhalation solution.
Nasal Spray	A nonpressurized, accurately metered, liquid drug dosage form for local application into the nasal passages that is packaged in a container▲–closure system▲ 2S (USP41) that upon activation ▲aerosolizes and▲ 2S (USP41) delivers ▲in droplets of formulation an accurately metered amount of active ingredient(s).▲ 2S (USP41)
Nasal Aerosol	A drug product for local application into the nasal passages that is packaged under pressure, ▲aerosolizes,▲ 2S (USP41) and delivers a specified amount of ▲▲ 2S (USP41) active ingredient(s) upon activation of an ▲accurate metering▲ 2S (USP41) valve system ▲in association with an actuator mechanism.▲ 2S (USP41)
Nasal Solution	A nonpressurized, liquid drug dosage form for local application into the nasal passages.
Nasal Powder	Drug powder for local application into the nasal passages with the use of a device that aerosolizes and delivers an accurately metered amount of ▲▲ 2S (USP41) active ingredient(s).

This chapter provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This general chapter applies, in part or in its entirety, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*), and includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the *USP–NF*. If a validated performance test procedure is available for the specific drug product, it is identified in a general chapter below 1000. Additional information, or information on promising technologies that have not yet been fully validated, may be presented in informational chapters above 1000.

Drug Product General Quality Tests and Performance Quality Tests

A *USP* drug product monograph contains tests, analytical procedures, and acceptance criteria. Drug product tests are divided into two categories: 1) ▲▲ 2S (USP41) general quality ▲tests;▲ 2S (USP41) and 2) ▲▲ 2S (USP41) product performance ▲tests,▲ 2S (USP41) e.g., delivered dose uniformity and its physical characteristics, such as aerodynamic particle size distribution, ▲and/or droplet size distribution.▲ 2S (USP41) General quality tests assess the integrity of the dosage form, whereas ▲product▲ 2S (USP41) performance quality tests assess delivery of the drug and other attributes that may relate to in vivo drug performance.

Taken together, ▲general▲ 2S (USP41) quality and ▲product▲ 2S (USP41) performance tests ensure the identity, strength, quality, and purity of inhalation and nasal drug products.

The next two sections of this chapter list product quality attributes for inhalation drug products and for nasal drug products, respectively. The final section describes in greater detail the quality tests for inhalation and nasal drug products. *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩ contains product performance tests for inhalation and nasal drug products and should be used in conjunction with this chapter.

Change to read:

2. GENERAL QUALITY TESTS FOR INHALATION DRUG PRODUCTS

Inhalation Aerosol

DESCRIPTION

Aerosol in this context is a dosage form consisting of a liquid preparation ▲▲ 2S (USP41) packaged under pressure and intended for administration as a fine mist. The descriptive term aerosol also refers to the fine mist of small droplets ▲and/or▲ 2S (USP41) solid particles that are emitted from the product. Inhalation aerosols, also known as MDIs, are preparations characterized by dispersion of the active pharmaceutical ingredient into the airways during oral inspiration for either local or systemic effect, and nasal aerosols, also known as nasal MDIs, are characterized by deposition in the nasal cavity for either local or systemic effect.

An aerosol formulation typically contains a drug substance(s) dissolved or suspended in a propellant or a mixture of propellant(s) and co-solvent(s) and possibly other suitable excipients. An inhalation aerosol drug product ▲▲ 2S (USP41) delivers a specified amount and quality of therapeutically active ingredient(s) upon activation of an ▲accurate metering▲ 2S (USP41) valve system. General quality tests for inhalation aerosol drug products ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Identification*
- *Assay*
- *Impurities and Degradation Products*
- *Water Content*
- *Foreign Particulate Matter*
- *Leachables*
- *Spray Pattern*

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- ▲*Valve Delivery*▲ 2S (USP41)
- *Microbial Limits*
- ▲*Co-Solvent*▲ 2S (USP41) *Content* ▲*[(e.g., ethanol)*▲ 2S (USP41) *if present]*
- ▲*Elemental Impurities—Limits* ⟨232⟩▲ 2S (USP41)
- *Net Fill Weight*
- *Leak Rate*
- For performance quality tests, refer to ⟨601⟩

Inhalation Solution

DESCRIPTION

Inhalation solution drug products typically are water-based ▲▲ 2S (USP41) sterile preparations. They are intended for delivery to the lungs by nebulization with a specified ▲▲ 2S (USP41) nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers ▲within▲ 2S (USP41) protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing a drug solution by means of ultrasonic energy, Venturi effect, or other appropriate mechanical/electrical means. General quality tests for inhalation solutions ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Identification*
- *Assay*
- *Impurities and Degradation Products*
- *Content Uniformity* ▲*for Premetered Dosage Forms*▲ 2S (USP41)
- *Assay for* ▲*Antimicrobial*▲ 2S (USP41) *Preservative and Stabilizing Excipients (if present)*
- *Sterility*
- *Foreign Particulate Matter*
- *pH*
- ▲*Viscosity,* if relevant▲ 2S (USP41)
- *Osmolality,* ▲if relevant▲ 2S (USP41)
- *Leachables*
- *Net Fill Weight*
- ▲*Elemental Impurities—Limits* ⟨232⟩▲ 2S (USP41)
- *Weight Loss*
- For information on performance quality tests, refer to *Products for Nebulization—Characterization Tests* ⟨1601⟩

Inhalation Suspension

DESCRIPTION

Inhalation suspension drug products typically are water-based ▲▲ 2S (USP41) sterile preparations. They are intended for delivery to the lungs by nebulization with a specified ▲▲ 2S (USP41) nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers ▲within▲ 2S (USP41) protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing the formulation components by means of ultrasonic energy, Venturi effect, or other appropriate mechanical means. General quality tests for inhalation suspensions ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- ▲*Primary*▲ 2S (USP41) *Particle Size Distribution*▲▲ 2S (USP41)
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes

Solution for Inhalation

DESCRIPTION

Solution for inhalation drug products typically are water-based ▲▲ 2S (USP41) sterile preparations. Upon dilution, in accordance with labeling, including identity and amount of dilution vehicle, they are intended for delivery to the lungs by nebulization ▲with a specified▲ 2S (USP41) nebulizer. Such drug preparations typically are packaged in single-dose semipermeable containers ▲within▲ 2S (USP41) protective packaging to minimize ingress of volatile foreign contaminants, loss of solvent, and exposure to oxygen and light. Nebulization involves continuous generation and delivery to the patient of a fine mist of aqueous droplets containing the formulation components by means of ultrasonic energy, Venturi effect, or other appropriate mechanical/electrical means. General quality tests for solutions for inhalation ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Clarity and Color of Solution upon Dilution* in accordance with the labeling

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- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes

Drug for Inhalation Solution

DESCRIPTION (POWDER)

Drug for inhalation solution is a (specified color) ▲sterile▲ 2S (USP41) drug powder formulation that upon the addition of a suitable vehicle, in accordance with labeling, including identity and amount of dilution vehicle, yields a ▲sterile▲ 2S (USP41) solution conforming in all respects to the *Inhalation Solution* requirements. General quality tests for drug for inhalation solutions ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- ▲*Reconstitution Time (powder)*▲ 2S (USP41)
- *Water Content*
- Clarity, color, and completeness of solution within specified time, upon reconstitution
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes upon (re)constitution of the drug product

Inhalation Spray

DESCRIPTION

Inhalation spray drug products typically are water-based ▲sterile▲ 2S (USP41) formulations packaged in a compact container–closure system containing an integral spray pump unit that, upon activation, delivers an accurately metered amount of fine ▲droplets of▲ 2S (USP41) the formulation. The droplets can be generated by various means such as mechanical action, power assistance, or energy from the patient's inspiration. The mechanisms by which they generate droplets distinguish the various types of inhalation sprays. These drug products may be unit-dose or multidose presentations. Inhalation spray drug products may be designed as premetered or device-metered presentations. A premetered unit contains a previously measured amount of liquid formulation in an individual container (e.g., a blister) that is inserted in the device by the patient before use. A device-metered product contains a sufficient amount of liquid formulation for a prescribed number of doses in a reservoir, and each dose is delivered as an accurately metered spray by the device throughout the unit's life. General quality tests for inhalation sprays ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Plume Geometry*
- For all other general quality attributes, refer to the previous *Inhalation Solution* attributes
- For performance quality tests, refer to ⟨601⟩

Inhalation Powder

DESCRIPTION

Inhalation powder drug products, commonly known as DPIs, dispense powders for inhalation with the use of a device that aerosolizes and delivers an accurately metered amount of active ingredient(s) with consistent physical characteristics alone or with a suitable excipient(s). Current designs include premetered and device-metered DPIs, all of which rely on various energy sources to create and disperse the aerosol during patient inspiration.

Premetered DPIs contain previously measured amounts of formulation in individual containers (e.g., capsules or blisters) that are inserted into the device before use. Premetered DPIs also may contain premetered dose units as ordered multidose assemblies in the delivery system. Device-metered DPIs have an internal reservoir that contains a sufficient quantity of formulation for multiple doses that are metered by the device itself during actuation by the patient. General quality tests for inhalation powders ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Identification*
- *Assay*
- *Impurities and Degradation Products*
- *Content Uniformity* ▲*for Premetered Dosage Forms*▲ 2S (USP41)
- *Water Content*
- *Foreign Particulate Matter*
- *Microbial Limits*
- ▲*Elemental Impurities—Limits* ⟨232⟩▲ 2S (USP41)
- Net Content (device-metered)
- *Residual Solvent*
- ▲▲ 2S (USP41)*Leachables*
- For performance quality tests, refer to ⟨601⟩

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Change to read:

3. GENERAL QUALITY TESTS FOR NASAL DRUG PRODUCTS

Nasal Aerosol

Refer to the above *Inhalation Aerosol* attributes.

Nasal Spray

DESCRIPTION

Nasal spray drug products typically are water-based ▲▲ 2S (USP41) formulations applied to the nasal cavity for local and/or systemic effects. They contain therapeutically active ingredient(s) dissolved or suspended in solution or mixtures of excipients in a nonpressurized compact container–closure system. The container–closure system includes an integral spray pump unit that upon activation delivers ▲▲ 2S (USP41) an accurately metered amount of ▲▲ 2S (USP41) the formulation ▲as a mist.▲ 2S (USP41) Dispersion of the formulation as a spray typically is accomplished by forcing the formulation through the nasal actuator and its orifice. Often, such drug products are multidose device-metered (see *Inhalation Spray*) presentations in which the dose is metered by the spray pump. Nasal spray drug products also may be designed as premetered presentations. General quality tests for nasal sprays ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Identification*
- *Assay*
- *Impurities and Degradation Products*
- *Assay for* ▲*Antimicrobial*▲ 2S (USP41) *Preservative and Stabilizing Excipients (if present)*
- *Content Uniformity* ▲*for Premetered Dosage Forms*▲ 2S (USP41)
- ▲*Primary*▲ 2S (USP41) *Particle Size Distribution* (for suspensions)
- *Foreign Particulate Matter*
- *Spray Pattern*
- *Microbial Limits*
- *Leachables*
- *Net Fill Weight*
- *pH*
- ▲*Pump Delivery*▲ 2S (USP41)
- *Osmolality*
- *Viscosity*
- ▲*Elemental Impurities—Limits* ⟨232⟩▲ 2S (USP41)
- ▲▲ 2S (USP41)
- For performance quality tests, refer to ⟨601⟩

Nasal Powder

Refer to the previous *Inhalation Powder* quality attributes ▲as applicable.▲ 2S (USP41)

Nasal Solution

DESCRIPTION

Nasal solutions are drug products that typically are water-based ▲▲ 2S (USP41) formulations applied to the nasal cavity for local effect. They may contain drug substance(s) dissolved in solution or mixtures of excipients in a nonpressurized compact container–closure system. The container–closure system includes a delivery system that administers nonmetered amounts of drops or a fine mist of droplets of the formulation. Typically, such drug products are multidose presentations. General quality tests for nasal solution drug products ▲▲ 2S (USP41) include the following ▲attributes▲ 2S (USP41) (see *4. Description of Product Quality Tests* for more detailed discussions of each test):

- *Identification*
- *Assay*
- *Impurities and Degradation Products*
- *Assay for* ▲*Antimicrobial*▲ 2S (USP41) *Preservative and Stabilizing Excipients (if present)*
- *Foreign Particulate Matter*
- *Microbial Limits*
- *Leachables*
- *Net Fill Weight*

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- *pH*
- ▲*Elemental Impurities—Limits* ⟨232⟩▲ 2S (USP41)
- *Osmolality*
- *Viscosity*

Change to read:

4. DESCRIPTION OF PRODUCT QUALITY TESTS

Product quality tests are listed as follows, and should be applied to inhalation and nasal drug products and to products for nebulization. Product-specific quality tests are addressed in product monographs.

Assay (strength and content uniformity)

The USP Assay test of a drug substance in the drug product container is determined by means of a validated stability-indicating procedure following *Validation of Compendial Procedures* ⟨1225⟩. The USP Assay test should measure ▲the amount of▲ 2S (USP41) drug ▲substance(s)in the unit▲ 2S (USP41) and its stability ▲throughout the expiration dating period,▲ 2S (USP41) including adherence of the drug substance to the container–closure components. Appropriate acceptance criteria can provide added assurance of manufacturing reproducibility and may ensure better conformance in ▲▲ 2S (USP41) performance attributes (e.g., delivered dose uniformity).

If a drug product is labeled to contain a single enantiomer of a chiral drug substance, analysts can use a chiral assay or a combination of an achiral assay and a validated procedure to control the presence of the undesired enantiomer as an impurity.

Assay for ▲Antimicrobial▲ 2S (USP41) Preservative and Stabilizing Excipients (if present)

The assay of any preservative (e.g., an antimicrobial) ▲in a multidose container▲ 2S (USP41) or stabilizing excipient (e.g., an antioxidant, an agent specifically added to minimize or prevent degradation) in a ▲formulation▲ 2S (USP41) should be determined analytically, typically with a validated stability-indicating procedure following ▲▲ 2S (USP41) International Council for Harmonisation (ICH) ▲current▲ 2S (USP41) Q2 guidance. The ▲assay▲ 2S (USP41) acceptance criteria ▲for antimicrobial preservatives▲ 2S (USP41) normally are based on appropriate preservative effectiveness demonstrated by a microbial challenge test.

Clarity and Color of Solution upon Dilution

Solution for inhalation and drug for inhalation solution dosage forms must be diluted and reconstituted in accordance with labeling before administration by nebulization. The type and amount of the vehicle used for dilution and reconstitution must be specified on the labeling. Appropriate studies must be undertaken to fully assess ▲quantitatively▲ 2S (USP41) the clarity and color of the solution upon dilution and reconstitution. The studies also should include appropriate physical and chemical stability studies, as well as studies of performance characteristics.

Content Uniformity for Premetered Dosage Forms

See *Uniformity of Dosage Units* ⟨905⟩.

▲Co-Solvent▲ 2S (USP41) Content ▲[(e.g., ethanol)▲ 2S (USP41) if present]

If ▲a co-solvent▲ 2S (USP41) is used in a drug product formulation, a specific assay with appropriate acceptance criteria should be included.

Description

See the previous ▲descriptions▲ 2S (USP41) corresponding ▲to applicable▲ 2S (USP41) dosage forms, ▲outlined in *2. General Quality Tests for Inhalation Drug Products* and *3. General Quality Tests for Nasal Drug Products* of this chapter, in association with their container–closure delivery systems▲ 2S (USP41) and the respective ▲labeling statement of▲ 2S (USP41) the monograph of a drug product. ▲This test should address the appearance of the contents of the container (i.e., dosage form) and the appearance and integrity of the components of the container closure system for conformance to their respective descriptions.▲ 2S (USP41)

▲Elemental Impurities

Drug products should be evaluated for elemental impurities (see ⟨232⟩). Validated analytical procedures should be used as described in *Elemental Impurities—Procedures* ⟨233⟩.▲ 2S (USP41)

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Foreign Particulate Matter

Foreign particulate matter in these drug products should be adequately controlled. Particulate matter in inhalation and nasal drug products may originate during manufacturing and from formulation and container–closure components. ▲Following a detailed toxicological assessment of the type, origin, amount, and size of any foreign particulates, including fine particulates (e.g., less than 10 µm), appropriate specifications should be established throughout the stability storage period to confirm overall quality.▲ 2S (USP41)

Identification

A specific identification test or tests are used to verify the identity of the drug substance in the drug product. If a nonspecific method is used for identification, then it should be combined with a second independent and complementary method. A specific identification test for polymorphic forms should be carried out, ▲if applicable.▲ 2S (USP41) Moreover, if the drug substance is a salt, an appropriate identification test also should be included for the counterion.

Impurities and Degradation Products

Validated stability-indicating analytical procedure(s) following ⟨1225⟩ should be used to determine the levels of impurities and degradation products in a drug product. Typically, the acceptance criteria are set for individual, ▲▲ 2S (USP41) unspecified, and total impurities and degradation products following current ICH Q3B guidance. For reporting, identification, and qualification thresholds and other relevant information, follow current ICH Q3B ▲and ICH M7 guidelines.▲ 2S (USP41)

Leachables

Inhalation and nasal drug products should be evaluated for compounds that may leach from elastomeric and plastic components and from coatings of components of the container–closure system in direct contact with the formulation. Additionally, the drug product inadvertently may contain other residual contaminants from manufacturing and processing. Leachables may include polynuclear aromatics, nitrosamines, monomers, plasticizers, accelerators, antioxidants, and vulcanizing agents. Processing contaminants may include surface-treatment or processing agents that may dissolve, chemically associate, or become suspended in the formulation.

Thus, throughout the expiration-dating period, the drug product should be evaluated for compounds that can migrate into the formulation from a variety of sources. The type of testing that should be performed depends on whether the formulation is a powder or a liquid and the composition of the container–closure system, e.g., a drug product packaged in a semipermeable container should be evaluated for ingress of volatile leachables. Appropriate specifications using validated analytical procedures should be applied to identify, monitor, and quantify the compounds in the drug product with appropriate minimum levels of quantification. Corresponding acceptance criteria should be established and justified from toxicological and safety perspectives.

Leak Rate

Leak rate studies of inhalation and nasal aerosol drug products can be used during drug product development and characterization to support the selection of appropriate container–closure components (e.g., valve and canister) and drug product manufacturing parameters, including the crimping process ▲and 100% heat stress test prior to equilibration/quarantine period. Release and stability▲ 2S (USP41) specifications for the USP Leak Rate ▲test▲ 2S (USP41) can include multiple units from each batch based on weight difference determination with time at a specified temperature. See *Leak Rate* ⟨604⟩ for additional information.

Microbial Limits

The microbial quality of dosage forms where indicated in *2. General Quality Tests for Inhalation Drug Products* and *3. General Quality Tests for Nasal Drug Products* normally is controlled by appropriate validated test(s) and acceptance criteria for total aerobic count, total yeasts and molds count, and freedom from designated indicator pathogens. Acceptance criteria can be expressed on a per-container basis. Refer to *Microbial Enumeration Tests* ⟨61⟩, *Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products* ⟨610⟩, ▲and *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* ⟨1111⟩▲ 2S (USP41) for additional information.

Net Fill Weight

The total net weight of the formulation in the container should be assessed and controlled with a test and acceptance criteria. ▲See *Minimum Fill* ⟨755⟩ for additional information.▲ 2S (USP41)

Osmolality

To control the tonicity of the formulation of dosage forms where indicated in *2. General Quality Tests for Inhalation Drug Products*, the product should be tested for osmolality with appropriate specifications as described in *Osmolality and Osmolarity* ⟨785⟩.

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pH

Appropriate specification for the pH of the formulation of dosage forms where indicated in *2. General Quality Tests for Inhalation Drug Products* and *3. General Quality Tests for Nasal Drug Products* should be established as described in *pH* ⟨791⟩.

▲Primary▲ 2S (USP41) Particle Size Distribution

For ▲▲ 2S (USP41) suspension ▲preparation▲ 2S (USP41) drug products, appropriate method(s) and corresponding acceptance criteria ▲should▲ 2S (USP41) be used for the determination of the particle size distribution of the drug substance particles in the formulation within the container. ▲Microscopic examination of the product formulation provides information and changes over product stability on the presence of large particles, morphology of the drug substance and carrier particles, extent of agglomerates, crystal growth, and foreign particulate matters. Additionally, where the crystalline form of the drug substance can affect the bioavailability, performance, stability, or other properties of the drug product, microscopic evaluation or other appropriate methods are recommended to control and monitor the crystalline and amorphous forms if changes are observed on stability. See *Optical Microscopy* ⟨776⟩ for additional information. For aerodynamic particle size distribution and droplet size distribution tests of inhalation and nasal drug products, as applicable, refer to ⟨601⟩.▲ 2S (USP41)

Plume Geometry

Because various factors can affect the plume characteristics of the spray of an inhalation aerosol, inhalation spray, nasal aerosol, or nasal spray drug product, its full characterization is important for assessing the performance of the delivery system. Plume geometry can be determined by a variety of procedures using appropriately validated methods. Plume geometry also can be controlled by appropriate acceptance criteria that measure spray pattern characteristics, including shape and size of the evolving spray plume under defined experimental and instrumental test conditions.

▲Pump Delivery

A test for individual pump spray weight delivery should be performed to better ensure reproducible performance of nasal spray drug products. Appropriate specifications for the pump delivery should be set.▲ 2S (USP41)

Reconstitution Time (powder)

Drug for inhalation solution dosage forms must be (re)constituted before administration with the use of a specified nebulization system. Hence, appropriate compatibility studies should be undertaken to fully assess the type and amount of the solvent(s), as well as (re)constitution time for preparation of the final solution for patient administration. The compatibility studies also should include appropriate physical and chemical stability studies on the reconstituted solution, including its performance characterization.

Residual Solvent

Suitable and validated tests should be used to determine the levels of any solvent(s) in the drug product. Refer to *Residual Solvents* ⟨467⟩ for additional information.

Spray Pattern

Because various factors can affect the spray pattern of an inhalation aerosol, nasal aerosol, or nasal spray drug product, full spray pattern characterization is important for assessing the performance of the specific valve and the actuator or the pump. The spray pattern can be determined using appropriately validated methods and corresponding acceptance criteria that measure the shape, density, and size of the pattern. The test procedure for spray patterns normally is specific to the drug product and may include, among others, the distance between the mouthpiece and the measurement plane or collection surface, minimum number of actuations per spray pattern to enable discrimination, orientation of the collection surface relative to the mouthpiece, and visualization procedure(s).

Sterility

All ▲aqueous-based ▲ 2S (USP41) inhalation ▲▲ 2S (USP41) dosage forms are sterile preparations and should meet the requirements of *Sterility Tests* ⟨71⟩.

Viscosity

A test for viscosity with appropriate acceptance criteria should be included for dosage forms where indicated in *2. General Quality Tests for Inhalation Drug Products* and *3. General Quality Tests for Nasal Drug Products* as appropriate (see *Viscosity—Capillary Methods* ⟨911⟩, ▲*Viscosity—Rotational Methods* ⟨912⟩, and *Viscosity—Rolling Ball Method* ⟨913⟩).▲ 2S (USP41)

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Water Content

Appropriate specification for water content of dosage forms where indicated in *2. General Quality Tests for Inhalation Drug Products* and *3. General Quality Tests for Nasal Drug Products* should be established to ensure the drug product's continued stability and acceptable performance▲ ▲ 25 (USP41). Validated analytical procedures should be used as described in *Water Determination* ⟨921⟩. Proceed as directed in ⟨921⟩ with the following modification: provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sampling cylinder.

Weight Loss

Drug products should be evaluated for weight loss ▲where applicable,▲ 25 (USP41) e.g., drug products packaged in semipermeable containers, to assess the moisture-loss protective properties of the overall container–closure system.

⟨7⟩ LABELING

INTRODUCTION

This general chapter provides definitions and standards for labeling of official articles. Labeling standards for an article recognized in *USP–NF* are expressed in the article's monograph and applicable general chapters. It is intended that all articles in *USP* or *NF* will be subject to the labeling requirements specified in this chapter by means of a provision in *General Notices, 10 Preservation, Packaging, Storage, and Labeling*, unless different requirements are provided in a specific monograph. As with compendial standards for naming, identity, strength, quality, and purity, compendial requirements for labeling have a role in the adulteration and misbranding provisions of federal law [see the Federal Food, Drug, and Cosmetic Act (FDCA) sections 501(b), 502(e)(3)(b), 502(g), and 502(h)]. Exceptions or additional requirements specific to animal drug products and compounded preparations are provided in separate sections. Vaccine labeling is not included in this general chapter.

DEFINITIONS

The term "labeling" includes all labels and other written, printed, or graphic matter on an article's immediate container or on, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term "label" is that part of the labeling on the immediate container.

A shipping container that contains a single article, unless the container also is essentially the immediate container or the outside of the consumer package, must be labeled with a minimum of product identification (except for controlled substances), lot number, expiration date, and conditions for storage and distribution.

Beyond-use dates (BUDs) and expiration dates are not the same. An expiration date identifies the time during which a conventionally manufactured product, active ingredient, or excipient can be expected to meet the requirements of a compendial monograph, if one exists, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the conventionally manufactured product, active pharmaceutical ingredient (API), or excipient may be dispensed or used. Expiration dates are assigned by manufacturers of conventionally manufactured products based on analytical and performance testing of the sterility, chemical and physical stability, and packaging integrity of the product. Expiration dates are specific for a particular formulation in its container and at stated exposure conditions of illumination and temperature.

The beyond-use date (BUD) is the date or time beyond which a compounded preparation must be discarded. The date or time is determined from the date the preparation was compounded.

LABELS AND LABELING FOR DRUG PRODUCTS AND COMPOUNDED PREPARATIONS EXPRESSED AS ACTIVE MOIETY IN NAME AND STRENGTH

The names and strengths of drug products and compounded preparations formulated with a salt of an acid or base are to be expressed in terms of the active moiety on the label (see *Nomenclature* ⟨1121⟩, *Monograph Naming Policy for Salt Drug Substances in Drug Products and Compounded Preparations*).

Labeling

The labeling clearly states the specific salt form of the active moiety that is present in the product or preparation because this information may be useful to practitioners and patients. The names and strengths of both the active moiety and specific salt form (when applicable) are provided in the labeling.

Exceptions

In rare cases in which the use of the specific salt form of the active moiety in the title provides vital information from a clinical perspective, an exception to this policy may be considered. In such cases, when the monograph title contains the specific salt form of the active moiety, the strength of the product or preparation is also expressed in terms of the specific salt form.

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LABELS AND LABELING FOR INJECTABLE PRODUCTS

The labels[1] and the labeling state the following information:

- Name of the product
  - In the case of a liquid, the quantity or proportion of each active moiety or drug substance in a specified volume
  - In the case of any product to which a diluent must be added before use, the quantity or proportion of each active moiety or drug substance, name and volume of diluent to be added, the concentration after the diluent is added, directions for proper storage of the constituted solution, and a BUD (see *Expiration Date and Beyond-Use Date*)
- Route(s) of administration
- Name and quantity or proportion of all inactive ingredients except ingredients added to adjust the pH or to make the drug isotonic may be declared by name with a statement of their effect; if the vehicle is *Water for Injection* it need not be named
- Statement of storage conditions
- Name and place of business of the manufacturer, packer, or distributor
- Identifying lot number and expiration date
- "Rx only" for human drugs
- The recommended or usual dosage.

The container must be labeled so that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents.

The lot number must be traceable to the complete manufacturing history of the specific package, including all manufacturing, filling, sterilizing, and labeling operations.

If the individual monograph permits varying concentrations of active moiety or drug substance in a large-volume injection (LVI), the concentration of each active moiety or drug substance named in the official title is stated as if it were part of the official title (e.g., *5% Dextrose Injection*, or *Dextrose Injection 5%*, or *5% Dextrose and 0.2% Sodium Chloride Injection* or *Dextrose (5%) and Sodium Chloride (0.2%) Injection*).

Quantity and Total Volume for Injectable Drug Products Packaged in Single- and Multiple-Dose Containers

For injectable drug products greater than 1 mL, whether packaged in single- or multiple-dose containers, the quantity per total volume should be the primary and prominent expression on the principal display panel of the label, followed in close proximity by quantity per milliliter enclosed by parentheses (quantity/mL).

For containers that hold a volume of less than 1 mL, the quantity per fraction of a milliliter should be the only expression of strength. For containers that hold a volume equal to 1 mL, the strength should be expressed as quantity per milliliter (quantity/mL), not quantity/1 mL.

The following example formats are acceptable:

1. For containers less than 1 mL: 12.5 mg/0.625 mL
2. For containers equal to 1 mL: 5 mg/mL (not 5 mg/1 mL)
3. For containers greater than 1 mL:

Example 1: 500 mg/10 mL (50 mg/mL)

Example 2: 25,000 Units/5 mL (5,000 Units/mL)

In certain cases, the primary and prominent expression of the total drug content per container is not effective in preventing medication errors and therefore in those cases, the total drug content per container should not be the primary and prominent expression of strength. Insulin products are an example of a product class that is an exception from the total drug content per container requirement. Another exception to expressing strength as quantity per total volume is lidocaine (or similar drugs for local anesthesia) where the product may be ordered and administered by percentage (e.g., 1% or 2%). In such cases, the percentage strength as well as the quantity per total volume followed in close proximity by quantity per milliliter enclosed by parentheses must be used.

Example 1: 1% (100 mg/10 mL) (10 mg/mL)

Example 2: 2% (1000 mg/50 mL) (20 mg/mL)

Dry solids that must be constituted should follow the same format with the exception that only the quantity of the drug in the container should be listed as the primary expression of strength, not the quantity per total volume or quantity per milliliter (quantity/mL).

[1] If there are space limitations, see 21 CFR§ 201.10(i), 21 CFR§ 201.105(b), 21 CFR§ 610.60.

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Example: 500 mg/vial

Ratio Expression of Strength

Single-entity injectable drug products must be labeled in terms of quantity per milliliter (quantity/mL) and not as a ratio expression.

Examples:

Epinephrine Injection, 1:1000 must be expressed as 1 mg/mL.

Epinephrine Injection, 1:10,000 must be expressed as 0.1 mg/mL.

Isoproterenol Hydrochloride Injection, 1:5000 must be expressed as 0.2 mg/mL.

Neostigmine Methylsulfate Injection, 1:1000 must be expressed as 1 mg/mL.

Single-entity injectable drug products greater than 1 mL should be formatted as quantity per total volume on the principal display panel of the label followed in close proximity by quantity per milliliter (quantity/mL) enclosed by parentheses.

When combined with a local anesthetic, the concentration of epinephrine will be expressed as a ratio.

Examples:

Lidocaine Hydrochloride and Epinephrine Injection 1%/1:100,000

or

Lidocaine Hydrochloride 1%

and

Epinephrine Injection 1:100,000

Pharmacy Bulk Package

Where a container is offered as a Pharmacy Bulk Package, the label must: 1) state prominently "Pharmacy Bulk Package—Not for Direct Infusion"; 2) contain or refer to information on proper techniques to help assure safe use of the product; and 3) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under labeled storage conditions (see *Packaging and Storage Requirements* ⟨659⟩).

Imaging Bulk Package

Where a container is offered as an Imaging Bulk Package, the label must: 1) state prominently "Imaging Bulk Package" and, in juxtaposition with this statement, include the following use statement: "For use only with an automated contrast injection system, contrast management system, or contrast media transfer set approved or cleared for use with this contrast agent in this Imaging Bulk Package"; 2) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under the labeled storage conditions; 3) bear the statement "See drug and device labeling for information on devices indicated for use with this Imaging Bulk Package and techniques to help assure safe use." (See ⟨659⟩.)

Ferrules and Cap Overseals

Healthcare practitioners using injectable products must be able to easily see and act on labeling statements that convey important safety messages critical for the prevention of imminent life-threatening situations. These cautionary labeling statements must be simple, concise, and devoid of nonessential information. Products that do not require cautionary statements should be free of information, so that those with cautionary statements are immediately apparent. Accomplishing this requires a systematic approach to the labeling of injectable products, and one that ensures that the ferrule and cap overseal—an area of these products that is highly visible to practitioners as they use these medicines—is reserved for critical safety messages. Accordingly:

1. Only cautionary statements may appear on the top (circle) surface of the ferrule and cap overseal of a vial containing an injectable product. The cautionary statement should appear on both the ferrule and cap, but may appear solely on the ferrule if the cap overseal is transparent and the cautionary statement beneath the cap is readily legible. A cautionary statement is one intended to prevent an imminent life-threatening situation and may include instructional statements that provide potency or other safety-related instructions if warranted. Examples of such statements include, but are not limited to: "Warning—Paralyzing Agent" and "Dilute before Using". The cautionary statement should be printed in a contrasting color and should be clearly visible under ordinary conditions of use.
2. If no cautionary statement is necessary, the top surface of the vial, including the ferrule and cap overseal, must remain blank.
3. Other statements or features including, but not limited to, identifying numbers or letters, such as code numbers, lot numbers, company names, logos, or product names, etc., may appear on the side (skirt) surface of the ferrule on vials containing injectable products, but not on the top (circle) surface of the ferrule or cap overseal. The appearance of such statements or features on the skirt surface of the ferrule should not detract from, or interfere with, the cautionary statement on the top surface.

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Potassium Chloride for Injection Concentrate

The use of a black closure system on a vial (e.g., a black cap overseal and a black ferrule to hold the elastomeric closure) or the use of a black band or series of bands above the constriction on an ampule is prohibited, except for Potassium Chloride for Injection Concentrate (see ⟨659⟩).

Neuromuscular Blocking and Paralyzing Agents

All injectable neuromuscular blocking agents and paralyzing agents must be packaged in vials with a cautionary statement printed on the ferrules and cap overseals. Both the container cap ferrule and the cap overseal must bear in black or white print (whichever provides the greatest color contrast with the ferrule or cap color) the words: "Warning: Paralyzing Agent" or "Paralyzing Agent" (depending on the size of the closure system). Alternatively, the overseal may be transparent and without words, allowing for visualization of the warning labeling on the closure ferrule.

Aluminum in Large-Volume Injections (LVIs), Small-Volume Injections (SVIs), and Pharmacy Bulk Packages (PBPs) Used in Parenteral Nutrition (PN) Therapy[2]

1. The aluminum content of LVIs used in PN therapy must not exceed 25 mcg/L.
2. The package insert of LVIs used in PN therapy must state that the drug product contains no more than 25 mcg of aluminum per liter. This information must be contained in the *Precautions* section of the labeling of all LVIs used in PN therapy.
3. If the maximum amount of aluminum in SVIs and PBPs is 25 mcg/L or less, instead of stating the exact amount of aluminum that each contains, as in paragraph (4), the immediate container label for SVIs and PBPs used in the preparation of PN admixtures or formulations (with exceptions as noted below) may state: "Contains no more than 25 mcg/L of aluminum." If the SVI or PBP is a lyophilized powder, the immediate container label may state the following: "When reconstituted in accordance with the package insert instructions, the concentration of aluminum will be no more than 25 mcg/L."
4. The maximum level of aluminum at expiry must be stated on the immediate container label of all SVIs and PBPs used in the preparation of PN admixtures or formulations. The aluminum content must be stated as follows: "Contains no more than ___ mcg/L of aluminum." The immediate container label of all SVIs and PBPs that are lyophilized powders used in the preparation of PN solutions must contain the following statement: "When reconstituted in accordance with the package insert instructions, the concentration of aluminum will be no more than ___ mcg/L." This maximum amount of aluminum must be stated as the highest one of the following three levels:
   - The highest level for the batches produced during the past 3 years
   - The highest level for the latest 5 batches
   - The maximum level in terms of historical levels, but only until completion of production of the first 5 batches.

The package insert for all LVIs, SVIs, and PBPs used in the preparation of PN admixtures or formulations must contain the following statement in the *Warnings* section of the labeling:

> WARNING: This product contains aluminum that may be toxic. Aluminum may reach toxic levels with prolonged parenteral administration if kidney function is impaired. Premature neonates are particularly at risk because their kidneys are immature, and they require large amounts of calcium and phosphate solutions, which contain aluminum.
>
> Research indicates that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 4 to 5 mcg/kg/day, accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration.

Change to read:

LABELS AND LABELING FOR PRODUCTS IN OTHER CATEGORIES

Labels and Labeling of Liquids and Constituted Products

The labels[1] and labeling state the following information:

1. In the case of a liquid product, the percentage content of each active moiety or drug substance or the amount of each active moiety or drug substance in a specified volume.
2. In the case of a product to which a diluent must be added before use, the amount of each active moiety or drug substance, the name and volume of diluent to be added, the final volume of solution, the concentration after the diluent is added (e.g., quantity/mL or quantity/5 mL), directions for proper storage of the constituted solution, and an expiration or BUD (see *Expiration Date and Beyond-Use Date*).

[2] See 21 CFR §201.323. USP uses the following terms: large-volume injections (LVIs), small-volume injections (SVIs), and parenteral nutrition (PN), rather than terminology used in 21 CFR §201.323: large-volume parenterals, small-volume parenterals, and total parenteral nutrition.

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Amount of Active Moiety or Drug Substance per Dosage Unit

The strength of a drug product is expressed on the container label in terms of micrograms, milligrams, grams, or percentage of the therapeutically active moiety or drug substance, whichever form is used in the title, unless otherwise indicated in an individual monograph. Both the active moiety and drug substance names and their equivalent amounts are then provided on the container label and in the labeling (see *Nomenclature* ⟨1121⟩, *Monograph Naming Policy for Salt Drug Substances in Drug Products and Compounded Preparations*).

Official articles in capsule, tablet, or other dosage forms must be labeled to express the quantity of each active moiety or drug substance contained in each unit. Unit-dose oral solutions or suspensions (whether supplied as liquid products or as liquid products that are constituted from solids upon addition of a designated volume of a specific diluent) must be labeled to express the quantity of each active moiety or drug substance delivered under the conditions prescribed in *Deliverable Volume* ⟨698⟩. Official drug products not in unit-dose packaging must be labeled to show the quantity of each active moiety or drug substance in each milliliter or in each gram, or to express the percentage of each such ingredient (see *General Notices, 8.140 Percentage Concentrations*). Exceptions are oral liquids or solids intended to be constituted to yield oral liquids that, alternatively, can be labeled in terms of each 5-mL portion of the liquid or resulting liquid. Unless otherwise required by regulation [e.g., over-the-counter (OTC) regulation 21 CFR §201.62(b)] or indicated in a monograph or chapter, declarations of strength or quantity must be stated only in metric units [see also *General Notices, 5.50.10 Units of Potency (Biological)*].

▲Expiration Date and Beyond-Use Date

The label of an official drug product or nutritional or dietary supplement product shall bear an expiration date. All products shall display the expiration date so that it can be read by an ordinary individual under customary conditions of purchase and use. The expiration date shall be prominently displayed in high contrast to the background or it shall be sharply embossed, and easily understood (e.g., "EXP 6/13," "Exp. June 13," or "Expires 6/2013").

[NOTE—For additional information and guidance, refer to the Consumer Products Association's *Voluntary Codes and Guidelines of the Consumer Healthcare Products Industry*.]

The monographs for some preparations state how the labeled expiration date shall be determined. In the absence of a specific requirement in the individual monograph for a drug product or nutritional supplement, the label shall bear an expiration date assigned for the particular formulation and package of the product, with the following exceptions: the label need not show an expiration date if the drug product or nutritional supplement is packaged in a container that is intended for sale without prescription, and the labeling states no dosage limitations, and if the product or supplement is stable for NLT 3 years when stored under the prescribed conditions.

If an official product is required to bear an expiration date, the product shall be dispensed solely in or from a container labeled with an expiration date, and the date on which the article is dispensed shall be within the labeled expiry period. The expiration date identifies the time during which the article can be expected to meet the requirements of the compendial monograph, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the article may be dispensed or used. If an expiration date is stated only in terms of the month and the year, then the intended expiration date is the last day of the stated month.

The beyond-use date is the date after which a product shall not be used. The dispenser shall place on the label of the prescription container a suitable beyond-use date to limit the patient's use of the article based on any information supplied by the manufacturer or this subsection. The beyond-use date shall not be later than the expiration date on the manufacturer's container. Also see the section *Compounded Preparations* below.

For articles that require constitution before use, a suitable beyond-use date for the constituted product shall be identified in the labeling.

For all other dosage forms, in determining a beyond-use date the dispenser shall take into account, in addition to any other relevant factors:

- Nature of the drug
- Container in which it was packaged by the manufacturer and the expiration date thereon
- Characteristics of the patient's container, if the article is repackaged for dispensing
- Expected storage conditions to which the article may be exposed
- Unusual storage conditions to which the article may be exposed
- Expected length of the course of therapy.

After considering these factors, the dispenser shall label a container with a suitable beyond-use date to limit the patient's use of the article. Unless otherwise specified in the individual monograph or in the absence of stability data to the contrary, the beyond-use date shall be not later than: (a) the expiration date on the manufacturer's container; or (b) 1 year from the date the drug is dispensed, whichever is earlier. For nonsterile solid and liquid dosage forms that are packaged in single-unit and unit-dose containers, the beyond-use date shall be 1 year from the date the drug is packaged into the single-unit or unit-dose container or the expiration date on the manufacturer's container, whichever is earlier, unless stability data or the manufacturer's labeling indicates otherwise.▲ (RB 1-Sep-2019)

Labels and Labeling for Compounded Preparations

The labels and labeling state the following information:

In the case of a compounded preparation, list the name(s) and amount(s) or concentrations of active moiety(ies) or drug substance(s) on the immediate container (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩ and *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩.)

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In the case of a compounded preparation prepared in 503A facilities as defined by FDCA §503A, the labeling should indicate that "this is a compounded preparation."

The label on the container or package of a compounded preparation must bear a BUD. The BUD is determined from the date the preparation is compounded. Because compounded preparations are intended for administration immediately or following short-term storage, their BUDs may be assigned based on criteria different from those applied to assigning expiration dates to manufactured drug products.

The monograph for an official compounded preparation typically includes a BUD that states the time period following the date of compounding during which the preparation, properly stored, may be used. For guidance regarding the BUD for compounded sterile and nonsterile preparations, see ⟨797⟩ and ⟨795⟩, respectively.

The label on the official compounded preparation must include the word "compounded" after the drug portion of a non-proprietary name (e.g., "*Baclofen Compounded Oral Suspension*").

Dialysis, Hemofiltration, and Irrigation

Solutions that are intended for use as dialysis, hemofiltration, or irrigation, and are packaged in a container with a volume of more than 1 L, must be labeled to indicate that the contents are not to be administered either intravenously or intra-arterially.

Use of Leading and Terminal Zeros

To help minimize the possibility of errors in drug dispensing and administration, when the quantity of active moiety or drug substance is expressed in whole numbers it must be shown without a decimal point followed by a terminal zero (e.g., express as 4 mg, not 4.0 mg). When the quantity of active moiety or drug substance is expressed as a decimal number smaller than 1, it must be shown with a zero preceding the decimal point (e.g., express as 0.2 mg, not .2 mg).

Units

Abbreviations for the terms "Units" or "International Units" must not be used for labeling or prescribing purposes. Examples include "U", "u", and "IU". Medication errors have occurred when these abbreviations have been used. See *General Notices, 9.10 Use of Metric Units*.

Alcohol

The alcohol content in a liquid formulation must be stated on the label as a percentage (v/v) of alcohol (C_2H_5OH).

Botanicals

The label of an herb or other botanical intended for use as a dietary supplement shall bear the statement, "If you are pregnant or nursing a baby, seek the advice of a health professional before using this product."

Electrolytes

The concentration of each electrolyte for replacement therapy (e.g., sodium, potassium, chloride) must be stated on the label in milliequivalents per volume (mEq/volume). Phosphorus containing injections must be expressed in milliMoles per volume (e.g., mM/volume). The label of the product must also indicate the quantity of ingredient(s) in terms of weight or percentage concentration.

Non-Oral Products

Non-oral product labels and labeling must state the names of added substances (as defined in *General Notices, 5.20 Added Substances*) in compliance with 21 CFR §201.100(b)(5).

Salts of Drugs

It is an established principle that official articles must have only one official title (see *General Notices, 2.20 Official Articles* and compendial nomenclature requirements in ⟨1121⟩). For purposes of saving space on labels and because chemical symbols for the most common inorganic salts of drugs are well known to practitioners, the following alternatives are permitted in labeling official articles that are salts: HCl for hydrochloride; HBr for hydrobromide; Na for sodium; and K for potassium. The symbols Na and K are intended for use in abbreviating names of the salts of organic acids, but these symbols are not used when the word Sodium or Potassium appears at the beginning of an official title (e.g., Phenobarbital Na is acceptable, but Na Salicylate is not).

Special Capsules and Tablets

The label of any form of capsule or tablet intended for administration other than by swallowing intact must bear a prominent indication of the manner in which it should be used (see *Compendial Nomenclature, Nomenclature Guidelines* on the USP website at www.usp.org).

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Products That Contain Vitamins

The vitamin content of a drug product must be stated on the label in metric units per dosage unit. The amounts of vitamins A, D, and E may also be stated in USP Units. Quantities of vitamin A declared in metric units refer to the equivalent amounts of retinol (vitamin A alcohol).

Controlled Room Temperature

Articles may be labeled for storage at "controlled room temperature" or at "20° to 25°", or other equivalent wording based on the same mean kinetic temperature. All three labeling options must ensure not to exceed the mean kinetic temperature of 25° with excursions between 15° and 30° (see ⟨659⟩).

Light-Resistant Container

When an opaque covering is used to provide protection from light for a light-sensitive product packaged in a clear or colorless or translucent container, the label of the container bears a statement that the opaque covering is needed until the contents are to be used or administered (see *Containers—Performance Testing* ⟨671⟩, *Spectral Transmission* and ⟨659⟩).

Single-Unit Container

Each single-unit container must be labeled to indicate the identity; quantity and/or strength; name of the manufacturer, packer, or distributor; lot number; and expiration date of the article (see ⟨659⟩).

Single-Dose Container

When space permits a single-dose container must be labeled as such, and should include on the label appropriate discard instructions (see ⟨659⟩).

Multiple-Dose Container

When space permits, a multiple-dose container must be labeled as such (see ⟨659⟩).

Unit-of-Use Container

A unit-of-use container must be labeled as such (see ⟨659⟩).

Protection from Freezing

The container label must bear an appropriate instruction to protect the article from freezing if subject to loss of strength or potency, or to destructive alteration of its characteristics (see ⟨659⟩).

Prescription Container Labeling

At a minimum, a prescription container must be labeled in a patient-centered manner. The label must contain essential information that is important for the patient's safe and effective use of the medicine. Labels should be designed and formatted to optimize readability and understanding (see *Prescription Container Labeling* ⟨17⟩).

GENERAL LABELING

Users are reminded to always refer to the *General Notices* in assessing or applying any compendial standards. *General Notices* addresses a number of labeling-related aspects, including *3.20 Indicating Conformance* (when an article may be labeled *USP*, *NF*, or *USP–NF*, and requirements related to differences in identity, naming, strength, quality, or purity); *General Notices, 5.20.20 Added Substances (Excipients and Ingredients) in Official Products*; *6.70 Reagents*; and *8.240 Weights and Measures* (e.g., microgram may be represented as either µg or mcg. For labeling or prescribing purposes, only "mcg" is to be used).

LABELS AND LABELING FOR ANIMAL DRUG PRODUCTS

This section provides exceptions or additions to the previous requirements in this chapter. The following requirements are specific to the labeling for animal drug products.

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Definitions

The written printed or graphic matter on the outer shipping container for animal drugs is considered to be part of "labeling". Shipping labeling for animal drugs should contain, at minimum, product identification, lot number, expiration date, and conditions for storage and handling.

Labeling for Animal Drug Products

The labeling for animal drugs should identify the animal species and, if applicable, specific subset(s) of the animal species for which the drug is approved, conditionally approved, or indexed. In the case of a compounded preparation for animals, the labeling should indicate that "This is a compounded preparation."

The labeling for prescription animal drug products must include the following statement:

- "Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian."

Labeling for Injectable Animal Drug Products

Labeling for injectable animal drug products includes the following information:

- A statement limiting the time frame in which the container may be used once it has been entered (e.g., needle-punctured), provided it is held under the labeled storage conditions.

Quantity and Total Volume for Single- and Multiple-Dose Injectable Animal Drug Products

Because of considerable variability in body weight within and among animal species, most injectable animal drugs are approved to be dosed on a mg/kg body weight basis. Thus, strength on labeling for single- and multiple-dose injectable animal drugs should be expressed on the basis of quantity per milliliter (quantity/mL), usually mg/mL. An exception would be for single-dose injectable animal drugs that are dosed regardless of the weight of the animal, in which case strength on labeling should be expressed as quantity per total volume (e.g., 50 mg/5 mL). For single-dose containers holding less than 1 mL, strength on labeling should be expressed as quantity per fraction of a mL (e.g., 12.5 mg/0.625 mL).

Dry solids that must be constituted should provide on labeling the total quantity followed by the quantity per milliliter (quantity/mL) after constitution.

Compounded Veterinary Preparations

The label on the official compounded veterinary preparation must include the word "compounded" after the drug portion of a non-proprietary name and the word "veterinary" at the end of the full official name (e.g., Atenolol Compounded Oral Suspension, Veterinary).

⟨11⟩ USP REFERENCE STANDARDS

Reference Standards provided by the United States Pharmacopeial Convention (USP Reference Standards, or RS) are highly characterized specimens reflective of specified drugs and foods (drug substances, biologics, excipients, dietary supplements, food ingredients, impurities, degradation products, reagents, and performance verification standards). When approved as suitable for use as comparison standards for documentary tests or assays (i.e., as a monograph component) in the *United States Pharmacopeia (USP)* or *National Formulary (NF)*, USP RS also assume official status and legal recognition in the United States. Assessment of the suitability for use in other applications rests with the user. Official USP RS are primary standards in jurisdictions that so recognize them as such and, when appropriate, are calibrated relative to international reference materials such as those provided by the World Health Organization. USP RS are never intended for therapeutic use. USP's RS are provided for legal metrology purposes and can help ensure comparability of results and traceability to Système International d'Unités (SI) units whether certified or not. USP RS are Reference Materials as defined in the *International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM)*: 3rd Edition 2007.

TYPES OF REFERENCE STANDARDS

Reference Standards for *USP* or *NF* Articles

Reference Standards for official articles in *USP* or *NF* are provided as pure materials or as mixtures of chemicals reflective of the corresponding drug substances or excipients. The use of these materials is specified in the article's monograph, and these materials generally are necessary for use in the *Assay* and/or the *Identification* tests. The suitability of a USP RS for uses outside those specified in a monograph is the responsibility of the user. The property value or calculation value of the Reference Standard is stated on the label and should be included in calculations used in the monograph and applicable general chapters. For Reference Standards that do not bear a property value or calculation value on the label or in accompanying documentation, assume the Reference Standard is 100.0% pure for compendial quantitative applications.

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Impurity Reference Standards

Reference Standards for impurities may include the following:

- Organic impurities that may arise either during the manufacturing process or during the shelf-life storage of an article and may include starting materials, intermediates, by-products, reagents, catalysts, and/or degradation products.
- Inorganic impurities that normally result from a synthesis process and may include reagents, catalysts, heavy metals, or inorganic salts
- Residual solvents that may be either inorganic or organic liquids that are used to prepare solutions or suspensions during the synthesis of an article

Impurity Reference Standards may be presented as purified single-component materials or as mixtures of more than one impurity. Other options for controlling impurities may include presenting the official article with a labeled impurity content; using relative chromatographic retention times and response factors; or providing theoretical values such as UV absorptivities at selected wavelengths.

In earlier editions of the compendium, impurities were designated by their chemical names. For ease of indexing and searching, these have been gradually replaced with the designation "X related compound Y RS", where X is the name of the official article, and Y is a sequential alphabetical letter. The assignment of this letter does not necessarily match the naming schemes of other compendia. Reference Standard impurity mixtures may also be designated by their intended use, such as "X System Suitability RS". The conventional names and the chemical names are reproduced in the catalog and on the RS product label.

Certified Reference Materials

USP's Certified Reference Materials (CRMs) are Reference Standards that provide certified property values with associated uncertainties and metrological traceability, in accordance with International Organization for Standardization (ISO) Guides 30–35. Correct use of these CRMs support traceability of results to SI units and comparability of procedures.

USP Reference Standards for Biologicals

USP provides RS for biologic drugs and ancillary materials. USP RS for biologicals may diverge in unitage, by definition, or otherwise from other internationally recognized standards as noted in *General Notices 5.50.10 Units of Potency (Biological)*. Unless so noted in the documentary standard, international reference standards generally are not interchangeable and the USP RS is required in the tests and assays of *USP–NF*.

NF Reference Standards

Reference Standards currently labeled as "NF Reference Standards" are intended to be designated and labeled as "USP Reference Standards" pursuant to the consolidation of *USP* and *NF* within the USP as of January 2, 1975. Where a USP Reference Standard is called for, the corresponding substance labeled as an "NF Reference Standard" may be used.

Transition of Authentic Substances to USP Reference Standards

Previously, highly characterized reference materials not required for use in a *USP–NF* monograph or general chapter were developed by USP as a service and were distributed as Authentic Substances (AS). AS typically are highly characterized chemicals that are collaboratively tested and made available as a service primarily to analytical, clinical, pharmaceutical, and research laboratories. Such materials may be used for identification, method development, evaluation of method performance, or other applications as found suitable and validated by the user. USP will no longer introduce materials labeled "Authentic Substances." All reference materials released, whether or not required for use in a *USP–NF* monograph or general chapter, will be "USP Reference Standards."

Authentic Visual References

Authentic Visual References are USP Reference Standards, but unlike chemical reference materials, Authentic Visual References (AVR) are not used in chemical analyses. Instead, AVR are visual images used by analysts to compare certain test articles to ensure that they meet compendial requirements. AVR are incorporated by reference into the monograph.

USP Performance Verification Test Standards

These materials are provided to analyze and where appropriate to facilitate adjustment of the operation of an instrument to ensure that the results obtained are accurate and/or precise or otherwise give acceptable results. The use of these Reference Standards is generally described in associated general test chapters and allied information.

APPLICATIONS OF USP REFERENCE STANDARDS

Official applications of USP RS are specified in *USP–NF* monographs and general chapters. They include the following:

- quantitative uses in assays for drug substances and formulations, limit tests, or blanks and controls

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- qualitative uses, (e.g., identification tests, system suitability tests, or chromatographic peak markers)
- method-specific uses, (e.g., performance verification standards, AVR, melting point standards, and the particle count set)

As described above, USP also provides Authentic Substances, not specified for use in a *USP* monograph or general chapter, which are used at the user's discretion.

PACKAGING

The amount of material per individual USP RS container depends on the compendial application of the standard and is generally sufficient for several replicates. Some standards (mainly materials with significant handling requirements or materials that are available only in small amounts) are provided in single-use containers. Such single-use products generally are lyophilized, and their content is labeled in mass or activity units per container. If so labeled, the content of the container should be reconstituted in its entirety without any additional weighing. Instructions for reconstitution are given either on the label or in the monographs where the standard is used.

LABELING

The label text provides all the information needed for the correct storage and use of the USP RS in monograph applications. The label includes directions for use, safety warnings, required information for controlled substances, and a property value or calculation value for standards with quantitative applications. For performance verification standards, acceptance ranges are provided. Where necessary, USP RS are accompanied by additional documentation such as Technical Data Sheets or Typical Chromatograms.

Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP RS should be used in accordance with the instructions on the label of the Reference Standard. Material Safety Data Sheets for all USP reference materials are available on the USP Web site.

Although USP RS undergo retesting on a predefined schedule to determine continued suitability for use, USP RS do not carry an expiration date on the label. A lot of USP RS may be used in its official applications as long as it is listed as "Current Lot" in the current USP Reference Standards Catalog or has not reached its Valid Use Date. Upon depletion, the lot is designated in the catalog as "Previous Lot" and a "Valid Use Date" is assigned. USP publishes the Catalog of Reference Standards bimonthly. The most current version of the catalog can be found on the USP Web site at www.usp.org. The user is responsible for ascertaining before use that the USP RS lot of interest currently carries official status, either as a "Current Lot" or as a "Previous Lot" within the Valid Use Date.

PROPER USE

Many compendial tests and assays are based on comparison of a test specimen with a USP RS. In such cases, measurements are made on preparations of both the test specimen and the Reference Standard. Where it is directed that a Standard solution or a Standard preparation be prepared for a quantitative determination by stepwise dilution or otherwise, it is intended that the Reference Standard substance be accurately weighed (see *Balances* ⟨41⟩ and *Volumetric Apparatus* ⟨31⟩). Due account should also be taken of the potential errors associated with weighing small masses (see also *General Notices, 6.50.20.1 Adjustments to Solutions*). Reference Standards that are defined on a content-per-container basis are an exception, as noted above.

USP RS instructions for use include the following:

- **As Is:** Use without any prior treatment or correction for volatiles. This is the preferred option, and is selected whenever valid data indicate that the volatiles content is constant over time.
- **Dry Before Use:** Use immediately after drying under stated conditions. Drying should not be performed in the original container. A portion of the material should be transferred to a separate drying vessel.
- **Determine Water Content Titrimetrically At Time of Use:** Use with a correction for the water content or the loss on drying, determined on a separate portion of material. Where the titrimetric determination of water is required at the time a Reference Standard is to be used, proceed as directed for *Method I* under *Water Determination* ⟨921⟩. Instrumental or microanalytical methods are acceptable for this purpose. When using typical amounts (about 50 mg of the Reference Standard), titrate with a 2- to 5-fold dilution of the reagent. Where the determination of the loss on drying on a separate portion of USP RS is required, proceed as directed on the label. Sample sizes smaller than those required in the general test chapter *Loss on Drying* ⟨731⟩ may be used for a USP RS provided that the user can obtain a sufficiently accurate result.

Whenever the labeled directions for use require drying or a correction for volatiles, it should be performed at the time of use. Further experimental details should be controlled by the user's Standard Operating Procedures and good laboratory practices.

STORAGE

USP RS should be stored in the packaging configuration provided by USP (e.g., vials that are packaged in hermetically sealed bags). When special storage conditions are specified, label directions should be followed. Unopened vials should be stored as indicated on the label. The user is responsible for ensuring that the contents of opened vials continue to be suitable for their intended use and that value assignment and uncertainty information are maintained.

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Apparatus for Tests and Assays

⟨17⟩ PRESCRIPTION CONTAINER LABELING

INTRODUCTION

This chapter applies to labeling instructions and information on prescription containers that are directly dispensed to the patient to promote better patient understanding. These standards do not apply when a prescription drug will be administered to a patient by licensed personnel who are acting within their scope of practice. Medication misuse has resulted in more than 1 million adverse drug events per year in the United States. Patients' best source (and often only source) of information regarding the medications they have been prescribed is on the prescription container label. Although other written information and oral counseling may be available, the prescription container label must fulfill the professional obligations of the prescriber and pharmacist. These obligations include giving patients the essential information they will need to understand how to safely and appropriately use the medication and how to adhere to the prescribed medication regimen.

Inadequate understanding of prescription directions for use and auxiliary information provided on dispensed containers is widespread. Studies have found that 46% of patients misunderstood one or more dosage instructions, and 56% misunderstood one or more auxiliary warnings. The problem of misunderstanding is particularly common and troublesome in patients with low or marginal literacy and in patients receiving multiple medications that are scheduled for administration using complex, nonstandardized time periods. In one study, patients with low literacy were 34 times more likely to misinterpret prescription medication warning labels than patients with adequate literacy. However, even patients with adequate literacy often misunderstand common prescription directions and warnings. In addition, there is great variability in the actual auxiliary warning and supplemental instructional information that have been applied by individual practitioners to the same prescription. The specific evidence to support a given auxiliary statement often is unclear, and patients often ignore such information. The need for auxiliary label information requires further study in comparison to explicit, simplified language alone.

Lack of universal standards for labeling on dispensed prescription containers is a root cause of patient misunderstanding, nonadherence, and medication errors.

USP developed patient-centered label standards for the format, appearance, content, and language of prescription medication instructions to promote patient understanding. These recommendations form the basis of this general chapter.

PRESCRIPTION CONTAINER LABEL STANDARDS TO PROMOTE PATIENT UNDERSTANDING

Organize the Prescription Label in a Patient-Centered Manner

Information shall be organized in a way that best reflects how most patients seek out and understand medication instructions. Prescription container labeling should feature only the most important patient information needed for safe and effective understanding and use.

Emphasize Instructions and Other Information Important to Patients

Prominently display information that is critical for the patient's safe and effective use of the medicine. At the top of the label, specify the patient's name, the drug name (spell out full generic and brand name) and drug strength, and explicit clear directions for use in simple language.

The prescription directions should follow a standard format so that the patient can expect that each element will be presented in a specific, predictable order each time a prescription is received. Use of a methodology that simplifies the administration instructions for the patient's medication should improve the individual's ability to understand prescription instructions, to take the correct dose, and to organize multi-drug regimens. Employing best practices of patient-centered instructions—which utilize the principles of health literacy, medication therapy management, and education, to explicitly describe how to take daily-use, solid dose-form medications—has resulted in improved patient understanding. One such patient-centered method is the universal medication schedule (UMS). The UMS shifts medication-taking into four standardized time periods (morning, noon, evening, bedtime) and uses simplified language and formatting to promote understanding (e.g., "take 1 pill in the morning and 1 pill at bedtime" rather than "take one tablet twice daily"). Such methods may be particularly useful for simplifying daily medication regimens that include multiple oral therapies.

[NOTE—The word "pill" is used in the UMS to enhance health literacy and may not reflect a USP definition for an oral dosage form (see *Compendial Nomenclature, USP Nomenclature Guidelines* on the USP website at www.usp.org/usp-nf/development-process).]

When oral liquid dosage forms are prescribed, the appropriate dosing component (e.g., oral syringe, dosing cup) shall be provided to the patient or caregiver to accurately measure and administer the oral medication. The graduations on the component shall be legible and indelible, and the associated volume markings shall be in metric units and limited to a single measurement scale that corresponds with the dose instructions on the prescription container label (see *Packaging and Storage Requirements* ⟨659⟩).

Other, less-critical but important content (e.g., pharmacy name and phone number, prescriber name, fill date, refill information, expiration date, prescription number, drug quantity, physical description, and evidence-based auxiliary information) should not supersede critical patient information. Such less-critical information should be placed away from dosing

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instructions (e.g., at the bottom of the label or in another less prominent location) because it can distract patients, which can impair their recognition and understanding.

Simplify Language

The language on the label should be clear, simplified, concise, and familiar, and should be used in a standardized manner. Only common terms and sentences should be used. Do not use unfamiliar words (including Latin terms) or medical jargon.

Use of readability formulas and software is not recommended for simplifying short excerpts of text such as those on prescription labels. Instead, use simplified, standardized sentences that were developed by seeking feedback from samples of diverse consumers. Such language will promote correct understanding of the instructions.

Give Explicit Instructions

Instructions for use (i.e., the SIG or signatur) should clearly separate the dose itself from the timing of each dose in order to explicitly convey the number of dosage units to be taken and when (e.g., specific time periods each day such as morning, noon, evening, and bedtime). Instructions shall include specifics on time periods. Do not use alphabetic characters for numbers. For example, write "Take 2 tablets in the morning and 2 tablets in the evening" rather than "Take two tablets twice daily").

Whenever available, use standardized directions (e.g., write "Take 1 tablet in the morning and 1 tablet in the evening" if the prescription reads b.i.d.). Vague instructions based on dosing intervals such as twice daily or 3 times daily, or hourly intervals such as every 12 hours, generally should be avoided because such instructions are implicit rather than explicit, they may involve numeracy skills, and patient interpretation may differ from prescriber intent. Although instructions that use specific hourly times (e.g., 8 a.m. and 10 p.m.) may seem to be more easily understood than implicit vague instructions, recommending dosing at precise hours of the day is less readily understood and may present greater adherence issues (due to individual lifestyle patterns such as shift work) than more general time frames such as in the morning, in the evening, after breakfast, with lunch, or at bedtime. Consistent use of the same terms should help avoid patient confusion. A set of standardized, explicit instructions (the universal medication schedule, UMS) were developed and tested in English and other languages to improve patient understanding.[1]

Ambiguous directions such as "take as directed" should be avoided unless clear and unambiguous supplemental instructions and counseling are provided (e.g., directions for use that will not fit on the prescription container label). A clear statement referring the patient to such supplemental materials should be included on the container label.

Include Purpose for Use

If the purpose of the medication is included on the prescription, it should be included on the prescription container label unless the patient prefers that it not appear. Practitioners should always ask patients their preference when writing the prescription. Confidentiality and FDA approval for intended use (e.g., labeled versus off-label use) may limit inclusion of the purpose on labels. Current evidence supports inclusion of purpose-for-use language in clear, simple terms (e.g., "for high blood pressure" rather than "for hypertension").

Limit Auxiliary Information

Auxiliary information on the prescription container label should be evidence-based and presented in simple explicit language that is minimized to avoid distracting patients with nonessential information. Most patients, particularly those with limited literacy, pay little attention to auxiliary information. The information should be presented in a standardized manner and should be critical for patient understanding and safe medication use (e.g., warnings and critical administration alerts). Icons are frequently misunderstood by patients. In addition, icons that provide abstract imagery for messages that are difficult to depict visually may be ineffective at improving understanding compared with simplified text alone. Use only icons for which there is adequate evidence, through consumer testing, that they improve patient understanding about correct use. Evidence-based auxiliary information, both text and icons, should be standardized so that it is applied consistently and does not depend on individual practitioner choice.

Address Limited English Proficiency

Whenever possible, the directions for use on a prescription container label should be provided in the patient's preferred language. Otherwise there is a risk of misinterpretation of instructions by patients with limited English proficiency, which could lead to medication errors and adverse health outcomes. Additionally, whenever possible, directions for use should appear in English as well, to facilitate counseling; the drug name(s) shall be in English so that emergency personnel and other intermediaries can have quick access to the information. Standardized translations of universal medication schedule instructions are available.[2]

Translations of prescription medication labels should be produced using a high-quality translation process. An example of a high-quality translation process is:

- Translation by a trained translator who is a native speaker of the target language
- Review of the translation by a second trained translator and reconciliation of any differences

[1] Explicit and Standardized Prescription Medicine Instructions. December 2014. Agency for Healthcare Research and Quality, Rockville, MD. http://www.ahrq.gov/professionals/quality-patient-safety/pharmhealthlit/prescriptionmed-instr.html.

[2] Available at http://www.ahrq.gov/professionals/quality-patient-safety/pharmhealthlit/prescriptionmed-instr.html.

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- Review of the translation by a pharmacist who is a native speaker of the target language and reconciliation of any differences
- Testing of comprehension with target audience

If a high-quality translation process cannot be provided, labels should be printed in English, with the use of trained interpreter services whenever possible to ensure patient comprehension. The use of computer-generated translations should be limited to programs with demonstrated quality because dosage instructions can be translated inconsistently or incorrectly, which is potentially hazardous. Standardized translated instructions and technology advances are needed to ensure the accuracy and safety of prescription container labeling for patients with limited English proficiency.

Improve Readability

Labels should be designed and formatted so they are easy to read. Currently, no strong evidence supports the superiority, in terms of legibility, of serif versus sans serif typefaces, therefore simple uncondensed fonts of either type can be used.

Optimize typography by using the following techniques:

- High-contrast print (e.g., black print on white background)
- Simple, uncondensed familiar fonts with sufficient space within letters and between letters (e.g., Times New Roman or Arial)
- Sentence case (i.e., punctuated like a sentence in English: Initial capital letter followed by lower-case words except proper nouns)
- Large font size (e.g., minimum 12-point Times New Roman or 11-point Arial) for critical information. Note that point size is not the actual size of the letter, so two fonts with the same nominal point size can have different actual letter sizes. The height of the typeface, x-height, has been used as a more accurate indicator of apparent size than point size. For example, for a given point size, the x-height and apparent size of Arial are actually bigger than those for Times New Roman. Do not use type smaller than 10-point Times New Roman or the equivalent size in another font. Older adults, in particular, have difficulty reading small print.
- Adequate white space between lines of text (25%–30% of the point size)
- White space to separate sections on the label such as directions for use versus pharmacy information
- Horizontal text only

Other measures that can also improve readability:

- Minimize the need to turn the container in order to read lines of text
- Never truncate or abbreviate critical information
- Use highlighting, bolding, and other cues to preserve readability (e.g., high-contrast print and light color for highlighting) and emphasize patient-centered information or information that facilitates adherence (e.g., refill ordering)
- Limit the number of colors used for highlighting (i.e., no more than two)
- Use of separate lines to distinguish when each dose should be taken

Alternative-Access Methods to Address Visual Impairment

Patients with visual impairment who are unable to read printed prescription container labels often report inadvertently taking the wrong medication or amount, or taking it at the wrong time or under the wrong instructions, compromising their own safety. Similarly, if a caregiver is visually impaired, the patient they care for is at risk for medication errors. The magnitude of this problem increases with aging, as the risk of visual impairment and the number of prescribed medications both increase with age.

- Follow standards for patient-centered prescription labels
- Provide alternative access for visually impaired patients (alternative-access methods include tactile, auditory, or enhanced visual systems that may employ advanced mechanics of assistive technology)[3]
- Enhance communication between the pharmacist and visually impaired patients (and their designated representatives) such that the pharmacist can explain alternative-access options and together they can identify those best suited to the patient's needs
- Once an alternative-access method is identified for the individual patient, the pharmacist shall provide the service or direct the patient to a pharmacy that offers that type of alternative access
- Ensure that duplicate accessible labels preserve the integrity of the print prescription drug container label and provide the same sequence of information as the printed label
- Follow specific best practices for each respective alternative-access format employed

⟨31⟩ VOLUMETRIC APPARATUS

Most of the volumetric apparatus available in the United States is calibrated at 20°, although the temperatures generally prevailing in laboratories more nearly approach 25°. To minimize volumetric error, the temperature should be the same for the

[3] See Working Group Recommendations from Access Board Working Group on Accessible Prescription Drug Container Labels: http://www.access-board.gov/guidelines-and-standards/health-care/about-prescription-drug-container-labels/working-group-recommendations.

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volumetric apparatus, the material being prepared, the solvents being used to prepare the volumetric solutions, the area in which they are prepared, and the final volume adjustment.

USE

To attain the degree of precision required in many Pharmacopeial assays involving volumetric measurements and directing that a quantity be "accurately measured", the apparatus must be chosen and used with care. A buret should be of such size that the titrant volume represents not less than 30% of the nominal volume. Where less than 10 mL of titrant is to be measured, a 10-mL buret or a microburet generally is required.

STANDARDS OF ACCURACY

The capacity tolerances for volumetric flasks, transfer pipets, and burets are those accepted by the National Institute of Standards and Technology (Class A),[1] as indicated in *Table 1*, *Table 2*, and *Table 3*, respectively. [NOTE—The tables in this chapter list the tolerances for the most commonly used sizes. See the referenced ASTM standards for a complete list of tolerances and other applicable criteria.] Use Class A volumetric apparatus unless otherwise specified in the individual monograph. When plastic volumetric apparatus is specified, the accepted capacity tolerances are equal to Class B glass.[2]

The capacity tolerances for measuring (i.e., "graduated") pipets of up to and including 10-mL capacity are somewhat larger than those for the corresponding sizes of transfer pipets, namely, 10, 20, and 30 µL for the 2-, 5-, and 10-mL sizes, respectively.

Transfer and measuring pipets calibrated "to deliver" should be drained in a vertical position and then touched against the wall of the receiving vessel to drain the tips. Volume readings on burets should be estimated to the nearest 0.01 mL for 25- and 50-mL burets and to the nearest 0.005 mL for 5- and 10-mL burets. Pipets calibrated "to contain" are called for in special cases, generally for measuring viscous fluids such as syrups; however, a volumetric flask may be substituted for a "to contain" pipet. In such cases, the pipet or flask should be washed clean after draining, and the washings should be added to the measured portion.

Table 1. Volumetric Flasks

Designated volume, mL	10	25	50	100	250	500	1000
Limit of error, mL	0.02	0.03	0.05	0.08	0.12	0.20	0.30
Limit of error, %	0.20	0.12	0.10	0.08	0.05	0.04	0.03

Table 2. Transfer Pipets

Designated volume, mL	1	2	5	10	25	50	100
Limit of error, mL	0.006	0.006	0.01	0.02	0.03	0.05	0.08
Limit of error, %	0.60	0.30	0.20	0.20	0.12	0.10	0.08

Table 3. Burets

Designated volume, mL	10 ("micro" type)	25	50
Subdivisions, mL	0.02	0.1	0.1
Limit of error, mL	0.02	0.03	0.05

⟨41⟩ BALANCES

This chapter states the requirements for balances used for materials that must be accurately weighed (see *General Notices, 8.20 About*). Unless otherwise specified, when substances must be "accurately weighed", the weighing shall be performed using a balance that is calibrated over the operating range and meets the requirements defined for repeatability and accuracy. For balances used for other applications, the balance repeatability and accuracy should be commensurate with the requirements for its use.

For a discussion of the theoretical basis of these requirements, see *Weighing on an Analytical Balance* ⟨1251⟩, which may be a helpful—but not mandatory—resource.

[1] See ASTM 288-06, ASTM E287-02, ASTM E1189-00, and ASTM E969-02.
[2] See ASTM E288 and ISO Standard 384.

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Change to read:

REPEATABILITY

Repeatability is assessed by weighing one test weight NLT 10 times. [NOTE—The test weight must be within the balance's operating range, but the weight need not be calibrated. Because the standard deviation is virtually independent of sample mass within the balance's capacity, use of a small test weight, which may be difficult to handle, is not required.]

Repeatability is satisfactory if ▲twice▲ (USP 1-Aug-2019) the standard deviation of the weighed value, ▲s_r,▲ (USP 1-Aug-2019) divided by the desired smallest net weight (i.e., smallest net weight that the users plan to use on that balance), does not exceed 0.10%. ▲The repeatability measurement establishes the smallest net amount of material that may be weighed on the balance in conformance with the 0.10% limit. The minimum weight, M_{min}, is described by the inequality $M_{min} \geq 2000 s_r$. For example, if s_r is found to be 0.0015, then M_{min} must be ≥ 0.3000 g or 300 mg.▲ (USP 1-Aug-2019) If the standard deviation, s, obtained is less than $0.41d$, where d is the scale ▲interval of the balance, then the inequality becomes $M_{min} \geq 2000\,(0.41d)$. For example, for a 4-place analytical balance, d is 0.0001 so that M_{min} must be ≥ 0.0820 g or 82 mg.▲ (USP 1-Aug-2019)

ACCURACY

The accuracy of a balance is satisfactory if its weighing value, when tested with a suitable weight(s), is within 0.10% of the test weight value.

A test weight is suitable if it has a mass between 5% and 100% of the balance's capacity. The test weight's maximum permissible error (mpe), or alternatively its calibration uncertainty, shall be NMT one-third of the applied test limit of the accuracy test.

[NOTE—Applicable standards are the following: American Society for Testing and Materials (ASTM) E617 (available from http://www.astm.org) and International Organization of Legal Metrology (OIML) R 111 (available from http://www.oiml.org).]

Microbiological Tests

⟨51⟩ ANTIMICROBIAL EFFECTIVENESS TESTING

INTRODUCTION

Antimicrobial preservatives are substances added to aqueous pharmaceutical products. Nonsterile dosage forms may have preservatives added to protect them from growth of microorganisms inadvertently introduced during or subsequent to the manufacturing process. In the case of sterile articles packaged in multiple-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. One or more antimicrobial preservative(s) are expected in all sterile multidose units.

Antimicrobial preservatives should not be used as a substitute for good manufacturing practices, solely to reduce the viable microbial population of a nonsterile product, or control the presterilization bioburden of a multidose formulation during manufacturing. Antimicrobial preservatives in compendial dosage forms meet the requirements for *General Notices, 5.20 Added Substances*.

All useful antimicrobial agents are toxic substances. For maximum protection of patients, the concentration of the preservative shown to be effective in the final packaged product should be below a level that may be toxic to human beings based on the recommended dosage of the medicinal product.

The concentration of an added antimicrobial preservative can be kept to a minimum if the active ingredients of the formulation possess an intrinsic antimicrobial activity. Antimicrobial effectiveness, whether inherent in the product or produced because of the addition of an antimicrobial preservative, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives. Antimicrobial effectiveness must be demonstrated for aqueous-based, multiple-dose topical and oral dosage forms and for other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids (see *Pharmaceutical Dosage Forms* ⟨1151⟩). For the purpose of the test, aqueous is defined as a water activity of more than 0.6 (see *Application of Water Activity Determination to Nonsterile Pharmaceutical Products* ⟨1112⟩).

Challenge organisms are generally based on likely contaminants to a drug product while considering its physical attributes, formulation, and intended use. The standard battery of challenge organisms described in this test need not prevent the inclusion of other species of microorganisms if deemed useful to measure the biological activity of the preservative system for a specific product. These supplemental challenge organisms are not within the scope of this chapter, but may be added in addition to the described test organisms.

GENERAL PROCEDURES

This chapter provides procedures to demonstrate the effectiveness of added antimicrobial preservatives. Such antimicrobial preservatives must be declared on the label. The procedures and acceptance criteria for effectiveness apply to a product in the original, sealed container in which it was distributed by the manufacturer (see *Table 1* for categories of products). The test need

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not be conducted in these containers, but care should be taken to avoid using materials that can interact with the preservative in the containers that are used for antimicrobial effectiveness testing.

Growth Promotion Procedure and Suitability of the Recovery Method

GENERAL CONSIDERATIONS

The ability of the procedure to detect challenge microorganisms in the presence of a suitably neutralized product to be tested must be established. The suitability of the procedure must be reconfirmed if a change is made in materials or methods or if a change is made in the product or direct product contact materials that may affect the outcome of the test.

The growth-promoting capabilities of media used in this procedure must be established.

PREPARATION OF TEST STRAINS

Use standardized suspensions of test strains or prepare as stated below. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are NMT five passages removed from the original master seed lot. Grow each of the bacterial and fungal test strains separately (see *Table 2*).

Use cultures of the following microorganisms:[1] *Candida albicans* (ATCC No. 10231), *Aspergillus brasiliensis* (ATCC No. 16404), *Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), and *Staphylococcus aureus* (ATCC No. 6538). The viable microorganisms used in the procedure should be part of a freshly growing culture (e.g., in logarithmic growth phase) with the exception of *A. brasiliensis* spores. The culture conditions for the inoculum culture are described (see *Table 2*) in which the suitable media are Soybean–Casein Digest or Sabouraud Dextrose Agar Medium.

To harvest the bacterial and *C. albicans* cultures, use sterile saline TS to wash the surface growth, and collect it in a suitable vessel. To harvest the spores of *A. brasiliensis,* use sterile saline TS containing 0.05% of polysorbate 80. The spore suspension should be aseptically treated (e.g., filtration through sterile glass wool) to remove hyphae. All microbial suspensions should be prepared to ensure that there is no carry over of residual growth medium from the inoculum (e.g., centrifugation followed by resuspension in appropriate sterile suspending fluid.)

Alternatively, the stock culture organisms may be grown in a suitable liquid medium (i.e., Soybean–Casein Digest Broth or Sabouraud Dextrose Broth) and the cells harvested by centrifugation, then washed and resuspended in appropriate sterile suspending fluid. The microbial suspensions used for inoculations should be adjusted to obtain a microbial count of about 1×10^8 cfu/mL. Use the bacterial and yeast suspensions within 2 h, or within 24 h if stored between 2° and 8°. A stable spore suspension can be prepared and then may be maintained at 2°–8° for up to 7 days. [NOTE—The estimate of inoculum concentration may be obtained by turbidimetric procedures for the challenge microorganisms and later confirmed by plate count.]

GROWTH PROMOTION OF THE MEDIA

Media used in this procedure must be capable of supporting microbial growth. Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients described.

For solid media, counts obtained must be at least 50% of the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms occurs comparable to that previously obtained with a previously tested and approved batch of medium.

Suitability of the Counting Method in the Presence of Product

Prepare a 10^{-1} dilution by adding 1 mL of product (by volume) to 9 mL of saline or other neutralizing diluent. Continue this dilution scheme to 10^{-2} and 10^{-3} dilution levels. Add an appropriate number of challenge organisms to each tube of diluted product, mix, and then plate a suitable volume from each dilution to yield less than 250 cfu/plate for bacteria and yeast (ideally between 25 and 250 cfu) or less than 80 cfu/plate for *A. brasiliensis* (ideally between 8 and 80 cfu). This plating should be performed minimally in duplicate (although a greater number of replicates can be useful to minimize variability in the plate count estimate). A positive control for this procedure is to introduce the same inocula into saline, and transfer similar volumes of saline to agar plates. A suitable recovery scheme is the one that provides at least 50% of this saline control count (averaged).

If the diluted product exhibits antimicrobial properties, specific neutralizers may need to be incorporated into the diluents or the recovery media. See *Validation of Microbial Recovery from Pharmacopeial Articles* ⟨1227⟩ for more information.

The ability of the procedure to measure preservative efficacy may be compromised if the method suitability requires significant dilution (10^{-2} or 10^{-3}) as this will affect the measured recovery (e.g., it may be difficult to measure a 3 log unit reduction for a 10^5–10^6 inoculum). If no suitable neutralizing agent or method is found and method suitability requires significant dilution, a higher level of inoculum (e.g., 10^7–10^8) may be used so that a 3 log unit reduction can be measured. Reported recovery cannot be less than 1 cfu/plate on average (or 100 cfu/mL if 1 mL is plated in duplicate at the 10^{-2} dilution).

Membrane filtration may be used to filter larger volumes of dilutions to overcome this difficulty or to assist in the neutralization of antimicrobial properties.

[1] Available from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 (http://www.atcc.org).

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Testing of Products

PRODUCT CATEGORIES

For the purpose of testing, compendial articles have been divided into four categories (see *Table 1*). The criteria of antimicrobial effectiveness for these products are a function of the route of administration. It is expected that formulations containing preservatives will meet minimal efficacy standards, whether packaged as multidoses or unit doses.

Table 1. Compendial Product Categories

Category	Product Description
1	Injections; other parenterals including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles
2	Topically used products made with aqueous bases or vehicles; nonsterile nasal products and emulsions, including those applied to mucous membranes
3	Oral products other than antacids, made with aqueous bases or vehicles
4	Antacids made with an aqueous base

PROCEDURE

The procedure can be conducted either in five original containers if a sufficient volume of product is available in each container and if the product container can be entered aseptically (i.e., needle and syringe through an elastomeric rubber stopper), or in five sterile, capped bacteriological containers [inert relative to the antimicrobial agent(s)] of suitable size into which a sufficient volume of product has been transferred. Inoculate each container with one of the prepared and standardized inocula, and mix. The volume of the suspension inoculum used is between 0.5% and 1.0% of the volume of the product to minimize potential effects on the product. The concentration of test microorganisms that is added to the product (*Category 1, 2,* or *3*) is such that the final concentration of the test preparation after inoculation is between 1×10^5 and 1×10^6 cfu/mL of the product. For *Category 4* products (antacids), the final concentration of the test preparation after inoculation is between 1×10^3 and 1×10^4 cfu/mL of the product.

The initial concentration of viable microorganisms in each test preparation is estimated based on the concentration of microorganisms in each of the standardized inocula as determined by the plate-count method. Incubate the inoculated containers at 22.5 ± 2.5°. Sample each container at the appropriate intervals (specified in *Table 3*). Record any changes observed in appearance at these intervals. Determine, by the plate-count procedure, the number of cfu present in each test preparation for the applicable intervals (see *General Procedures* in *Microbial Enumeration Tests* ⟨61⟩). Plate counts will be conducted using a minimum of duplicate plates, with the cfu averaged before determination of deduced cfu/mL. If membrane filtration is used, duplicate membrane filters will be used for each estimate. Using the calculated concentrations of cfu/mL present at the start of the test, calculate the change in $\log_{10}$ values of the concentration of cfu/mL for each microorganism at the applicable test intervals, and express the changes in concentration in terms of log reductions. The log reduction is defined as the difference between the $\log_{10}$ unit value of the starting concentration of cfu/mL in the suspension and the $\log_{10}$ unit value of cfu/mL of the survivors at that time point.

Table 2. Culture Conditions for Inoculum Preparation

Organism	Suitable Medium	Incubation Temperature	Inoculum Incubation Time	Microbial Recovery Incubation Time
Escherichia coli (ATCC No. 8739)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5°	18–24 h	3–5 days
Pseudomonas aeruginosa (ATCC No. 9027)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5°	18–24 h	3–5 days
Staphylococcus aureus (ATCC No. 6538)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5°	18–24 h	3–5 days
Candida albicans (ATCC No. 10231)	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5°	44–52 h	3–5 days
Aspergillus brasiliensis (ATCC No. 16404)	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5°	6–10 days	3–7 days

Criteria for Antimicrobial Effectiveness

The requirements for antimicrobial effectiveness are met if the criteria specified in *Table 3* are met (see *General Notices, 7. Test Results*). "No increase" in counts is defined as NMT 0.5 $\log_{10}$ unit more than the value to which it is compared.

Table 3. Criteria for Tested Microorganisms

For Category 1 Products	
Bacteria	NLT 1.0 log reduction from the initial calculated count at 7 days, NLT 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days

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Table 3. Criteria for Tested Microorganisms *(continued)*

Yeast and molds	No increase from the initial calculated count at 7, 14, and 28 days
For Category 2 Products	
Bacteria	NLT 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
Yeast and molds	No increase from the initial calculated count at 14 and 28 days
For Category 3 Products	
Bacteria	NLT 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
Yeast and molds	No increase from the initial calculated count at 14 and 28 days
For Category 4 Products	
Bacteria, yeast, and molds	No increase from the initial calculated count at 14 and 28 days

⟨55⟩ BIOLOGICAL INDICATORS—RESISTANCE PERFORMANCE TESTS

INTRODUCTION

A biological indicator (BI) is a well-characterized preparation of a specific microorganism with a known resistance to a specific sterilization process. The correct use of BIs in the development, validation, and control of sterilization processes requires that their population and resistance be accurately known. The population and resistance can be selected to confirm the adequacy of individual sterilization process conditions for an article. The recommendations of *Sterilization of Compendial Articles* ⟨1229⟩ should be followed for effective BI usage. The methods described below can be used to establish population and resistance, such that the response of the BI to the subject sterilization process is appropriate. Although the BI manufacturers are required to maintain rigorous control of population and resistance using the number of replicates as specified below, the end users are not required to use the same number of replicates for verification of those determinations. Conduct all of the tests described in this chapter under appropriate microbiological laboratory conditions (see *Microbiological Best Laboratory Practices* ⟨1117⟩).

TOTAL VIABLE SPORE COUNT

Sample Collection/Recovery

PAPER/FIBER INDICATORS

For paper/fiber carrier BIs, remove at least four test samples from their individual containers. Disperse the indicator into component fibers by placing the test samples in a sterile vessel containing 100 mL of sterilized *Purified Water* chilled to 2°–8°, and mechanically disrupt to achieve a homogeneous suspension. For self-contained BIs, aseptically remove four BI carriers from their containers and proceed as directed above.

INDICATORS ON OTHER SUBSTRATES

For all other biological indicators, remove at least four samples from their individual containers. Place the test samples in a sterile vessel containing 100 mL of sterilized *Purified Water* chilled to 2°–8°, and mechanically disrupt to achieve a homogeneous suspension of the spores in the water.

SPORE SUSPENSIONS

For spore suspensions of BIs, prepare an appropriate serial dilution of the original spore suspension in sterilized *Purified Water* chilled to 2°–8°, in a sterile container, and follow the viable spore count procedures as specified below. The requirements of the tests are met if the average number of viable spores is within 50%–300% of the labeled count of the spore suspension.

Viable Spore Count

Transfer a 10-mL aliquot of the suspension to a sterile tube. For BIs using spores of *Geobacillus stearothermophilus*, *Bacillus coagulans*, and other thermophilic spore formers, heat the tube containing the suspension in a water bath at 95°–100° for 15 min (heat shock), starting the timing when the temperature reaches 95°. For BIs containing nonthermophilic spore formers, heat the tube containing the suspension in a water bath at 80°–85° for 10 min, starting the timing when the temperature of the spore suspension reaches 80°. Cool suspensions rapidly in an ice-water bath at 0°–4°. Transfer two 1-mL aliquots to suitable tubes, and make appropriate serial dilutions in sterilized *Purified Water*. Calculate the dilutions to yield 30–300 colonies on each plate in a pair, when treated as described below. Where the BI has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution.

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Prepare a separate series of plates for each aliquot. Place 1.0 mL of each selected dilution in each of two 15 × 100-mm Petri dishes. Within 20 min, add to each plate 20 mL of *Soybean–Casein Digest Agar Medium* that has been melted and cooled to approximately 45°. Swirl to attain a homogeneous suspension, and allow it to solidify. Incubate the plates in an inverted position at 55°–60° for thermophilic spore formers and at 30°–35° for nonthermophilic spore formers or at the optimal recovery temperature specified by the manufacturer. Examine the plates after at least 48 h, recording for each plate the number of colonies. Calculate the average number of spores per test sample from the results, using the appropriate dilution factor. When evaluating vendor-supplied BIs, the viable spore count shall be between 50% and 300% of the manufacturer's stated value.

D-VALUE DETERMINATION

Apparatus

The test equipment used for the determination of microbial resistance ("D-value") is described in substantial detail in ISO 18472, Sterilization of Health Care Products—Biological and Chemical Indicators—Test Equipment (*1*). The details of individual Biological Indicator Evaluation Resistometers (BIERs) vary with the specifics of their design and the particular sterilization process for which they are used. Provided that the performance of the BIER vessel meets the requirements of the ISO standard for exposure of the BI, design differences are acceptable. For single-phase sterilization processes where an acceptable BIER has not been defined, the D-value determination can be accomplished by adapting a BIER design intended for a sterilization process operating in the same phase. There are no current methods available for D-value determination for multiple-phase sterilization processes.

Procedure

Carry out the tests for D-value at sterilization conditions consistent with those intended for use. Use 20 replicate test sample BIs in their original individual containers, subjected to at least five exposure conditions for a total of 100 tests. The number of exposure conditions is chosen to provide a range of observations from NLT one labeled D-value below the expected survival time through NLT one labeled D-value above the expected kill time. Place each group on a separate suitable sample holder that permits each sample to be exposed to the prescribed sterilizing condition at a specific location in the sterilizing chamber of the BIER. Check the BIER apparatus for operating parameters using sample holders without test samples. Select a series of sterilizing times in increments from the shortest time for the samples to be tested. The differences in sterilizing times over the series are as constant as feasible, and the difference between adjacent times is NMT 75% of the expected D-value. Test procedures for the use of BIER vessels for the evaluation of microbial resistance are defined in a series of ISO standards under the 11138 series (*2–5*). The appropriate standard should be followed for the BI. The test methods and carriers used with the BIER may be adapted to the specifics of the BI. The method and apparatus used for paper carriers may differ from those for other carriers and will be substantially different from those used for suspensions of BIs.

The D-value exposure conditions for alternative material carriers are the same as the conditions used to determine the D-value for paper carriers. If a manufacturer's label permits usage of the BI carrier with multiple sterilization methods, then data on D-value, survival time, and kill time will need to be provided by the manufacturer for each sterilization method.

For BIs that are spore suspensions, conduct D-value determinations for each of the microorganisms that are provided as a liquid spore crop suspension. The test is conducted using appropriate serial dilutions predicated upon the stated spore filter of the suspension in *Purified Water* in a sterile tube. Where the suspension is placed on or in a substrate such as an elastomeric closure or formulated product, its resistance may differ from that determined in *Purified Water*. That difference may be significant to the usage of the BIs and appropriate measurements made before use in sterilization validation activities.

Recovery

After completion of the sterilizing procedure for BIs and within a noted time (NMT 4 h), aseptically remove and add each BI to a suitable medium (see media in *Sterility Tests* ⟨71⟩) to submerge the BI completely in a suitable tube. For self-contained BIs, the paper strip is immersed in the self-contained medium according to manufacturers' instructions, within a noted time NMT 4 h. For insoluble items inoculated with a spore suspension, aseptically transfer these items individually to a suitable medium (see media in ⟨71⟩) to submerge the item completely in the medium. When a sealed aqueous filled container has been inoculated, test the units individually, as described within ⟨71⟩.

Incubate each tube at the optimal recovery temperature appropriate for the BI. Observe each inoculated medium-containing tube at appropriate intervals for a total of 7 days after inoculation. Where growth is observed at any particular observation time, further incubation of the test sample(s) concerned may be omitted. Note the number of samples showing no evidence of growth at any time.

Where *Clostridium sporogenes* or another anaerobic microorganism is used as a BI, methods for preparation, inoculation, and recovery methods and media must be adapted to accommodate the use of these anaerobic spore formers.

Calculation

The determination of D-values of BIs can be performed using the Limited Spearman-Karber, Survival Curve Method, or Stumbo-Murphy-Cochran procedures (*6–8*). When the BI has been purchased, use the same method as that defined by the BI manufacturer to subsequently determine D-values. The use of an alternate method can result in differences that are more an artifact of the method than a variation in the performance of the BI.

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Survival Time and Kill Time

Take two groups of BIs, each consisting of 10 test samples, in their original, individual containers. Place the samples of each group in suitable sample holders that permit each sample to be exposed to the sterilizing conditions at a specific location in the BIER chamber.

Expose the samples for the required survival time, enter the chamber, and remove the holder(s) containing the 10 test samples. Repeat the above procedure immediately, or preheat if a substantial interval has elapsed, so as to subject the second holder(s) containing 10 test samples similarly to the first conditions, but for the required kill time. Recover BIs as described above. The *Survival Time and Kill Time* should be provided by the BI manufacturer and verified by the end user.

REFERENCES

1. American National Standards Institute (ANSI)/Association for the Advancement of Medical Instrumentation (AAMI)/ International Organization for Standardization (ISO) 18472:2006. Sterilization of health care products—biological and chemical indicators—test equipment. 1st ed. Arlington, VA: AAMI.
2. ANSI/AAMI/ISO 11138-1:2006. Sterilization of health care products—biological indicators—part 1: general requirements. 2nd ed. Arlington, VA: AAMI.
3. ANSI/AAMI/ISO 11138-2:2006. Sterilization of health care products—biological indicators—part 2: biological indicators for ethylene oxide sterilization processes. 3rd ed. Arlington, VA: AAMI.
4. ANSI/AAMI/ISO 11138-3:2006. Sterilization of health care products—biological indicators—part 3: biological indicators for moist heat sterilization processes. 1st ed. Arlington, VA: AAMI.
5. ANSI/AAMI/ISO 11138-4:2006. Sterilization of health care products—biological indicators—part 4: biological indicators for dry heat sterilization processes. 1st ed. Arlington, VA: AAMI.
6. Pflug IJ. Syllabus for an introductory course in the microbiology and engineering of sterilization processes. 4th ed. St. Paul, MN: Environmental Sterilization Services; 1980.
7. Pflug IJ, Smith GM. The use of biological indicators for monitoring wet-heat sterilization processes. In: Gaughran ERL, Kereluk K, eds. Sterilization of medical products. New Brunswick, NJ: Johnson and Johnson; 1977:193–230.
8. Holcomb RG, Pflug IJ. The Spearman-Karber method of analyzing quantal assay microbial destruction data. In: Pflug IJ, ed. Microbiology and engineering sterilization processes. St. Paul, MN: Environmental Sterilization Services; 1979.

Add the following:

▲⟨60⟩ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS —TESTS FOR *BURKHOLDERIA CEPACIA* COMPLEX

INTRODUCTION

The tests described in this chapter will allow determination of the absence of *Burkholderia cepacia* complex (Bcc), which can be detected under the conditions described.

The tests are designed to determine whether a substance or preparation complies with an established specification for microbiological quality and/or to evaluate whether products—especially those for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use—contain members of the Bcc.

GROWTH-PROMOTING AND INHIBITORY PROPERTIES OF THE MEDIA AND SUITABILITY OF TESTS FOR ABSENCE OF Bcc

Test each batch of ready-prepared medium and each batch of medium prepared from either dehydrated medium or ingredients.

Preparation of Test Strains

Use standardized stable suspensions of test strains (see *Table 1*) NMT 5 passages removed from the original strain culture.

Table 1. Test Strains of Microorganisms for Growth Promotion and Suitability Testing

Microorganism	Standard Strain
Burkholderia cepacia	ATCC 25416, NCTC 10743, or CIP 80.24
Burkholderia cenocepacia	ATCC BAA-245 or LMG 16656
Burkholderia multivorans	ATCC BAA-247, LMG 13010, CCUG 34080, CIP 105495, DSM 13243, or NCTC 13007

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Table 1. Test Strains of Microorganisms for Growth Promotion and Suitability Testing *(continued)*

Microorganism	Standard Strain
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, or NBRC 13275
Staphylococcus aureus	ATCC 6538, NCIMB 9518, CIP 4.83, or NBRC 13276

Microorganisms

Grow each of the test strains separately in *Soybean–Casein Digest Broth* or on Soybean–Casein Digest Agar at 30°–35° for 18–24 h.

Use *Buffered Sodium Chloride–Peptone Solution pH 7.0* or *Phosphate Buffer Solution pH 7.2* to make the test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2°–8°. If purchased, follow the supplier's instructions. If self-prepared cultures are used, follow a validated procedure (such as in *Microbial Enumeration Tests* ⟨61⟩) for preparation. Use a challenge inoculum of NMT 100 colony-forming units (cfu) for growth promotion and suitability testing.

NEGATIVE CONTROLS

Include a negative control to verify the testing conditions. There must be no growth of microorganisms. A negative control is also performed when testing the products as described in *Testing of Products*.

TEST FOR GROWTH-PROMOTING PROPERTIES, SOLID MEDIA

Perform the *Surface-Spread Method* (see *Microbial Enumeration Tests* ⟨61⟩, *Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Recovery of Microorganisms in the Presence of Product, Plate-Count Methods*), inoculating each plate with a small number (NMT 100 cfu) of the appropriate microorganism (see *Table 2*). Incubate at the specified temperature for NMT the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Table 2. Microorganisms for the Growth-Promoting, Inhibitory, and Indicative Properties of the Media

Medium	Property	Microorganism
Burkholderia cepacia selective agar	Growth-Promoting and Indicative	*Burkholderia cepacia, Burkholderia cenocepacia,* or *Burkholderia multivorans*
	Inhibitory	*Pseudomonas aeruginosa, Staphylococcus aureus*

TEST FOR INHIBITORY PROPERTIES, SOLID MEDIA

Inoculate the appropriate medium with at least 100 cfu of the appropriate microorganism. Incubate at the specified temperature for NLT the longest period of time specified in the test. Inhibition of growth of the indicated microorganisms occurs (see *Table 2*).

TEST FOR INDICATIVE PROPERTIES

Perform the *Surface-Spread Method* (see *Microbial Enumeration Tests* ⟨61⟩, *Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Recovery of Microorganisms in the Presence of Product, Plate-Count Methods*), inoculating each plate with a small number (NMT 100 cfu) of the indicated microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium (see *Table 2*).

Suitability of the Test Method

The ability of the test to detect Bcc in the presence of the product to be tested must be established. The incubation time for the method suitability should not exceed the shortest incubation period specified. Suitability must be confirmed if there is a change in testing performance or a change in the product that may affect the outcome of the test.

For each new product to be tested, perform the sample preparation as described in *Testing of Products*. At the time of mixing, add each test strain in the prescribed growth medium. Inoculate the test strains individually. Use a number of microorganisms equivalent to NMT 100 cfu in the inoculated test preparation.

Perform the test as described in *Testing of Products*, using the shortest incubation period prescribed. Bcc microorganisms must be detected with the indication reactions described in *Interpretation*.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see *Microbial Enumeration Tests* ⟨61⟩, *Growth Promotion Test, Suitability of the Counting Method and Negative Controls, Suitability of the Counting Method in the Presence of Product, Neutralization/Removal of Antimicrobial Activity*).

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TESTING OF PRODUCTS

Sample Preparation and Pre-Incubation

Prepare a sample using a 1-in-10 dilution of NLT 1 g of the product to be examined. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in *Suitability of the Test Method*) of *Soybean–Casein Digest Broth* or an appropriate dilution of *Soybean–Casein Digest Broth* as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Then mix and incubate at 30°–35° for 48–72 h.

Selection and Subculture

Subculture by streaking on a plate of *Burkholderia cepacia* selective agar (BCSA), and incubate at 30°–35° for 48–72 h.

Interpretation

The possible presence of Bcc is indicated by the growth of greenish–brown colonies with yellow halos, or white colonies surrounded by a pink–red zone on BCSA. Any growth on BCSA is confirmed by identification tests. See *Microbial Characterization, Identification, and Strain Typing* ⟨1113⟩ for additional information.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

RECOMMENDED CULTURE MEDIA

[NOTE—This section is given for information.]

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the tests in this Pharmacopeia. Other media may be used provided that their suitability can be demonstrated.

Stock Buffer Solution

Transfer 34 g of potassium dihydrogen phosphate to a 1000-mL volumetric flask, dissolve in 500 mL of *Purified Water*, adjust with sodium hydroxide to a pH of 7.2 ± 0.2, add *Purified Water* to volume, and mix. Dispense in containers, and sterilize. Store at a temperature of 2°–8°.

Phosphate Buffer Solution pH 7.2

Prepare a mixture of *Purified Water* and *Stock Buffer Solution* (800:1 v/v), and sterilize.

Buffered Sodium Chloride–Peptone Solution pH 7.0

Prepare *Buffered Sodium Chloride–Peptone Solution pH 7.0* as directed in *Table 3*. Sterilize in an autoclave using a validated cycle.

Table 3

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g (equivalent to 0.067 M of phosphate)
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified Water	1000 mL

Soybean–Casein Digest Broth

Prepare *Soybean–Casein Digest Broth* as directed in *Table 4*. Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Table 4

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Dibasic hydrogen phosphate	2.5 g

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Table 4 *(continued)*

Glucose monohydrate	2.5 g
Purified Water	1000 mL

Burkholderia cepacia Selective Agar

Prepare BCSA as directed in *Table 5*. When preparing media in-house, first prepare the base ingredients without the antibiotics. Adjust the pH so that after sterilization it is 6.8 ± 0.3 at 25°. Sterilize in an autoclave using a validated cycle. Cool the base medium to 45°–50° and add a 1% solution of the sterile filtered antibiotics, mix, and pour into the plates.

Table 5

Casein peptone	10.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Yeast extract	1.5 g
Phenol red	0.08 g
Gentamicin	10.0 mg
Vancomycin	2.5 mg
Crystal violet	2.0 mg
Polymyxin B	600,000 U
Agar	14.0 g
Demineralized water	1000 mL▲ (USP 1-Dec-2019)

⟨61⟩ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS

INTRODUCTION

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken, and interpret the results as stated below.

The methods are not applicable to products containing viable microorganisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopeial method has been demonstrated.

GENERAL PROCEDURES

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any microorganisms that are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is, insofar as possible, removed or neutralized. If inactivators are used for this purpose, their efficacy and their absence of toxicity for microorganisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with any inactivators used must be demonstrated.

ENUMERATION METHODS

Use the *Membrane Filtration* method or one of the *Plate-Count Methods*, as directed. The *Most-Probable-Number (MPN) Method* is generally the least accurate method for microbial counts; however, for certain product groups with very low bioburden, it may be the most appropriate method.

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The choice of a method is based on factors such as the nature of the product and the required limit of microorganisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

GROWTH PROMOTION TEST, SUITABILITY OF THE COUNTING METHOD AND NEGATIVE CONTROLS

General Considerations

The ability of the test to detect microorganisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance or a change in the product that may affect the outcome of the test, is introduced.

Preparation of Test Strains

Use standardized stable suspensions of test strains or prepare as stated below. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in *Table 1*.

Table 1. Preparation and Use of Test Microorganisms

		Growth Promotion		Suitability of Counting Method in the Presence of Product	
Microorganism	**Preparation of Test Strain**	**Total Aerobic Microbial Count**	**Total Yeasts and Molds Count**	**Total Aerobic Microbial Count**	**Total Yeasts and Molds Count**
Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83, or NBRC 13276	Soybean–Casein Digest Agar or Soybean–Casein Digest Broth 30°–35° 18–24 hours	Soybean–Casein Digest Agar and Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days		Soybean–Casein Digest Agar/MPN Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days	
Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118, or NBRC 13275	Soybean–Casein Digest Agar or Soybean–Casein Digest Broth 30°–35° 18–24 hours	Soybean–Casein Digest Agar and Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days		Soybean–Casein Digest Agar/MPN Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days	
Bacillus subtilis such as ATCC 6633, NCIMB 8054, CIP 52.62, or NBRC 3134	Soybean–Casein Digest Agar or Soybean–Casein Digest Broth 30°–35° 18–24 hours	Soybean–Casein Digest Agar and Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days		Soybean–Casein Digest Agar/MPN Soybean–Casein Digest Broth ≤100 cfu 30°–35° ≤3 days	
Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72, or NBRC 1594	Sabouraud Dextrose Agar or Sabouraud Dextrose Broth 20°–25° 2–3 days	Soybean–Casein Digest Agar ≤100 cfu 30°–35° ≤5 days	Sabouraud Dextrose Agar ≤100 cfu 20°–25° ≤5 days	Soybean–Casein Digest Agar ≤100 cfu 30°–35° ≤5 days MPN: not applicable	Sabouraud Dextrose Agar ≤100 cfu 20°–25° ≤5 days
Aspergillus brasiliensis such as ATCC 16404, IMI 149007, IP 1431.83, or NBRC 9455	Sabouraud Dextrose Agar or Potato–Dextrose Agar 20°–25° 5–7 days, or until good sporulation is achieved	Soybean–Casein Digest Agar ≤100 cfu 30°–35° ≤5 days	Sabouraud Dextrose Agar ≤100 cfu 20°–25° ≤5 days	Soybean–Casein Digest Agar ≤100 cfu 30°–35° ≤5 days MPN: not applicable	Sabouraud Dextrose Agar ≤100 cfu 20°–25° ≤5 days

Use *Buffered Sodium Chloride–Peptone Solution pH 7.0* or *Phosphate Buffer Solution pH 7.2* to make test suspensions; to suspend *A. brasiliensis* spores, 0.05% of polysorbate 80 may be added to the buffer. Use the suspensions within 2 hours, or within 24 hours if stored between 2° and 8°. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *A. brasiliensis* or *B. subtilis,* a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period of time.

Negative Control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of microorganisms. A negative control is also performed when testing the products as described under *Testing of Products*. A failed negative control requires an investigation.

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Growth Promotion of the Media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of *Soybean–Casein Digest Broth* and *Soybean–Casein Digest Agar* with a small number (not more than 100 cfu) of the microorganisms indicated in *Table 1*, using a separate portion/plate of medium for each. Inoculate plates of *Sabouraud Dextrose Agar* with a small number (not more than 100 cfu) of the microorganisms indicated in *Table 1*, using a separate plate of medium for each. Incubate according to the conditions described in *Table 1*.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Suitability of the Counting Method in the Presence of Product

PREPARATION OF THE SAMPLE

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, a suitable alternative procedure must be developed.

Water-Soluble Products—Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in *Buffered Sodium Chloride–Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2,* or *Soybean–Casein Digest Broth*. If necessary, adjust to a pH of 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Nonfatty Products Insoluble in Water—Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in *Buffered Sodium Chloride–Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2,* or *Soybean–Casein Digest Broth*. A surface-active agent such as 1 g per L of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary, adjust to a pH of 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Fatty Products—Dissolve in isopropyl myristate sterilized by filtration, or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another noninhibitory sterile surface-active reagent heated, if necessary, to not more than 40° or, in exceptional cases, to not more than 45°. Mix carefully and if necessary maintain the temperature in a water bath. Add a sufficient quantity of the prewarmed chosen diluent to make a 1 in 10 dilution of the original product. Mix carefully, while maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial 10-fold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another noninhibitory sterile surface-active reagent.

Fluids or Solids in Aerosol Form—Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

Transdermal Patches—Remove the protective cover sheets ("release liners") of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with a suitable sterile porous material (e.g., sterile gauze) to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 minutes.

INOCULATION AND DILUTION

Add to the sample prepared as directed above and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than than 100 cfu. The volume of the suspension of the inoculum should not exceed 1% of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution, or filtration.

NEUTRALIZATION/REMOVAL OF ANTIMICROBIAL ACTIVITY

The number of microorganisms recovered from the prepared sample diluted as described in *Inoculation and Dilution* and incubated following the procedure described in *Recovery of Microorganisms in the Presence of Product,* is compared to the number of microorganisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example,

1. An increase in the volume of the diluent or culture medium;
2. Incorporation of a specific or general neutralizing agents into the diluent;
3. Membrane filtration; or
4. A combination of the above measures.

Neutralizing Agents—Neutralizing agents may be used to neutralize the activity of antimicrobial agents (see *Table 2*). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for microorganisms must be demonstrated by carrying out a blank with neutralizer and without product.

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Table 2. Common Neutralizing Agents/Methods for Interfering Substances

Interfering Substance	Potential Neutralizing Agents/Method
Glutaraldehyde, mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, alcohol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary ammonium compounds (QACs), parahydroxybenzoates (parabens), bis-biguanides	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, halogens, aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the microorganism. However, it is possible that the product inhibits only some of the microorganisms specified herein, but does not inhibit others not included among the test strains or those for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

RECOVERY OF MICROORGANISMS IN THE PRESENCE OF PRODUCT

For each of the microorganisms listed, separate tests are performed. Only microorganisms of the added test strain are counted.

Membrane Filtration—Use membrane filters having a nominal pore size not greater than 0.45 µm. The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the microorganisms listed, one membrane filter is used.

Transfer a suitable quantity of the sample prepared as described under *Preparation of the Sample, Inoculation and Dilution,* and *Neutralization/Removal of Antimicrobial Activity* (preferably representing 1 g of the product, or less if large numbers of cfu are expected) to the membrane filter, filter immediately, and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of the *Soybean–Casein Digest Agar.* For the determination of total combined yeasts and molds count (TYMC), transfer the membrane to the surface of the *Sabouraud Dextrose Agar.* Incubate the plates as indicated in *Table 1.* Perform the counting.

Plate-Count Methods—Perform plate-count methods at least in duplicate for each medium, and use the mean count of the result.

Pour-Plate Method—For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under *Preparation of the Sample, Inoculation and Dilution,* and *Neutralization/Removal of Antimicrobial Activity* and 15 to 20 mL of *Soybean–Casein Digest Agar* or *Sabouraud Dextrose Agar,* both media maintained at not more than 45°. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the microorganisms listed in *Table 1,* at least two Petri dishes are used.

Incubate the plates as indicated in *Table 1.* Take the arithmetic mean of the counts per medium, and calculate the number of cfu in the original inoculum.

Surface-Spread Method—For Petri dishes 9 cm in diameter, add 15 to 20 mL of *Soybean–Casein Digest Agar* or *Sabouraud Dextrose Agar* at about 45° to each Petri dish, and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example, in a laminar-airflow cabinet or in an incubator. For each of the microorganisms listed in *Table 1,* at least two Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample, prepared as directed under *Preparation of the Sample, Inoculation and Dilution,* and *Neutralization/Removal of Antimicrobial Activity* over the surface of the medium. Incubate and count as directed for *Pour-Plate Method.*

Most-Probable-Number (MPN) Method—The precision and accuracy of the *MPN Method* is less than that of the *Membrane Filtration* method or the *Plate-Count Method.* Unreliable results are obtained particularly for the enumeration of molds. For these reasons, the *MPN Method* is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least three serial 10-fold dilutions of the product as described for *Preparation of the Sample, Inoculation and Dilution,* and *Neutralization/Removal of Antimicrobial Activity.* From each level of dilution, three aliquots of 1 g or 1 mL are used to inoculate three tubes with 9 to 10 mL of *Soybean–Casein Digest Broth.* If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added to the medium. Thus, if three levels of dilution are prepared, nine tubes are inoculated.

Incubate all tubes at 30° to 35° for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth or in *Soybean–Casein Digest Agar* for 1 to 2 days at the same temperature, and use these results. From *Table 3,* determine the most probable number of microorganisms per g or mL of the product to be examined.

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Table 3. Most-Probable-Number Values of Microorganisms

Observed Combinations of Numbers of Tubes Showing Growth in Each Set			MPN per g or per mL of Product	95% Confidence Limits
Number of g or mL of Product per Tube				
0.1	0.01	0.001		
0	0	0	<3	0–9.4
0	0	1	3	0.1–9.5
0	1	0	3	0.1–10
0	1	1	6.1	1.2–17
0	2	0	6.2	1.2–17
0	3	0	9.4	3.5–35
1	0	0	3.6	0.2–17
1	0	1	7.2	1.2–17
1	0	2	11	4–35
1	1	0	7.4	1.3–20
1	1	1	11	4–35
1	2	0	11	4–35
1	2	1	15	5–38
1	3	0	16	5–38
2	0	0	9.2	1.5–35
2	0	1	14	4–35
2	0	2	20	5–38
2	1	0	15	4–38
2	1	1	20	5–38
2	1	2	27	9–94
2	2	0	21	5–40
2	2	1	28	9–94
2	2	2	35	9–94
2	3	0	29	9–94
2	3	1	36	9–94
3	0	0	23	5–94
3	0	1	38	9–104
3	0	2	64	16–181
3	1	0	43	9–181
3	1	1	75	17–199
3	1	2	120	30–360
3	1	3	160	30–380
3	2	0	93	18–360
3	2	1	150	30–380
3	2	2	210	30–400
3	2	3	290	90–990
3	3	0	240	40–990
3	3	1	460	90–1980
3	3	2	1100	200–4000
3	3	3	>1100	

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RESULTS AND INTERPRETATION

When verifying the suitability of the *Membrane Filtration* method or the *Plate-Count Method,* a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in *Inoculation and Dilution* in the absence of product must be obtained. When verifying the suitability of the *MPN Method,* the calculated value from the inoculum must be within 95% confidence limits of the results obtained with the control.

If the above criteria cannot be met for one of more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

TESTING OF PRODUCTS

Amount Used for the Test

Unless otherwise directed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g., tablet, capsule, injection) is less than or equal to 1 mg, or the amount per g or mL (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product.

For materials used as active substances where the sample quantity is limited or batch size is extremely small (i.e., less than 1000 mL or 1000 g), the amount tested shall be 1% of the batch unless a lesser amount is prescribed or justified and authorized.

For products where the total number of entities in a batch is less than 200 (e.g., samples used in clinical trials), the sample size may be reduced to two units, or one unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

Examination of the Product

MEMBRANE FILTRATION

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown to be suitable as described in *Growth Promotion Test and Suitability of the Counting Method,* transfer the appropriate amount to each of two membrane filters, and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of *Soybean–Casein Digest Agar.* For the determination of TYMC, transfer the other membrane to the surface of *Sabouraud Dextrose Agar.* Incubate the plate of *Soybean–Casein Digest Agar* at 30° to 35° for 3 to 5 days and the plate of *Sabouraud Dextrose Agar* at 20° to 25° for 5 to 7 days. Calculate the number of cfu per g or per mL of product.

When examining transdermal patches, separately filter 10% of the volume of the preparation described for *Preparation of the Sample* through each of two sterile filter membranes. Transfer one membrane to *Soybean–Casein Digest Agar* for TAMC and the other membrane to *Sabouraud Dextrose Agar* for TYMC.

PLATE-COUNT METHODS

Pour-Plate Method—Prepare the sample using a method that has been shown to be suitable as described in *Growth Promotion Test and Suitability of the Counting Method.* Prepare for each medium at least two Petri dishes for each level of dilution. Incubate the plates of *Soybean–Casein Digest Agar* at 30° to 35° for 3 to 5 days and the plates of *Sabouraud Dextrose Agar* at 20° to 25° for 5 to 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts, and calculate the number of cfu per g or per mL of product.

Surface-Spread Method—Prepare the sample using a method that has been shown to be suitable as described in *Growth Promotion Test and Suitability of the Counting Method.* Prepare at least two Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of cfu, proceed as directed for the *Pour-Plate Method.*

MOST-PROBABLE-NUMBER METHOD

Prepare and dilute the sample using a method that has been shown to be suitable as decribed in *Growth Promotion Test and Suitability of the Counting Method.* Incubate all tubes for 3 to 5 days at 30° to 35°. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of microorganisms per g or mL of the product to be examined from *Table 3.*

Interpretation of the Results

The total aerobic microbial count (TAMC) is considered to be equal to the number of cfu found using *Soybean–Casein Digest Agar;* if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts and molds count (TYMC) is considered to be equal to the number of cfu found using *Sabouraud Dextrose Agar;* if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance

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criterion due to the bacterial growth, *Sabouraud Dextrose Agar* containing antibiotics may be used. If the count is carried out by the *MPN Method,* the calculated value is TAMC.

When an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows:

- 10^1 cfu: maximum acceptable count = 20;
- 10^2 cfu: maximum acceptable count = 200;
- 10^3 cfu: maximum acceptable count = 2000;

and so forth.

The recommended solutions and media are described in *Tests for Specified Microorganisms* ⟨62⟩.

⟨62⟩ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR SPECIFIED MICROORGANISMS

INTRODUCTION

The tests described hereafter will allow determination of the absence of, or limited occurrence of, specified microorganisms that may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken, and interpret the results as stated below.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopeial method has been demonstrated.

GENERAL PROCEDURES

The preparation of samples is carried out as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩.

If surface-active substances are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with any inactivators used must be demonstrated as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩.

GROWTH-PROMOTING AND INHIBITORY PROPERTIES OF THE MEDIA, SUITABILITY OF THE TEST AND NEGATIVE CONTROLS

The ability of the test to detect microorganisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance or a change in the product that may affect the outcome of the test is introduced.

Preparation of Test Strains

Use standardized stable suspensions of test strains as stated below. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

AEROBIC MICROORGANISMS

Grow each of the bacterial test strains separately in containers containing *Soybean–Casein Digest Broth* or on *Soybean–Casein Digest Agar* at 30° to 35° for 18 to 24 hours. Grow the test strain for *Candida albicans* separately on *Sabouraud Dextrose Agar* or in *Sabouraud Dextrose Broth* at 20° to 25° for 2 to 3 days.

Staphylococcus aureus	such as ATCC 6538, NCIMB 9518, CIP 4.83, or NBRC 13276
Pseudomonas aeruginosa	such as ATCC 9027, NCIMB 8626, CIP 82.118, or NBRC 13275
Escherichia coli	such as ATCC 8739, NCIMB 8545, CIP 53.126, or NBRC 3972
Salmonella enterica subsp. *enterica* serovar Typhimurium or, as an alternative,	such as ATCC 14028
Salmonella enterica subsp. *enterica* serovar Abony	such as NBRC 100797, NCTC 6017, or CIP 80.39
Candida albicans	such as ATCC 10231, NCPF 3179, IP 48.72, or NBRC 1594

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Use *Buffered Sodium Chloride–Peptone Solution pH 7.0* or *Phosphate Buffer Solution pH 7.2* to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2° to 8°.

CLOSTRIDIA

Use *Clostridium sporogenes* such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under anaerobic conditions in *Reinforced Medium for Clostridia* at 30° to 35° for 24 to 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Cl. sporogenes,* a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period.

Negative Control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of microorganisms. A negative control is also performed when testing the products as described under *Testing of Products*. A failed negative control requires an investigation.

Growth Promotion and Inhibitory Properties of the Media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Verify suitable properties of relevant media as described in *Table 1*.

Table 1. Growth Promoting, Inhibitory, and Indicative Properties of Media

Test/Medium	Property	Test Strains
Test for bile-tolerant Gram-negative bacteria		
Enterobacteria Enrichment Broth Mossel	Growth promoting	*E. coli*
		P. aeruginosa
	Inhibitory	*S. aureus*
Violet Red Bile Glucose Agar	Growth promoting + Indicative	*E. coli*
		P. aeruginosa
Test for Escherichia coli		
MacConkey Broth	Growth promoting	*E. coli*
	Inhibitory	*S. aureus*
MacConkey Agar	Growth promoting + Indicative	*E. coli*
Test for Salmonella		
Rappaport Vassiliadis Salmonella Enrichment Broth	Growth promoting	*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* or
		Salmonella enterica subsp. *enterica* serovar *Abony*
	Inhibitory	*S. aureus*
Xylose Lysine Deoxycholate Agar	Growth promoting + Indicative	*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* or
		Salmonella enterica subsp. *enterica* serovar *Abony*
Test for Pseudomonas aeruginosa		
Cetrimide Agar	Growth promoting	*P. aeruginosa*
	Inhibitory	*E. coli*
Test for Staphylococcus aureus		
Mannitol Salt Agar	Growth promoting + Indicative	*S. aureus*
	Inhibitory	*E. coli*
Test for Clostridia		
Reinforced Medium for Clostridia	Growth promoting	*Cl. sporogenes*
Columbia Agar	Growth promoting	*Cl. sporogenes*
Test for Candida albicans		
Sabouraud Dextrose Broth	Growth promoting	*C. albicans*
Sabouraud Dextrose Agar	Growth promoting + Indicative	*C. albicans*

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Test for Growth-Promoting Properties, Liquid Media

Inoculate a portion of the appropriate medium with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for Growth-Promoting Properties, Solid Media

Perform *Surface-Spread Method* (see *Plate-Count Methods* under *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for Inhibitory Properties, Liquid or Solid Media

Inoculate the appropriate medium with at least 100 cfu of the appropriate microorganism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test microorganism occurs.

Test for Indicative Properties

Perform *Surface-Spread Method* (see *Plate-Count Methods* under *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

Suitability of the Test Method

For each new product to be tested perform sample preparation as described in the relevant paragraph under *Testing of Products*. At the time of mixing, add each test strain in the prescribed growth medium. Inoculate the test strains individually. Use a number of microorganisms equivalent to not more than 100 cfu in the inoculated test preparation.

Perform the test as described in the relevant paragraph under *Testing of Products* using the shortest incubation period prescribed.

The specified microorganisms must be detected with the indication reactions as described under *Testing of Products*.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see *Neutralization/Removal of Antimicrobial Activity* under *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩).

For a given product, if the antimicrobial activity with respect to a microorganism for which testing is prescribed cannot be neutralized, then it is to be assumed that the inhibited microorganism will not be present in the product.

TESTING OF PRODUCTS

Bile-Tolerant Gram-Negative Bacteria

Sample Preparation and Pre-Incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, but using *Soybean–Casein Digest Broth* as the chosen diluent, mix, and incubate at 20° to 25° for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

Test for Absence

Unless otherwise prescribed, use the volume corresponding to 1 g of the product, as prepared in *Sample Preparation and Pre-Incubation,* to inoculate *Enterobacteria Enrichment Broth Mossel.* Incubate at 30° to 35° for 24 to 48 hours. Subculture on plates of *Violet Red Bile Glucose Agar.* Incubate at 30° to 35° for 18 to 24 hours.

The product complies with the test if there is no growth of colonies.

Quantitative Test

Selection and Subculture—Inoculate suitable quantities of *Enterobacteria Enrichment Broth Mossel* with the preparation as directed under *Sample Preparation and Pre-Incubation* and/or dilutions of it containing respectively 0.1 g, 0.01 g, and 0.001 g (or 0.1 mL, 0.01 mL, and 0.001 mL) of the product to be examined. Incubate at 30° to 35° for 24 to 48 hours. Subculture each of the cultures on a plate of *Violet Red Bile Glucose Agar.* Incubate at 30° to 35° for 18 to 24 hours.

Interpretation—Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from *Table 2* the probable number of bacteria.

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Table 2. Interpretation of Results

Results for Each Quantity of Product			
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	Probable Number of Bacteria per g or mL of Product
+	+	+	more than 10^3
+	+	–	less than 10^3 and more than 10^2
+	–	–	less than 10^2 and more than 10
–	–	–	less than 10

Escherichia coli

Sample Preparation and Pre-Incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, and use 10 mL or the quantity corresponding to 1 g or 1 mL, to inoculate a suitable amount (determined as described under *Suitability of the Test Method*) of *Soybean–Casein Digest Broth,* mix, and incubate at 30° to 35° for 18 to 24 hours.

Selection and Subculture

Shake the container, transfer 1 mL of *Soybean–Casein Digest Broth* to 100 mL of *MacConkey Broth,* and incubate at 42° to 44° for 24 to 48 hours. Subculture on a plate of *MacConkey Agar* at 30° to 35° for 18 to 72 hours.

Interpretation

Growth of colonies indicates the possible presence of *E. coli.* This is confirmed by identification tests.
The product complies with the test if no colonies are present or if the identification tests are negative.

Salmonella

Sample Preparation and Pre-Incubation

Prepare the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, and use the quantity corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under *Suitability of the Test Method*) of *Soybean–Casein Digest Broth,* mix, and incubate at 30° to 35° for 18 to 24 hours.

Selection and Subculture

Transfer 0.1 mL of *Soybean–Casein Digest Broth* to 10 mL of *Rappaport Vassiliadis Salmonella Enrichment Broth,* and incubate at 30° to 35° for 18 to 24 hours. Subculture on plates of *Xylose Lysine Deoxycholate Agar.* Incubate at 30° to 35° for 18 to 48 hours.

Interpretation

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers. This is confirmed by identification tests.
The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

Pseudomonas aeruginosa

Sample Preparation and Pre-Incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under *Suitability of the Test Method*) of *Soybean–Casein Digest Broth,* and mix. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation (see *Transdermal Patches* under *Preparation of the Sample* in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩) through a sterile filter membrane, and place in 100 mL of *Soybean–Casein Digest Broth.* Incubate at 30° to 35° for 18 to 24 hours.

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Selection and Subculture

Subculture on a plate of *Cetrimide Agar,* and incubate at 30° to 35° for 18 to 72 hours.

Interpretation

Growth of colonies indicates the possible presence of *P. aeruginosa.* This is confirmed by identification tests.
The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

Staphylococcus aureus

Sample Preparation and Pre-Incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under *Suitability of the Test Method*) of *Soybean–Casein Digest Broth,* and homogenize. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation (see *Transdermal Patches* under *Preparation of the Sample* in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩) through a sterile filter membrane, and place in 100 mL of *Soybean–Casein Digest Broth.* Incubate at 30° to 35° for 18 to 24 hours.

Selection and Subculture

Subculture on a plate of *Mannitol Salt Agar,* and incubate at 30° to 35° for 18 to 72 hours.

Interpretation

The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests.
The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

Clostridia

Sample Preparation and Heat Treatment

Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩. Divide the sample into two portions of at least 10 mL. Heat one portion at 80° for 10 minutes, and cool rapidly. Do not heat the other portion.

Selection and Subculture

Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under *Suitability of the Test Method*) of *Reinforced Medium for Clostridia.* Incubate under anaerobic conditions at 30° to 35° for 48 hours. After incubation, make subcultures from each container on *Columbia Agar,* and incubate under anaerobic conditions at 30° to 35° for 48 to 72 hours.

Interpretation

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of Clostridia.
This is confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

Candida albicans

Sample Preparation and Pre-Incubation

Prepare the product to be examined as described in *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* ⟨61⟩, and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL, to inoculate 100 mL of *Sabouraud Dextrose Broth,* and mix. Incubate at 30° to 35° for 3 to 5 days.

Selection and Subculture

Subculture on a plate of *Sabouraud Dextrose Agar,* and incubate at 30° to 35° for 24 to 48 hours.

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INTERPRETATION

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.
The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

RECOMMENDED SOLUTIONS AND CULTURE MEDIA

[NOTE—This section is given for information.]
The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopeia. Other media may be used provided that their suitability can be demonstrated.

Stock Buffer Solution—Transfer 34 g of potassium dihydrogen phosphate to a 1000-mL volumetric flask, dissolve in 500 mL of Purified Water, adjust with sodium hydroxide to a pH of 7.2 ± 0.2, add Purified Water to volume, and mix. Dispense in containers, and sterilize. Store at a temperature of 2° to 8°.

Phosphate Buffer Solution pH 7.2—Prepare a mixture of Purified Water and *Stock Buffer Solution* (800:1 v/v), and sterilize.

Buffered Sodium Chloride–Peptone Solution pH 7.0	
Potassium Dihydrogen Phosphate	3.6 g
Disodium Hydrogen Phosphate Dihydrate	7.2 g (equivalent to 0.067 M phosphate)
Sodium Chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified Water	1000 mL

Sterilize in an autoclave using a validated cycle.

Soybean–Casein Digest Broth	
Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean	3.0 g
Sodium Chloride	5.0 g
Dibasic Hydrogen Phosphate	2.5 g
Glucose Monohydrate	2.5 g
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Soybean–Casein Digest Agar	
Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Sabouraud Dextrose Agar	
Dextrose	40.0 g
Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1)	10.0 g
Agar	15.0 g
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

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Potato Dextrose Agar	
Infusion from potatoes	200 g
Dextrose	20.0 g
Agar	15.0 g
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Sabouraud Dextrose Broth	
Dextrose	20.0 g
Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1)	10.0 g
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Enterobacteria Enrichment Broth Mossel	
Pancreatic Digest of Gelatin	10.0 g
Glucose Monohydrate	5.0 g
Dehydrated Ox Bile	20.0 g
Potassium Dihydrogen Phosphate	2.0 g
Disodium Hydrogen Phosphate Dihydrate	8.0 g
Brilliant Green	15 mg
Purified Water	1000 mL

Adjust the pH so that after heating it is 7.2 ± 0.2 at 25°. Heat at 100° for 30 minutes, and cool immediately.

Violet Red Bile Glucose Agar	
Yeast Extract	3.0 g
Pancreatic Digest of Gelatin	7.0 g
Bile Salts	1.5 g
Sodium Chloride	5.0 g
Glucose Monohydrate	10.0 g
Agar	15.0 g
Neutral Red	30 mg
Crystal Violet	2 mg
Purified Water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°. Heat to boiling; do not heat in an autoclave.

MacConkey Broth	
Pancreatic Digest of Gelatin	20.0 g
Lactose Monohydrate	10.0 g
Dehydrated Ox Bile	5.0 g
Bromocresol Purple	10 mg
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

MacConkey Agar	
Pancreatic Digest of Gelatin	17.0 g
Peptones (meat and casein)	3.0 g

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MacConkey Agar	
Lactose Monohydrate	10.0 g
Sodium Chloride	5.0 g
Bile Salts	1.5 g
Agar	13.5 g
Neutral Red	30.0 mg
Crystal Violet	1 mg
Purified Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 ± 0.2 at 25°. Boil for 1 minute with constant shaking, then sterilize in an autoclave using a validated cycle.

Rappaport Vassiliadis Salmonella Enrichment Broth	
Soya Peptone	4.5 g
Magnesium Chloride Hexahydrate	29.0 g
Sodium Chloride	8.0 g
Dipotassium Phosphate	0.4 g
Potassium Dihydrogen Phosphate	0.6 g
Malachite Green	0.036 g
Purified Water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°. The pH is to be 5.2 ± 0.2 at 25° after heating and autoclaving.

Xylose Lysine Deoxycholate Agar	
Xylose	3.5 g
L-Lysine	5.0 g
Lactose Monohydrate	7.5 g
Sucrose	7.5 g
Sodium Chloride	5.0 g
Yeast Extract	3.0 g
Phenol Red	80 mg
Agar	13.5 g
Sodium Deoxycholate	2.5 g
Sodium Thiosulfate	6.8 g
Ferric Ammonium Citrate	0.8 g
Purified Water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°. Heat to boiling, cool to 50°, and pour into Petri dishes. Do not heat in an autoclave.

Cetrimide Agar	
Pancreatic Digest of Gelatin	20.0 g
Magnesium Chloride	1.4 g
Dipotassium Sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified Water	1000 mL
Glycerol	10.0 mL

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Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is 7.2 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Mannitol Salt Agar	
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Beef Extract	1.0 g
D-Mannitol	10.0 g
Sodium Chloride	75.0 g
Agar	15.0 g
Phenol Red	0.025 g
Purified Water	1000 mL

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is 7.4 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Reinforced Medium for Clostridia	
Beef Extract	10.0 g
Peptone	10.0 g
Yeast Extract	3.0 g
Soluble Starch	1.0 g
Glucose Monohydrate	5.0 g
Cysteine Hydrochloride	0.5 g
Sodium Chloride	5.0 g
Sodium Acetate	3.0 g
Agar	0.5 g
Purified Water	1000 mL

Hydrate the agar, and dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is about 6.8 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Columbia Agar	
Pancreatic Digest of Casein	10.0 g
Meat Peptic Digest	5.0 g
Heart Pancreatic Digest	3.0 g
Yeast Extract	5.0 g
Maize Starch	1.0 g
Sodium Chloride	5.0 g
Agar, according to gelling power	10.0–15.0 g
Purified Water	1000 mL

Hydrate the agar, and dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle. Allow to cool to 45° to 50°; add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base, and pour into Petri dishes.

⟨63⟩ MYCOPLASMA TESTS

INTRODUCTION

The genus Mycoplasma represents a group of minute bacteria which have no cell walls. The genus comprises more than 120 species. They are the smallest self-replicating prokaryotic organisms. The cells vary in size and morphology and cannot be Gram stained, but impressions of colonies on solid agar can be stained with methylene blue or equivalent stain. Mycoplasma are

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parasites and commensals, and some may be pathogenic to a variety of animal and plant hosts. In humans, Mycoplasma are usually surface parasites that colonize the epithelial lining of the respiratory and urogenital tracts. Mycoplasma are common and may cause serious contamination in cell and/or tissue cultures used to generate compendial articles. They may also cause contamination of filtered sterilized soybean casein digest broth. A cell culture infection may persist for an extended period of time without causing apparent cell damage. Infection of cells in a culture can affect nearly every pathway of cell metabolism, including alteration of the cells' phenotypical characteristics and normal growth. The presence of Mycoplasma species does not always result in turbid growth in cultures or visible alteration of the cells.

Testing for Mycoplasma is a necessary quality control requirement to assure reliably pure biotechnological products and allied materials used to generate these products. This general test chapter describes two methods required to detect Mycoplasma contamination of test articles, tissues and/or cell cultures used to produce test articles, digest broth, or any other material in which Mycoplasma contamination is suspected. These are: (A) the agar and broth media procedure and (B) the indicator cell culture procedure. These tests require careful aseptic technique and suitable laboratory conditions. In order to ensure appropriate testing and interpretation of results, personnel should be properly trained and qualified. A validated nucleic acid amplification technique (NAT) or an enzymatic activity based method may be used to detect Mycoplasma, provided such a method is shown to be comparable to both methods (A) and (B). Alternative methods must be suitably validated. Validation requirements for alternate methods will not be addressed in this chapter.

CULTURE METHOD

Choice of Media

The test is carried out using a sufficient number of both solid and liquid media to ensure growth in the chosen incubation conditions of small numbers (approximately 100 colony-forming units, cfu; or 100 color-changing units, ccu) of Mycoplasmas that may be present in the test article/material. Liquid media must contain phenol red. The range of media chosen is shown to have satisfactory nutritive properties for at least the microorganisms shown in *Quality Control Test Strain Organisms* (below). The nutritive properties of each new batch of medium are verified for the appropriate microorganisms in the list. When testing for Mycoplasmas include in each test at least two known Mycoplasma species or strains (listed in *Quality Control Test Strain Organisms*) as positive controls, one of which should be a dextrose fermenter (i.e., *M. pneumoniae* or equivalent species and strain) and one of which should be an arginine hydrolyzer (i.e., *M. orale* or equivalent species and strain). Only when testing insect cell lines should one include a *Spiroplasma* control strain (e.g., *S. citri* ATCC 29747, *S. melliferum* ATCC 29416, or equivalent species and strains). Additionally, these strains may be a little more fastidious in their nutritional requirements. They require lower incubation temperatures (as do insect cell lines).

Quality Control Test Strain Organisms

Positive control cultures should be not more than 15 passages from isolation. Mycoplasma species or strains suitable for use are listed below:

- *Acholeplasma laidlawii* (vaccines and/or cell-derived materials/cultures for human and veterinary use when an antibiotic has been used during production)
- *M. gallisepticum* (when avian material has been used during production or when the vaccine or cell culture is intended for use in poultry)
- *M. hyorhinis* (nonavian veterinary vaccines or cell cultures)
- *M. orale* (vaccines for human and veterinary use)
- *M. pneumoniae* (vaccines or cell banks for human use) or another suitable species of D-glucose fermenter such as *M. fermentans*
- *M. synoviae* (when avian material has been used during production or when the vaccine or cell bank is intended for use in poultry)

The test strains may be field isolates that have undergone a limited number of subcultures (not more than 15), are stored frozen (−20° or lower) or freeze-dried, and are identified as being of the required species by comparison with type cultures, for example, those shown in *Table 1*.

Table 1. Type Cultures for Identifying Field Isolates Used as Test Strains

Test Organism	NCTC Number	CIP Number	ATCC Number
A. laidlawii	NCTC 10116	CIP 75.27	ATCC 23206
M. gallisepticum	NCTC 10115	CIP 104967	ATCC 19610
M. fermentans	NCTC 10117	CIP 105680	ATCC 19989
M. hyorhinis	NCTC 10130	CIP 104968	ATCC 17981
M. orale	NCTC 10112	CIP 104969	ATCC 23714
M. pneumoniae	NCTC 10119	CIP 103766	ATCC 15531
M. synoviae	NCTC 10124	CIP 104970	ATCC 25204

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Incubation Conditions

Incubate liquid media in tightly stoppered containers at 36 ± 1°. Incubate solid media in microaerophilic conditions (hydrogen atmosphere containing < 0.5% oxygen and/or nitrogen containing 5%–10% carbon dioxide in nitrogen). Sufficient humidity should be available to prevent desiccation of the agar surface at 36 ± 1°.

Nutritive Properties

Carry out the test for nutritive properties for each new batch of medium. Inoculate the chosen media with the appropriate test microorganisms; use not more than 100 cfu per plate containing at least 9 mL of solid media and per 100-mL container of liquid medium; use a separate plate and container for each species of microorganism. Incubate the media and make subcultures from 0.2 mL of liquid medium to solid medium at the specified intervals (see below under *Test for Mycoplasma in the Test Article/Material*). The solid medium complies with the test if a count within a 0.5-log unit range of the inoculate amount is found for each test microorganism. The liquid medium complies with the test if growth is found on agar plates subcultured from the broth, for at least 1 subculture for each test microorganism. The use of a microscope at 100× or greater may be helpful.

Inhibitory Substances

The test for inhibitory substances is carried out once for a given product and is repeated whenever there is a change in production method that may affect the detection of Mycoplasma. To demonstrate absence of inhibitory substances, carry out the test for nutritive properties in the presence and absence of the test article/material. If growth of a test microorganism occurs more than 1 subculture sooner in the absence of the test article/material than in its presence, inhibitory substances are present. The same is true if plates directly inoculated with the test article/material are not within a 0.5-log unit range of the number of colonies of those inoculated without the test article/material. In both cases, inhibitory substances must be neutralized or their effect otherwise countered, by an appropriate method, for example, by passage in substrates not containing inhibitors or dilution in a larger volume of medium, before the test. If dilution is used, larger medium volumes may be used or the inoculums' volume may be divided among several 100-mL flasks. The effectiveness of the neutralization or other process is checked by repeating the test for inhibitory substances after neutralization.

Test for Mycoplasma in the Test Article/Material

Inoculate no less than 10 mL of the test article/material per 100 mL of each liquid medium. If a significant pH change occurs upon the addition of the test article/material, the liquid medium is restored to its original pH value by the addition of a sterile solution of either sodium hydroxide or hydrochloric acid. Inoculate 0.2 mL of the test article/material on each plate of each solid medium. Incubate liquid media for 20–21 days. Incubate solid media for not less than 14 days, except those plates corresponding to the 20–21 day subculture, which are incubated for 7 days. Concurrently, incubate an uninoculated 100-mL portion of each liquid medium and agar plate, as a negative control. On days 2–4 after inoculation, subculture each liquid medium by inoculating 0.2 mL on at least 1 plate of each solid medium. Repeat the procedure between days 6 and 8, again between days 13 and 15, and again between days 19 and 21 of the test. Observe the liquid media every 2 or 3 days and if a color change occurs, subculture. If a liquid medium shows bacterial or fungal contamination, the test is invalid. The test is valid if at least 1 plate per medium and per inoculation day can be read. Include in the test positive controls prepared by inoculation of not more than 100 cfu of at least 1 test microorganism on agar medium or into broth medium. Where the test for Mycoplasmas is carried out regularly, it is recommended to use the test microorganisms in regular rotation. The test microorganisms used are those listed under *Choice of Media*. Incubate broths and plates in a humidified atmosphere with microaerophilic conditions (5%–10% CO_2).

Interpretation of Results

At the end of the prescribed incubation period, examine all inoculated solid media for the presence of Mycoplasma colonies. The product complies with the test if growth of typical Mycoplasma colonies has not occurred. The product does not comply with the test if growth of typical Mycoplasma colonies has occurred on any of the solid media. The test is invalid if 1 or more of the positive controls do not show growth of Mycoplasmas on at least 1 subculture plate. The test is invalid if 1 or more of the negative controls show growth of Mycoplasmas. If suspect colonies are observed, use a suitable validated method to determine whether they are due to Mycoplasmas.

Recommended Solutions and Media for the Culture Method

[NOTE—This section is provided for information.]

SOLUTIONS

Beef Heart Infusion Broth

Beef heart (for preparation of the infusion)	500 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	to 1000 mL

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Essential Vitamins

Biotin	100 mg
Calcium pantothenate	100 mg
Choline chloride	100 mg
Folic acid	100 mg
i-Inositol	200 mg
Nicotinamide	100 mg
Pyridoxal hydrochloride	100 mg
Riboflavine	10 mg
Thiamine hydrochloride	100 mg
Distilled water	to 1000 mL

Agar, Purified

A highly refined agar for use in microbiology and immunology, prepared by an ion-exchange procedure that results in a product having superior purity, clarity, and gel strength. It contains the following ingredients:	
Water	12.2%
Ash	1.5%
Acid-insoluble ash	0.2%
Chlorine	0
Phosphate (calculated as P_2O_5)	0.3%
Total nitrogen	0.3%
Copper	8 ppm
Iron	170 ppm
Calcium	0.28%
Magnesium	0.32%

Hanks' Balanced Salt Solution (modified)

Sodium chloride	6.4 g
Potassium chloride	0.32 g
Magnesium sulphate heptahydrate	0.08 g
Magnesium chloride hexahydrate	0.08 g
Calcium chloride, anhydrous	0.112 g
Disodium hydrogen phosphate dihydrate	0.0596 g
Potassium dihydrogen phosphate, anhydrous	0.048 g
Distilled water	to 800 mL

Brain Heart Infusion

Calf-brain infusion	200 g
Beef-heart infusion	250 g
Proteose peptone	10 g
Glucose monohydrate	2 g
Sodium chloride	5 g
Disodium hydrogen phosphate, anhydrous	2.5 g
Distilled water	to 1000 mL

PPLO Broth

Beef-heart infusion	50 g
Peptone	10 g
Sodium chloride	5 g

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PPLO Broth *(continued)*

Distilled water	to 1000 mL

MEDIA

The following media are recommended. Other media may be used, provided they meet the criteria given in the sections *Choice of Culture Media, Incubation Conditions, Nutritive Properties,* and *Inhibitory Substances.*

Hayflick Media (Recommended for the general detection of Mycoplasmas)

Liquid Medium	
Beef heart infusion broth	90.0 mL
Horse serum (unheated)	20.0 mL
Yeast extract (250 g/L) (fresh yeast extract is recommended)	10.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL
Deoxyribonucleic acid (2 g/L solution)	1.2 mL
Adjust to a pH of 7.8	
Solid Medium	
Prepare as described above replacing beef heart infusion broth by beef heart infusion agar containing 15 g/L of agar.	

Frey Media (Recommended for the detection of *M. synoviae*)

Liquid Medium	
Beef heart infusion broth	90.0 mL
Essential vitamins	0.025 mL
Glucose monohydrate (500 g/L solution)	2.0 mL
Swine serum (inactivated at 56° for 30 min)	12.0 mL
β-Nicotinamide adenine dinucleotide (10 g/L solution)	1.0 mL
Cysteine hydrochloride (10 g/L solution)	1.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL
Mix the solutions of β-nicotinamide adenine dinucleotide and cysteine hydrochloride and after 10 min add to the other ingredients. Adjust to a pH of 7.8.	
Solid Medium	
Beef heart infusion broth	90.0 mL
Agar, purified	1.4 g
Adjust to pH 7.8, sterilize by autoclaving then add:	
Essential vitamins	0.025 mL
Glucose monohydrate (500 g/L solution)	2.0 mL
Swine serum (unheated)	12.0 mL
β-Nicotinamide adenine dinucleotide (10 g/L solution)	1.0 mL
Cysteine hydrochloride (10 g/L solution)	1.0 mL
Phenol red (0.6 g/L solution)	5.0 mL
Penicillin (20,000 IU/mL)	0.25 mL

Friis Media (Recommended for the detection of nonavian Mycoplasmas)

Liquid Medium	
Hanks' balanced salt solution (modified)	800 mL
Distilled water	67 mL
Brain heart infusion	135 mL
PPLO Broth	248 mL
Yeast extract (170 g/L)	60 mL

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Friis Media (Recommended for the detection of nonavian Mycoplasmas) *(continued)*

Bacitracin	250 mg
Meticillin	250 mg
Phenol red (5 g/L)	4.5 mL
Horse serum	165 mL
Swine serum	165 mL
Adjust to a pH of 7.40–7.45	
Solid Medium	
Hanks' balanced salt solution (modified)	200 mL
DEAE-dextran	200 mg
Agar, purified	15.65 g
Mix well and sterilize by autoclaving. Cool to 100°. Add to 1740 mL of *Liquid Medium* as described above.	

INDICATOR CELL CULTURE METHOD

Cell cultures are stained with a fluorescent dye that binds to DNA. Mycoplasmas are detected by their characteristic particulate or filamentous pattern of fluorescence on the cell surface and, if contamination is heavy, in surrounding areas. Mitochondria in the cytoplasm may be stained but are readily distinguished from Mycoplasmas. For viral suspensions, if the interpretation of results is affected by marked cytopathic effects, neutralize the virus using a specific antiserum that has no inhibitory effects on Mycoplasmas, or use a cell culture substrate that does not allow growth of the virus. To demonstrate the absence of inhibitory effects of serum, carry out the positive control tests in the presence and absence of the antiserum.

Verification of the Substrate

Use Vero cells or equivalent cell culture (for example, the production cell line) that is equivalent in effectiveness for detecting Mycoplasmas. Test the effectiveness of the cells to be used by applying the procedure shown below and inoculating not more than 100 cfu or ccu microorganisms of suitable reference strains of *M. hyorhinis* and *M. orale*. The cells are suitable if both reference strains are detected. The indicator cells must be subcultured without an antibiotic before use in the test.

Test Method

[NOTE—The following is provided for information.]

SOLUTIONS

Phosphate Buffered Saline

2.0 M Monobasic Potassium Phosphate—Dissolve 13.61 g of anhydrous monobasic potassium phosphate in 50 mL of water.

2.0 M Dibasic Potassium Phosphate—Dissolve 17.42 g of anhydrous dibasic potassium phosphate in 50 mL of water.

Phosphate Buffered Saline Solution (pH 7.4)—Combine 3.6 mL of *2.0 M Monobasic Potassium Phosphate,* 16.4 mL of *2.0 M Dibasic Potassium Phosphate,* 8 g of sodium chloride, and 1 L of water. Mix thoroughly. Adjust the pH if necessary.

Bisbenzimide Stock Solution—Dissolve 5 mg of bisbenzimide in water, and dilute with the same solvent to 100 mL. Store in the dark.

Bisbenzimide Working Solution—Immediately before use, dilute 100 µL of *Bisbenzimide Stock Solution* with *Phosphate Buffered Saline Solution (pH 7.4)* to 100 mL.

Phosphate-Citrate Buffer Solution pH 5.5—Mix 56.85 mL of a 28.4-g/L solution of anhydrous disodium hydrogen phosphate and 43.15 mL of a 21-g/L solution of citric acid.

METHOD

1. Seed the indicator cell culture at a suitable density (for example, 2×10^4 to 2×10^5 cells/mL, 4×10^3 to 2.5×10^4 cells/cm^2) that will yield confluence after 3 days of growth. Inoculate 1 mL of the product to be examined into the cell culture vessel, and incubate at 36 ± 1°.
2. After at least 3 days of incubation, when the cells have grown to confluence, make a subculture on cover slips in suitable containers or on some other surface (for example, chambered slides) suitable for the test procedure. Seed the cells at low density so that they reach 50% confluence after 3–5 days of incubation. Complete confluence impairs visualization of Mycoplasmas after staining and must be avoided.
3. Remove the medium and rinse the indicator cells with phosphate buffered saline, pH 7.4, then add a suitable fixing solution (a freshly prepared mixture of 1 volume of acetic acid, glacial, TS and 3 volumes of methanol, is suitable when bisbenzimide is used for staining).
4. Remove the fixing solution and wash the cells with sterile Purified Water. Dry the slides completely if they are to be stained more than 1 hour later (particular care is needed for staining of slides after drying owing to artifacts that may be produced).

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5. Add a suitable DNA stain and allow standing for a suitable time (bisbenzimide working solution and a standing time of 10 minutes are suitable).
6. Remove the stain and rinse the monolayer with Purified Water.
7. Mount each coverslip, where applicable (a mixture of equal volumes of glycerol and *Phosphate-Citrate Buffer Solution pH 5.5* is suitable for mounting). Examine by fluorescence (for bisbenzimide stain a 330 nm/380 nm excitation filter and an LP 440 nm barrier filter are suitable) at 400× magnification or greater.
8. Compare the microscopic appearance of the test cultures with that of the negative and positive controls, examining for extranuclear fluorescence. Mycoplasmas produce pinpoints or filaments over the indicator cell cytoplasm. They may also produce pinpoints and filaments in the intercellular spaces. Multiple microscopic fields are examined according to the protocol established during validation.

Interpretation of Results

The product to be examined complies with the test if fluorescence typical of Mycoplasmas is not present. The test is invalid if the positive controls do not show fluorescence typical of Mycoplasmas. The test is invalid if the negative controls show fluorescence typical of Mycoplasmas.

Add the following:

▲⟨64⟩ PROBIOTIC TESTS

INTRODUCTION

This chapter provides testing procedures for identification, enumeration, contamination, and other requirements for probiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Probiotics are typically identified at the strain level as their characteristics are considered strain-specific.

This chapter applies to probiotics produced in specialized fermenters under strict hygiene conditions for dietary supplements or pharmaceutical applications. Fermentation media are tailored to the specific requirements of the microbial species or strain and typically contain nutrients such as proteins, carbohydrates, vitamins, and minerals. The culture is allowed to multiply and grow under carefully defined conditions. After the microbial cells are grown, the cultured cells are harvested, often by centrifugation. Suitable protectants may be added to the concentrated probiotic microorganisms (biomass), and the biomass is freeze-dried or spray-dried to a powdered form. The dried biomass then undergoes formulation, which may involve blending one or more strains with suitable excipients. Formulated probiotic ingredients can be further processed into dosage forms, e.g., tablets, capsules, softgels, powders, or gels.

IDENTIFICATION

Probiotics as defined in this chapter can be identified at the genus and species level or at the strain level according to the monograph requirements. Since regulatory filings for commercial probiotics are commonly made at the strain level supported by information from safety assessment and human studies, identification at the strain level may be required to verify the strain declared in labeling of commercial products. In this chapter, strain-specific identification using polymerase chain reaction (PCR) with strain-specific primers is recommended for the identification of probiotics. Currently, a testing procedure for identification of *Lactobacillus* and *Bifidobacterium* strains by PCR with specific primers is listed in this chapter. Additional testing procedures for identification of probiotic strains including non-spore-forming bacteria, spore-forming bacteria, and yeasts or molds will be listed in this chapter once they become available.

• **IDENTIFICATION FOR NON-SPORE-FORMING BACTERIAL STRAINS**

Identification for *Lactobacillus* and *Bifidobacterium* strains by PCR with specific primers

Unless otherwise specified in the individual monograph, use the following general procedure. All reagents and solutions used in this test should be molecular biology grade.

Buffer: 10 mM tris-hydrochloride, 1 mM sodium ethylenediaminetetraacetic acid (EDTA) buffer[1]

PCR master mix: *Taq* DNA polymerase (62.5 U/mL), 2.5X *Taq* reaction buffer [125 mM potassium chloride (KCl), 75 mM tris-hydrochloride pH 8.3, and 4 mM magnesium (Mg^{+2})], and 500 µM of each deoxynucleotide (dNTP). Prepared *PCR master mix* solutions are commercially available.[2]

Sample: 100 mg/mL suspension of the probiotic powder in *Buffer*

Primer set: Use the primers for the strain specified in the individual monograph. Dilute the forward and reverse primers in *Buffer* to a stock concentration of 100 µM, and dilute further with *Buffer* to 25 µM. The diluted forward primer and diluted reverse primer 25-µM dilutions can be stored at −20°.[3]

PCR sample preparations: For each *Primer set*, prepare a solution containing 1 µL of the *Sample*, 10 µL of *PCR master mix*, 1 µL of the diluted forward primer (25 µM), 1 µL of the diluted reverse primer (25 µM), and 12 µL of *Sterile Purified Water*.

PCR negative control: Prepare as directed for the *PCR sample preparations*, replacing the *Sample* with 1 µL of *Sterile Purified Water*.

[1] Suitable buffers (e.g., TE Buffer 1X, molecular biology grade) are available from Promega (www.promega.com).

[2] 5 PRIME MasterMix Polymerase from VWR Scientific (www.vwr.com), AmpliTaq Gold DNA Polymerases from ThermoFisher Scientific (www.thermofisher.com), or other chemical/microbiology suppliers.

[3] DNA primers are commercially available (custom manufacture) from Integrated DNA Technologies (www.idtdna.com) and other commercial sources.

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PCR positive control (if available): Prepare as directed for the *PCR sample preparations*, replacing the *Sample* with 1 µL of the corresponding Reference Standard.

Analysis: Perform PCR amplification on each of the *PCR sample preparations*, *PCR positive control*, and the *PCR negative control* using an appropriate thermal cycler.[4] Unless otherwise specified in the individual monograph, incubate at 95° for 7 min (step 1) for direct heat-induced DNA extraction.

Thermal cycle: 95° for 30 s (step 2); annealing temperature specified in the individual monograph for 30 s (step 3); and at 72° for 30 s (step 4). Repeat steps 2–4 for between 30 and 34 cycles, incubate at 72° for 5 min, and hold at 4°. Perform electrophoretic separation of the PCR amplification products for each of the *PCR sample preparations*, *PCR positive control*, and for the *PCR negative control*. An automated on-chip electrophoresis system with a DNA kit can be used.

Prepare or use a commercially available 1% (w/v) agarose gel in a 1X tris-acetic acid–EDTA buffer (40 mM tris-hydrochloride, 1% glacial acetic acid, and 1 mM EDTA). Stain the gel with 0.5 mg/mL of ethidium bromide in water and de-stain with water. [**CAUTION**—Ethidium bromide is considered a toxic substance and a potential mutagen. Use appropriate personal protective equipment (including nitrile gloves) when handling this reagent.]

Use a DNA ladder standard (1 KB plus)[5] suitable for determining the size of linear double-stranded DNA fragments between 100 and 12,000 base pairs. The ladder standard should be used in the first and last lanes on the gel to allow for proper comparison of amplicons. Analysis of the *PCR negative control* must result in the absence of any amplification products. If amplification occurs, repeat the *PCR sample preparations*, and the *PCR negative control*, followed by PCR amplification and analysis.

Acceptance criteria: Refer to the acceptance criteria in the individual monograph.

ENUMERATION

Currently, a testing procedure for enumeration of *Lactobacillus* and *Bifidobacterium* species is listed in this chapter. Additional testing procedures for enumeration of probiotic strains, including non-spore-forming bacteria, spore-forming bacteria, and yeasts and molds, will be listed in this chapter once they become available. Alternative microbiological procedures, including automated methods, may be used provided that their equivalence has been demonstrated.

• **ENUMERATION FOR NON-SPORE-FORMING BACTERIA STRAINS**

***Lactobacillus* and *Bifidobacterium* species**

Unless otherwise specified in the individual monograph, use the following general procedure.

***Lactobacilli* MRS agar** (medium for *Lactobacillus* and *Bifidobacterium* species): Prepare according to *Table 1* or use a commercially available mixture.[6]

Table 1. *Lactobacilli* MRS Agar

Reagent	Quantity (g)
Proteose peptone #3[a]	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Polysorbate 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.1
Manganese sulfate[b]	0.05
Dipotassium phosphate	2.0
Agar	15.0

[a] A suitable peptone for microbiological analysis is available from BD Bacto™ (www.bd.com).
[b] A suitable manganese sulfate is available from Sigma-Aldrich (www.sigmaaldrich.com).

Suspend the *Lactobacilli MRS agar* in 1 L of *Purified Water* in an appropriately sized conical flask or beaker (sufficiently large to not boil over). Cover the flask or beaker with aluminum foil and heat to boiling with stirring on a hot plate. Allow to boil for 1 min to completely dissolve the medium, and then autoclave the solution at 121° for 15 min. Cool to 45° and use immediately. Boiled agar medium may also be aseptically transferred into individual media bottles in 100- or 200-mL aliquots before sterilizing, and then autoclaved and stored for later use. The agar can be stored at a temperature between 2° and 8° in a sterile well-closed container unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated. Allow the agar to come to room temperature before use.

[4] Suitable thermal cyclers are available from Eppendorf (www.eppendorf.com/OC-en/).
[5] Suitable 1 KB plus DNA ladders are available from ThermoFisher Scientific (www.thermofisher.com).
[6] Difco™ *Lactobacilli* MRS Agar, or equivalent. Suitable *Lactobacilli* MRS agars are available from VWR Scientific (www.vwr.com) or other chemical/microbiological suppliers.

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***Lactobacilli* MRS broth:** Prepare according to *Table 2* or use a suitable commercially available broth.[7]

Table 2. *Lactobacilli* MRS Broth

Reagent	Quantity (g)
Proteose peptone #3[a]	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Polysorbate 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.1
Manganese sulfate[b]	0.05
Dipotassium phosphate	2.0

[a] A suitable peptone for microbiological analysis is available from BD Bacto™ (www.bd.com).
[b] A suitable manganese sulfate is available from Sigma-Aldrich (www.sigmaaldrich.com).

Suspend *Lactobacilli MRS broth* in 1 L of *Purified Water* in an appropriately sized conical flask or beaker (sufficiently large to not boil over). Cover the flask or beaker with aluminum foil and heat to boiling with stirring on a hot plate. Allow to boil for 1 min to completely dissolve the broth ingredients, and then autoclave the solution at 121° for 15 min. Broth may also be aseptically transferred into individual media bottles in 100- or 200-mL aliquots before sterilizing, and then autoclaved and stored for later use. The broth can be stored at a temperature between 2° and 8° in a sterile well-closed container unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated. Allow broth to come to room temperature before use.

Peptone diluent: Prepare a solution of 0.1% peptone[8] in water (w/v) and adjust with a lactic acid solution to a pH of 7.0. Using an autoclave, steam sterilize the solution at 121° for NLT 15 min, and then allow to cool in the unopened autoclave. Dispense into sterile containers as needed for preparing samples.

Sample preparation: Unless otherwise specified in the individual monograph, transfer an amount equivalent to 11.0 g of probiotic powder into a sterile polyethylene bag, commercially known as a stomacher bag. Add 99 mL of previously sterilized (room temperature) *Lactobacilli MRS broth* to the bag. Blend in a mechanical blender until a uniform mixture is achieved or in a stomacher[9] at 230 rpm for 30 s. Hold the mixture at room temperature for 30 min to allow rehydration of the sample, and then blend for an additional 30 s at 230 rpm. This is the primary 10^{-1} dilution. Using sterilized, filtered pipet tips, make serial dilutions by aseptically transferring 1.0 mL of the primary 10^{-1} dilution to sterile media bottles, each containing 99.0 mL of *Peptone diluent* (10^{-3} dilution). Repeat this operation until the desired dilution series is obtained. Dilutions are expected to contain 25–250 cfu/mL. Shake the media bottles to mix completely before proceeding with the *Analysis*.

Analysis: Using three sterile, filtered 1-mL pipet tips, aseptically transfer 1.0 mL of the *Sample preparation* separately into three appropriately labeled, sterile 15-mm × 100-mm Petri plates, and then pour about 10–15 mL of the *Lactobacilli MRS agar* at about 45° onto each plate, flaming the lip of the bottle between pours. Place the lid on each plate after adding the agar medium, and then gently swirl the plates to mix the *Sample preparation* and the agar medium. Avoid spillage onto the lid of the dish when swirling the plates. Repeat this procedure for additional dilutions of the *Sample preparation*. Prepare a blank plate containing only *Lactobacilli MRS agar* and a second blank plate containing 1.0 mL of *Peptone diluent* mixed with *Lactobacilli MRS agar*. Allow the plates to sit at room temperature on a level surface until the agar solidifies, and then incubate the plates at 36°–40° for 3–5 days under anaerobic conditions.[10] After the incubation period, count the colonies and record the results as viable colony-forming units (cfu) per gram, taking into account the appropriate dilution factor of the *Sample preparation*. Only count plates containing 25–250 colonies. Determine the average plate count, in colony-forming units per gram.

Acceptance criteria: Refer to the acceptance criteria in the individual monograph.

PERFORMANCE TESTS

Performance tests are recommended for any dosage forms of probiotics, including tablets, capsules, softgels, gelcaps, powders, or chewable gels, etc.

- **Disintegration and Dissolution** ⟨2040⟩, *Disintegration*: Meet the requirements
- **Weight Variation** ⟨2091⟩: Meet the requirements

CONTAMINANTS

The level of the standard contaminant-indicator organisms as well as the absence of pathogenic microorganisms specified in the individual monographs must be determined to ensure the quality of probiotics. Recommended standard indicator

[7] Difco™ *Lactobacilli* MRS Broth or equivalent. Suitable *Lactobacilli* MRS broths are available from VWR Scientific (www.vwr.com) or other chemical/microbiological suppliers.
[8] Suitable peptone for microbiological analysis is available from BD Bacto (www.bd.com).
[9] Suitable stomacher (e.g., Stomacher 400 Circulator) for mechanical sample blending is available from Seward (www.seward.co.uk).
[10] Suitable anaerobic systems are available from BD GasPak EZ Container System (www.bd.com).

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organisms and specified microorganisms are listed in *Table 3* and *Table 4*. Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence has been demonstrated. Principles of validation of alternative microbiological methods are also described in *Validation of Alternative Microbiological Methods* ⟨1223⟩. Methods (e.g., dilution, buffer, or membrane filtration) may be suitably modified to overcome potential interferences such as acid production resulting from growth of probiotic organisms during the enrichment step, leading to possible false-negative results. Method suitability to detect the presence of microorganisms in the product to be tested must be established. Method suitability must be confirmed if changes in the testing performance occur, or changes in the product composition that may affect the outcome of the test are introduced.

• CONTAMINANT MICROORGANISMS

Unless otherwise specified in the individual monograph, the recommended tests and acceptance criteria for contaminant microorganisms in probiotic ingredients or finished products for oral use are listed in *Table 3*.

Table 3. Recommended Tests for Contaminant Microorganisms in Probiotic Ingredients or Finished Products for Oral Use

Probiotic Classification	Test	Test Methods	Acceptance Criteria (cfu/g)
Non-spore-forming bacteria	Non-lactic acid bacteria	ISO 13559 (IDF 153)[a]	NMT 5×10^3
	Total yeasts and molds	*Microbial Enumeration Tests* ⟨2021⟩	NMT 100
Spore-forming bacteria	Total yeasts and molds	*Microbial Enumeration Tests* ⟨2021⟩	NMT 100
Yeasts and molds	Total aerobic microbial count	*Microbial Enumeration Tests* ⟨2021⟩	NMT 1×10^3

[a] Available from the International Organization for Standardization (www.iso.org).

• SPECIFIED MICROORGANISMS

Unless otherwise specified in the individual monograph, the recommended tests and acceptance criteria for specified microorganisms in probiotics or finished products for oral use are listed in *Table 4*.

Table 4. Recommended Tests for Specified Microorganisms in Probiotic Ingredients or Finished Products for Oral Use

Probiotic Strain	Test	Test Methods	Acceptance Criteria
Non-spore-forming bacteria, spore-forming bacteria, or yeasts and molds	*Escherichia coli*	*Absence of Specified Microorganisms* ⟨2022⟩	None detected in 10 g
	Salmonella species	*Absence of Specified Microorganisms* ⟨2022⟩	None detected in 10 g

The absence of *Listeria monocytogenes*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa* in addition to the absence of the specified microorganisms listed in *Table 4* should be tested and confirmed if a probiotic ingredient or a finished product containing a probiotic ingredient(s) poses a risk associated with the contamination of the above microorganisms based on formal risk assessment programs such as Hazard Analysis and Critical Control Points (HACCP). The absence of *Clostridium perfringens* and *Cronobacter sakazakii* in addition to the absence of the listed specified microorganisms should be tested and confirmed if a probiotic ingredient or finished product is intended for infant use. The methods for *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Cronobacter sakazakii* must be officially accepted methods or appropriately validated methods.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE

Shipping and storage conditions will necessarily be adapted to the particular strains being sold. Unless otherwise specified in the individual monograph, probiotic ingredients should be stored in high-barrier foil-laminate bags and kept at or below 4° for long-term storage. Dosage forms of probiotics should be stored in tight containers in a cool, dry place.

• LABELING

For probiotic ingredients, the strain designation should be listed on the label for each strain. An ingredient or a dosage form of probiotics should be labeled with the genus, species, and strain names. In cases where there is suitable scientific rationale, such as scientific substantiation of health benefits that are not strain specific, a dosage form of probiotics may be labeled with the genus and species names. A total formulated enumeration of all probiotic ingredients throughout the product shelf life should be included at minimum in cfu/g or cfu/serving. ▲ (USP 1-Aug-2019)

⟨71⟩ STERILITY TESTS

•Portions of this general chapter have been harmonized with the corresponding texts of the European Pharmacopeia and/or the Japanese Pharmacopeia. Those portions that are not harmonized are marked with symbols (•,) to specify this fact.•

These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures.

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The test is applied to substances, preparations, or articles which, according to the Pharmacopeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test.

PRECAUTIONS AGAINST MICROBIAL CONTAMINATION

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any microorganisms that are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

CULTURE MEDIA AND INCUBATION TEMPERATURES

Media for the test may be prepared as described below or equivalent commercial media may be used provided that they comply with the requirements of the *Growth Promotion Test of Aerobes, Anaerobes, and Fungi.*

The following culture media have been found to be suitable for the test for sterility. *Fluid Thioglycollate Medium* is primarily intended for the culture of anaerobic bacteria. However, it will also detect aerobic bacteria. *Soybean–Casein Digest Medium* is suitable for the culture of both fungi and aerobic bacteria.

Fluid Thioglycollate Medium

L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose Monohydrate/Anhydrous	5.5/5.0 g
Agar	0.75 g
Yeast Extract (water-soluble)	5.0 g
Pancreatic Digest of Casein	15.0 g
Sodium Thioglycollate	0.5 g
or Thioglycolic Acid	0.3 mL
Resazurin Sodium Solution (1 in 1000), freshly prepared	1.0 mL
Purified Water	1000 mL

pH after sterilization: 7.1±0.2.

Mix the L-cystine, agar, sodium chloride, dextrose, yeast extract, and pancreatic digest of casein with the purified water, and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycolic acid in the solution and, if necessary, add 1 N sodium hydroxide so that, after sterilization, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling, and filter while hot through moistened filter paper. Add the resazurin sodium solution, mix, and place the medium in suitable vessels that provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2° and 25° in a sterile, airtight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and by cooling quickly, taking care to prevent the introduction of nonsterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid Thioglycollate Medium is to be incubated at 30°–35°. For products containing a mercurial preservative that cannot be tested by the membrane filtration method, *Fluid Thioglycollate Medium* incubated at 20°–25° may be used instead of *Soybean–Casein Digest Medium* provided that it has been validated as described in *Growth Promotion Test of Aerobes, Anaerobes, and Fungi.* Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the *Fluid Thioglycollate Medium,* but omitting the agar and the resazurin sodium solution. Sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30°–35° under anaerobic conditions.

Soybean–Casein Digest Medium

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean Meal	3.0 g
Sodium Chloride	5.0 g
Dibasic Potassium Phosphate	2.5 g
Dextrose Monohydrate/Anhydrous	2.5/2.3 g
Purified Water	1000 mL

pH after sterilization: 7.3±0.2.

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Dissolve the solids in the Purified Water, heating slightly to effect a solution. Cool the solution to room temperature, and adjust the pH with 1 N sodium hydroxide so that, after sterilization, it will have a pH of 7.3 ± 0.2. Filter, if necessary to clarify, dispense into suitable containers, and sterilize using a validated procedure. Store at a temperature between 2° and 25° in a sterile well-closed container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soybean–Casein Digest Medium is to be incubated at 22.5 ± 2.5°.

♦Media for Penicillins or Cephalosporins

Where sterility test media are to be used in the *Direct Inoculation of the Culture Medium* method under *Test for Sterility of the Product to be Examined,* modify the preparation of *Fluid Thioglycollate Medium* and the *Soybean–Casein Digest Medium* as follows. To the containers of each medium, transfer aseptically a quantity of β-lactamase sufficient to inactivate the amount of antibiotic in the specimen under test. Determine the quantity of β-lactamase required to inactivate the antibiotic by using a β-lactamase preparation that has been assayed previously for its penicillin- or cephalosporin-inactivating power. [NOTE—Supplemented β-lactamase media can also be used in the membrane filtration test.]

Alternatively (in an area completely separate from that used for sterility testing), confirm that an appropriate amount of β-lactamase is incorporated into the medium, following either method under *Method Suitability Test,* using less than 100 colony-forming units (cfu) of *Staphylococcus aureus* (see *Table 1*) as the challenge. Typical microbial growth of the inoculated culture must be observed as a confirmation that the β-lactamase concentration is appropriate.♦

Table 1. Strains of the Test Microorganisms Suitable for Use in the Growth Promotion Test and the Method Suitability Test

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
Pseudomonas aeruginosa♦[1]♦	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium	
Clostridium sporogenes♦[2]♦	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
Aspergillus brasiliensis (*Aspergillus Niger*)	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

♦[1] An alternative microorganism is *Kocuria rhizophila (Micrococcus luteus)* ATCC 9341.♦

♦[2] An alternative to *Clostridium sporogenes,* when a nonspore-forming microorganism is desired, is *Bacteroides vulgatus* (ATCC 8482).♦

The media used comply with the following tests, carried out before, or in parallel, with the test on the product to be examined.

Sterility

Incubate portions of the media for 14 days. No growth of microorganisms occurs.

Growth Promotion Test of Aerobes, Anaerobes, and Fungi

Test each lot of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of microorganisms are indicated in *Table 1*.

Inoculate portions of *Fluid Thioglycollate Medium* with a small number (not more than 100 cfu) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: *Clostridium sporogenes, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* ♦Inoculate portions of alternative thioglycollate medium with a small number (not more than 100 cfu) of *Clostridium sporogenes.*♦ Inoculate portions of *Soybean–Casein Digest Medium* with a small number (not more than 100 cfu) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: *Aspergillus brasiliensis, Bacillus subtilis,* and *Candida albicans.* Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the microorganisms occurs.

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♦DILUTING AND RINSING FLUIDS FOR MEMBRANE FILTRATION

Fluid A

PREPARATION

Dissolve 1 g of peptic digest of animal tissue in water to make 1 L, filter or centrifuge to clarify, if necessary, and adjust to a pH of 7.1 ± 0.2. Dispense into containers, and sterilize using a validated process.

PREPARATION FOR PENICILLINS OR CEPHALOSPORINS

Aseptically add to the above *Preparation*, if necessary, a quantity of sterile β-lactamase sufficient to inactivate any residual antibiotic activity on the membranes after the solution of the test specimen has been filtered (see *Media for Penicillins or Cephalosporins*).

Fluid D

To each L of *Fluid A* add 1 mL of polysorbate 80, adjust to a pH of 7.1 ± 0.2, dispense into containers, and sterilize using a validated process. Use this fluid for articles containing lecithin or oil, or for devices labeled as "sterile pathway."

Fluid K

Dissolve 5.0 g of peptic digest of animal tissue, 3.0 g of beef extract, and 10.0 g of polysorbate 80 in water to make 1 L. Adjust the pH to obtain, after sterilization, a pH of 6.9 ± 0.2. Dispense into containers, and sterilize using a validated process.♦

METHOD SUITABILITY TEST

Carry out a test as described below under *Test for Sterility of the Product to be Examined* using exactly the same methods, except for the following modifications.

Membrane Filtration

After transferring the content of the container or containers to be tested to the membrane, add an inoculum of a small number of viable microorganisms (not more than 100 cfu) to the final portion of sterile diluent used to rinse the filter.

Direct Inoculation

After transferring the contents of the container or containers to be tested (for catgut and other surgical sutures for veterinary use: strands) to the culture medium, add an inoculum of a small number of viable microorganisms (not more than 100 cfu) to the medium.

In both cases use the same microorganisms as those described above under *Growth Promotion Test of Aerobes, Anaerobes, and Fungi*. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of microorganisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity, and repeat the *Method Suitability Test*.

This method suitability is performed (a) when the test for sterility has to be carried out on a new product; and (b) whenever there is a change in the experimental conditions of the test. The method suitability may be performed simultaneously with the *Test for Sterility of the Product to be Examined*.

TEST FOR STERILITY OF THE PRODUCT TO BE EXAMINED

♦Number of Articles to Be Tested

Unless otherwise specified elsewhere in this chapter or in the individual monograph, test the number of articles specified in *Table 3*. If the contents of each article are of sufficient quantity (see *Table 2*), they may be divided so that equal appropriate portions are added to each of the specified media. [NOTE—Perform sterility testing employing two or more of the specified media.] If each article does not contain sufficient quantities for each medium, use twice the number of articles indicated in *Table 3*.♦

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Table 2. Minimum Quantity to be Used for Each Medium

Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)
Liquids	
Less than 1 mL	The whole contents of each container
1–40 mL	Half the contents of each container, but not less than 1 mL
Greater than 40 mL, and not greater than 100 mL	20 mL
Greater than 100 mL	10% of the contents of the container, but not less than 20 mL
Antibiotic liquids	1 mL
Insoluble preparations, creams, and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
Less than 50 mg	The whole contents of each container
50 mg or more, but less than 300 mg	Half the contents of each container, but not less than 50 mg
300 mg–5 g	150 mg
Greater than 5 g	500 mg
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30-cm long)
*Surgical dressing/cotton/gauze (in packages)	100 mg per package
Sutures and other individually packaged single-use material	The whole device
Other medical devices	The whole device, cut into pieces or disassembled.

Table 3. Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Number of Items in the Batch*	Minimum Number of Items to be Tested for Each Medium (unless otherwise justified and authorized)**
Parenteral preparations	
Not more than 100 containers	10% or 4 containers, whichever is the greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less
* *For large-volume parenterals*	2% or 10 containers, whichever is less
Antibiotic solids	
Pharmacy bulk packages (<5 g)	20 containers
Pharmacy bulk packages (≥5 g)	6 containers
Bulks and blends	See *Bulk solid products*.
Ophthalmic and other noninjectable preparations	
Not more than 200 containers	5% or 2 containers, whichever is the greater
More than 200 containers	10 containers
If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use.	
Catgut and other surgical sutures for veterinary use	2% or 5 packages, whichever is the greater, up to a maximum total of 20 packages
*Not more than 100 articles	10% or 4 articles, whichever is greater
More than 100, but not more than 500 articles	10 articles
More than 500 articles	2% or 20 articles, whichever is less.
Bulk solid products	
Up to 4 containers	Each container
More than 4 containers, but not more than 50 containers	20% or 4 containers, whichever is greater
More than 50 containers	2% or 10 containers, whichever is greater

* If the batch size is unknown, use the maximum number of items prescribed.
** If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

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The test may be carried out using the technique of *Membrane Filtration* or by *Direct Inoculation of the Culture Medium* with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits; that is, for filterable aqueous preparations, for alcoholic or oily preparations, and for preparations miscible with, or soluble in, aqueous or oily solvents, provided these solvents do not have an antimicrobial effect in the conditions of the test.

Membrane Filtration

Use membrane filters having a nominal pore size not greater than 0.45 µm, in which the effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily, and weakly alcoholic solutions; and cellulose acetate filters, for example, are used for strongly alcoholic solutions. Specially adapted filters may be needed for certain products (e.g., for antibiotics).

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used, the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions: it permits the aseptic removal of the membrane for transfer to the medium, or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

AQUEOUS SOLUTIONS

If appropriate, transfer a small quantity of a suitable, sterile diluent such as ·*Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration*)·, onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances, for example, in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary, after diluting to the volume used in the *Method Suitability Test* with the chosen sterile diluent, but using not less than the quantities of the product to be examined prescribed in *Tables 2* and *3*. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the *Method Suitability Test*. Do not exceed a washing cycle of five times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts, and transfer one half to each of two suitable media. Use the same volume of each medium as in the *Method Suitability Test*. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

SOLUBLE SOLIDS

Use for each medium not less than the quantity prescribed in *Tables 2* and *3* of the product dissolved in a suitable solvent, such as the solvent provided with the preparation, Sterile Water for Injection, sterile saline, or a suitable sterile solution such as ·*Fluid A* (*Diluting and Rinsing Fluids for Membrane Filtration*)·, and proceed with the test as described above for *Aqueous Solutions* using a membrane appropriate to the chosen solvent.

OILS and OILY SOLUTIONS

Use for each medium not less than the quantity of the product prescribed in *Tables 2* and *3*. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight, and then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as ·*Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration*)·, containing a suitable emulsifying agent at a concentration shown to be appropriate in the *Method Suitability Test,* for example polysorbate 80 at a concentration of 10 g per L ·(*Fluid K*)·. Transfer the membrane or membranes to the culture medium or media, or vice versa, as described above for *Aqueous Solutions*, and incubate at the same temperatures and for the same times.

OINTMENTS and CREAMS

Use for each medium not less than the quantities of the product prescribed in *Tables 2* and *3*. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1% in isopropyl myristate as described above, by heating, if necessary, to not more than 40°. In exceptional cases it may be necessary to heat to not more than 44°. Filter as rapidly as possible, and proceed as described above for *Oils and Oily Solutions*.

·PREFILLED SYRINGES

For prefilled syringes without attached sterile needles, expel the contents of each syringe into one or two separate membrane filter funnels or into separate pooling vessels prior to transfer. If a separate sterile needle is attached, directly expel the syringe contents as indicated above, and proceed as directed for *Aqueous Solutions*. Test the sterility of the needle, using *Direct Inoculation* under *Method Suitability Test*.

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SOLIDS FOR INJECTION OTHER THAN ANTIBIOTICS

Constitute the test articles as directed on the label, and proceed as directed for *Aqueous Solutions* or *Oils and Oily Solutions*, whichever applies. [NOTE—If necessary, excess diluent can be added to aid in the constitution and filtration of the constituted test article.]

ANTIBIOTIC SOLIDS FOR INJECTION

Pharmacy Bulk Packages, <5 g—From each of 20 containers, aseptically transfer about 300 mg of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of *Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration*), and mix; or constitute, as directed in the labeling, each of 20 containers and transfer a quantity of liquid or suspension, equivalent to about 300 mg of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of *Fluid A*, and mix. Proceed as directed for *Aqueous Solutions* or *Oils and Oily Solutions*, whichever applies.
Pharmacy Bulk Packages, ≥5 g—From each of 6 containers, aseptically transfer about 1 g of solids into a sterile 500-mL conical flask, dissolve in about 200 mL of *Fluid A*, and mix; or constitute, as directed in the labeling, each of 6 containers and transfer a quantity of liquid, equivalent to about 1 g of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of *Fluid A*, and mix. Proceed as directed for *Aqueous Solutions*.

ANTIBIOTIC SOLIDS, BULKS, and BLENDS

Aseptically remove a sufficient quantity of solids from the appropriate amount of containers (see *Table 2*), mix to obtain a composite, equivalent to about 6 g of solids, and transfer to a sterile 500-mL conical flask. Dissolve in about 200 mL of *Fluid A*, and mix. Proceed as directed for *Aqueous Solutions*.

STERILE AEROSOL PRODUCTS

For fluid products in pressurized aerosol form, freeze the containers in an alcohol-dry ice mixture at least at –20° for about 1 hour. If feasible, allow the propellant to escape before aseptically opening the container, and transfer the contents to a sterile pooling vessel. Add 100 mL of *Fluid D* to the pooling vessel, and mix gently. Proceed as directed for *Aqueous Solutions* or *Oils and Oily Solutions*, whichever applies.

DEVICES WITH PATHWAYS LABELED STERILE

Aseptically pass not less than 10 pathway volumes of *Fluid D* through each device tested. Collect the fluids in an appropriate sterile vessel, and proceed as directed for *Aqueous Solutions* or *Oils and Oily Solutions*, whichever applies.
In the case of sterile, empty syringes, draw sterile diluent into the barrel through the sterile needle, if attached, or through a sterile needle attached for the purpose of the test, and express the contents into a sterile pooling vessel. Proceed as directed above.

Direct Inoculation of the Culture Medium

Transfer the quantity of the preparation to be examined prescribed in *Tables 2* and *3* directly into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed.
If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product, it may be preferable to use a concentrated culture medium prepared in such a way that it takes into account the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container.

OILY LIQUIDS

Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the *Method Suitability Test*, for example polysorbate 80 at a concentration of 10 g per L.

OINTMENTS and CREAMS

Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as *Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration*). Transfer the diluted product to a medium not containing an emulsifying agent.
Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However, when *Fluid Thioglycollate Medium* is used for the detection of anaerobic microorganisms, keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

CATGUT and OTHER SURGICAL SUTURES FOR VETERINARIAN USE

Use for each medium not less than the quantities of the product prescribed in *Tables 2* and *3*. Open the sealed package using aseptic precautions, and remove three sections of the strand for each culture medium. Carry out the test on three sections, each 30-cm long, which have been cut off from the beginning, the center, and the end of the strand. Use whole strands from freshly opened cassette packs. Transfer each section of the strand to the selected medium. Use sufficient medium to cover adequately the material to be tested (20 mL to 150 mL).

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♦SOLIDS

Transfer a quantity of the product in the form of a dry solid (or prepare a suspension of the product by adding sterile diluent to the immediate container), corresponding to not less than the quantity indicated in *Tables 2* and *3*. Transfer the material so obtained to 200 mL of *Fluid Thioglycollate Medium,* and mix. Similarly, transfer the same quantity to 200 mL of *Soybean–Casein Digest Medium,* and mix. Proceed as directed above.

PURIFIED COTTON, GAUZE, SURGICAL DRESSINGS, and RELATED ARTICLES

From each package of cotton, rolled gauze bandage, or large surgical dressings being tested, aseptically remove two or more portions of 100- to 500-mg each from the innermost part of the sample. From individually packaged, single-use materials, aseptically remove the entire article. Immerse the portions or article in each medium, and proceed as directed above.

STERILE DEVICES

Articles can be immersed intact or disassembled. To ensure that device pathways are also in contact with the media, immerse the appropriate number of units per medium in a volume of medium sufficient to immerse the device completely, and proceed as directed above. For extremely large devices, immerse those portions of the device that are to come into contact with the patient in a volume of medium sufficient to achieve complete immersion of those portions.

For catheters where the inside lumen and outside are required to be sterile, either cut them into pieces such that the medium is in contact with the entire lumen or fill the lumen with medium, and then immerse the intact unit.♦

OBSERVATION AND INTERPRETATION OF RESULTS

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium, and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:

1. The data of the microbiological monitoring of the sterility testing facility show a fault.
2. A review of the testing procedure used during the test in question reveals a fault.
3. Microbial growth is found in the negative controls.
4. After determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid, it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility. If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.

APPLICATION OF THE TEST TO PARENTERAL PREPARATIONS, OPHTHALMIC, AND OTHER NONINJECTABLE PREPARATIONS REQUIRED TO COMPLY WITH THE TEST FOR STERILITY

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in *Table 2,* diluting where necessary to about 100 mL with a suitable sterile solution, such as ♦*Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration*).♦

When using the technique of direct inoculation of media, use the quantities shown in *Table 2,* unless otherwise justified and authorized. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

MINIMUM NUMBER OF ITEMS TO BE TESTED

The minimum number of items to be tested in relation to the size of the batch is given in *Table 3*.

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Biological Tests and Assays

⟨81⟩ ANTIBIOTICS—MICROBIAL ASSAYS

Change to read:

INTRODUCTION AND GENERAL INFORMATION

The activity (potency) of antibiotics can be demonstrated by their inhibitory effect on microorganisms under suitable conditions. A reduction in antimicrobial activity may not be adequately demonstrated by chemical methods. This chapter summarizes procedures for the antibiotics recognized in the *United States Pharmacopeia* (*USP*) for which the microbiological assay is the standard analytical method.

Two general techniques are employed: the cylinder-plate (or plate) assay and the turbidimetric (or tube) assay. *Table 1* lists all the antibiotics that contain microbial assays and specifies the type of assay (cylinder-plate or turbidimetric).

Table 1

Antibiotic	Type of Assay
▲Amoxicillin	Cylinder-plate ▲(USP 1-Dec-2019)
Amphotericin B	Cylinder-plate
Bacitracin	Cylinder-plate
Bleomycin	Cylinder-plate
Capreomycin	Turbidimetric
▲▲(USP 1-Dec-2019)	▲▲(USP 1-Dec-2019)
Chloramphenicol	Turbidimetric
Chlortetracycline	Turbidimetric
Cloxacillin	Cylinder-plate
Colistemethate	Cylinder-plate
Colistin	Cylinder-plate
Dihydrostreptomycin	Cylinder-plate Turbidimetric
Erythromycin	Cylinder-plate
Gentamicin	Cylinder-plate
Gramicidin	Turbidimetric
Nafcillin	Cylinder-plate
▲▲(USP 1-Dec-2019)	▲▲(USP 1-Dec-2019)
Neomycin	Cylinder-plate Turbidimetric
Novobiocin	Cylinder-plate
Nystatin	Cylinder-plate
Oxytetracycline	Turbidimetric
Paromomycin	Cylinder-plate
Penicillin G	Cylinder-plate
Polymyxin B	Cylinder-plate
▲▲(USP 1-Dec-2019)	▲▲(USP 1-Dec-2019)
Tetracycline	Turbidimetric
Thiostrepton	Turbidimetric
▲▲(USP 1-Dec-2019)	▲▲(USP 1-Dec-2019)
Tylosin	Turbidimetric
Vancomycin	Cylinder-plate

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[NOTE—Perform all procedures under conditions designed to avoid extrinsic microbial contamination. Take adequate safety precautions while performing these assays because of possible allergies to drugs and because live cultures of organisms are used in the procedures.]

Cylinder-Plate Assay

The cylinder-plate assay depends on diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a Petri dish or plate. The growth of the specific microorganisms inoculated into the agar is prevented in a circular area or "zone" around the cylinder containing the solution of the antibiotic.

Turbidimetric Assay

The turbidimetric assay depends on the inhibition of growth of a microorganism in a uniform solution of the antibiotic in a fluid medium that is favorable to the growth of the microorganism in the absence of the antibiotic.

Units and Reference Standards

▲For substances like the antibiotics quantified using the method in this chapter which are not easily characterized by chemical or physical means, it is still necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative reference standard. The potency of antibiotics is designated in either units (U) or µg of activity. In each case, the unit or µg of antibiotic activity was originally established against a US Federal Master Standard for that antibiotic. This practice started because originally the antibiotic selected as a reference standard was thought to consist entirely of a single chemical entity and was therefore assigned a potency of 1000 µg/mg. In several such instances, as the manufacturing and purification methods for particular antibiotics became more advanced, antibiotics containing more than 1000 µg of activity/mg became possible. Since relative potency was used to assign this value and not the mass of the antibiotics themselves, such antibiotics had an "activity" equivalent to a given number of µg of the original reference standard (rather than a µg of mass as would be expected). Over time, less complex antibiotics or those with a single primary active substance were demonstrated to have µg of activity equivalent numerically to the µg (weight) of the pure substance. In these cases, the antibiotics have moved away from the microbial assay methods and are assigned by a mass balance approach; until that exercise is completed for a given antibiotic, manufacturers cannot assume that the µg of activity corresponds to the µg (weight) of the antibiotic substance. Since the US Federal Master antibiotic standards are no longer available, USP Reference Standards for antibiotics that are assigned relative potency by the methods in this chapter are calibrated either against the World Health Organization (WHO) International Standard for Antibiotics (ISA), if it exists, or the previous USP lot when a WHO ISA or another authoritative reference standard calibrated against an ISA does not exist. Once assigned, the International Units (IUs), when used, are converted to USP units by the previously agreed upon bridging value between the two when the respective Reference Standards were originally assigned. Over time, it may be possible to convert the assay methods of more antibiotics from the microbial bioassay to a physicochemical method much like that suggested in *Validation of Alternative Methods to Antibiotic Microbial Assays* ⟨1223.1⟩ or other suitable strategies that include validated methods with associated bridging data.▲ (USP 1-Dec-2019)

Apparatus

Labware used for the storage and transfer of test dilutions and microorganisms must be sterile and free of interfering residues (see *Cleaning Glass Apparatus* ⟨1051⟩). Use a validated sterilization method, such as dry heat, steam, or radiation; or use sterile, disposable labware.

Temperature Control

Thermostatic control is required in several stages of a microbial assay: when culturing a microorganism and preparing its inoculum, and during incubation in plate and tube assays. Refer to specific temperature requirements below for each type of assay.

Test Organisms

The test organism for each antibiotic is listed in *Table 3* for the cylinder-plate assay and *Table 8* for the turbidimetric assay. The test organisms are specified by the American Type Culture Collection (ATCC) number.

In order to ensure acceptable performance of test organisms, store and maintain them properly. Establish the specific storage conditions during method validation or verification. Discard cultures if a change in the organism's characteristics is observed.

Prolonged Storage

For prolonged storage, maintain test organisms in a suitable storage solution such as 50% fetal calf serum in broth, 10%–15% glycerol in tryptic soy broth, defibrinated sheep blood, or skim milk. Prolonged-storage cultures are best stored in the freeze-dried state; temperatures of –60° or below are preferred; temperatures below –20° are acceptable.

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Primary Cultures

Prepare primary cultures by transferring test organisms from prolonged-storage vials onto appropriate media, and incubate under appropriate growth conditions. Store primary cultures at the appropriate temperature, usually 2°–8°, and discard after 3 weeks. A single primary culture can be used to prepare working cultures only for as many as 7 days.

Working Cultures

Prepare working cultures by transferring the primary culture onto appropriate solid media to obtain isolated colonies. Incubate working cultures under appropriate conditions to obtain satisfactory growth for preparation of test inocula. Prepare fresh working cultures for each test day.

Uncharacteristic Growth or Performance of a Test Organism

Use new stock cultures, primary cultures, or working cultures when a test organism shows uncharacteristic growth or performance.

Assay Designs

Suitable experimental designs are key to increasing precision and minimizing bias. Control of the incubation parameters, temperature distribution and time, is critical for minimizing bias; it can be accomplished by staging the plates and racks as described for each assay.

CYLINDER-PLATE ASSAY

The comparisons are restricted to relationships between zone diameter measurements within plates, excluding the variation between plates. Individual plate responses are normalized on the basis of the relative zone size of the standard compared to the mean zone size of the standard across all plates.

TURBIDIMETRIC ASSAY

To avoid systematic bias, place replicate tubes randomly in separate racks so that each rack contains one complete set of treatments. The purpose of this configuration is to minimize the influence of temperature distribution on the replicate samples. The turbidimetric assay, because of the configuration of the samples in test tube racks, is sensitive to slight variations in temperature. The influence of temperature variation can also be decreased by ensuring proper airflow or heat convection during incubation. At least 3 tubes for each sample and Standard concentration (one complete set of samples) should be placed in a single rack. The comparisons are restricted to relationships between the observed turbidities within racks.

Potency Considerations

Within the restrictions listed above, the recommended assay design employs a five-concentration standard curve and a single concentration of each sample preparation.

For the cylinder-plate assay, each plate includes only 2 treatments, the reference treatment (median level standard, i.e., S_3) and one of the other 4 concentrations of the standard (S_1, S_2, S_4, and S_5) or the sample (U_3). The concentration of the sample is an estimate based on the target concentration. The sample should be diluted to give a nominal concentration that is estimated to be equivalent to the median reference concentration (S_3) of the standard. The purpose of diluting to the median reference concentration is to ensure that the sample result will fall within the linear portion of the standard curve. The test determines the relative potency of U_3 against the standard curve. The sample (U_3) should have a relative potency of about 100%. The final potency of the sample is obtained by multiplying the U_3 result by the dilution factor.

An assay should be considered preliminary if the computed potency value of the sample is less than 80% or more than 125%. In this case, the results suggest that the sample concentration assumed during preparation of the sample stock solution was not correct. In such a case, one can adjust the assumed potency of the sample on the basis of the preliminary potency value and repeat the assay. Otherwise, the potency will be derived from a portion of the curve where the Standard and sample responses will likely not be parallel.

Microbial determinations of potency are subject to inter-assay as well as intra-assay variables; therefore ▲three▲ (USP 1-Dec-2019) or more independent assays are required for a reliable estimate of the potency of a given sample. Starting with separately prepared stock solutions and test dilutions of both the Standard and the sample, perform additional assays of a given sample▲▲ (USP 1-Dec-2019). The mean potency should include the results from all the valid independent assays. ▲▲ (USP 1-Dec-2019) The latter is assessed by the width of the confidence interval (refer to *Confidence Limits and Combination of Assays Calculations*). The combined result of a series of smaller, independent assays ▲▲ (USP 1-Dec-2019) is a more reliable estimate of potency than one from a single large assay with the same total number of plates or tubes. Note that additional assays or lower variability allows the product to meet tighter specification ranges. Reducing assay variability achieves the required confidence limit with fewer assays.

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Change to read:

CYLINDER-PLATE METHOD

Temperature Control

Use appropriately qualified and calibrated equipment to obtain the temperature ranges specified in *Table 3*.

Apparatus

PLATES

Use glass or disposable plastic Petri dishes (approximately 20 mm × 100 mm or other appropriate dimensions) with lids.

CYLINDERS

Use stainless steel or porcelain cylinders; 8 ± 0.1-mm o.d.; 6 ± 0.1-mm i.d.; 10 ± 0.1-mm high. [NOTE—Carefully clean cylinders to remove all residues; occasional cleaning in an acid bath, e.g., with about 2 N nitric acid or with chromic acid (see ⟨1051⟩) is required.]

Standard Solutions

To prepare a stock solution, dissolve a suitable quantity of the USP Reference Standard of a given antibiotic, or the entire contents of a vial of USP Reference Standard, where appropriate, in the solvent specified in *Table 2*; and dilute to the specified concentration. Store at 2°–8°, and use within the period indicated. On the day of the assay, prepare from the stock solution five or more test dilutions, in which the successive solutions increase stepwise in concentration, usually in the ratio of 1:1.25. Use the final diluent specified such that the median has the concentration suggested in *Table 2*.

Sample Solutions

Assign an assumed potency per unit weight or volume to the sample. On the day of the assay, prepare a stock solution in the same manner specified for the USP Reference Standard (see *Table 2*). Dilute the sample stock solution in the specified final diluent to obtain a nominal concentration equal to the median concentration of the standard (S_3).

Table 2

Antibiotic	Stock Solution					Test Dilution	
	Initial Solvent	Initial Concentration	Further Diluent	Final Concentration	Use Within (days)	Final Diluent	Median Concentration (S_3)[a, b]
▲Amoxicillin[c]▲	Water	—	—	100 µg/mL	7	B.3[d]	0.1 µg/mL▲ (USP 1-Dec-2019)
Amphotericin B[c, e]	Dimethyl sulfoxide	—	—	1 mg/mL	Same day	B.10[d]	1 µg/mL
Bacitracin[f]	0.01 N hydrochloric acid	—	—	100 U/mL	Same day	B.1[d]	1 U/mL
Bleomycin	B.16[d]	—	—	2 U/mL	14	B.16[d]	0.04 U/mL
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Cloxacillin	B.1[d]	—	—	1 mg/mL	7	B.1[d]	5 µg/mL
Colistimethate[c]	Water	10 mg/mL	B.6[d]	1 mg/mL	Same day	B.6[d]	1 µg/mL
Colistin	Water	10 mg/mL	B.6[d]	1 mg/mL	14	B.6[d]	1 µg/mL
Dihydrostreptomycin[g]	B.3[d]	—	—	1 mg/mL	30	B.3[d]	1 µg/mL
Erythromycin	Methanol	10 mg/mL	B.3[d]	1 mg/mL	14	B.3[d]	1 µg/mL
Gentamicin	B.3[d]	—	—	1 mg/mL	30	B.3[d]	0.1 µg/mL
Nafcillin	B.1[d]	—	—	1 mg/mL	2	B.1[d]	2 µg/mL
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Neomycin[g]	B.3[d]	—	—	1 mg/mL	14	B.3[d]	1 µg/mL
Novobiocin	alcohol	10 mg/mL	B.3[d]	1 mg/mL	5	B.6[d]	0.5 µg/mL
Nystatin[c, h]	Dimethylformamide	—	—	1000 U/mL	Same day	B.6[d]	20 U/mL

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Table 2 *(continued)*

Antibiotic	Stock Solution					Test Dilution	
	Initial Solvent	Initial Concentration	Further Diluent	Final Concentration	Use Within (days)	Final Diluent	Median Concentration (S_3)[a,b]
Paromomycin	B.3[d]	—	—	1 mg/mL	21	B.3[d]	1 µg/mL
Penicillin G	B.1[d]	—	—	1000 U/mL	4	B.1[d]	1 U/mL
Polymyxin B[i]	Water	—	B.6[d]	10,000 U/mL	14	B.6[d]	10 U/mL
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Vancomycin	Water	—	—	1 mg/mL	7	B.4[d]	10 µg/mL

[a] It is acceptable to adjust the median concentration to optimize zone sizes if the data remain in the linear range.
[b] µg in this column refers to µg of activity.
[c] Prepare the USP Reference Standard and sample test dilutions simultaneously.
[d] The letter B refers to buffer. See *Buffers* for a description of each buffer listed in this table.
[e] Further dilute the stock solution with dimethyl sulfoxide to give concentrations of 12.8, 16, 20, 25, and 31.2 µg/mL before making the test dilutions. The test dilution of the sample should contain the same amount of dimethyl sulfoxide as the test dilutions of the USP Reference Standard.
[f] Each of the standard test dilutions should contain the same amount of hydrochloric acid as the test dilution of the sample.
[g] The turbidimetric assay can be used as an alternative procedure.
[h] Further dilute the stock solution with dimethylformamide to give concentrations of 256, 320, 400, 500, and 624 U/mL before making the test dilutions. Prepare the standard test dilutions simultaneously with test dilutions of the sample to be tested. The test dilution of the sample should contain the same amount of dimethylformamide as the test dilutions of the standard. Use low-actinic glassware.
[i] Prepare the stock solution by adding 2 mL of water for each 5 mg of the USP Reference Standard.

Inocula

Suspend the test organism from a freshly grown slant or culture in 3 mL of sterile saline TS. Glass beads can be used to facilitate the suspension. Spread the saline suspension onto the surface of two or more agar plates (covering the entire surface) or onto the surface of a Roux bottle containing 250 mL of the specified medium (see *Table 3*).

Incubate for the specified time and at the temperature as specified in *Table 3*, or until growth is apparent.

After incubation, harvest the organism from the plates or Roux bottle with approximately 50 mL of sterile saline TS (except use *Medium 34* for bleomycin; see *Media and Solutions*), using a sterile bent glass rod or sterile glass beads. Pipet the suspension into a sterile glass container. This is the harvest suspension.

▲To make the stock suspension,▲ (USP 1-Dec-2019) dilute an appropriate amount of the harvest suspension with sterile saline TS. Using the UV-Vis spectrophotometer, measure the percentage transmittance at 580 nm. The target value is approximately 25% transmittance at 580 nm. This value is used to standardize the harvest suspension volume added to the seed layer agar.

Starting with the suggested volumes indicated in *Table 3*, determine during method verification the proportions of stock suspension to be added to the inoculum medium that result in satisfactory zones of inhibition of approximately 14–16 mm in diameter for the median concentration of the Standard (S_3).

[NOTE—Zone sizes that are outside the 11–19-mm range are not desirable, because these contribute to assay variability.]

If the dilution percentage transmittance is above 25%, a ratio may be used to normalize the addition of organism to the seed layer. The normalization factor can be determined by dividing the percentage transmittance obtained from the dilution by 25. This ratio can then be multiplied by the suggested inoculum amount to obtain the volume (mL) of harvest suspension that needs to be added to the seed layer. Adjust the quantity of inoculum on a daily basis, if necessary, to obtain an optimum concentration–response relationship.

Alternatively, determine during method verification the proportion of harvest suspension to be incorporated into the inoculum, starting with the volumes indicated in *Table 3*, that result in satisfactory demarcation of the zones of inhibition of about 14–16 mm in diameter for the median concentration of the Standard (S_3) and giving a reproducible concentration–response relationship. Prepare the inoculum by adding a portion of stock suspension to a sufficient amount of agar medium that has been melted and cooled to 45°–50°. Swirl the mixture without creating bubbles in order to obtain a homogeneous suspension.

Table 3

Antibiotic	Test Organism	ATCC[a] Number	Incubation Conditions			Suggested Inoculum Composition	
			Medium[b]	Temperature (°)	Time	Medium[b]	Amount (mL/100 mL)
▲Amoxicillin	*Kocuria rhizophila*	9341	1	32–35	24 h	11	0.5▲ (USP 1-Dec-2019)
Amphotericin B	*Saccharomyces cerevisiae*	9763	19	29–31	48 h	19	1.0
Bacitracin	*Micrococcus luteus*	10240	1	32–35	24 h	1	0.3
Bleomycin	*Mycobacterium smegmatis*	607	36	36–37.5	48 h	35	1.0

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Table 3 *(continued)*

Antibiotic	Test Organism	ATCC[a] Number	Incubation Conditions			Suggested Inoculum Composition	
			Medium[b]	Temperature (°)	Time	Medium[b]	Amount (mL/100 mL)
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Cloxacillin	*Staphylococcus aureus*	29737	1	32–35	24 h	1	0.1
Colistimethate	*Bordetella bronchiseptica*	4617	1	32–35	24 h	10	0.1
Colistin	*Bordetella bronchiseptica*	4617	1	32–35	24 h	10	0.1
Dihydrostreptomycin	*Bacillus subtilis*	6633	32	32–35	5 days	5	As required
Erythromycin	▲*Kocuria rhizophila*▲ (USP 1-Dec-2019)	9341	1	32–35	24 h	11	1.5
Gentamicin	*Staphylococcus epidermidis*	12228	1	32–35	24 h	11	0.03
Nafcillin	*Staphylococcus aureus*	29737	1	32–35	24 h	1	0.3
Neomycin	*Staphylococcus epidermidis*	12228	1	32–35	24 h	11	0.4
Novobiocin	*Staphylococcus epidermidis*	12228	1	32–35	24 h	1	4.0
Nystatin	*Saccharomyces* ▲*Kudriavzevii*▲ (USP 1-Dec-2019)	2601	19	29–31	48 h	19	1.0
Paromomycin	*Staphylococcus epidermidis*	12228	1	32–35	24 h	11	2.0
Penicillin G	*Staphylococcus aureus*	29737	1	32–35	24 h	1	1.0
Polymyxin B	*Bordetella bronchiseptica*	4617	1	32–35	24 h	10	0.1
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Vancomycin	*Bacillus subtilis*	6633	32	32–35	5 days	8	As required

▲ (USP 1-Dec-2019)

[a] American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 (www.atcc.org).

[b] See *Media*.

Analysis

Prepare the base layer for the required number of assay Petri plates, using the medium and volume shown in *Table 4*. Allow it to harden into a smooth base layer of uniform depth. Prepare the appropriate amount of seed layer inoculum (see *Table 5*) as directed for the given antibiotic (see *Table 3*) with any adjustments made based on the preparatory trial analysis. Tilt the plate back and forth to spread the inoculum evenly over the base layer surface, and allow it to harden.

Table 4. Base Layer

Antibiotic	Medium[a]	Target Volume (mL)
▲Amoxicillin	11	21 ▲ (USP 1-Dec-2019)
Amphotericin B[b]	—	—
Bleomycin	35	10
▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)	▲ (USP 1-Dec-2019)
Colistimethate	9	21
Colistin	9	21
Dihydrostreptomycin	5	21
Erythromycin	11	21

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Table 4. Base Layer *(continued)*

Antibiotic	Medium[a]	Target Volume (mL)
Gentamicin	11	21
Neomycin	11	21
Nystatin[b]	—	—
Paromomycin	11	21
Polymyxin B	9	21
▲ ▲ (USP 1-Dec-2019)	▲ ▲ (USP 1-Dec-2019)	▲ ▲ (USP 1-Dec-2019)
Vancomycin	8	10
All others	2	21

[a] See *Media*.
[b] No base layer is used. [NOTE—The base layer may be warmed to facilitate a uniform seed layer.]

Table 5. Seed Layer

Antibiotic	Medium[a]	Target Volume (mL)
Amphotericin B	Refer to *Table 3*	8
Bleomycin		6
Nystatin		8
All others		4

[a] See *Media*.

Drop 6 assay cylinders on the inoculated surface from a height of 12 mm, using a mechanical guide or other device to ensure even spacing on a radius of 2.8 cm, and cover the plates to avoid contamination. Fill the 6 cylinders on each plate with ▲the same volume of▲ (USP 1-Dec-2019) dilutions of antibiotic containing the test levels (S_1–S_5 and U_3) specified in the following paragraph. Incubate the plates as specified in *Table 6* for 16–18 h, and remove the cylinders. Measure and record the diameter of each zone of growth inhibition to the nearest 0.1 mm.

Table 6

Antibiotic	Incubation Temperature (°)
Amphotericin B	29–31
▲ ▲ (USP 1-Dec-2019)	▲ ▲ (USP 1-Dec-2019)
Colistimethate	36–37.5
Colistin	36–37.5
Dihydrostreptomycin	36–37.5
Gentamicin	36–37.5
Neomycin	36–37.5
Novobiocin	34–36
Nystatin	29–31
Paromomycin	36–37.5
Polymyxin B	36–37.5
▲ ▲ (USP 1-Dec-2019)	▲ ▲ (USP 1-Dec-2019)
Vancomycin	36–37.5
All others	32–35

The Standards (S_1–S_5) and a single test level of the sample (U_3) corresponding to S_3 of the standard curve, as defined in *Standard Solutions* and *Sample Solutions* will be used in the assay. For deriving the standard curve, fill alternate cylinders on each of 3 plates with the median test dilution (S_3) of the Standard and each of the remaining 9 cylinders with one of the other four test dilutions of the standard. Repeat the process for the three test dilutions of the Standard. For the sample, fill alternate cylinders on each of 3 plates with the median test dilution of the Standard (S_3), and fill the remaining 9 cylinders with the corresponding test dilution (U_3) of the sample.

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Change to read:

TURBIDIMETRIC METHOD

Temperature Control

Use appropriately qualified and calibrated equipment to obtain the temperature ranges specified in *Table 8*. [NOTE—Temperature control can be achieved using either circulating air or water. The greater heat capacity of water lends it some advantage over circulating air.]

Spectrophotometer

Measuring absorbance or transmittance within a fairly narrow frequency band requires a suitable spectrophotometer in which the wavelength can be varied or restricted by the use of 580- or 530-nm filters. Alternatively, a variable-wavelength spectrophotometer can be used and set to a wavelength of 580 or 530 nm.

The instrument may be modified as follows:

1. To accept the tube in which incubation takes place (see *Apparatus* below)
2. To accept a modified cell fitted with a drain that facilitates rapid change of contents
3. To contain a flow cell for a continuous flowthrough analysis

Autozero the instrument with clear, uninoculated broth prepared as specified for the particular antibiotic, including the same amount of test dilution (including formaldehyde if specified) as found in each sample.

Either absorbance or transmittance can be measured while preparing inocula.

Apparatus

Use glass or plastic test tubes, e.g., 16 mm × 125 mm or 18 mm × 150 mm. [NOTE—Use tubes that are relatively uniform in length, diameter, and thickness and substantially free from surface blemishes and scratches. In the spectrophotometer, use matched tubes that are free from scratches or blemishes. Clean tubes thoroughly to remove all antibiotic residues and traces of cleaning solution. Sterilize tubes before use.]

Standard Solutions

To prepare a stock solution, dissolve a quantity of the USP Reference Standard of a given antibiotic or the entire contents of a vial of USP Reference Standard, where appropriate, in the solvent specified in *Table 7*, and dilute to the required concentration. Store at 2°–8°, and use within the period indicated. On the day of the assay, prepare from the stock solution five or more test dilutions, the successive solutions increasing stepwise in concentration, usually in the ratio of 1:1.25. [NOTE—It may be necessary to use smaller ratios for the successive dilutions from the stock solution for the turbidimetric assay.] Use the final diluent specified such that the median level of the Standard (S_3) has the concentration suggested in *Table 7*.

Sample Solutions

Assign an assumed potency per unit weight or volume to the unknown, and on the day of the assay prepare a stock solution in the same manner specified for the USP Reference Standard (see *Table 7*). Dilute the sample stock solution in the specified final diluent at a nominal concentration equal to the median concentration of the Standard (S_3) as specified in *Table 7*.

Table 7

Antibiotic	Stock Solution					Test Dilution	
	Initial Solvent	Initial Concentration	Further Diluent	Final Stock Concentration	Use Within (days)	Final Diluent	Median Concentration (S_3)[a]
Capreomycin	Water	—	—	1 mg/mL	7	Water	100 µg/mL
Chloramphenicol	Alcohol	10 mg/mL	Water	1 mg/mL	30	Water	2.5 µg/mL
Chlortetracycline	0.1 N hydrochloric acid	—	—	1 mg/mL	4	Water	0.06 µg/mL
Dihydrostreptomycin[b]	Water	—	—	1 mg/mL	30	Water	30 µg/mL
Gramicidin	Alcohol	—	—	1 mg/mL	30	Alcohol	0.04 µg/mL
Neomycin[b, c]	B.3[d]	—	—	100 µg/mL	14	B.3[d]	1.0 µg/mL
Oxytetracycline	0.1 N hydrochloric acid	—	—	1 mg/mL	4	Water	0.24 µg/mL
Tetracycline	0.1 N hydrochloric acid	—	—	1 mg/mL	1	Water	0.24 µg/mL

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Table 7 *(continued)*

Antibiotic	Stock Solution					Test Dilution	
	Initial Solvent	Initial Concentration	Further Diluent	Final Stock Concentration	Use Within (days)	Final Diluent	Median Concentration (S_3)[a]
Thiostrepton	Dimethyl sulfoxide	—	—	▲100 U/mL▲ (USP 1-Dec-2019)	Same day	Dimethyl sulfoxide	0.80 U/mL
▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)
Tylosin	Methanol	10 mg/mL	B.16[d]	1 mg/mL	30	Methanol and B. 3[d] (1:1)	4 µg/mL

[a] µg in this column refers to µg of activity.
[b] The cylinder-plate assay can be used as an alternative procedure.
[c] Dilute the 100-µg/mL stock solution with *Buffer B.3* to obtain a solution having a concentration equivalent to 25 µg/mL of neomycin. To separate 50-mL volumetric flasks add 1.39, 1.67, 2.00, 2.40, and 2.88 mL of this solution. Add 5.0 mL of 0.1 N hydrochloric acid to each flask, dilute with *Buffer B.3* to volume, and mix to obtain solutions having concentrations of 0.69, 0.83, 1.0, 1.2, and 1.44 µg/mL of neomycin. Use these solutions to prepare the standard response line.
[d] The letter B refers to buffer. See *Buffers* for a description of each buffer listed in this table.

Inocula

Suspend the test organism from a freshly grown slant or culture in 3 mL of sterile saline TS. Glass beads can be used to facilitate the suspension. *Enterococcus hirae* (ATCC 10541) ▲(when used for the gramicidin assay),▲ (USP 1-Dec-2019) and *Staphylococcus aureus* (ATCC 9144) are grown in a liquid medium, not on agar. Spread the saline suspension onto the surface of two or more agar plates (covering the entire surface) or onto the surface of a Roux bottle containing 250 mL of the specified medium (see *Table 8*). Incubate at the time and temperature specified in *Table 8*, or until growth is apparent.

After incubation, harvest the organism from the plates or Roux bottle with approximately 50 mL of sterile saline TS, using a sterile bent glass rod or sterile glass beads. Pipet the suspension into a sterile glass bottle. This is the harvest suspension.

Determine during method verification the quantity of harvest suspension that will be used as the inoculum, starting with the volume suggested in *Table 8*. Prepare also an extra Standard (S_3) as a test of growth. Incubate the trial tests for the times indicated in *Table 11*. Adjust the quantity of inoculum daily, if necessary, to obtain the optimum concentration–response relationship from the amount of growth of the test organism in the assay tubes. At the completion of the specified incubation periods, tubes containing the median concentration of the Standard should have absorbance values as specified in *Table 9*. Determine the exact duration of incubation by observing the growth in the reference concentration (median concentration) of the standard (S_3).

Table 8

Antibiotic	Test Organism	ATCC[a] Number	Incubation Conditions			Suggested Inoculum Composition	
			Medium[b]	Temperature (°)	Time (h)	Medium[b]	Amount (mL/100 mL)
Capreomycin	*Klebsiella pneumoniae*	10031	1	36–37.5	16–24	3	0.05
Chloramphenicol	*Escherichia coli*	10536	1	32–35	24	3	0.7
Chlortetracycline	*Staphylococcus aureus*	29737	1	32–35	24	3	0.1
Dihydrostreptomycin	*Klebsiella pneumoniae*	10031	1	36–37.5	16–24	3	0.1
Gramicidin	*Enterococcus hirae*	10541	3	36–37.5	16–18	3	1.0
Neomycin	*Klebsiella pneumoniae*	10031	1	36–37.5	16–24	39	2
Oxytetracycline	*Staphylococcus aureus*	29737	1	32–35	24	3	0.1
Tetracycline	*Staphylococcus aureus*	29737	1	32–35	24	3	0.1
Thiostrepton	*Enterococcus hirae*	10541	40	36–37.5	18–24	41	0.2
▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)
Tylosin	*Staphylococcus aureus*	9144	3	35–39	16–18	39	2–3

[a] American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 (www.atcc.org).
[b] See *Media*.

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Table 9

Antibiotic	Absorbance, NLT (a.u.)
Capreomycin	0.4
Chlortetracycline	0.35
Gramicidin	0.35
Tetracycline	0.35
All others	0.3

Analysis

On the day of the assay, prepare the necessary concentration of antibiotic by dilution of stock solutions of the Standard and of each sample as specified under *Standard Solutions* and *Sample Solutions*. Prepare 5 test levels, each in triplicate, of the Standard (S_1–S_5) and a single test level (U_3), also in triplicate, of up to 20 samples corresponding to S_3 (median concentration) of the Standard.

Place the tubes in test tube racks or other carriers. Include in each rack 1–2 control tubes containing ▲0.1 mL of the test diluent for gramicidin, thiostrepton, and tylosin, or▲ (USP 1-Dec-2019) 1 mL of the test diluent ▲for all others▲ (USP 1-Dec-2019) (see *Table 7*), but no antibiotic. Add the volumes of the Standard and sample test dilutions as indicated in *Table 10*. Randomly distribute one complete set, including the controls, in a tube rack. Add the volume of inoculum specified in *Table 10* to each tube in the rack in turn, and place the completed rack immediately in an incubator or a water bath maintained at 36.0°–37.5° for the time specified in *Table 11*.

Table 10

Antibiotic	Volume of Test Dilution (mL)	Volume of Inoculum (mL)
Gramicidin	0.10	9.0
Thiostrepton	0.10	10.0
Tylosin	0.10	9.0
All others	1.0	9.0

Table 11

Antibiotic	Incubation Time (h)
Capreomycin	3–4
Chloramphenicol	3–4
Cycloserine	3–4
Dihydrostreptomycin	3–4
▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)
▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)
Tylosin	3–5
All others	4–5

After incubation, immediately inhibit the growth of the organism by adding 0.5 mL of dilute formaldehyde to each tube, except for tylosin. For tylosin, heat the rack in a water bath at 80°–90° for 2–6 min or in a steam bath for 5–10 min, and bring to room temperature. Read absorbance or transmittance at 530 or 580 nm, analyzing one rack at a time.

MEDIA AND SOLUTIONS

The media required for the preparation of test organism inocula are made from the ingredients listed herein. Minor modifications of the individual ingredients are acceptable; and reconstituted dehydrated media can be substituted, provided that the resulting media possess equal or better growth-promoting properties and give a similar standard curve response.

Media

Dissolve the ingredients in water to make 1 L, and adjust the solutions with either 1 N sodium hydroxide or 1 N hydrochloric acid as required, so that after steam sterilization the pH is as specified.

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Medium 1

Peptone	6.0 g
Pancreatic digest of casein	4.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	6.6 ± 0.1

Medium 2

Peptone	6.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	6.6 ± 0.1

Medium 3

Peptone	5.0 g
Yeast extract	1.5 g
Beef extract	1.5 g
Sodium chloride	3.5 g
Dextrose	1.0 g
Dibasic potassium phosphate	3.68 g
Monobasic potassium phosphate	1.32 g
Water	1000 mL
pH after sterilization	7.0 ± 0.05

Medium 4

Peptone	6.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	6.6 ± 0.1

Medium 5

Peptone	6.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	7.9 ± 0.1

Medium 8

Peptone	6.0 g
Yeast extract	3.0 g

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Medium 8 *(continued)*

Beef extract	1.5 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	5.9 ± 0.1

Medium 9

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Dextrose	2.5 g
Agar	20.0 g
Water	1000 mL
pH after sterilization	7.2 ± 0.1

Medium 10

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Dextrose	2.5 g
Agar	12.0 g
Water	1000 mL
Polysorbate 80 (added after boiling the medium to dissolve the agar)	10 mL
pH after sterilization	7.2 ± 0.1

Medium 11

Peptone	6.0 g
Pancreatic digest of casein	4.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	8.3 ± 0.1

Medium 13

Peptone	10.0 g
Dextrose	20.0 g
Water	1000 mL
pH after sterilization	5.6 ± 0.1

Medium 19

Peptone	9.4 g
Yeast extract	4.7 g
Beef extract	2.4 g
Sodium chloride	10.0 g
Dextrose	10.0 g
Agar	23.5 g

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Medium 19 *(continued)*

Water	1000 mL
pH after sterilization	6.1 ± 0.1

Medium 32

Peptone	6.0 g
Pancreatic digest of casein	4.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Manganese sulfate	0.3 g
Dextrose	1.0 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	6.6 ± 0.1

Medium 34

Glycerol	10.0 g
Peptone	10.0 g
Beef extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL
pH after sterilization	7.0 ± 0.1

Medium 35

Glycerol	10.0 g
Peptone	10.0 g
Beef extract	10.0 g
Sodium chloride	3.0 g
Agar	17.0 g
Water	1000 mL
pH after sterilization	7.0 ± 0.1

Medium 36

Pancreatic digest of casein	15.0 g
Papaic digest of soybean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL
pH after sterilization	7.3 ± 0.1

Medium 39

Peptone	5.0 g
Yeast extract	1.5 g
Beef extract	1.5 g
Sodium chloride	3.5 g
Dextrose	1.0 g
Dibasic potassium phosphate	3.68 g
Monobasic potassium phosphate	1.32 g
Water	1000 mL
pH after sterilization	7.9 ± 0.1

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Medium 40

Yeast extract	20.0 g
Polypeptone	5.0 g
Dextrose	10.0 g
Monobasic potassium phosphate	2.0 g
Polysorbate 80	0.1 g
Agar	10.0 g
Water	1000 mL
pH after sterilization	6.7 ± 0.2

Medium 41

Pancreatic digest of casein	9.0 g
Dextrose	20.0 g
Yeast extract	5.0 g
Sodium citrate	10.0 g
Monobasic potassium phosphate	1.0 g
Dibasic potassium phosphate	1.0 g
Water	1000 mL
pH after sterilization	6.8 ± 0.1

Solutions

BUFFERS

Prepare as directed in *Table 12*, or by other suitable means. The buffers are sterilized after preparation; the pH specified in each case is the pH after sterilization.

Table 12. Buffers

Buffer	Concentration of Dibasic Potassium Phosphate (g/L)	Concentration of Monobasic Potassium Phosphate (g/L)	Volume of 10 N Potassium Hydroxide (mL)	pH after Sterilization[a]
Buffer B.1 (1%, pH 6.0)	2	8	—	6.0 ± 0.05
Buffer B.3 (0.1 M, pH 8.0)	16.73	0.523	—	8.0 ± 0.1
Buffer B.4 (0.1 M, pH 4.5)	—	13.61	—	4.5 ± 0.05
Buffer B.6 (10%, pH 6.0)	20	80	—	6.0 ± 0.05
Buffer B.10 (0.2 M, pH 10.5)	35	—	2	10.5 ± 0.1
Buffer B.16 (0.1 M, pH 7.0)	13.6	4	—	7.0 ± 0.2

[a] Adjust the pH with 18 N phosphoric acid or 10 N potassium hydroxide.

Other solutions: See *Reagents, Indicators, and Solutions*.
Water: Use *Purified Water*.
Saline: Use saline TS.
Dilute formaldehyde: Formaldehyde solution and water (1:3)

Change to read:

CALCULATIONS

Introduction

Antibiotic potency is calculated by interpolation from a standard curve using a log-transformed straight-line method with a least-squares fitting procedure (see below for calculation details). The analyst must consider three essential concepts in interpreting antibiotic potency results:

1. Biological concentration–response relationships generally are not linear. The antibiotic potency method allows fitting the data to a straight line by evaluating a narrow concentration range where the results approach linearity. The assay results

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can be considered valid only if the computed potency is 80%–125% of that assumed in preparing the sample stock solution. When the calculated potency value falls outside 80%–125%, the result for the sample may fall outside the narrow concentration range where linearity has been established. In such a case, adjust the assumed potency of the sample accordingly, and repeat the assay to obtain a valid result.

2. The most effective means of reducing the variability of the reportable value (the geometric mean potency across runs and replicates) is through independent runs of the assay procedure. The combined result of a series of smaller, independent assays ▲(USP 1-Dec-2019)▲ is a more reliable estimate of potency than that from a single large assay with the same total number of plates or tubes. Three or more independent assays are required for antibiotic potency determinations.
3. The number of assays needed in order to obtain a reliable estimate of antibiotic potency depends on the required specification range and the assay variability. The confidence limit calculation described below is determined from several estimated log potencies that are approximately equal in precision. If the value calculated for the ▲half-▲(USP 1-Dec-2019) width of the confidence interval, *W*, is too wide, no useful decision can be made about whether the potency meets its specification.

The laboratory should predetermine in its standard operating procedures a maximum acceptable value for the confidence interval ▲half-▲(USP 1-Dec-2019) width. This maximum value should be determined during development and confirmed during validation or verification. If the calculated confidence interval ▲half-▲(USP 1-Dec-2019) width exceeds this limit, the analyst must perform additional independent potency determinations to meet the limit requirement. Note that the decision to perform additional determinations does not depend on the estimated potency but only on the uncertainty in that estimate as determined by the confidence interval ▲half-▲(USP 1-Dec-2019) width. *Assay* variability has a greater impact on the calculated confidence limit than does the number of independent potency determinations. As a result, the analyst should first consider decreasing variability to the extent possible before conducting potency determinations.

The following sections describe the calculations for determining antibiotic potency as well as for performing the confidence limit calculation. Methods for calculating standard error are also shown in order to allow estimates of assay variance. Where logarithms are used, any base log is acceptable. *Appendix 1: Formulas for Manual Calculations of Regression and Sample Concentration* provides formulas for hand calculations applicable when the concentrations are equally spaced in the log scale. Alternative statistical methods may be used if appropriately validated.

Cylinder-Plate Assay

This section details analysis of the sample data and determination of the potency of an unknown, using the cylinder-plate assay.

SAMPLE DATA

Table 13 shows the data from one assay that will be used as an example throughout this section. For each of the ▲15▲(USP 1-Dec-2019) plates, zones 1, 3, and 5 are ▲for▲(USP 1-Dec-2019) the reference concentration, ▲(S_3),▲(USP 1-Dec-2019) and the other three zones are for one of the other four ▲standard▲(USP 1-Dec-2019) concentrations, ▲(S_1, S_2, S_4, and S_5) or the sample (U_3),▲(USP 1-Dec-2019) as shown. Other columns are needed for calculations and are explained below.

Step 1: Perform initial calculations and variability suitability check.

For each set of 3 plates, average the 9 reference values and average the 9 Standard values.

Example: See *Table 13*.

$$15.867 = \overline{X}(16.1, 15.6, \ldots, 15.8)$$
$$14.167 = \overline{X}(14.6, 14.1, \ldots, 14.8)$$

For each set of 3 plates, determine the standard deviation of the 9 reference values and the standard deviation of the 9 Standard values. For each standard deviation, determine the corresponding relative standard deviation.

Example: See *Table 13*.

$$0.200 = \sigma(16.1, \ldots, 15.8)$$
$$1.3\% = (0.200/15.867) \times 100$$
$$0.324 = \sigma(14.6, \ldots, 14.1)$$
$$2.3\% = (0.324/14.167) \times 100$$

For a variability suitability criterion, each laboratory should determine a maximum acceptable value for the relative standard deviation. If any of the eight relative standard deviations (four for the reference and four for the Standard) exceed this predetermined maximum, the assay data are not suitable and should be discarded. [NOTE—The suggested limit for relative standard deviation is NMT 10%.]

Step 2: Perform a plate-to-plate variation correction.

This correction is applied to convert the average zone measurement obtained for each concentration to the value it would be if the average reference concentration measurement for that set of 3 replicate plates were the same as the value of the correction point:

$$\overline{X}_C = \overline{X}_S - (\overline{X}_R - P)$$

$\overline{X}_C$ = corrected standard mean
$\overline{X}_S$ = original standard mean

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$\overline{X}_R$ = reference mean
P = correction point

Example: For the first set of 3 plates in *Table 13* (S_1), the correction is:

$$14.022 = 14.167 - (15.867 - 15.722) = 14.167 - 0.145$$

Step 3: Determine the standard curve line.

Generate the standard curve line by plotting the corrected zone measurements versus the log of the standard concentration values. Calculate the equation of the standard curve line by performing a standard unweighted linear regression on these values, using appropriate software or the manual calculations of *Appendix 1: Formulas for Manual Calculations of Regression and Sample Concentration*. [NOTE—Use either the natural log or the base 10 log to plot the standard curve and determine the regression equation; both provide the same final test result.] Each laboratory should determine a minimum value of the coefficient of determination (%R^2) for an acceptable regression. The regression is acceptable only if the obtained %R^2 exceeds this predetermined value. [NOTE—The suggested limit for the percentage coefficient of determination is NLT 95%.]

Table 13. Sample Data (Cylinder-Plate Assay)

Standard	Concentration (U/mL)	Plate Replicate	Reference (S_3) Zone 1 (mm)	Zone 3 (mm)	Zone 5 (mm)	Mean (mm)	SD	%RSD	▲Standard▲ (USP 1-Dec-2019) Zone 2 (mm)	Zone 4 (mm)	Zone 6 (mm)	Mean (mm)	SD	%RSD	Corrected Mean (mm)
		1	16.1	15.6	15.8				14.6	14.1	13.5				
		2	16.0	15.9	16.2				14.5	14.1	14.4				
S_1	3.20	3	15.7	15.7	15.8	15.867	0.200	1.3	14.0	14.2	14.1	14.167	0.324	2.3	14.022
		1	15.8	15.6	15.5				14.7	15.1	14.8				
		2	15.7	15.5	15.6				14.7	14.9	15.2				
S_2	4.00	3	15.7	15.4	15.3	15.567	0.158	1.0	14.8	15.0	14.3	14.833	0.265	1.8	14.989
		1	15.6	15.8	16.0				16.6	16.8	16.3				
		2	15.8	15.6	15.7				16.6	16.5	16.2				
S_4	6.25	3	16.1	15.7	15.8	15.789	0.169	1.1	16.9	16.5	16.8	16.578	0.233	1.4	16.511
		1	15.6	15.6	15.5				17.3	17.0	17.0				
		2	15.6	15.7	15.5				17.3	17.4	17.2				
S_5	7.8125	3	15.9	15.8	15.8	15.667	0.141	0.9	17.3	17.3	16.7	17.167	0.224	1.3	17.222
						15.722 [a]									
▲Sample			Reference (S_3) ▲(USP 1-Dec-2019)						▲Sample▲ (USP 1-Dec-2019)						
		1	15.7	15.8	15.7				15.3	15.8	15.7				
		2	15.9	15.7	15.7				15.8	15.8	15.5				
U_3	Unknown	3	15.5	15.8	15.3	15.678	0.179	1.1	15.2	15.1	15.1	15.478	0.307	2.0	15.522

[a] This is the value of the overall reference mean, referred to as the "correction point" below.

Example: Table 14 summarizes the portion of *Table 13* needed for this part of the calculation.

Table 14

Standard Set	Corrected Zone Measurements (mm)	Concentration (U/mL)
S_1	14.022	3.2
S_2	14.989	4.0
Reference (S_3)	15.722	5
S_4	16.511	6.25
S_5	17.222	7.8125

LINEAR REGRESSION RESULTS

Standard curve line:

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$$Z = [3.551 \times \ln(C)] + 9.978$$

Z = corrected zone measurement
C = concentration
$\%R^2$ = 99.7

SAMPLE POTENCY DETERMINATION

To estimate the potency of the unknown sample, average the zone measurements of the Standard and the zone measurements of the sample on the 3 plates used. Correct for plate-to-plate variation using the correction point determined above to obtain a corrected average for the unknown, $\overline{U}$. [NOTE—An acceptable alternative to using the correction point is to correct using the value on the estimated regression line corresponding to the log concentration of S_3.] Use the corrected average zone measurement in the equation of the standard curve line to determine the log concentration of the sample, L_U, by:

$$L_U = (\overline{U} - a)/b$$

a = intercept of the regression line
b = slope of the regression line

To obtain the potency of the unknown, take the antilog of L_U and multiply the result by any applicable dilution factor. This value can also be expressed as a percentage of the reference concentration value.

Example: Corrected sample zone measurement (see *Table 13*) = 15.522

Natural log of the sample concentration:

$$L_U = (15.522 - 9.978)/3.551 = 1.561$$

Sample concentration:

$$C_U = e^{1.561} = 4.765$$

Percentage of reference concentration:

$$\text{Result} = (4.765/5.000) \times 100 = 95.3\%$$

Turbidimetric Assay

This section details analysis of the sample data and determination of the potency of an unknown using the turbidimetric assay. The method assumes that the tubes are randomly distributed within the heat block or other temperature control device. If the device has a temperature profile that is not uniform, a randomized blocks design is preferred. In such a design, the rack is divided into areas ("blocks") of relatively uniform temperature and at least one tube of each Standard concentration and of each unknown is placed in each area. The data analysis of a randomized block design is different from the following.

SAMPLE DATA

Table 15 shows the data from one assay that will be used for an example throughout this section. Other columns are needed for calculations and are explained below.

Table 15. Sample Data (Turbidimetric Assay)

Standard	Concentration (µg/mL)	Replicate	Absorbance (a.u.)	Average (a.u.)	Standard Deviation
S_1	64	1	0.8545	0.8487	0.0062
		2	0.8422		
		3	0.8495		
S_2	80	1	0.8142	0.8269	0.0125
		2	0.8273		
		3	0.8392		
S_3	100	1	0.6284	0.6931	0.0640
		2	0.6947		
		3	0.7563		
S_4	125	1	0.6933	0.6827	0.0119
		2	0.6850		
		3	0.6699		

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Table 15. Sample Data (Turbidimetric Assay) *(continued)*

Standard	Concentration (µg/mL)	Replicate	Absorbance (a.u.)	Average (a.u.)	Standard Deviation
		1	0.5299		
		2	0.5779		
S_5	156	3	0.5316	0.5465	0.0272
		1	0.7130		
		2	0.7960		
U_3	Unknown	3	0.7201	0.7430	0.0460

Step 1: Perform initial calculations and variability suitability check.

For each concentration (including the sample), average the three absorbance values.

Example: See S_1 in *Table 15*.

$$0.8487 = \overline{X}(0.8545, 0.8422, 0.8495)$$

For each concentration, determine the standard deviation of the three readings and a combined standard deviation for all the concentrations.

Example: See S_1 in *Table 15*.

$$0.0062 = SD(0.8545, 0.8422, 0.8495)$$

The combined value is calculated by taking the square root of the average of the five variances:

$$0.0322 = \{[(0.0062)^2 + (0.0125)^2 + (0.0640)^2 + (0.0119)^2 + (0.0272)^2]/5\}^{1/2}$$

For a variability suitability criterion, each laboratory should determine a maximum acceptable combined standard deviation. If the combined standard deviation exceeds this predetermined maximum, the assay data are not suitable and should be discarded. [NOTE—The suggested limit for the combined standard deviation is NMT 10% of the average absorbance value across the five concentrations.] If the number of replicates per concentration is at least 5, then a relative standard deviation can be computed for each concentration after checking for outliers and compared to a maximum acceptable relative standard deviation. [NOTE—The suggested limit for the relative standard deviation is NMT 10%.]

Step 2: Determine the standard curve line.

Generate the standard curve line by plotting the average absorbance values versus the log of the standard concentration values. Calculate the equation of the standard curve line by performing an unweighted linear regression on these values using appropriate software or the manual calculations of *Appendix 1: Formulas for Manual Calculations of Regression and Sample Concentration*. [NOTE—Use either the natural log or the base 10 log to plot the standard curve and determine the regression equation; both provide the same final test result.] Each laboratory should determine a minimum value of the percentage coefficient of determination (%R^2) for an acceptable regression. The regression is acceptable only if the %R^2 value obtained exceeds this predetermined value. [NOTE—The suggested limit for the percentage coefficient of determination is NLT 90%.]

Example: Table 16 summarizes the portion of *Table 15* needed for this part of the calculation.

Table 16

Set of Standards	Average Absorbance Values (a.u.)	Concentration (µg/mL)
S_1	0.8487	64
S_2	0.8269	80
S_3	0.6931	100
S_4	0.6827	125
S_5	0.5465	156

LINEAR REGRESSION RESULTS

Standard curve line:

$$\text{Absorbance} = 2.2665 - [0.7735 \times \log_{10}(\text{concentration})]$$

%R^2 = 93.0%

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SAMPLE POTENCY DETERMINATION

To estimate the potency of the unknown sample, average the three absorbance measurements to obtain an average for the unknown, $\overline{U}$. Use this average measurement in the equation of the standard curve line to determine the log concentration of the unknown sample, L_U, by:

$$L_U = (\overline{U} - a)/b$$

a = intercept of the regression line
b = slope of the regression line

To obtain the potency of the unknown, take the antilog of L_U and multiply the result by any applicable dilution factor. This value can also be expressed as a percentage of the reference concentration value.
Example: Average sample absorbance (see *Table 15*) = 0.7430.

$$\log_{10}(C_U) = (0.7430 - 2.2665)/(-0.7735) = 1.9696$$
$$C_U = 10^{1.9696} = 93.2$$
$$\text{Percentage of reference concentration} = (93.2/100.0) \times 100 = 93.2\%$$

C_U = concentration of the sample

Confidence Limits and Combination of Assays Calculations

Because of interassay variability, three or more independent determinations are required for a reliable estimate of the sample potency. For each independent determination, start with separately prepared stock solutions and test dilutions of both the Standard and the sample, and repeat the assay of a given sample ▲▲ (USP 1-Dec-2019).

Given a set of at least three determinations of the unknown potency, use the method of *Appendix 2: Procedure for Checking for Outliers; Rejection of Outlying or Aberrant Measurements* to check for any outlier values. This determination should be done in the log scale.

To obtain a combined estimate of the unknown potency, calculate the average, M, and the standard deviation of the accepted log potencies. [NOTE—Use either the natural log or the base 10 log.] Determine the confidence interval for the potency as follows:

$$\text{antilog}[M - t(0.05, N - 1) \times SD/\sqrt{N}],\ \text{antilog}[M + t(0.05, N - 1) \times SD/\sqrt{N}]$$

M = average
$t(0.05, N-1)$ = the two-sided 5% point of a Student's t-distribution with $N-1$ degrees of freedom
[NOTE—The t value is available in spreadsheets, statistics texts, and statistics software.]
SD = standard deviation
N = number of assays

$$W = \text{antilog}\{[t(0.05, N - 1) \times SD/\sqrt{N}]\}$$

W = half-width of the confidence interval

Compare the half-width of the confidence interval to a predetermined maximum acceptable value. If the half-width is larger than the acceptance limit, continue with additional assays.

EXAMPLE

Suppose the sample is assayed four times, with potency results in the natural log scale of 1.561, 1.444, 1.517, and 1.535. Then:

$$N = 4$$
$$M = \overline{X}(1.561, 1.444, 1.517, 1.535) = 1.514$$
$$SD = \sigma(1.561, 1.444, 1.517, 1.535) = 0.050$$
$$t = 3.182$$

The confidence interval in the log scale is

$$1.514 \pm (3.182 \times 0.050/\sqrt{4}) = (1.434, 1.594)$$

Taking antilogs, the estimated potency is

$$e^{1.514} = 4.546$$

with a 95% confidence interval for the potency of $e^{1.434}$, $e^{1.594}$ = (4.197, 4.924).
The half-width of the confidence interval to compare to an acceptance value is the ratio 4.924/4.546 = 1.083.

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Change to read:

APPENDICES

Appendix 1: Formulas for Manual Calculations of Regression and Sample Concentration

If the concentrations are equally spaced in the logarithmic scale, the calculations can be performed using the following formula. Let:

$\bar{S}_k$ = mean corrected zone measurement (cylinder-plate assay) or average absorbance value (turbidimetric assay) for standard set k
k = 1, 2, 3, 4, 5
$\bar{S}$ = mean of the five $\bar{S}_k$ values
L_k = logarithm of the kth concentration. [NOTE—Use either the natural log or the base 10 log. Slope of the regression line is calculated by:]

$$b = (Y_{high} - Y_{low})/(X_{high} - X_{low})$$
$$Y_{high} = \tfrac{1}{8}(3\bar{S}_5 + 2\bar{S}_4 + \bar{S}_3 - \bar{S}_1)$$
$$Y_{low} = \tfrac{1}{8}(3\bar{S}_1 + 2\bar{S}_2 + \bar{S}_3 - \bar{S}_5)$$

$X_{high} = L_5$
$X_{low} = L_1$

Combine and simplify to:

$$b = (4\bar{S}_5 + 2\bar{S}_4 - 2\bar{S}_2 - 4\bar{S}_1)/[5(L_5 - L_1)]$$

The log of the concentration of the sample is found using:

$$L_U = L_{reference} + [(\bar{U} - \bar{S})/b]$$

For example, using the data for the cylinder-plate assay in *Table 13* and natural logarithms:

$$b = [(4 \times 17.222) + (2 \times 16.511) - (2 \times 14.989) - (4 \times 14.020)]/\{5[\ln(7.81) - \ln(3.2)]\} = 3.551$$
$$\bar{S} = (14.020 + 14.989 + 15.722 + 16.511 + 17.222)/5 = 15.693$$
$$\text{Natural log of sample concentration} = \ln(5) + [(15.522 - 15.693)/3.551] = 1.561$$
$$\text{Sample concentration} = e^{1.561} = 4.765$$

Appendix 2: Procedure for Checking for Outliers—Rejection of Outlying or Aberrant Measurements

A measurement that is clearly questionable because of a failure in the assay procedure should be rejected, whether it is discovered during the measuring or tabulation procedure. The arbitrary rejection or retention of an apparently aberrant measurement can be a serious source of bias. In general, the rejection of measurements solely on the basis of their relative magnitudes is a procedure that should be used sparingly.

Each suspected potency measurement, or outlier, may be tested against the following criterion. This criterion is based on the variation within a single group of supposedly equivalent measurements from a normal distribution. ▲At a confidence level of 99%, a valid observation will be rejected once in 100 trials (when the suspected outlier can occur at only one end) or once in 50 trials (when the suspected outlier can occur at either end), provided that relatively few, if any, responses within the group are identical. Arrange the responses in order of magnitude from y_1 to y_N, where N is the number of observations in the group.▲ (USP 1-Dec-2019) Compute the relative gap by using *Table A2-1*, and the formulas below:

When N = 3–7:

$G_1 = (y_2 - y_1)/(y_N - y_1)$ ▲Candidate Outlier is Smallest (y_1)
$G_1 = (y_N - y_{N-1})/(y_N - y_1)$ Candidate Outlier is Largest (y_N)▲ (USP 1-Dec-2019)

When N = 8–10:

$G_2 = (y_2 - y_1)/(y_{N-1} - y_1)$ ▲Candidate Outlier is Smallest (y_1)
$G_2 = (y_N - y_{N-1})/(y_N - y_2)$ Candidate Outlier is Largest (y_N)▲ (USP 1-Dec-2019)

When N = 11–13:

$G_3 = (y_3 - y_1)/(y_{N-1} - y_1)$ ▲Candidate Outlier is Smallest (y_1)
$G_3 = (y_N - y_{N-2})/(y_N - y_2)$ Candidate Outlier is Largest (y_N)▲ (USP 1-Dec-2019)

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If G_1, G_2, or G_3, as appropriate, exceeds the critical value in *Table A2-1*, for the observed N, there is a statistical basis ▲for identifying the discordant measurement as an outlier and considering its removal.▲ (USP 1-Dec-2019)

Table A2-1. Test for Outlier Measurements

In samples from a normal population, gaps equal to or larger than the following values of G_1, G_2, and G_3 occur with a probability $P = 0.01$, when outlier measurements can occur only at one end; or with $P = 0.02$, when they may occur at either end.					
N	3	4	5	6	7
G_1	▲0.988▲ (USP 1-Dec-2019)	0.889	▲0.780▲ (USP 1-Dec-2019)	0.698	0.637
N	8	9	10	—	—
G_2	▲0.683	0.635▲ (USP 1-Dec-2019)	0.597	—	—
N	11	12	13	—	—
G_3	▲0.679	0.642	0.615▲ (USP 1-Dec-2019)	—	—

EXAMPLE

Estimated potencies of sample in log scale = 1.561, 1.444, 1.517, 1.535.
Check the lowest potency for outlier:

$$G_1 = (1.517 - 1.444)/(1.561 - 1.444) = 0.624 < 0.889$$

Therefore, 1.444 is not an outlier.
Check the highest potency for outlier:

$$G_1 = (1.561 - 1.535)/(1.561 - 1.444) = 0.222 < 0.889$$

Therefore, 1.561 is not an outlier.
Outlier potencies should be marked as outlier values and excluded from the assay calculations. NMT 1 potency can be excluded as an outlier.

⟨85⟩ BACTERIAL ENDOTOXINS TEST

•Portions of this general chapter have been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. Those portions that are not harmonized are marked with symbols (• •) to specify this fact.•

The Bacterial Endotoxins Test (BET) is a test to detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*).

There are three techniques for this test: the gel-clot technique, which is based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. Proceed by any of the three techniques for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot limit test unless otherwise indicated in the monograph for the product being tested. The test is carried out in a manner that avoids endotoxin contamination.

APPARATUS

Depyrogenate all glassware and other heat-stable materials in a hot air oven using a validated process.•[1]• A commonly used minimum time and temperature is 30 min at 250°. If employing plastic apparatus, such as microplates and pipet tips for automatic pipetters, use apparatus that is shown to be free of detectable endotoxin and does not interfere in the test. [NOTE—In this chapter, the term "tube" includes any other receptacle such as a microtiter well.]

REAGENTS AND TEST SOLUTIONS

Amoebocyte Lysate

A lyophilized product obtained from the lysate of amoebocytes (white blood cells) from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). This reagent refers only to a product manufactured in accordance with the regulations of the competent authority. [NOTE—*Amoebocyte Lysate* reacts to some β-glucans in addition to endotoxins. *Amoebocyte Lysate* preparations that do not react to glucans are available: they are prepared by removing the G factor reacting to glucans from

•[1] For a validity test of the procedure for inactivating endotoxins, see *Dry-Heat Sterilization* under *Sterility Assurance* ⟨1211⟩. Use *Lysate TS* having a sensitivity of not less than 0.15 Endotoxin Unit per mL.•

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Amoebocyte Lysate or by inhibiting the G factor reacting system of *Amoebocyte Lysate* and may be used for endotoxin testing in the presence of glucans.]

Water for Bacterial Endotoxins Test (BET)

Use Water for Injection or water produced by other procedures that shows no reaction with the lysate employed, at the detection limit of the reagent.

Lysate TS

Dissolve *Amoebocyte Lysate* in *Water for BET*, or in a buffer recommended by the lysate manufacturer, by gentle stirring. Store the reconstituted lysate, refrigerated or frozen, according to the specifications of the manufacturer.

PREPARATION OF SOLUTIONS

Standard Endotoxin Stock Solution

A *Standard Endotoxin Stock Solution* is prepared from a USP Endotoxin Reference Standard that has been calibrated to the current WHO International Standard for Endotoxin. Follow the specifications in the package leaflet and on the label for preparation and storage of the *Standard Endotoxin Stock Solution*. Endotoxin is expressed in Endotoxin Units (EU). [NOTE—One USP Endotoxin Unit (EU) is equal to one International Unit (IU) of endotoxin.]

Standard Endotoxin Solutions

After mixing the *Standard Endotoxin Stock Solution* vigorously, prepare appropriate serial dilutions of *Standard Endotoxin Solution*, using *Water for BET*. Use dilutions as soon as possible to avoid loss of activity by adsorption.

Sample Solutions

Prepare the *Sample Solutions* by dissolving or diluting drugs using *Water for BET*. Some substances or preparations may be more appropriately dissolved, or diluted in other aqueous solutions. If necessary, adjust the pH of the solution to be examined (or dilution thereof) so that the pH of the mixture of the lysate and *Sample Solution* falls within the pH range specified by the lysate manufacturer, usually 6.0–8.0. The pH may be adjusted by use of an acid, base, or suitable buffer as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with *Water for BET* in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

DETERMINATION OF MAXIMUM VALID DILUTION (MVD)

The maximum valid dilution is the maximum allowable dilution of a specimen at which the endotoxin limit can be determined. Determine the MVD from the following equation:

$$MVD = (\text{endotoxin limit} \times \text{concentration of } \textit{Sample Solution})/(\lambda)$$

Endotoxin Limit

The endotoxin limit for parenteral drugs, defined on the basis of dose, equals K/M [♦2] ,, where K is a threshold pyrogenic dose of endotoxin per kg of body weight, and M is equal to the maximum recommended bolus dose of product per kg of body weight. When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period. The endotoxin limit for parenteral drugs is specified in the individual monograph in units such as EU/mL, EU/mg, EU/Unit of biological activity, etc.

Concentration of Sample Solution

mg/mL: in the case of endotoxin limit specified by weight (EU/mg);
Units/mL: in the case of endotoxin limit specified by unit of biological activity (EU/Unit);
mL/mL: when the endotoxin limit is specified by volume (EU/mL).
λ: the labeled sensitivity in the *Gel-Clot Technique* (EU/mL) or the lowest concentration used in the standard curve for the *Turbidimetric Technique* or *Chromogenic Technique*.

[♦2] K is 5 USP-EU/kg of body weight for any route of administration other than intrathecal (for which K is 0.2 USP-EU/kg of body weight). For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as 175 EU/V, where V is the maximum recommended dose in mL. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula 14 EU/V. For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is K/M, where K = 100 EU/m² and M is the maximum dose/m². ♦

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GEL-CLOT TECHNIQUE

The gel-clot technique is used for detecting or quantifying endotoxins based on clotting of the lysate reagent in the presence of endotoxin. The minimum concentration of endotoxin required to cause the lysate to clot under standard conditions is the labeled sensitivity of the lysate reagent. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate sensitivity and for interfering factors as described in *Preparatory Testing*, immediately below.

Preparatory Testing

TEST FOR CONFIRMATION OF LABELED LYSATE SENSITIVITY

Confirm in four replicates the labeled sensitivity, λ, expressed in EU/mL of the lysate prior to use in the test. The test for confirmation of lysate sensitivity is to be carried out when a new batch of lysate is used or when there is any change in the test conditions that may affect the outcome of the test. Prepare standard solutions having at least four concentrations equivalent to 2λ, λ, 0.5λ, and 0.25λ by diluting the USP Endotoxin RS with *Water for BET*.

Mix a volume of the *Lysate TS* with an equal volume (such as 0.1-mL aliquots) of one of the *Standard Endotoxin Solutions* in each test tube. When single test vials or ampuls containing lyophilized lysate are used, add solutions directly to the vial or ampul. Incubate the reaction mixture for a constant period according to the directions of the lysate manufacturer (usually at 37 ± 1° for 60 ± 2 min), avoiding vibration. To test the integrity of the gel, take each tube in turn directly from the incubator, and invert it through about 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed. The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The endpoint is the smallest concentration in the series of decreasing concentrations of standard endotoxin that clots the lysate. Determine the geometric mean endpoint by calculating the mean of the logarithms of the endpoint concentrations of the four replicate series and then taking the antilogarithm of the mean value, as indicated in the following formula:

$$\text{geometric mean endpoint concentration} = \text{antilog}\ (\Sigma e/f)$$

where Σe is the sum of the log endpoint concentrations of the dilution series used, and f is the number of replicate test tubes. The geometric mean endpoint concentration is the measured sensitivity of the lysate (in EU/mL). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate.

TEST FOR INTERFERING FACTORS

Usually prepare solutions *(A–D)* as shown in *Table 1*, and perform the inhibition/enhancement test on the *Sample Solutions* at a dilution less than the MVD, not containing any detectable endotoxins, operating as described for *Test for Confirmation of Labeled Lysate Sensitivity*. The geometric mean endpoint concentrations of *Solutions B* and *C* are determined using the formula described in the *Test for Confirmation of Labeled Lysate Sensitivity*. The test for interfering factors must be repeated when any condition changes that is likely to influence the result of the test.

Table 1. Preparation of Solutions for the Inhibition/Enhancement Test for Gel-Clot Techniques

Solution	Endotoxin Concentration/ Solution to Which Endotoxin Is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
A[a]	None/*Sample Solution*	—	—	—	4
B[b]	2λ/*Sample Solution*	*Sample Solution*	1	2λ	4
			2	1λ	4
			4	0.5λ	4
			8	0.25λ	4
C[c]	2λ/*Water for BET*	*Water for BET*	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D[d]	None/*Water for BET*	—	—	—	2

[a] *Solution A*: A *Sample Solution* of the preparation under test that is free of detectable endotoxins.
[b] *Solution B*: Test for interference.
[c] *Solution C*: Control for labeled lysate sensitivity.
[d] *Solution D*: Negative control of *Water for BET*.

The test is considered valid when all replicates of *Solutions A* and *D* show no reaction and the result of *Solution C* confirms the labeled sensitivity.

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If the sensitivity of the lysate determined in the presence of *Solution B* is not less than 0.5λ and not greater than 2λ, the *Sample Solution* does not contain factors that interfere under the experimental conditions used. Otherwise, the *Sample Solution* to be examined interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined, and this may contribute to the elimination of interference.

Interference may be overcome by suitable treatment such as filtration, neutralization, dialysis, or heating. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

Limit Test

PROCEDURE

Prepare *Solutions A, B, C,* and *D* as shown in *Table 2*, and perform the test on these solutions following the procedure above for *Preparatory Testing, Test for Confirmation of Labeled Lysate Sensitivity*.

Table 2. Preparation of Solutions for the Gel-Clot Limit Test

Solution*	Endotoxin Concentration/ Solution to Which Endotoxin Is Added	Number of Replicates
A	None/Diluted *Sample Solution*	2
B	2λ/Diluted *Sample Solution*	2
C	2λ/*Water for BET*	2
D	None/*Water for BET*	2

* Prepare *Solution A* and the positive product control *Solution B* using a dilution not greater than the MVD and treatments as described for the *Test for Interfering Factors* in *Preparatory Testing*. The positive control *Solutions B* and *C* contain the *Standard Endotoxin Solution* at a concentration corresponding to twice the labeled lysate sensitivity. The negative control *Solution D* consists of *Water for BET*.

INTERPRETATION

The test is considered valid when both replicates of *Solutions B* and *C* are positive and those of *Solution D* are negative. When a negative result is found for both replicates of *Solution A*, the preparation under test complies with the test. When a positive result is found for both replicates of *Solution A*, the preparation under test does not comply with the test.

When a positive result is found for one replicate of *Solution A* and a negative result is found for the other, repeat the test. In the repeat test, the preparation under test complies with the test if a negative result is found for both replicates of *Solution A*. The preparation does not comply with the test if a positive result is found for one or both replicates of *Solution A*. However, if the preparation does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

Quantitative Test

PROCEDURE

The test quantifies bacterial endotoxins in *Sample Solutions* by titration to an endpoint. Prepare *Solutions A, B, C,* and *D* as shown in *Table 3*, and test these solutions by following the procedure in *Preparatory Testing, Test for Confirmation of Labeled Lysate Sensitivity*.

Table 3. Preparation of Solutions for the Gel-Clot Assay

Solution	Endotoxin Concentration/ Solution to Which Endotoxin Is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
A[a]	None/*Sample Solution*	*Water for BET*	1	—	2
			2	—	2
			4	—	2
			8	—	2
B[b]	2λ/*Sample Solution*	—	1	2λ	2
C[c]	2λ/*Water for BET*	*Water for BET*	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2

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Table 3. Preparation of Solutions for the Gel-Clot Assay *(continued)*

Solution	Endotoxin Concentration/ Solution to Which Endotoxin Is Added	Diluent	Dilution Factor	Endotoxin Concentration	Number of Replicates
D[d]	None/*Water for BET*	—	—	—	2

[a] *Solution A*: *Sample Solution* under test at the dilution, not to exceed the MVD, with which the *Test for Interfering Factors* was completed. Subsequent dilution of the *Sample Solution* must not exceed the MVD. Use *Water for BET* to make a dilution series of four tubes containing the *Sample Solution* under test at concentrations of 1, ½, ¼, and ⅛ relative to the concentration used in the *Test for Interfering Factors*. Other dilutions up to the MVD may be used as appropriate.
[b] *Solution B*: *Solution A* containing standard endotoxin at a concentration of 2λ (positive product control).
[c] *Solution C*: Two replicates of four tubes of *Water for BET* containing the standard endotoxin at concentrations of 2λ, λ, 0.5λ, and 0.25λ, respectively.
[d] *Solution D*: *Water for BET* (negative control).

CALCULATION AND INTERPRETATION

The test is considered valid when the following three conditions are met: (1) Both replicates of negative control *Solution D* are negative; (2) Both replicates of positive product control *Solution B* are positive; and (3) The geometric mean endpoint concentration of *Solution C* is in the range of 0.5λ to 2λ.

To determine the endotoxin concentration of *Solution A*, calculate the endpoint concentration for each replicate by multiplying each endpoint dilution factor by λ. The endotoxin concentration in the *Sample Solution* is the endpoint concentration of the replicates. If the test is conducted with a diluted *Sample Solution*, calculate the concentration of endotoxin in the original *Sample Solution* by multiplying by the dilution factor. If none of the dilutions of the *Sample Solution* is positive in a valid assay, report the endotoxin concentration as less than λ (if the diluted sample was tested, report as less than λ times the lowest dilution factor of the sample). If all dilutions are positive, the endotoxin concentration is reported as equal to or greater than the greatest dilution factor multiplied by λ (e.g., initial dilution factor times eight times λ in *Table 3*).

The preparation under test meets the requirements of the test if the concentration of endotoxin in both replicates is less than that specified in the individual monograph.

PHOTOMETRIC QUANTITATIVE TECHNIQUES

Turbidimetric Technique

This technique is a photometric assay measuring increases in reactant turbidity. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-turbidimetric assay or a kinetic-turbidimetric assay. The endpoint-turbidimetric assay is based on the quantitative relationship between the concentration of endotoxins and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation period. The kinetic-turbidimetric assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance or transmission of the reaction mixture, or the rate of turbidity development. The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually 37 ± 1°).

Chromogenic Technique

This technique is an assay to measure the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with lysate. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-chromogenic assay or a kinetic-chromogenic assay. The endpoint-chromogenic assay is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period. The kinetic-chromogenic assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance of the reaction mixture, or the rate of color development. The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually 37 ± 1°).

Preparatory Testing

To assure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to verify that the criteria for the standard curve are valid and that the sample solution does not interfere with the test. Validation for the test method is required when conditions that are likely to influence the test result change.

ASSURANCE OF CRITERIA FOR THE STANDARD CURVE

The test must be carried out for each lot of lysate reagent. Using the *Standard Endotoxin Solution*, prepare at least three endotoxin concentrations within the range indicated by the lysate manufacturer to generate the standard curve. Perform the assay using at least three replicates of each standard endotoxin concentration according to the manufacturer's instructions for the lysate (volume ratios, incubation time, temperature, pH, etc.). If the desired range is greater than two logs in the kinetic methods, additional standards should be included to bracket each log increase in the range of the standard curve. The absolute value of the correlation coefficient, *r*, must be greater than or equal to 0.980 for the range of endotoxin concentrations set up.

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TEST FOR INTERFERING FACTORS

Select an endotoxin concentration at or near the middle of the endotoxin standard curve. Prepare *Solutions A, B, C,* and *D* as shown in *Table 4*. Perform the test on *Solutions A, B, C,* and *D* at least in duplicate, according to the instructions for the lysate employed, for example, concerning volume of *Sample Solution* and *Lysate TS,* volume ratio of *Sample Solution* to *Lysate TS,* incubation time, etc.

Table 4. Preparation of Solutions for the Inhibition/Enhancement Test for Photometric Techniques

Solution	Endotoxin Concentration	Solution to Which Endotoxin Is Added	Number of Replicates
A[a]	None	*Sample Solution*	Not less than 2
B[b]	Middle concentration of the standard curve	*Sample Solution*	Not less than 2
C[c]	At least three concentrations (lowest concentration is designated λ)	*Water for BET*	Each not less than 2
D[d]	None	*Water for BET*	Not less than 2

[a] *Solution A*: The *Sample Solution* may be diluted not to exceed MVD.
[b] *Solution B*: The preparation under test at the same dilution as *Solution A,* containing added endotoxin at a concentration equal to or near the middle of the standard curve.
[c] *Solution C*: The standard endotoxin at the concentrations used in the validation of the method described for *Assurance of Criteria for the Standard Curve* under *Preparatory Testing* (positive controls).
[d] *Solution D*: *Water for BET* (negative control).

The test is considered valid when the following conditions are met.

1. The absolute value of the correlation coefficient of the standard curve generated using *Solution C* is greater than or equal to 0.980.
2. The result with *Solution D* does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution, if any (*Solution A, Table 4*), from that containing the added endotoxin (*Solution B, Table 4*). In order to be considered free of factors that interfere with the assay under the conditions of the test, the measured concentration of the endotoxin added to the *Sample Solution* must be within 50%–200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified range, the *Sample Solution* under test is considered to contain interfering factors. Then, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the *Sample Solution* or diluted *Sample Solution* not to exceed the MVD may be eliminated by suitable validated treatment such as filtration, neutralization, dialysis, or heat treatment. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described above, using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

Test Procedure

Follow the procedure described for *Test for Interfering Factors* under *Preparatory Testing,* immediately above.

Calculation

Calculate the endotoxin concentration of each of the replicates of *Solution A,* using the standard curve generated by the positive control *Solution C*. The test is considered valid when the following three requirements are met.

1. The results of the control *Solution C* comply with the requirements for validation defined for *Assurance of Criteria for the Standard Curve* under *Preparatory Testing*.
2. The endotoxin recovery, calculated from the concentration found in *Solution B* after subtracting the concentration of endotoxin found in *Solution A,* is within the range of 50%–200%.
3. The result of the negative control *Solution D* does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Interpretation

In photometric assays, the preparation under test complies with the test if the mean endotoxin concentration of the replicates of *Solution A,* after correction for dilution and concentration, is less than the endotoxin limit for the product.

USP Reference Standards ⟨11⟩

USP Endotoxin RS

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⟨87⟩ BIOLOGICAL REACTIVITY TESTS, IN VITRO

The following tests are designed to determine the biological reactivity of mammalian cell cultures following contact with the elastomeric plastics and other polymeric materials with direct or indirect patient contact or of specific extracts prepared from the materials under test. It is essential that the tests be performed on the specified surface area. When the surface area of the specimen cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic or other material for every mL of extraction fluid. Exercise care in the preparation of the materials to prevent contamination with microorganisms and other foreign matter.

Three tests are described (i.e., the *Agar Diffusion Test*, the *Direct Contact Test*, and the *Elution Test*).[1] The decision as to which type of test or the number of tests to be performed to assess the potential biological response of a specific sample or extract depends upon the material, the final product, and its intended use. Other factors that may also affect the suitability of a sample for a specific use are the polymeric composition; processing and cleaning procedures; contacting media; inks; adhesives; absorption, adsorption, and permeability of preservatives; and conditions of storage. Evaluation of such factors should be made by appropriate additional specific tests before determining that a product made from a specific material is suitable for its intended use. Materials that fail the *in vitro* tests are candidates for the *in vivo* tests described in *Biological Reactivity Tests, In Vivo* ⟨88⟩.

PROCEDURES

- **TEST CONTROL**

Positive control: Polyurethane film containing zinc diethyldithiocarbamate (ZDEC)[2] or zinc dibutyldithiocarbamate (ZDBC)

Cell culture preparation: Prepare multiple cultures of L-929 (ATCC cell line CCL 1, NCTC clone 929; alternative cell lines obtained from a standard repository may be used with suitable validation) mammalian fibroblast cells in serum-supplemented minimum essential medium having a seeding density of about 10^5 cells per mL. Incubate the cultures at 37 ± 1° in a humidified incubator for NLT 24 h in a 5 ± 1% carbon dioxide atmosphere until a monolayer, with greater than 80% confluence, is obtained. Examine the prepared cultures under a microscope to ensure uniform, near-confluent monolayers. [NOTE—The reproducibility of the in vitro biological reactivity tests depends upon obtaining uniform cell culture density.]

Extraction solvents: *Sodium Chloride Injection* [see monograph—use *Sodium Chloride Injection* containing 0.9% of sodium chloride (NaCl)]. Alternatively, serum-free mammalian cell culture media or serum-supplemented mammalian cell culture media may be used. Serum supplementation is used when extraction is done at 37° for 24 h.

- **APPARATUS**

Autoclave: Employ an autoclave capable of maintaining a temperature of 121 ± 2°, equipped with a thermometer, a pressure gauge, a vent cock, a rack adequate to accommodate the test containers above the water level, and a water cooling system that will allow for cooling of the test containers to about 20°, but not below 20°, immediately following the heating cycle.

Oven: Use an oven, preferably a mechanical convection model, that will maintain operating temperatures in the range of 50°–70° within ±2°.

Incubator: Use an incubator capable of maintaining a temperature of 37 ± 1° and a humidified atmosphere of 5 ± 1% carbon dioxide in air.

Extraction containers: Use only containers, such as ampuls or screw-cap culture test tubes, or their equivalent, of Type I glass. If used, culture test tubes, or their equivalent, are closed with a screw cap having a suitable elastomeric liner. The exposed surface of the elastomeric liner is completely protected with an inert solid disk 50–75 µm in thickness. A suitable disk can be fabricated from polytef.

Preparation of apparatus: Cleanse all glassware thoroughly with chromic acid cleansing mixture and, if necessary, with hot nitric acid followed by prolonged rinsing with *Sterile Water for Injection*. Sterilize and dry by a suitable process for containers and devices used for extraction, transfer, or administration of test material. If ethylene oxide is used as the sterilizing agent, allow NLT 48 h for complete degassing.

- **PROCEDURE**

Preparation of sample for extracts: Prepare as directed in the *Procedure* in ⟨88⟩.

Preparation of extracts: Prepare as directed for *Preparation of extracts* in ⟨88⟩ using either *Sodium Chloride Injection* [0.9% sodium chloride (NaCl)] or serum-free mammalian cell culture media as *Extraction solvents*. [NOTE—If extraction is done at 37° for 24 h in an incubator, use cell culture media supplemented by serum. The extraction conditions should not in any instance cause physical changes, such as fusion or melting of the material pieces, other than a slight adherence.]

- **AGAR DIFFUSION TEST**

This test is designed for elastomeric closures in a variety of shapes. The agar layer acts as a cushion to protect the cells from mechanical damage while allowing the diffusion of leachable chemicals from the polymeric specimens. Extracts of materials that are to be tested are applied to a piece of filter paper.

Sample preparation: Use extracts prepared as directed, or use portions of the test specimens having flat surfaces NLT 100 mm^2 in surface area.

Positive control preparation: Proceed as directed for *Sample preparation*.

Negative control preparation: Proceed as directed for *Sample preparation*.

Procedure: Using 7 mL of cell suspension prepared as directed in *Cell culture preparation*, prepare the monolayers in plates having a 60-mm diameter. Following incubation, aspirate the culture medium from the monolayers, and replace it with serum-supplemented culture medium containing NMT 2% of agar. [NOTE—The quality of the agar must be adequate to

[1] Further details are given in the following publications of the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103: Standard test method for agar diffusion cell culture screening for cytotoxicity, ASTM Designation F 895-84; Standard practice for direct contact cell culture evaluation of materials for medical devices, ASTM Designation F 813-83.

[2] ZDEC and ZDBC polyurethanes are available from the Food and Drug Safety Center, Hatano Research Institute, Ochiai 729–5, Hadanoshi, Kanagawa 257, Japan.

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support cell growth. The agar layer must be thin enough to permit diffusion of leached chemicals.] Place the flat surfaces of *Sample preparation*, *Positive control preparation*, and *Negative control preparation* or their extracts in an appropriate extracting medium, in duplicate cultures in contact with the solidified agar surface. Use no more than three specimens per prepared plate. Incubate all cultures for NLT 24 h at 37 ± 1°, preferably in a humidified incubator containing 5 ± 1% of carbon dioxide. Examine each culture around each sample, negative control, and positive control under a microscope, using a suitable stain, if desired.

Interpretation of results: The biological reactivity (cellular degeneration and malformation) is described and rated on a scale of 0–4 (see *Table 1*). Measure the responses of the cell cultures to the *Sample preparation*, the *Positive control preparation*, and the *Negative control preparation*. The cell culture test system is suitable if the observed responses to the *Negative control preparation* is grade 0 (no reactivity) and to the *Positive control preparation* is at least grade 3 (moderate). The sample meets the requirements of the test if the response to the *Sample preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed.

Table 1. Reactivity Grades for Agar Diffusion Test and Direct Contact Test

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen and less than 0.45 cm beyond specimen
3	Moderate	Zone extends 0.45–1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

- **DIRECT CONTACT TEST**

This test is designed for materials in a variety of shapes. The procedure allows for simultaneous extraction and testing of leachable chemicals from the specimen with a serum-supplemented medium. The procedure is not appropriate for very low- or high-density materials that could cause mechanical damage to the cells.

Sample preparation: Use portions of the test specimen having flat surfaces NLT 100 mm^2 in surface area.

Positive control preparation: Proceed as directed for *Sample preparation*.

Negative control preparation: Proceed as directed for *Sample preparation*.

Procedure: Using 2 mL of cell suspension prepared as directed in *Cell culture preparation*, prepare the monolayers in plates having a 35-mm diameter. Following incubation, aspirate the culture medium from the cultures, and replace it with 0.8 mL of fresh culture medium. Place a single *Sample preparation*, a *Positive control preparation*, and a *Negative control preparation* in each of the duplicate cultures. Incubate all cultures for NLT 24 h at 37 ± 1° in a humidified incubator containing 5 ± 1% of carbon dioxide. Examine each culture around each *Sample preparation*, a *Positive control preparation*, and a *Negative control preparation*, under a microscope, using a suitable stain, if desired.

Interpretation of results: Proceed as directed for *Interpretation of results* in *Agar Diffusion Test*. The sample meets the requirements of the test if the response to the *Sample preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed.

- **ELUTION TEST**

This test is designed for the evaluation of extracts of polymeric materials. The procedure allows for extraction of the specimens at physiological or nonphysiological temperatures for varying time intervals. It is appropriate for high-density materials and for dose-response evaluations.

Sample preparation: Prepare as directed in *Preparation of extracts*, using either *Sodium Chloride Injection* [0.9% sodium chloride (NaCl)] or serum-free mammalian cell culture media as *Extraction solvents*. If the size of the sample cannot be readily measured, a mass of NLT 0.1 g of elastomeric material or 0.2 g of plastic or polymeric material per mL of extraction medium may be used. Alternatively, use serum-supplemented mammalian cell culture media as the extracting medium to simulate more closely physiological conditions. Prepare the extracts by heating for 24 h in an incubator containing 5 ± 1% of carbon dioxide. Maintain the extraction temperature at 37 ± 1°, because higher temperatures may cause denaturation of serum proteins.

Positive control preparation: Proceed as directed for *Sample preparation*.

Negative control preparation: Proceed as directed for *Sample preparation*.

Procedure: Using 2 mL of cell suspension prepared as directed in *Cell culture preparation*, prepare the monolayers in plates having a 35-mm diameter. Following incubation, aspirate the culture medium from the monolayers, and replace it with extracts of the *Sample preparation*, *Positive control preparation*, or *Negative control preparation*. The serum-supplemented and serum-free cell culture media extracts are tested in duplicate without dilution (100%). The *Sodium Chloride Injection* extract is diluted with serum-supplemented cell culture medium and tested in duplicate at 25% extract concentration. Incubate all cultures for 48 h at 37 ± 1° in a humidified incubator preferably containing 5 ± 1% of carbon dioxide. Examine each culture at 48 h, under a microscope, using a suitable stain, if desired.

Interpretation of results: Proceed as directed for *Interpretation of results* in *Agar Diffusion Test* but use *Table 2*. The sample meets the requirements of the test if the response to the *Sample preparation* is not greater than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed. For dose-response evaluations, repeat the procedure, using quantitative dilutions of the sample extract.

Table 2. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of All Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis

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Table 2. Reactivity Grades for Elution Test *(continued)*

Grade	Reactivity	Conditions of All Cultures
1	Slight	Less than or equal to 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Greater than 20% to less than or equal to 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Greater than 50% to less than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP High-Density Polyethylene RS
  (Negative Control)

⟨88⟩ BIOLOGICAL REACTIVITY TESTS, IN VIVO

The following tests are designed to determine the biological response of animals to elastomerics, plastics, and other polymeric material with direct or indirect patient contact, or by the injection of specific extracts prepared from the material under test. It is essential to make available the specific surface area for extraction. When the surface area of the specimen cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic or other material for every mL of extraction fluid. Also, it is essential to exercise care in the preparation of the materials to be injected or instilled to prevent contamination with microorganisms and other foreign matter. Three tests are described. The *Systemic Injection Test* and the *Intracutaneous Test* are used for elastomeric materials, especially to elastomeric closures for which the appropriate *Biological Reactivity Tests, In Vitro* ⟨87⟩ have indicated significant biological reactivity. These two tests are used for plastics and other polymers, in addition to a third test, the *Implantation Test*, to test the suitability of these materials intended for use in fabricating containers and accessories thereto, for use in parenteral preparations, and for use in medical devices, implants, and other systems.

These three tests are applied to materials or medical devices, if there is a need for classification of plastics and other polymers based on in vivo biological reactivity testing.

For the purpose of this chapter, these definitions apply: the *Sample* is the specimen under test or an extract prepared from such a specimen. A *Blank* consists of the same quantity of the same extracting medium that is used for the extraction of the specimen under test, treated in the same manner as the extracting medium containing the specimen under test. A *Negative Control*[1] is a specimen that gives no reaction under the conditions of the test.

CLASSIFICATION OF PLASTICS

Six Plastic Classes are defined (see *Table 1*). This classification is based on responses to a series of in vivo tests for which extracts, materials, and routes of administration are specified. These tests are directly related to the intended end-use of the plastic articles. The choice of extractants is representative of the vehicles in preparations with which the plastics are likely to be in contact. The *Table 1* classification facilitates communication among suppliers, users, and manufacturers of plastics by summarizing the tests to be performed for containers for injections and medical devices if a need for classification exists.

Table 1. Classification of Plastics

Plastic Classes[a]						Tests to be Conducted			
I	II	III	IV	V	VI	Test Material	Animal	Dose	Procedure[b]
x	x	x	x	x	x	Extract of *Sample* in *Sodium Chloride Injection*	Mouse	50 mL/kg	A (IV)
x	x	x	x	x	x		Rabbit or Guinea Pig	0.2 mL/animal at each of 10 or 6 sites	B (IC)
	x	x	x	x	x	Extract of *Sample* in *1 in 20 Solution of Alcohol in Sodium Chloride Injection*	Mouse	50 mL/kg	A (IP)
	x	x	x	x	x		Rabbit or Guinea Pig	0.2 mL/animal at each of 10 or 6 sites	B (IC)
		x		x	x	Extract of *Sample* in *Polyethylene Glycol 400*	Mouse	10 g/kg	A (IP)
				x	x		Rabbit or Guinea Pig	0.2 mL/animal at each of 10 or 6 sites	B (IC)
		x	x	x	x	Extract of *Sample* in *Vegetable Oil*	Mouse	50 mL/kg	A (IP)
			x	x	x		Rabbit or Guinea Pig	0.2 mL/animal at each of 10 or 6 sites	B (IC)
			x		x	Implant strips of *Sample*	Rabbit	4 strips/animal	C

[1] USP High-Density Polyethylene RS.

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Table 1. Classification of Plastics *(continued)*

Plastic Classes[a]						Tests to be Conducted			
I	II	III	IV	V	VI	Test Material	Animal	Dose	Procedure[b]
			x		x	Implant *Sample*	Rat	2 *Samples*/animal	C

[a] Tests required for each class are indicated by "x" in appropriate columns.
[b] Legend: A (IP)—Systemic Injection Test (intraperitoneal); B (IC)—Intracutaneous Test (intracutaneous); C—Implantation Test (intramuscular or subcutaneous implantation).

With the exception of the *Implantation Test,* the procedures are based on the use of extracts that, depending on the heat resistance of the material, are prepared at one of three standard temperatures: 50°, 70°, and 121°. Therefore, the class designation of a plastic must be accompanied by an indication of the temperature of extraction (e.g., IV-121°, which represents a class IV plastic extracted at 121°, or I-50°, which represents a class I plastic extracted at 50°).

Plastics may be classified as USP Plastic Classes I–VI only on the basis of the response criteria prescribed in *Table 1*.

This classification does not apply to plastics that are intended for use as containers for oral or topical products, or that may be used as an integral part of a drug formulation. *Table 1* does not apply to natural elastomers, which are to be tested in Sodium Chloride Injection and vegetable oils only.

The *Systemic Injection Test* and the *Intracutaneous Test* are designed to determine the systemic and local, respectively, biological responses of animals to plastics and other polymers by the single-dose injection of specific extracts prepared from a *Sample*. The *Implantation Test* is designed to evaluate the reaction of living tissue to the plastic and other polymers by the implantation of the *Sample* itself into animal tissue. The proper preparation and placement of the specimens under aseptic conditions are important in the conduct of the *Implantation Test*.

These tests are designed for application to plastics and other polymers in the condition in which they are used. If the material is to be exposed to any cleansing or sterilization process prior to its end-use, then the tests are to be conducted on a *Sample* prepared from a specimen preconditioned by the same processing.

Factors such as material composition, processing and cleaning procedures, contacting media, inks, adhesives, absorption, adsorption and permeability of preservatives, and conditions of storage may also affect the suitability of a material for a specific use. Evaluation of such factors should be made by appropriate additional specific tests to determine the suitability of a material for its intended use.

USP Reference Standards ⟨11⟩—*USP High-Density Polyethylene RS.*

Extracting Media—

SODIUM CHLORIDE INJECTION (see monograph). Use Sodium Chloride Injection containing 0.9% of NaCl.

1 IN 20 SOLUTION OF ALCOHOL IN SODIUM CHLORIDE INJECTION.

POLYETHYLENE GLYCOL 400 (see monograph).

VEGETABLE OIL—Use freshly refined Sesame Oil (see monograph) or Cottonseed Oil (see monograph) or other suitable vegetable oils.

DRUG PRODUCT VEHICLE (where applicable).

WATER FOR INJECTION (see monograph).

[NOTE—The Sesame Oil or Cottonseed Oil or other suitable vegetable oil meets the following additional requirements. Obtain, if possible, freshly refined oil. Use three properly prepared animals, and inject the oil intracutaneously in a dose of 0.2 mL into each of 10 sites per animal, and observe the animals at 24, 48, and 72 h following injection. Rate the observations at each site on the numerical scale indicated in *Table 2*. For the 3 rabbits or guinea pigs (30 or 18 injection sites), at any observation time, the average response for erythema is not greater than 0.5 and for edema is not greater than 1.0, and no site shows a tissue reaction larger than 10 mm in overall diameter. The residue of oil at the injection site should not be misinterpreted as edema. Edematous tissue blanches when gentle pressure is applied.]

Table 2. Evaluation of Skin Reactions[a]

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema Formation[b]	**Score**
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

[a] Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* 1944;82:377–390.
[b] Excludes noninflammatory (mechanical) edema from the blank or extraction fluid.

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Apparatus—The apparatus for the tests includes the following.

AUTOCLAVE—Use an autoclave capable of maintaining a temperature of 121 ± 2.0°, equipped with a thermometer, a pressure gauge, a vent cock, a rack adequate to accommodate the test containers above the water level, and a water cooling system that will allow for cooling of the test containers to about, but not below, 20° immediately following the heating cycle.

OVEN—Use an oven, preferably a forced-circulation model, that will maintain operating temperatures of 50° or 70° within ±2°.

EXTRACTION CONTAINERS—Use only containers, such as ampuls or screw-cap culture test tubes, of Type I glass. If used, culture test tubes are closed with screw caps having suitable elastomeric liners. The exposed surface of the elastomeric liner is completely protected with an inert solid disk 0.05–0.075 mm in thickness. A suitable disk may be fabricated from a polytef resin.

Preparation of Apparatus—Cleanse all glassware thoroughly with chromic acid cleansing mixture, or if necessary, with hot nitric acid, followed by prolonged rinsing with water. Clean cutting utensils by an appropriate method (e.g., successive cleaning with acetone and methylene chloride) prior to use in subdividing a specimen. Clean all other equipment by thorough scrubbing with a suitable detergent and prolonged rinsing with water.

Render containers and equipment used for extraction, and in transfer and administration of test material, sterile and dry by a suitable process. [NOTE—If ethylene oxide is used as the sterilizing agent, allow adequate time for complete degassing.]

Procedure—

PREPARATION OF SAMPLE—Both the *Systemic Injection Test* and the *Intracutaneous Test* may be performed using the same extract, if desired, or separate extracts may be made for each test. Select and subdivide into portions a *Sample* of the size indicated in *Table 3*. Remove particulate matter, such as lint and free particles, by treating each subdivided *Sample* or *Negative Control* as follows. Place the *Sample* into a clean, glass-stoppered, 100-mL graduated cylinder of Type I glass, and add about 70 mL of *Water for Injection*. Agitate for about 30 s, and drain off the water. Repeat this step, and dry those pieces prepared for the extraction with *Vegetable Oil* in an oven at a temperature not exceeding 50°. [NOTE—Do not clean the *Sample* with a dry or wet cloth or by rinsing or washing with an organic solvent, surfactant, etc.]

PREPARATION OF EXTRACTS—Place a properly prepared *Sample* to be tested in an extraction container, and add 20 mL of the appropriate extracting medium. Repeat these directions for each extracting medium required for testing. Also, prepare one 20-mL blank of each medium for parallel injections and comparisons. Extract by heating in an autoclave at 121° for 60 min, in an oven at 70° for 24 h, or at 50° for 72 h. Allow adequate time for the liquid within the container to reach the extraction temperature.

[NOTE—The extraction conditions should not in any instance cause physical changes such as fusion or melting of the *Sample* pieces, which result in a decrease in the available surface area. A slight adherence of the pieces can be tolerated. Always add the cleaned pieces individually to the extracting medium. If culture tubes are used for autoclave extractions with *Vegetable Oil*, seal screw caps adequately with pressure-sensitive tape.]

Cool to about room temperature but not below 20°, shake vigorously for several minutes, and decant each extract immediately, using aseptic precautions, into a dry, sterile vessel. Store the extracts at a temperature of 20°–30°, and do not use for tests after 24 h. Of importance are the contact of the extracting medium with the available surface area of the plastic and the time and temperature during extraction, the proper cooling, agitation, and decanting process, and the aseptic handling and storage of the extracts following extraction.

SYSTEMIC INJECTION TEST

This test is designed to evaluate systemic responses to the extracts of materials under test following injection into mice. Alternate routes of injection may be used with justification.

Test Animals—Use healthy, not previously used albino mice weighing 17–23 g. For each test group use only mice of the same source. Allow water and food, commonly used for laboratory animals and of known composition, ad libitum.

Procedure— [NOTE—Agitate each extract vigorously prior to withdrawal of injection doses to ensure even distribution of the extracted matter.] Inject each of the five mice in a test group with the *Sample* or the *Blank* as outlined in *Table 4*, except to dilute each g of the extract of the *Sample* prepared with *Polyethylene Glycol 400*, and the corresponding *Blank*, with 4.1

Table 3. Surface Area of Specimen To Be Used[a]

Form of Material	Thickness	Amount of Sample for each 20 mL of Extracting Medium	Subdivided into
Film or sheet	<0.5 mm	Equivalent of 120 cm^2 total surface area (both sides combined)	Strips of about 5 × 0.3 cm
	0.5–1 mm	Equivalent of 60 cm^2 total surface area (both sides combined)	
Tubing	<0.5 mm (wall)	Length (in cm) = 120 cm^2/(sum of ID and OD circumferences)	Sections of about 5 × 0.3 cm
	0.5–1 mm (wall)	Length (in cm) = 60 cm^2/(sum of ID and OD circumferences)	
Slabs, tubing, and molded items	>1 mm	Equivalent of 60 cm^2 total surface area (all exposed surfaces combined)	Pieces up to about 5 × 0.3 cm
Elastomers	>1 mm	Equivalent of 25 cm^2 total surface area (all exposed surfaces combined)	Do not subdivide[b]

[a] When surface area cannot be determined due to the configuration of the specimen, use 0.1 g of elastomer or 0.2 g of plastic or other polymers for every 1 mL of extracting fluid.

[b] Molded elastomeric closures are tested intact.

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volumes of *Sodium Chloride Injection* to obtain a solution having a concentration of about 200 mg of polyethylene glycol per mL.

Table 4. Injection Procedure—Systemic Injection Test

Extract or Blank	Dose per kg	Route[a]
Sodium Chloride Injection	50 mL	IV
1 in 20 solution of Alcohol in Sodium Chloride Injection	50 mL	IV
Polyethylene Glycol 400	10 g	IP
Drug product vehicle (where applicable)	50 mL	IV
	50 mL	IP
Vegetable Oil	50 mL	IP

[a] IV = intravenous (aqueous sample and blank); IP = intraperitoneal (oleaginous sample and blank).

Observe the animals immediately after injection, again 4 h after injection, and then at least at 24, 48, and 72 h. If during the observation period none of the animals treated with the extract of the *Sample* shows a significantly greater biological reactivity than the animals treated with the *Blank*, the *Sample* meets the requirements of this test. If two or more mice die, or if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a body weight loss greater than 2 g occurs in three or more mice, the *Sample* does not meet the requirements of the test. If any animals treated with the *Sample* show only slight signs of biological reactivity, and not more than one animal shows gross symptoms of biological reactivity or dies, repeat the test using groups of 10 mice. On the repeat test, all 10 animals treated with the *Sample* show no significant biological reactivity above the *Blank* animals during the observation period.

INTRACUTANEOUS TEST

This test is designed to evaluate local responses to the extracts of materials under test following intracutaneous injection into rabbits or guinea pigs.

Test Animals—Select healthy, rabbits or guinea pigs with fur that can be clipped closely and skin that is free from mechanical irritation or trauma. In handling the animals, avoid touching the injection sites during observation periods, except to discriminate between edema and an oil residue.

Procedure— [NOTE—Agitate each extract vigorously prior to withdrawal of injection doses to ensure even distribution of the extracted matter.] On the day of the test, closely clip the fur on the animal's back on both sides of the spinal column over a sufficiently large test area. Avoid mechanical irritation and trauma. Remove loose hair by means of vacuum. If necessary, swab the skin lightly with diluted alcohol, and dry the skin prior to injection. More than one extract from a given material can be used per rabbit or guinea pig, if it is determined that the test results will not be affected. For each *Sample* use two animals and inject each intracutaneously, using one side of the animal for the *Sample* and the other side for the *Blank*, as outlined in *Table 5*. [NOTE—Dilute each g of the extract of the *Sample* prepared with *Polyethylene Glycol 400*, and the corresponding *Blank*, with 7.4 volumes of *Sodium Chloride Injection* to obtain a solution having a concentration of about 120 mg of polyethylene glycol per mL.]

Table 5. Intracutaneous Test

Extract or Blank	Number of Sites (per animal)	Dose (µL per site)
Sample	5	200
Blank	5	200

Examine injection sites for evidence of any tissue reaction such as erythema, edema, and necrosis. Swab the skin lightly, if necessary, with diluted alcohol to facilitate reading of injection sites. Observe all animals at 24, 48, and 72 h after injection. Rate the observations on a numerical scale for the extract of the *Sample* and for the *Blank*, using *Table 2*. Reclip the fur as necessary during the observation period. The average erythema and edema scores for *Sample* and *Blank* sites are determined at every scoring interval (24, 48, and 72 h) for each rabbit or guinea pig. After the 72-hour scoring, all erythema scores plus edema scores are totalled separately for each *Sample* and *Blank*. Divide each of the totals by 12 (2 animals × 3 scoring periods × 2 scoring categories) to determine the overall mean score for each *Sample* versus each corresponding *Blank*. The requirements of the test are met if the difference between the *Sample* and the *Blank* mean score is 1.0 or less. If at any observation period the average reaction to the *Sample* is questionably greater than the average reaction to the *Blank*, repeat the test using three additional rabbits or guinea pigs. The requirements of the test are met if the difference between the *Sample* and the *Blank* mean score is 1.0 or less.

IMPLANTATION TEST

The implantation test is designed for the evaluation of plastic materials and other polymeric materials in direct contact with living tissue. Of importance are the proper preparation of the implant strips and their proper implantation under aseptic conditions. The intramuscular implantation test requires healthy adult New Zealand rabbits. The test specimens are placed into

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needles as the delivery system for implantation. Although most materials lend themselves readily to this method, there are a number of materials that are unsuitable for intramuscular implantation. For materials with physical characteristics unsuitable for routine intramuscular implantation, the subcutaneous rat implantation model is a viable alternative.

Intramuscular Implantation in Rabbits

Prepare for implantation 8 strips of the *Sample* and 4 strips of USP High-Density Polyethylene RS. Each strip should measure not less than 10 × 1 mm. The edges of the strips should be as smooth as possible to avoid additional mechanical trauma upon implantation. Strips of the specified minimum size are implanted by means of a hypodermic needle (15–19 gauge) with intravenous point and a sterile trocar. Use either presterilized needles into which the sterile plastic strips are aseptically inserted, or insert each clean strip into a needle, the cannula and hub of which are protected with an appropriate cover, and then subjected to the appropriate sterilization procedure. [NOTE—Allow for proper degassing if agents such as ethylene oxide are used.]

Test Animals—Select healthy, adult rabbits weighing not less than 2.5 kg, and with paravertebral muscles that are sufficiently large in size to allow for implantation of the test strips. Do not use any muscular tissue other than the paravertebral site. The animals must be anesthetized with a commonly used anesthetic agent to a degree deep enough to prevent muscular movements, such as twitching. See the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines.

Procedure—Perform the test in a clean area. On the day of the test or up to 20 h before testing, clip the fur of the animals on both sides of the spinal column. Remove loose hair by means of vacuum. Swab the skin lightly with diluted alcohol, and dry the skin prior to injection.

Implant four strips of the *Sample* into the paravertebral muscle on one side of the spine of each of two rabbits, 2.5–5 cm from the midline and parallel to the spinal column, and about 2.5 cm apart from each other. In a similar fashion implant two strips of USP High-Density Polyethylene RS in the opposite muscle of each animal. Insert a sterile stylet into the needle to hold the implant strip in the tissue while withdrawing the needle. If excessive bleeding is observed after implantation of a strip, place a duplicate strip at another site.

Keep the animals for a period of not less than 120 h, and sacrifice them at the end of the observation period by administering an overdose of an anesthetic agent or other suitable agents. Allow sufficient time to elapse for the tissue to be cut without bleeding. Examine macroscopically the area of the tissue surrounding the center portion of each implant strip. Use a magnifying lens and auxiliary light source. Observe the *Sample* and *Control* implant sites for hemorrhage, necrosis, discolorations, and infections, and record the observations. Measure encapsulation, if present, by recording the width of the capsule (from the periphery of the space occupied by the implant *Control* or *Sample* to the periphery of the capsule) rounded to the nearest 0.1 mm. Score encapsulation according to *Table 6*.

Table 6. Evaluation of Encapsulation in the Implantation Test

Capsule Width	Score
None	0
Up to 0.5 mm	1
0.6–1.0 mm	2
1.1–2.0 mm	3
Greater than 2.0 mm	4

Calculate the differences between average scores for the *Sample* and *Control* sites. The requirements of the test are met if the difference does not exceed 1.0, or if the difference between the *Sample* and *Control* mean scores for more than one of the four implant sites does not exceed 1 for any implanted animal.

Subcutaneous Implantation in Rats

Prepare for implantation 10 sample specimens and 10 control specimens. The size and shape of the control specimens shall be as similar to that of the test specimens as practically possible. For example, specimens made of sheeting material shall be 10–12 mm in diameter and from 0.3–1 mm in thickness. The edges of the specimens should be as smooth as possible to avoid additional mechanical trauma upon implantation.

Test Animals—Select healthy albino rats weighing 225–350 g at the time of implantation.

Procedure—Perform the test in a clean area. Anesthetize (see AAALAC guidelines) the animal until a surgical plane is achieved. Clip the fur of the animals on both sides of the spinal column. Remove loose hair by means of vacuum. Clean the clipped area with povidone–iodine solution. Using aseptic technique, make two midline incisions (approximately 1.0 cm long) through the skin at the cranial and caudal regions on the dorsal surface. Using blunt dissection, separate the fascia connecting skin to muscle to form a pocket underneath the skin lateral to each side of the incision (base of pocket approximately 20 mm from the line of implant). Insert a sterile sample into each pocket, and close the incision with wound clips or sutures. Implant two test samples and two control samples in each of five rats. Keep the animals for a period of at least seven days, and sacrifice them at the end of the observation period by CO_2 induced hypoxia or administering an overdose of an anesthetic agent. Allow sufficient time to elapse for the tissue to be cut without bleeding. Cut the skin (dorsal surface) longitudinally and lay back. Carefully examine macroscopically the area of the tissue surrounding the implant. Cut the sample in half and remove for close examination of the tissue in direct contact with the sample. Use a magnifying lens and auxiliary light source, if appropriate. Observe the *Sample* and *Control* implant sites for hemorrhage, necrosis, discolorations, and infections, and record the

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observations. Measure encapsulation, if present, by recording the width of the capsule (from the periphery of the space occupied by the implant *Control* or *Sample* to the periphery of the capsule) rounded to the nearest 0.1 mm. Score encapsulation according to *Table 6*. Calculate the differences between average scores for the *Sample* and *Control* sites. The requirements of the test are met if the difference does not exceed 1.0.

SAFETY TESTS—BIOLOGICALS

The safety test set forth here is intended to detect in an article any unexpected, unacceptable biological reactivity. This in vivo test is provided for the safety assessment of biotechnology-derived products.

Safety Test

Select five healthy mice not previously used for testing, weighing 17–23 g, unless otherwise directed in the individual monograph or elsewhere in this chapter, and maintained on an adequate balanced diet. Prepare a test solution as directed in the individual monograph. Unless otherwise directed in the individual monograph or elsewhere in this chapter, inject a dose of 0.5 mL of the test solution into each of the mice, using a 26-gauge needle of suitable length, or of the length specified below as applicable. Observe the animals over the 48 h following the injection. If, at the end of 48 h, all of the animals survive and not more than one of the animals shows outward symptoms of a reaction not normally expected of the level of toxicity related to the article, the requirements of this test are met. If one or more animals die or if more than one of the animals shows signs of abnormal or untoward toxicity of the article under test, repeat the test using at least another 10 mice similar to those used in the initial test, but weighing 20 ± 1 g. In either case, if all of the animals survive for 48 h and show no symptoms of a reaction indicative of an abnormal or undue level of toxicity of the article, the requirements of the test are met. Body weights of mice before and at the end of the test should be obtained to detect any untoward effects. Animals that show signs of toxicity should be grossly necropsied and subjected to histopathology if necessary.

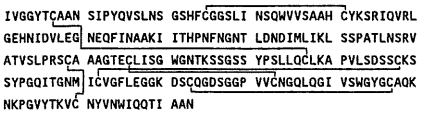
For biologics, perform the test according to the procedures prescribed in the *Code of Federal Regulations,* Section 610.11.

⟨89⟩ ENZYMES USED AS ANCILLARY MATERIALS IN PHARMACEUTICAL MANUFACTURING

INTRODUCTION

The purpose of this chapter is to describe the quality attributes and associated tests with acceptance criteria for enzymatic preparations used in biopharmaceutical manufacturing. The quality of ancillary materials, including enzymes, used in biopharmaceutical manufacturing can have an impact on the therapeutic products. Several enzymes are used in this type of cell processing. Examples include trypsin, collagenase, pepsin, and papain. This chapter does not discuss applications of these enzymes but rather focuses on tests to assess their quality as process materials.

Recombinant Trypsin



$C_{1020}H_{1597}N_{287}O_{321}S_{14}$

23,463 (for β-Trypsin)

[9002-07-7]

DEFINITION

Recombinant trypsin, a key raw material in biopharmaceutical manufacturing, is a serine protease that cleaves peptide chains mainly at the carboxyl end of the amino acids arginine and lysine. The amino acid sequence of recombinant trypsin is identical to that of trypsin from porcine pancreas, and the recombinant trypsin is produced by methods based on recombinant DNA technology in the yeast *Pichia pastoris*. Therefore, the specifications described in this chapter apply only to recombinant porcine trypsin produced in yeast. Because of the recombinant production process, recombinant trypsin is free of chymotrypsin. Two active forms of trypsin are known: β-trypsin (23,463 daltons) and α-trypsin (23,481 daltons). Autolysis of β-trypsin at the peptide bond between Arg^{99} and Val^{100}, Lys^{125} and Ser^{126}, or Lys^{139} and Ala^{140} results in three possible isoforms of α-trypsin. All isoforms are held together by disulfide bridges and remain correctly folded. As a consequence of hydrolysis of a peptide bond, the molecular weight of α-trypsin is more than that of β-trypsin by 18 daltons. The peak area for β-trypsin is NLT 70%, and the peak area for α-trypsin is NMT 20% as determined by the HPLC procedure described in the test for *Purity*. The specific activity is NLT 180 Units/mg of protein using carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate as the substrate described in the *Assay*.

[NOTE—One Unit of trypsin activity using carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate as the substrate corresponds to 21 USP Trypsin Units. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in the *Assay* in the Crystallized Trypsin monograph using *N*-benzoyl-L-arginine ethyl ester hydrochloride as the substrate. Therefore, the specific activity of 180 Units/mg of protein using carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate as the substrate for recombinant trypsin corresponds to 3800 USP Trypsin Units/mg of protein.]

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IDENTIFICATION

- **A.** It meets the requirements in the *Assay*.
- **B.** The retention time of the major peak for β-trypsin in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Purity*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 1.21 g of tris(hydroxymethyl)aminomethane and 0.29 g of calcium chloride dihydrate in 100 mL of water, and adjust with 2 N hydrochloric acid to a pH of 8.0 (at 25 ± 1°).

Substrate stock solution: Dissolve 20 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate[1], accurately weighed, in 3.0 mL of water. [NOTE—Use a freshly prepared solution only.]

Substrate solution: Prepare a solution by mixing 28 mL of *Buffer* and 2.8 mL of *Substrate stock solution*. [NOTE—Use a freshly prepared solution only.]

Standard solutions: Precool USP Trypsin Recombinant Porcine RS and water to approximately 4°. Start preparing *Standard solutions* immediately when the temperature has reached 4°. Prepare each *Standard solution* by diluting USP Trypsin Recombinant Porcine RS with water at 1:68,921 dilution. Prepare at least five *Standard solutions* in parallel. Assay each *Standard solution* in duplicate. [NOTE—Use an adjustable pipettor for each measurement and dilution operation. Use polystyrene test tubes for preparing *Standard solutions* and *Sample solutions*, and use polystyrene pipet tips containing polyethylene filters to transfer samples. The pipet tip should not be wet before transfer, and each pipet tip should be used only for transferring one sample.] [NOTE—A dilution of 1:68,921 can be achieved by three dilution steps in which each step has a 1:41 dilution (1:41/1:41/1:41). For example, for the initial dilution withdraw 0.1 mL of USP Trypsin Recombinant Porcine RS using a pipettor with a polystyrene tip containing polyethylene filters, wipe off the outside of the tip to remove any residual solution, add the Reference Standard to a polystyrene test tube containing 4.0 mL of precooled water, rinse the tip by pipetting the solution up and down for 2–3 times, discard the tip, and mix the solution on a vortex mixer for approximately 2 s with maximum speed. For the second and third dilution steps, proceed as described for the initial dilution except for transferring 0.1 mL of solution from the previous dilution step.]

Sample solutions: Prepare at least five *Sample solutions* of recombinant trypsin in parallel as directed for the *Standard solutions* to obtain a final concentration of at least 0.16 Units/mL using precooled water as the diluent. Each *Sample solution* is assayed in duplicate.

Instrumental conditions

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

Mode: UV

Analytical wavelength: 405 nm

Pathlength: 1 cm

Temperature: 25°

Analysis

Samples: *Standard solutions* and *Sample solutions*

Transfer 1.10 mL of *Substrate solution* into a polystyrene semimicro cuvette, allow the temperature to stabilize, check the cuvette for the specified temperature, and wait for 10 min. Start the reaction by adding 0.020 mL of *Standard solution* or *Sample solution*. Record the absorbance for at least 5 min, and determine the change in absorbance (Δ*A*/min) from the linear range of the reaction.

Calculate the activity of recombinant trypsin in Units/mL:

$$\text{Result} = [V_T/(\varepsilon \times V \times B)] \times (\Delta A/\text{min}) \times D$$

V_T = volume of the reaction mixture, 1.12 mL
ε = extinction coefficient for 405 nm, 10.4 ($\text{mmol}^{-1} \cdot 1\ \text{cm}^{-1}$)
V = volume of *Standard solution* or *Sample solution*, 0.020 mL
B = absorption pathlength, 1 cm
D = dilution factor

[NOTE—One Unit will release the equivalent of 1 mmol of 4-nitril aniline from carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate per min under the conditions of the *Assay*.]

Calculate the specific activity of recombinant trypsin in Units/mg of protein:

$$\text{Result} = \text{Activity}/C$$

C = protein concentration of recombinant trypsin (mg/mL)

System suitability

Samples: *Standard solutions* and *Sample solutions*

Suitability requirement: Δ*A*/min should be 0.03–0.07 for *Standard solutions* and *Sample solutions*. The average calculated activity for the *Standard solutions* is 90%–110% of the value on the label.

Acceptance criteria

Specific activity: NLT 180 Units/mg of protein

Relative standard deviation: NMT 5% for the activities determined from 5 replicates

[1] A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog number 10378496103) or equivalent.

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PURITY

• PROCEDURE

Solution A: Dilute 1 mL of phosphoric acid (85%) with water to 1000 mL.
Solution B: Dilute 1 mL of phosphoric acid (85%) with acetonitrile to 1000 mL.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
25	55	45
30	10	90
34	10	90
35	75	25
45	75	25

Standard solution: Thaw 100 µL of USP Trypsin Recombinant Porcine RS at room temperature for about 1 h, mix, and transfer to an HPLC vial. The target protein concentration should be 70 ± 10 mg/mL.
Sample solution: Thaw 100 µL of recombinant trypsin at room temperature for about 1 h, mix, and transfer to an HPLC vial. The target protein concentration should be 70 ± 10 mg/mL.
[NOTE—Keep *Standard solution* and *Sample solution* at 2°–8° if they are not ready to be injected immediately after preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1 with a 200 Å pore size
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for the main peak of recombinant trypsin is 12–17 min.]
Suitability requirements
Resolution: NLT 1 between the peaks of α-trypsin and β-trypsin
Analysis
Sample: *Sample solution*
Record the chromatograms, and measure the peak areas. Evaluate the purity of trypsin using the area-% method. Time of integration is 25 min. The blank should be considered for integration. Peaks that elute as a fronting shoulder of α-trypsin are integrated by perpendicular dropping if and only if a minimum is formed. Peaks that elute as a tailing shoulder of β-trypsin are evaluated by tangential integration if and only if a minimum is formed.
Acceptance criteria: NLT 70% for the peak area of β-trypsin and NMT 20% for the peak area of α-trypsin

SPECIFIC TESTS

• PROTEIN CONTENT

4 N hydrochloric acid solution: Mix 10.4 mL of 25% hydrochloric acid with 9.6 mL of water.
Storage buffer: Dissolve 2.9 g of calcium chloride dihydrate in water, add 2.5 mL of *4 N hydrochloric acid solution*, and dilute with water to a final volume of 1000 mL. Adjust with *4 N hydrochloric acid solution* to a pH of 2.0 ± 0.2, if necessary.
Sample solutions: Add 0.025 mL of recombinant trypsin to 3 mL of *Storage buffer*. Prepare at least in triplicate.
Blank solution: *Storage buffer*, 3 mL
Instrumental conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: UV
Analytical wavelength: 280 nm
Pathlength: 1 cm
System suitability
Sample: *Sample solutions*
Suitability requirement: ΔA (as defined below) is in the range of 0.13–1.8.
Analysis
Samples: *Sample solutions* and *Blank solution*
Calculate the protein concentration in mg/mL:

$$\text{Result} = \left[\frac{(\Delta A \times F)}{A_{280}^{\frac{1\%}{1\text{cm}}}}\right] \times D$$

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$$\Delta A = A_U - A_B$$

A_U = absorbance from the *Sample solution*
A_B = absorbance from the *Blank solution*
F = conversion factor from 1% to mg/mL, 10
$A_{280}^{1\%}{}_{1cm}$ = extinction coefficient for trypsin, 13.6
D = dilution factor

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩: The total bacterial count does not exceed 100 cfu/mL, the test being performed on 1 mL of recombinant trypsin in duplicate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in closed containers at –15° to –25°.
- **LABELING:** The labeling states that the material is of recombinant DNA origin, along with the product number and lot number, storage conditions, and expiration date.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Trypsin Recombinant Porcine RS

⟨89.1⟩ COLLAGENASE I

```
IANTNSEKYD FEYLNGLSYT ELTNLIKNIK WNQINGLFNY STGSQKFFGD
KNRVQAIINA LQESGRTYTA NDMKGIETFT EVLRAGFYLG YYNDGLSYLN
DRNFQDKCIP AMIAIQKNPN FKLGTAVQDE VITSLGKLIG NASANAEVVN
NCVPVLKQFR ENLNQYAPDY VKGTAVNELI KGIEFDFSGA AYEKDVKTMP
WYGKIDPFIN ELKALGLYGN ITSATEWASD VGIYYLSKFG LYSTNRNDIV
QSLEKAVDMY KYGKTAFVAM ERITWDYDGI GSNGKKVDHD KFLDDAEKHY
LPKTYTFDNG TFIIRAGDKV SEEKIKRLYW ASREVKSQFH RVVGNDKALE
VGNADDVLTM KIFNSPEEYK FNTNINGVST DNGGLYIEPR GTFYTYERTP
QQSIFSLEEL FRHEYTHYLQ ARYLVDGLWG QGPFYEKNRL TWFDEGTAEF
FAGSTRTSGV LPRKSILGYL AKDKVDHRYS LKKTLNSGYD DSDWMFYNYG
FAVAHYLYEK DMPTFIKMNK AILNTDVKSY DEIIKKLSDD ANKNTEYQNH
IQELADKYQG AGIPLVSDDY LKDHGYKKAS EVYSEISKAA SLTNTSVTAE
KSQYFNTFTL RGTYTGETSK GEFKDWDENS KKLDGTLESL AKNSWSGYKT
LTAYFTNYRV TSDNKVQYDV VFHGVLTDNA DISNNKAPIA KVTGPSTGAV
GRNIEFSGKD SKDEDGKIVS YDWDFGDGAT SRGKNSVHAY KKAGTYNVTL
KVTDDKGATA TESFTIEIKN EDTTTPITKE MEPNDDIKEA NGPIVEGVTV
KGDLNGSDDA DTFYFDVKED GDVTIELPYS GSSNFTWLVY KEGDDQNHIA
SGIDKNNSKV GTFKSTKGRH YVFIYKHDSA SNISYSLNIK GLGNEKLKEK
ENNDSSDKAT VIPNFNTTMQ GSLLGDDSRD YYSFEVKEEG EVNIELDKKD
EFGVTWTLHP ESNINDRITY GQVDGNKVSN KVKLRPGKYY LLVYKYSGSG
NYELRVNK
```

$C_{5099}H_{7771}N_{1329}O_{1610}S_{14}$
113,897 daltons (for β subtype)
[9001-12-1]

DEFINITION

Collagenase I (EC 3.4.24.3), isolated from *Clostridium histolyticum* and encoded by *colG* gene (GenBank accession number BAA77453.1), is a key raw material used in the dissociation or destruction of a broad range of tissue types. Collagenase I is a metalloprotease that acts as an endoprotease and also exhibits a tripeptidylcarboxypeptidase activity. It shows endopeptidic activity with the main cleavage site found in front of the human collagen duplex amino acids glycine–proline. Hydrolysis takes place near the ends of the triple helical domains of collagen.

Collagenase I is also known as class I collagenase and consists of three subtypes: α, β, and γ. Collagenase I β is the full-length enzyme while collagenase I α (68,000 Da) and collagenase I γ (79,000 Da) are thought to be proteolytic degradation products of collagenase I β caused by other proteases present in *C. histolyticum* (mainly a trypsin-like enzyme and clostripain/endoproteinase Arg C).

Collagenase I can be provided in a liquid formulation consisting of 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and 1 mM calcium chloride pH 7.5, and stored as a frozen liquid. The specific activity of collagenase I is 0.10–0.60 units/mg of protein using 4-phenylazobenzyloxycarbonyl (PZ)-Pro-Leu-Gly-Pro-D-Arg as the substrate described in the *Assay*. The peak area for collagenase I is NLT 90% as determined by HPLC described in the test for *Purity*. The test for *Clostripain Activity* is used to assess the activity of the clostripain impurity and the acceptance criterion is NMT 0.5 units/mg of protein. The test for *Trypsin Activity* is used to assess the activity of the trypsin-like enzyme impurity and the acceptance criterion is NMT 0.5 units/mg of protein.

IDENTIFICATION

- **A.** It meets the requirements in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Purity*.

ASSAY

- **PROCEDURE**

Tris buffer: 0.1 M Tris pH 7.1, prepared as follows. Dissolve 6.05 g of tris(hydroxymethyl)aminomethane (Tris) in 400 mL of water, and adjust with 2 N hydrochloric acid to a pH of 7.1 (at 25 ± 1°). Dilute with water to a final volume of 500 mL.

Substrate solution: Dissolve 10 mg of PZ-Pro-Leu-Gly-Pro-D-Arg in 0.2 mL of methanol, and dilute this solution with *Tris buffer* to a final volume of 10 mL. [NOTE—Use a freshly prepared solution only.]

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Calcium chloride solution: 0.1 M, prepared as follows. Weigh 1.47 g of calcium chloride dihydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.
Citric acid solution: 0.025 M, prepared as follows. Weigh 525 mg of citric acid monohydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.
Extraction mixture: To one test tube per sample to be assayed, pipette 5.0 mL of ethyl acetate and 1.0 mL of *Citric acid solution*. [NOTE—Use a freshly prepared mixture only.]
Drying tube: Into one test tube per sample to be assayed, add 0.35–0.40 g of sodium sulfate anhydrous. Seal the test tube with parafilm.
Standard solution: Dilute USP Collagenase I RS with *Tris buffer* in the range of 1:50 to 1:100 (v/v). [NOTE—Avoid freezing and thawing USP Collagenase I RS. After withdrawing USP Collagenase I RS, wipe off the outside of the plastic pipette tips to remove any residual solution.]
Sample solutions: Dilute collagenase I with *Tris buffer* to an appropriate dilution to achieve the absorbance range of 0.3–0.9 from the *Analysis*. Prepare in triplicate. [NOTE—Avoid freezing and thawing the collagenase I sample. After withdrawing the collagenase I sample, wipe off the outside of the tip to remove any residual solution.]
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 320 nm
Path length: 1 cm
Temperature: 25°
Analysis
Samples: *Standard solution* and *Sample solutions*
Transfer 1.0 mL of *Substrate solution* and 0.2 mL of *Calcium chloride solution* into a test tube, and equilibrate the test tube to 25°. Start the reaction by adding 0.05 mL of *Standard solution* or each *Sample solution*. Prepare a blank by replacing the *Standard solution* or *Sample solution* with 0.05 mL of *Tris buffer*. Mix and incubate for exactly 15 min at 25°. Transfer 0.5 mL of the reaction to the test tube containing 6.0 mL of *Extraction mixture*. Vortex immediately for 20 s. Transfer 3 mL of the ethyl acetate phase (upper layer) into a *Drying tube* using a glass pipette, and vortex thoroughly. Transfer the supernatant to a disposable, semi-micro cuvette suitable for UV absorbance with a Pasteur pipette. Record the absorbance.
Calculate the activity of collagenase I in units/mL:

$$\text{Result} = (A - A_B) \times [V_T \times V_E/(\varepsilon \times V \times V_R \times B \times T)] \times D$$

A = absorbance of the *Standard solution* or *Sample solution*
A_B = absorbance of the blank
V_T = volume of the reaction mixture, 1.25 mL
V_E = volume of ethyl acetate in the *Extraction mixture*, 5.0 mL
ε = extinction coefficient for 320 nm, 21 ($1\ cm^2 \cdot \mu mol^{-1}$)
V = volume of the *Standard solution* or *Sample solution*, 0.05 mL
V_R = volume of the reaction transferred to *Extraction mixture*, 0.5 mL
B = absorption path length, 1 cm
T = incubation time, 15 min
D = dilution factor

[NOTE—One unit will release the equivalent of 1 µmol of PZ-Pro-Leu from PZ-Pro-Leu-Gly-Pro-D-Arg per minute under the conditions of the *Assay*.]
Calculate the specific activity of collagenase I in units/mg of protein:

$$\text{Result} = Activity/C$$

Activity = activity of collagenase I (units/mL)
C = protein concentration (mg/mL)

System suitability
Samples: *Standard solution* and *Sample solutions*
Suitability requirements
Average calculated activity: 85%–115% of the value on the label, *Standard solution*
Absorbance: 0.3–0.9, *Standard solution* and *Sample solutions*
Acceptance criteria: 0.10–0.60 units/mg of protein

PURITY

• **PROCEDURE**
Solution A: 20 mM Tris and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.
Solution B: 20 mM Tris, 1 mM calcium chloride, and 1 M sodium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris, 147 mg of calcium chloride dihydrate, and 58.44 g of sodium chloride in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.
Mobile phase: See *Table 1*.

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Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
22	85	15
32	0	100
34	100	0
40	100	0

Storage buffer: 5 mM HEPES and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 1.19 g of HEPES and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 4 N sodium hydroxide solution to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Standard solution: Thaw USP Collagenase I RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate, and inject each duplicate once.

Collagenase II solution: Thaw USP Collagenase II RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate, and inject each duplicate once.

Sample solution: Dilute collagenase I with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL and keep at 5°.

Blank: *Storage buffer*

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 5-mm × 5-cm; 10-µm packing L91

Temperatures

Column: 25°

Autosampler: 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirement: The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided with USP Collagenase I RS.

Analysis

Sample: *Sample solution*

The *Blank* should be considered for integration. Identify the peak corresponding to collagenase II by comparing its retention time to the main peak from the *Collagenase II solution*. Evaluate the purity of collagenase I using the area–% method but excluding peaks associated with the *Blank*. All shoulders in the fronting and tailing of the main peak are integrated by dropping a perpendicular line at the inflection points and considered as separate impurities. Disregard any peaks having retention times greater than 25 min.

Acceptance criteria: NLT 90% for the main peak of collagenase I and NMT 3% of collagenase II

IMPURITIES

• CLOSTRIPAIN ACTIVITY

Potassium phosphate buffer: 0.1 M pH 7.6, prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute to 100 mL. Dissolve 2.28 g of dibasic potassium phosphate trihydrate in water, and dilute to 100 mL. Adjust the pH of the second solution to 7.6 with the first solution.

Dithiothreitol solution: 0.194 M, prepared as follows. Dissolve 60 mg of dithiothreitol (DTT) in 2 mL of *Potassium phosphate buffer*.

Calcium chloride solution: 0.01 M, prepared as follows. Dissolve 147 mg of calcium chloride dihydrate in 100 mL of water.

Substrate stock solution: 38 mM, prepared as follows. Dissolve 13 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride (BAEE · HCl) in 1 mL of *Potassium phosphate buffer*.

Substrate solution: 0.73 mM BAEE · HCl, 7.8 mM DTT, and 0.4 mM calcium chloride, prepared as follows. Transfer 0.5 mL of *Substrate stock solution*, 1.0 mL of *Dithiothreitol solution*, and 1.0 mL of *Calcium chloride solution* to a 25-mL volumetric flask, and dilute with *Potassium phosphate buffer* to volume.

Sample solution: Prepare in such a way that ΔA/min lies in the 0.02–0.06 range. Dilute with ice-cold *Potassium phosphate buffer* if necessary.

Instrumental conditions

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

Mode: UV

Analytical wavelength: 255 nm

Path length: 1 cm

Temperature: 25°

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Analysis

Sample: *Sample solution*

Transfer 3.0 mL of *Substrate solution* into a cuvette, and equilibrate the cuvette to 25°. Start the reaction by adding 0.05 mL of *Sample solution*. Prepare a blank by replacing the *Sample solution* with 0.05 mL of *Potassium phosphate buffer*. Mix well. Determine the change in absorbance (ΔA/min) from the linear range of the reaction. Assay the *Sample solution* in triplicate.

System suitability

Sample: *Sample solution*

Suitability requirement: 0.02–0.06 for ΔA/min

Calculate the activity of clostripain in units/mL in the portion of collagenase I taken:

$$\text{Result} = [V_T/(\varepsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 3.05 mL
ε = extinction coefficient for 255 nm, 0.81 (1 $cm^2 \cdot mmol^{-1}$)
V_U = volume of the *Sample solution*, 0.05 mL
B = absorption path length, 1 cm
ΔA/min = change in absorbance from the linear range of the reaction
D = dilution factor

Calculate the specific activity in units/mg of protein:

$$\text{Result} = \textit{Activity}/C$$

Activity = activity of clostripain (units/mL)
C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 units of clostripain activity per mg of protein

• **TRYPSIN ACTIVITY**

Buffer: 0.1 M Tris and 0.02 M calcium chloride pH 8.0, prepared as follows. Dissolve 6.05 g of Tris and 1.45 g of calcium chloride dihydrate in 400 mL of water. Adjust with 2 N hydrochloric acid to a pH of 8.0 (at 25 ± 1°). Dilute with water to a final volume of 500 mL.

Substrate stock solution: Dissolve 10 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate,[1] accurately weighed, in 1.5 mL of water. Store on ice. [NOTE—Use freshly prepared solution only.]

Substrate solution: Prepare a solution by mixing 9.2 mL of *Buffer* and 1.0 mL of *Substrate stock solution*. Store on ice. [NOTE—Use freshly prepared solution only.]

Sample solution: Undiluted collagenase I solution

Instrumental conditions

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

Mode: Vis

Analytical wavelength: 405 nm

Path length: 1 cm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 1.02 mL of *Substrate solution* into a polystyrene, semi-micro cuvette, allow the temperature to stabilize, equilibrate the cuvette to 25°, and wait for 10 min. Start the reaction by adding 0.10 mL of *Sample solution*. Start recording the absorbance and continue for at least 5 min after the addition of the *Sample solution*. Determine the change in absorbance (ΔA/min) from the linear range of the reaction. Assay the *Sample solution* in triplicate. [NOTE—Use polyethylene pipette tips to transfer the *Sample solution*. The pipette tip should not be wet before transfer, and each pipette tip should be used only for transferring one sample. After withdrawing the *Sample solution*, wipe off the outside of the tip to remove any residual solution. After adding the *Sample solution* to the *Substrate solution*, rinse the tip by pipetting the solution up and down 2–3 times, discard the tip, and mix.]

System suitability

Sample: *Sample solution*

Suitability requirement: >0.01 for ΔA/min

Calculate the activity of trypsin in units/mL in the portion of collagenase I taken:

$$\text{Result} = [V_T/(\varepsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 1.12 mL
ε = extinction coefficient for 405 nm, 10.4 (1 $cm^2 \cdot mmol^{-1}$)
V_U = volume of the *Sample solution*, 0.10 mL
B = absorption path length, 1 cm
ΔA/min = change in absorbance from the linear range of the reaction
D = dilution factor

[1] A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog number 10378496103) or equivalent.

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Calculate the specific activity in units/mg of protein:

$$\text{Result} = \textit{Activity}/C$$

Activity = activity of trypsin (units/mL)
C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 units of trypsin activity per mg of protein

SPECIFIC TESTS

- **PROTEIN CONTENT**

Sample solutions: Dilute collagenase I in water. Prepare at least in triplicate. [NOTE—Prepare the dilution using plastic pipette tips and not glass pipettes. Carefully wipe off the outside of the tip to remove any residual solution.]

Blank solution: Water

Instrumental conditions

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

Mode: UV

Analytical wavelength: 280 nm

Path length: 1 cm

System suitability

Samples: *Sample solutions*

Suitability requirement: Absorbance is in the range of 0.10–1.00.

Analysis

Samples: *Sample solutions* and *Blank solution*

Determine the net absorbance of *Sample solutions* by subtracting the absorbance of the *Blank solution* from the absorbance of each *Sample solution*. Determine the average net absorbance of *Sample solutions*.

Calculate the protein concentration in mg/mL:

$$\text{Result} = A_U \times D/\varepsilon$$

A_U = average net absorbance of the *Sample solutions*
D = dilution factor
ε = extinction coefficient (A_{280}0.1%/cm) for collagenase, 1.4

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50 USP Endotoxin Units/mg of protein
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩: The total bacterial count is NMT 100 cfu/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in closed containers at –15° to –25°.
- **LABELING:** The labeling states that the material is derived from *Clostridium histolyticum* along with the lot number, product or catalog number, and storage conditions.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Collagenase I RS
USP Collagenase II RS
USP Endotoxin RS

⟨89.2⟩ COLLAGENASE II

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VQNESKRYTV SYLKTLNYYD LVDLLVKTEI ENLPDLFQYS SDAKEFYGNK
TRMSFIMDEI GRRAPQYTEI DHKGIPTLVE VVRAGFYLGF HNKELNEINK
RSFKERVIPS ILATQKNPNF KLGTEVQDKI VSATGLLAGN ETAPPEVVNN
FTPILQDCIK NIDRYALDDL KSKALFNVLA APTYDITEYL RATKEKPENT
PWYGKIDGFI NELKKLALYG KINDNNSWII DNGIYHIAPL GKLHSNNKIG
IETLTEVMKV YPYLSMQHLQ SADQIKRHYD SKDAEGNKIP LDKFKKEGKE
KYCPKTYTFD DGKVIIKAGA RVEEEKVKRL YWASKEVNSQ FFRVYGIDKP
LEEGNPDDIL TMVIYNSPEE YKLNSVLYGY DTNNGGMYIE PEGTFFTYER
EAQESTYTLE ELFRHEYTHY LQGRYAVPGQ WGRTKLYDND RLTWYEEGGA
ELFAGSTRTS GILPRKSIVS NIHNTTRNNR YKLSDTVHSK YGASFEFYNY
ACMFMDYMYN KDMGILNKLN DLAKNNDVDG YDNYIRDLSS NYALNDKYQD
HMQERIDNYE NLTVPFVADD YLVRHAYKNP NEIYSEISEV AKLKDAKSEV
KKSQYFSTFT LRGSYTGGAS KGKLEDQKAM NKFIDDSLKK LDTYSWSGYK
TLTAYFTNYK VDSSNRVTYD VVFHGYLPNE GDSKNSLPYG KINGTYKGTE
KEKIKFSSEG SFDPDGKIVS YEWDFGDGNK SNEENPEHSY DKVGTYTVKL
KVTDDKGESS VSTTTAEIKD LSENKLPVIY MHVPKSGALN QKVVFYGKGT
YDPDGSIAGY QWDFGDGSDF SSEQNPSHVY TKKGEYTVTL RVMDSSGQMS
EKTMKIKITD PVYPIGTEKE PNNSKETASG PIVPGIPVSG TIENTSDQDY
FYFDVITPGE VKIDINKLGY GGATWVVYDE NNNAVSYATD DGQNLSGKFK
ADKPGRYYIH LYMFNGSYMP YRINIEGSVG R
```

$C_{5028}H_{7666}N_{1300}O_{1564}S_{21}$
112,023 (for ζ subtype)
[9001-12-1]

DEFINITION

Collagenase II (EC 3.4.24.3), isolated from *Clostridium histolyticum* and encoded by *colH* gene (GenBank accession number BAA06251.1), is a key raw material used in the dissociation or destruction of a broad range of tissue types. Collagenase II

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is a metalloprotease that acts as an endoprotease and also exhibits a tripeptidylcarboxypeptidase activity. It shows endopeptidic activity with the main cleavage site found in front of the human collagen duplex amino acids glycine–proline. Hydrolysis takes place in the interior of the triple helical domains of collagen.

Collagenase II is also known as class II collagenase and consists of three subtypes: δ, ε, and ζ. Collagenase II ζ is the full-length enzyme while collagenase II δ (100,000 Da) and collagenase II ε (110,000 Da) are thought to be proteolytic degradation products of collagenase II ζ caused by other proteases present in *C. histolyticum* (mainly a trypsin-like enzyme and clostripain/endoproteinase Arg C).

Collagenase II can be provided in a liquid formulation consisting of 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and 1 mM calcium chloride pH 7.5, and stored as a frozen liquid. The specific activity of collagenase II is 10–16 units/mg of protein using 4-phenylazobenzyloxycarbonyl (PZ)-Pro-Leu-Gly-Pro-D-Arg as the substrate described in the *Assay*. The peak area for collagenase II is NLT 90% as determined by HPLC described in the test for *Purity*. The test for *Clostripain Activity* is used to assess the activity of the clostripain impurity and the acceptance criterion is NMT 0.5 units/mg of protein. The test for *Trypsin Activity* is used to assess the activity of the trypsin-like enzyme impurity and the acceptance criterion is NMT 0.5 units/mg of protein.

IDENTIFICATION

- **A.** It meets the requirements in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Purity*.

ASSAY

- PROCEDURE

Tris buffer: 0.1 M Tris pH 7.1, prepared as follows. Dissolve 6.05 g of tris(hydroxymethyl)aminomethane (Tris) in 400 mL of water, and adjust with 2 N hydrochloric acid to a pH of 7.1 (at 25 ± 1°). Dilute with water to a final volume of 500 mL.

Substrate solution: Dissolve 10 mg of PZ-Pro-Leu-Gly-Pro-D-Arg in 0.2 mL of methanol, and dilute this solution with *Tris buffer* to a final volume of 10 mL. [NOTE—Use a freshly prepared solution only.]

Calcium chloride solution: 0.1 M, prepared as follows. Weigh 1.47 g of calcium chloride dihydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Citric acid solution: 0.025 M, prepared as follows. Weigh 525 mg of citric acid monohydrate in a volumetric flask, and dilute with water to a final volume of 100 mL.

Extraction mixture: To one test tube per sample to be assayed, pipette 5.0 mL of ethyl acetate and 1.0 mL of *Citric acid solution*. [NOTE—Use a freshly prepared mixture only.]

Drying tube: Into one test tube per sample to be assayed, add 0.35–0.40 g of sodium sulfate anhydrous. Seal the test tube with parafilm.

Standard solution: Dilute USP Collagenase II RS with *Tris buffer* in the range of 1:2000 to 1:4000 (v/v). [NOTE—Avoid freezing and thawing USP Collagenase II RS. After withdrawing USP Collagenase II RS, wipe off the outside of the plastic pipette tips to remove any residual solution.]

Sample solutions: Dilute collagenase II with *Tris buffer* to an appropriate dilution to achieve the absorbance range of 0.3–0.9 from the *Analysis*. Prepare in triplicate. [NOTE—Avoid freezing and thawing the collagenase II sample. After withdrawing the collagenase II sample, wipe off the outside of the tip to remove any residual solution.]

Instrumental conditions

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

Mode: UV

Analytical wavelength: 320 nm

Path length: 1 cm

Temperature: 25°

Analysis

Samples: *Standard solution* and *Sample solutions*

Transfer 1.0 mL of *Substrate solution* and 0.2 mL of *Calcium chloride solution* into a test tube, and equilibrate the test tube to 25°. Start the reaction by adding 0.05 mL of *Standard solution* or each *Sample solution*. Prepare a blank by replacing the *Standard solution* or *Sample solution* with 0.05 mL of *Tris buffer*. Mix and incubate for exactly 15 min at 25°. Transfer 0.5 mL of the reaction to the test tube containing 6.0 mL of *Extraction mixture*. Vortex immediately for 20 s. Transfer 3 mL of the ethyl acetate phase (upper layer) into a *Drying tube* using a glass pipette, and vortex thoroughly. Transfer the supernatant to a disposable, semi-micro cuvette suitable for UV absorbance with a Pasteur pipette. Record the absorbance.

Calculate the activity of collagenase II in units/mL:

$$\text{Result} = (A - A_B) \times [V_T \times V_E/(\varepsilon \times V \times V_R \times B \times T)] \times D$$

A = absorbance of the *Standard solution* or *Sample solution*
A_B = absorbance of the blank
V_T = volume of the reaction mixture, 1.25 mL
V_E = volume of ethyl acetate in the *Extraction mixture*, 5.0 mL
ε = extinction coefficient for 320 nm, 21 (1 $cm^2 \cdot \mu mol^{-1}$)
V = volume of the *Standard solution* or *Sample solution*, 0.05 mL
V_R = volume of the reaction transferred to *Extraction mixture*, 0.5 mL
B = absorption path length, 1 cm
T = incubation time, 15 min
D = dilution factor

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[NOTE—One unit will release the equivalent of 1 µmol of PZ-Pro-Leu from PZ-Pro-Leu-Gly-Pro-D-Arg per minute under the conditions of the *Assay*.]

Calculate the specific activity of collagenase II in units/mg of protein:

$$\text{Result} = \textit{Activity}/C$$

Activity = activity of collagenase II (units/mL)
C = protein concentration (mg/mL)

System suitability
Samples: *Standard solution* and *Sample solutions*
Suitability requirements
Average calculated activity: 85%–115% of the value on the label, *Standard solution*
Absorbance: 0.3–0.9, *Standard solution* and *Sample solutions*
Acceptance criteria: 10–16 units/mg of protein

PURITY

• PROCEDURE

Solution A: 20 mM Tris and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Solution B: 20 mM Tris, 1 mM calcium chloride, and 1 M sodium chloride pH 7.5, prepared as follows. Dissolve 2.42 g of Tris, 147 mg of calcium chloride dihydrate, and 58.44 g of sodium chloride in 900 mL of water. Adjust with 2 N hydrochloric acid to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
22	85	15
32	0	100
34	100	0
40	100	0

Storage buffer: 5 mM HEPES and 1 mM calcium chloride pH 7.5, prepared as follows. Dissolve 1.19 g of HEPES and 147 mg of calcium chloride dihydrate in 900 mL of water. Adjust with 4 N sodium hydroxide solution to a pH of 7.5. Dilute with water to a final volume of 1000 mL.

Standard solution: Thaw USP Collagenase II RS at room temperature shortly before use and mix. Store on ice or at 5°. Dilute with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL. Transfer to an HPLC vial and keep at 5°. Prepare in duplicate, and inject each duplicate once.

Sample solution: Dilute collagenase II with *Storage buffer* to achieve a protein concentration of 5.5 mg/mL and keep at 5°.

Blank: *Storage buffer*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 5-mm × 5-cm; 10-µm packing L91
Temperatures
Column: 25°
Autosampler: 5°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirement: The chromatogram from the *Standard solution* corresponds to the typical chromatogram provided with USP Collagenase II RS.

Analysis
Sample: *Sample solution*
The *Blank* should be considered for integration. Evaluate the purity of collagenase II using the area–% method but excluding peaks associated with the *Blank*. All shoulders in the fronting and tailing of the main peak are integrated by dropping a perpendicular line at the inflection points and considered as separate impurities. Disregard any peaks having retention times greater than 25 min.

Acceptance criteria: NLT 90% for the main peak of collagenase II

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IMPURITIES

• **CLOSTRIPAIN ACTIVITY**

Potassium phosphate buffer: 0.1 M pH 7.6, prepared as follows. Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute to 100 mL. Dissolve 2.28 g of dibasic potassium phosphate trihydrate in water, and dilute to 100 mL. Adjust the pH of the second solution to 7.6 with the first solution.

Dithiothreitol solution: 0.194 M, prepared as follows. Dissolve 60 mg of dithiothreitol (DTT) in 2 mL of *Potassium phosphate* buffer.

Calcium chloride solution: 0.01 M, prepared as follows. Dissolve 147 mg of calcium chloride dihydrate in 100 mL of water.

Substrate stock solution: 38 mM, prepared as follows. Dissolve 13 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride (BAEE · HCl) in 1 mL of *Potassium phosphate buffer.*

Substrate solution: 0.73 mM BAEE · HCl, 7.8 mM DTT, and 0.4 mM calcium chloride, prepared as follows. Transfer 0.5 mL of *Substrate stock solution*, 1.0 mL of *Dithiothreitol solution*, and 1.0 mL of *Calcium chloride solution* to a 25-mL volumetric flask, and dilute with *Potassium phosphate buffer* to volume.

Sample solution: Prepare in such a way that ΔA/min lies in the 0.02–0.06 range. Dilute with ice-cold *Potassium phosphate buffer* if necessary.

Instrumental conditions

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

Mode: UV

Analytical wavelength: 255 nm

Path length: 1 cm

Temperature: 25°

Analysis

Sample: *Sample solution*

Transfer 3.0 mL of *Substrate solution* into a cuvette, and equilibrate the cuvette to 25°. Start the reaction by adding 0.05 mL of *Sample solution*. Prepare a blank by replacing the *Sample solution* with 0.05 mL of *Potassium phosphate buffer*. Mix well. Determine the change in absorbance (ΔA/min) from the linear range of the reaction. Assay the *Sample solution* in triplicate.

System suitability

Sample: *Sample solution*

Suitability requirement: 0.02–0.06 for ΔA/min

Calculate the activity of clostripain in units/mL in the portion of collagenase II taken:

$$\text{Result} = [V_T/(\varepsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 3.05 mL
ε = extinction coefficient for 255 nm, 0.81 ($1\ cm^2 \cdot mmol^{-1}$)
V_U = volume of the *Sample solution*, 0.05 mL
B = absorption path length, 1 cm
ΔA/min = change in absorbance from the linear range of the reaction
D = dilution factor

Calculate the specific activity in units/mg of protein:

$$\text{Result} = \textit{Activity}/C$$

Activity = activity of clostripain (units/mL)
C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 units of clostripain activity per mg of protein

• **TRYPSIN ACTIVITY**

Buffer: 0.1 M Tris and 0.02 M calcium chloride pH 8.0, prepared as follows. Dissolve 6.05 g of Tris and 1.45 g of calcium chloride dihydrate in 400 mL of water. Adjust with 2 N hydrochloric acid to a pH of 8.0 (at 25 ± 1°). Dilute with water to a final volume of 500 mL.

Substrate stock solution: Dissolve 10 mg of carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate,[1] accurately weighed, in 1.5 mL of water. Store on ice. [NOTE—Use freshly prepared solution only.]

Substrate solution: Prepare a solution by mixing 9.2 mL of *Buffer* and 1.0 mL of *Substrate stock solution*. Store on ice. [NOTE—Use freshly prepared solution only.]

Sample solution: Undiluted collagenase II solution

Instrumental conditions

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

Mode: Vis

Analytical wavelength: 405 nm

Path length: 1 cm

Temperature: 25°

Analysis

Sample: *Sample solution*

[1] A suitable carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate is Chromozym TRY from Roche Applied Science (catalog number 10378496103) or equivalent.

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Transfer 1.02 mL of *Substrate solution* into a polystyrene semi-micro cuvette, allow the temperature to stabilize, equilibrate the cuvette to 25°, and wait for 10 min. Start the reaction by adding 0.10 mL of *Sample solution*. Start recording the absorbance and continue for at least 5 min after the addition of the *Sample solution*. Determine the change in absorbance (ΔA/min) from the linear range of the reaction. Assay the *Sample solution* in triplicate. [NOTE—Use polyethylene pipette tips to transfer the *Sample solution*. The pipette tip should not be wet before transfer, and each pipette tip should be used only for transferring one sample. After withdrawing the *Sample solution*, wipe off the outside of the tip to remove any residual solution. After adding the *Sample solution* to the *Substrate solution*, rinse the tip by pipetting the solution up and down 2–3 times, discard the tip, and mix.]

System suitability

Sample: *Sample solution*

Suitability requirement: >0.01 for ΔA/min

Calculate the activity of trypsin in units/mL in the portion of collagenase II taken:

$$\text{Result} = [V_T/(\varepsilon \times V_U \times B)] \times \Delta A/\text{min} \times D$$

V_T = volume of the reaction mixture, 1.12 mL
ε = extinction coefficient for 405 nm, 10.4 (1 $cm^2 \cdot mmol^{-1}$)
V_U = volume of the *Sample solution*, 0.10 mL
B = absorption path length, 1 cm
ΔA/min = change in absorbance from the linear range of the reaction
D = dilution factor

Calculate the specific activity in units/mg of protein:

$$\text{Result} = \textit{Activity}/C$$

Activity = activity of trypsin (units/mL)
C = protein concentration (mg/mL)

Acceptance criteria: NMT 0.5 units of trypsin activity per mg of protein

SPECIFIC TESTS

- **PROTEIN CONTENT**

Sample solutions: Dilute collagenase II in water. Prepare at least in triplicate. [NOTE—Prepare the dilution using plastic pipette tips and not glass pipettes. Carefully wipe off the outside of the tip to remove any residual solution.]

Blank solution: Water

Instrumental conditions

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

Mode: UV

Analytical wavelength: 280 nm

Path length: 1 cm

System suitability

Samples: *Sample solutions*

Suitability requirement: Absorbance is in the range of 0.10–1.00.

Analysis

Samples: *Sample solutions* and *Blank solution*

Determine the net absorbance of *Sample solutions* by subtracting the absorbance of the *Blank solution* from the absorbance of each *Sample solution*. Determine the average net absorbance of *Sample solutions*.

Calculate the protein concentration in mg/mL:

$$\text{Result} = A_U \times D/\varepsilon$$

A_U = average net absorbance of the *Sample solutions*
D = dilution factor
ε = extinction coefficient (A_{280}0.1%/cm) for collagenase, 1.4

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50 USP Endotoxin Units/mg of protein
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩: The total bacterial count is NMT 100 cfu/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in closed containers at –60° to –90°.
- **LABELING:** The labeling states that the material is derived from *Clostridium histolyticum* along with the lot number, product or catalog number, and storage conditions.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Collagenase II RS
USP Endotoxin RS

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⟨90⟩ FETAL BOVINE SERUM—QUALITY ATTRIBUTES AND FUNCTIONALITY TESTS

INTRODUCTION

Fetal bovine serum (FBS) is the light-brown liquid fraction of clotted fetal bovine blood. It is depleted of cells, fibrin, and clotting factors. Although the complete composition of FBS is undefined, FBS contains high levels of growth factors and low levels of immunoglobulins. In addition, it contains other key ingredients that are essential in supporting proliferation of cells in culture. This product is used both in life science basic research and industrial manufacturing. FBS is a by-product of the meat industry and is collected from bovine fetuses removed from cattle found to be pregnant at slaughter. FBS is harvested from abattoirs that are inspected by the competent authority in the country of origin. Trained personnel following written and approved procedures should perform collection and processing. Blood is collected in a closed system in a dedicated area within the facility, and processed quickly to prevent hemolysis. The blood is allowed to clot and then typically is centrifuged in a refrigerated centrifuge to separate the serum from the other components. Serum typically is removed from the clot, transferred to labeled containers, and frozen. All manufacturers employ sterile filtration before final packaging. Additionally, gamma irradiation provides the highest assurance of the absence of viral activity. Gamma irradiation doses of 25–40 kGy provide significant log reduction of viral and other adventitious agents while preserving cellular growth performance.

The screening of FBS for viral contamination is accomplished by using all applicable testing described in the Code of Federal Regulations 9 CFR 113.53 (known as full 9 CFR testing). Mycoplasma assays are performed as described in *Mycoplasma Tests* ⟨63⟩.

IDENTIFICATION

• **IDENTIFICATION—RADIAL IMMUNODIFFUSION**

Reagents

- FBS test samples
- Horse serum, negative control samples
- Bovine IgG calibrator (500 mg/L)
- Sheep albumin diluent (1% Sheep albumin, 0.18% EDTA, 1.75% NaCl, and 1.21% Tris/HCl pH 7.4).

Materials/Apparatus: Ring measuring device is calibrated in 0.1-mm increments. Radial immunodiffusion (RID) plates are commercially available and contain anti-bovine IgG antiserum in a 1.5% agarose gel, 0.1 M phosphate buffer, pH 7.0, 0.1% sodium azide as bacteriostatic agent, and 1 µg/mL amphotericin B as an antifungal agent. Store at 2°–8°. Use RID plates that can measure bovine IgG in the range of 50–500 mg/L.

Standard curve: Use the bovine IgG calibrators for system suitability and for generation of a calibration curve. Prepare two dilutions from a 500 mg/L bovine IgG stock solution. Dilute 120 µL of the 500 mg/L stock with 80 µL of diluent (medium dilution) and 25 µL of the 500 mg/L stock with 225 µL diluent (low dilution). Label each dilution respectively as 300 mg/L and 50 mg/L calibrators. Use the 500 mg/L, 300 mg/L, and 50 mg/L solutions to generate the standard curve. [NOTE—Prepare and analyse the calibrator bovine IgG solutions in duplicate.] Load 5 µL of each sample into the 2.5-mm wells of the plate. At 72 h of incubation, measure ring diameters to the nearest 0.1 mm using an appropriate ring measuring device. Record the results and proceed to the generation of a standard curve.

The ring diameter should develop to completion at room temperature for 72 h. Using the result from each data point of the standard curve, generate a single linearity plot where y is the squared diameter (mm^2) of precipitin ring around the well and x is the Bovine IgG concentration (mg/L). Calculate the linear least-squares-fit regression line of the form $y = m(x) + b$ with the help of suitable software and determine the values for slope (m), y-intercept (b), and coefficient of determination (R^2). The standard curve for the method is linear if R^2 is ≥ 0.98.

Analysis: Frozen undiluted samples of FBS are thawed and tested within 24 h if stored at 4°. Testing of FBS test and USP Fetal Bovine Serum RS samples is performed in triplicate. Prepare RID plates containing anti-bovine IgG to be tested for the various types of sera. Allow plates and reagents to equilibrate to room temperature before use by leaving the plates open for 10–15 min at room temperature to allow any condensation in the wells or on the gel surface to evaporate. Samples should not be applied to wells where moisture is visible. Prepare serial dilutions, if necessary, of FBS test and USP Fetal Bovine Serum RS samples in diluent. Dilute the negative control horse serum in diluent. Load 5 µL of each sample into the 2.5-mm wells of the plate, and incubate at room temperature for 72 h. [NOTE—The test samples and the negative control are loaded on the same plate.]

Calculation: After 72 h, measure the diameters of the rings using the ring measuring device, and record the results. Using the regression equation developed under standard curve deviation, calculate the concentration of bovine IgG in FBS samples. Concentration is expressed as mg/L.

Acceptance criteria: Horse serum is negative (should not give a precipitation ring). FBS test and USP Fetal Bovine Serum RS samples are positive and contain NMT 500 mg/L of IgG.

• **HEMOGLOBIN CONTENT**

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

Sample preparation: FBS samples are thawed, are stored at 4°, and are tested within the same day.

Analysis: Determine the absorbance of the serum sample using a spectrophotometric cell of 1-cm path length at the wavelengths of absorbance at 576, 623, and 700 nm and using water as a blank. Calculate the concentration of hemoglobin in mg/dL:

$$(Abs_{576} \times 115) - (Abs_{623} \times 102) - (Abs_{700} \times 39.1)$$

Acceptance criteria: NMT 30 mg/dL

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SPECIFIC TESTS

• **FBS FUNCTIONALITY TESTS**

In the absence of a user-defined functionality assay, the following tests are suitable to determine the functionality of specific lots of FBS and to aid in the optimization of the growth conditions of mammalian cell cultures in the presence of FBS. For valid functionality confirmation independent of user-specific applications, tests are performed on the specified cell lines. For in-house validation of specialized cell culture applications, cell line(s) specific to those applications should be used and characterized. Use appropriate tissue culture vessels. Two tests described in this chapter are the *Growth-Promotion Curve* and the *Clonal Assay*. The decision about which type of test or the number of tests to be performed to assess suitability of a specific lot of FBS depends on the type of cell line used. For adherent cell lines, the number of colonies at the end of the culturing period represents a good assessment of the capacity of these cells, at low concentration, to grow in the presence of a specific lot of FBS. For cell lines growing in suspension cultures, the optimum growth kinetics is measured by counting viable cells after 7 days of culture.

Cell lines: Five cell lines are recommended for use:

1. HFL1 (ATCC CCL-153) normal lung, fibroblast
2. Mv1 Lu (ATCC CCL-64) mink lung, epithelial
3. HL-60 (ATCC CCL-240) peripheral blood promyeloblast, suspension
4. VERO (ATCC CCL-81) monkey kidney fibroblast
5. CHO (CCL-61) Chinese hamster ovary

The functionality tests described are to be performed on three cell lines, two of which are drawn from the five recommended cell lines and the third of which is the cell line relevant to the user's application. Cell lines are cultured with specific media as recommended by ATCC.

Materials

- Suitable growth vessel/container
- Biological Safety Cabinet Class II, Type A
- Cell counter/hemacytometer
- Inverted microscope with digital camera accessory
- Tissue culture vessels: T25 cm^2

Preparation of cells for assays: Quick-thaw a vial in a 37° water bath, and determine cell count and viability. Prepare multiple cultures from each cell line in serum-supplemented growth medium. Incubate the cultures at 37° following instructions provided by ATCC for each of the cell lines used for the test. Examine the prepared cultures under a microscope to ensure uniform, near-confluent monolayers or suspensions. Expand cells until there are enough for assay (about 1×10^7 total cells; >90% viability).

Harvesting of cultures

1. Remove and discard the growth medium, and then rinse each culture with media lacking FBS.
2. For adherent cells, add 1 mL of Trypsin/EDTA for a few minutes for cells to disperse. Incubate at 37°, if necessary. Neutralize with 1 mL culture medium containing at least 10% FBS.
3. Spin down the cells in a centrifuge. Aspirate off wash media, and resuspend cells in an appropriate volume for seeding.

Seeding of cells

1. On day 0: For the three cell lines to be tested prepare multiple cultures using seeding densities that range between 2×10^3 and 2×10^4 viable cells/mL. (Different inocula are chosen initially to determine optimum growth conditions. Once the appropriate inoculum is chosen, that condition is used to propagate the cells.) Following are the recommended seeding densities:

   Low seeding density: 2×10^3 viable cells/mL
   Mid seeding density: 6×10^3 viable cells/mL
   High seeding density: 2×10^4 viable cells/mL
2. Prepare cultures in triplicate for at least five time points (in days or hours according to the cell line), to determine the seeding density that will yield the optimal growth conditions for each cell line used.
3. Incubate the cultures at 37° in a humidified incubator saturated with 5% CO_2.
4. For each time point of measurement (days 0, 1, 2, 3, 4, and 7), take a photograph of each culture, in triplicate, for both the FBS test material and the USP Fetal Bovine Serum RS at each of the three concentrations for each cell line, and record the percentage of confluency for each of the conditions. [NOTE—Perform this step before trypsinization and cell counting.]
5. Harvest the cells from the three different seeding density cultures for each specific time point. For adherent cultures, harvest cells as described above.
6. Perform and record total cell count and viability for each of the nine cultures for the FBS test and the USP Fetal Bovine Serum RS for each cell line using an appropriate cell counter or hemacytometer. [NOTE—The schedule for counting may have to be changed for fast-growing cell lines or large cells that would become confluent before day 7 and/or for slow-growing lines that need to be in culture 8–10 days before reaching a plateau. Some adherent cell lines will never reach confluency.]

• **GROWTH-PROMOTION CURVE**

Measurements of cell proliferation rates often are used to determine the response of cells to exogenous stimuli. Quantitative assessment of cell growth conditions is an important factor in monitoring consistency of culture conditions. The optimal cell concentration range for subculturing, optimum inoculum, and doubling time are parameters that can be quantified and trended. Information about the growth kinetics of a culture is critical in the design of cell-based experiments. Cultures

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vary significantly in their growth properties from lag phase, log phase, and stationary phase. Document the growth characteristics of the culture during the three growth stages to determine population doubling time and cell cycle time. Cells that have entered the stationary phase may demonstrate reduced growth potential and change in morphology. Cells may become polarized and may secrete more extracellular matrix, making them difficult to remove from the substratum. Cells at the end of the log phase give the highest yield and greatest reproducibility.

Reagents

- Growth media without FBS
- FBS test samples
- Growth medium + 10% FBS
- Trypsin/EDTA solution (0.25%/0.53 mM) in Hank's Balanced Salt Solution (HBSS)

Analysis: Once the cells have reached the end of the log phase, subculture the cells for the test. Follow the procedure described in *Specific Tests, FBS Functionality Tests, Seeding of cells* and prepare multiple cultures for the USP Fetal Bovine Serum RS, and test FBS for different cell lines at three seeding densities for which at least one growth curve displays a lag phase, log phase, and stationary phase and for which the log phase is linear at three or more time points. Viable cell counts are determined on days 0, 1, 2, 3, 4, and 7.

Calculation and **Data analysis:** Calculate the mean viable count [cells/cm^2 (adherent) or cells/mL (suspension)] and the mean percent viability for each data point. Plot the data on a semi-log scale graph with the viable count on the log scale on the *y*-axis and days (or hours) in culture on an arithmetic scale on the *x*-axis. Estimate the doubling time using a growth curve that is linear over three or more time points.

Acceptance criteria: The R^2 value of the line should be equal to or greater than 0.98 in order to support calculation of a valid doubling time. The doubling time of the test sample should be no less than 90% of the doubling time of USP Fetal Bovine Serum RS.

• **CLONAL ASSAY**

This assay is designed to assess the optimal growth for adherent cell lines. Plating efficiency or colony formation at low cell density is a preferred method for analyzing the proliferative capacity and survival of single cells under optimal growth conditions. This is a very sensitive test and is often used for assessing the quality of serum lots. This technique reveals differences in the growth rate within the cell population and is capable of distinguishing between changes in growth rate (colony size) and cell survival (colony number). Because of the heterogeneous cell population of some cell cultures, remember that cells grow differently as isolated colonies at low densities. Consequently, few cells survive even under ideal conditions because all cell interaction is lost. Cloning is a survival assay that is also used for optimizing growth conditions (selection of medium and serum). If it can be confirmed that a single colony arose from a single cell, then cloning efficiency can be determined.

Reagents

- Growth medium + 10% FBS (test serum)—Eagle minimum essential medium (EMEM) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium carbonate, 0.1 mM nonessential amino acids, and sodium pyruvate containing 100 U/mL penicillin and 100 g/mL streptomycin plus 10% FBS.
- Trypsin/EDTA solution (0.25%/0.53 mM) in HBSS.
- Dulbecco's Phosphate Buffer Saline without calcium or magnesium.
- Carbol Fuchsin–Methylene Blue Solution—Mix 20 g carbol fuchsin stock in 2 L methanol and stir for 10 min (1% carbol fuchsin). Mix 50 g methylene blue in 5 L methanol, and stir for 10 min (1% methylene blue). Prepare Carbol Fuschin–Methylene Blue working solution by mixing methylene blue, methanol, and carbol fuchsin in a ratio of 3:2:1. Mix for 20 min and filter through four folds of cheesecloth in a funnel. Aliquot and store in brown glass bottles at 15° to 25°.

Sample: Multiple lots of FBS are used for this assay. For each lot of serum to be tested, add 20 mL of FBS to 180 mL of EMEM, and use the same sample for the entire test. Sterilize using 0.22-m low protein binding filter units. Store growth medium at 4° until ready to use.

Cell preparation: This test is only for adherent cultures and is performed with the adherent cell lines described under *Cell Lines* (HFL1 and Mv 1 Lu). One week before testing serum, expand the cell lines as described under *Seeding of Cells*, change the medium every 2–3 days, and subculture the cells when they are about 90% confluent. Determine the cell count and viability (viability should be >90%) before performing the assay. Harvest cells as described under *Harvesting of Cultures*, wash twice, and resuspend cells in basal EMEM.

Analysis: The procedure involves plating single-cell suspension at low densities (2–50 cells/cm^2) from which discrete colonies will form. At the end of the assay, fix, stain, and count the number of colonies as directed below.

1. For each cell line label ten 60-mm × 15-mm tissue culture dishes for each serum lot that will be tested. Label the side of the lower half of each dish, including controls.
2. Transfer 5 mL of medium containing 10% of the appropriate test serum (10 replicates). Add 400 cells per culture dish (aim for a cell concentration of about 800 cells/mL).
3. Incubate for 10–14 days at 37° in a humidified incubator saturated with 5% CO^2.
4. Remove the supernatant and add enough Carbol Fuchsin–Methylene Blue Solution to cover each of the culture dishes for 10 min.
5. Remove the stain; rinse the culture dishes with several changes of distilled water; invert the dishes on paper towels; and allow to dry.
6. Count and record (1) the number of colonies and (2) the total surface of stained colonies (mm^2). Calculate means and standard deviations.

Acceptance criteria: Percent plating efficiency is expressed by counting the number of colonies in a defined area divided by the number of cells seeded multiplied by 100. Compare results between lots of FBS, and select a serum lot that is good for various types of cells and optimal for a specific cell culture application.

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- **pH** ⟨791⟩: 7.00–8.00, in undiluted serum samples
- **Osmolality and Osmolarity** ⟨785⟩, *Osmolality*: 280–360 mOsmol/kg
- **Bacterial Endotoxins Test** ⟨85⟩: It contains not more than 10 USP Endotoxin Units/mL of serum.
- **Total Protein Content** ⟨1057⟩: 30–45 mg/mL
- **Sterility Tests** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in sealed containers at a temperature of –10° or below.
- **Labeling:** Label it to indicate that contents are Fetal Bovine Serum, and indicate lot number, expiration date, and storage conditions. Also, indicate country of origin on product labeling.
- **USP Reference Standards** ⟨11⟩
  USP Endotoxin RS
  USP Fetal Bovine Serum RS

⟨91⟩ CALCIUM PANTOTHENATE ASSAY

INTRODUCTION

The following liquid chromatographic procedures are provided for the determination of calcium pantothenate as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in dietary supplements or pharmaceutical dosage forms.

While conducting these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from atmosphere and light, preferably with the use of low-actinic glassware. Use the appropriate USP Reference Standards.

ASSAY

- **Chromatographic Methods, Procedure 1**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This procedure involves the extraction of calcium pantothenate from the formulation by the *Internal standard solution*.

Unless specified in the individual monographs, the *Internal standard solution*, *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: Phosphoric acid and water (1:1000)

Internal standard solution: 80 mg of *p*-hydroxybenzoic acid in 3 mL of alcohol. Add 50 mL of water and 7.1 g of dibasic sodium phosphate, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.7.

Standard solution: 0.6 mg/mL of USP Calcium Pantothenate RS in *Internal standard solution*

Sample solution (for capsules): Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without losing shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a quantity of mixed capsule contents and a volume of *Internal standard solution* to a centrifuge tube to obtain a concentration of 0.6 mg/mL in the *Sample solution*.

Sample solution (for tablets): Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to a nominal amount of 15 mg of calcium pantothenate, to a centrifuge tube. Add 25.0 mL of the *Internal standard solution*, and shake vigorously for 10 min. Centrifuge, filter, and use the clear filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for calcium pantothenate and *p*-hydroxybenzoic acid are about 0.5 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

R_U = peak response ratio of calcium pantothenate to *p*-hydroxybenzoic acid from the *Sample solution*
R_S = peak response ratio of calcium pantothenate to *p*-hydroxybenzoic acid from the *Standard solution*
C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of calcium pantothenate in the *Sample solution* (mg/mL)

• **CHROMATOGRAPHIC METHODS, PROCEDURE 2**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This procedure involves dissolving an appropriate quantity of the sample equivalent to 10 mg of calcium pantothenate in 10 mL of methanol and diluting with water to 250 mL.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Buffer solution: Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Methanol and *Buffer solution* (1:9)

Standard stock solution: 0.25 mg/mL of USP Calcium Pantothenate RS in water. Prepare fresh every 4 weeks. Store in a refrigerator.

Standard solution: 40 µg/mL of USP Calcium Pantothenate RS from the *Standard stock solution* diluted with water

Sample solution (for capsules): Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without losing shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 10 mg of calcium pantothenate, to a 250-mL volumetric flask. Add 10 mL of methanol, and swirl the flask to disperse the capsule contents. Dilute with water to volume, mix, and filter.

Sample solution (for tablets): Finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 10 mg of calcium pantothenate, to a 250-mL volumetric flask. Add 10 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

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

• **CHROMATOGRAPHIC METHODS, PROCEDURE 3**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Oral Solution*

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- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Oral Solution*

This procedure involves diluting an appropriate quantity of the liquid sample with water to a final concentration of 80 µg/mL of calcium pantothenate.

Unless specified in the individual monographs, the *Standard solution*, *System suitability solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: 0.2 M monobasic sodium phosphate and methanol (97:3). Adjust with 1.7 M phosphoric acid to a pH of 3.2 ± 0.1.

Standard solution: 80 µg/mL of USP Calcium Pantothenate RS in *Mobile phase*

System suitability solution: 80 µg/mL of USP Racemic Panthenol RS in *Mobile phase*. Mix the resulting solution and *Standard solution* (1:1).

Sample solution: Equivalent to 80 µg/mL of calcium pantothenate from the oral solution in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between panthenol and calcium pantothenate, *System suitability solution*

Tailing factor: NMT 2.0 for the calcium pantothenate peak, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

r_U = peak response of calcium pantothenate from the *Sample solution*

r_S = peak response of calcium pantothenate from the *Standard solution*

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

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

• **CHROMATOGRAPHIC METHODS, PROCEDURE 4**

This procedure can be used to determine calcium pantothenate in:

- Active pharmaceutical ingredients
- Dietary ingredients

This procedure involves the dissolution of the sample directly into water.

Unless specified in the individual monographs, the *Standard solution*, *System suitability solution*, *Sample solution*, and reagent solutions are prepared as follows.

Buffer solution: Dissolve 3.2 g of monobasic sodium phosphate in 1 L of water and adjust with 1 N sodium hydroxide to a pH of 5.5.

Mobile phase: Acetonitrile and *Buffer solution* (2:98)

System suitability solution: 0.1 mg/mL of USP Calcium Pantothenate RS and 0.5 mg/mL each of USP Beta Alanine RS, USP Sodium D-Pantoate RS, and USP Pantolactone RS in water

Standard solution: 0.5 mg/mL of USP Calcium Pantothenate RS in water

Sample solution: 0.5 mg/mL of Calcium Pantothenate in water

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

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

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times for beta alanine, pantoic acid, pantothenic acid, and pantolactone are 0.3, 0.6, 1.0, and 1.9 min, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the pantothenic acid and pantoic acid peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

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

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

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

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Beta Alanine RS
USP Calcium Pantothenate RS
USP Pantolactone RS
USP Racemic Panthenol RS
USP Sodium D-Pantoate RS

⟨92⟩ GROWTH FACTORS AND CYTOKINES USED IN CELL THERAPY MANUFACTURING

INTRODUCTION

Qualification of reagents, source materials, and control of the manufacturing process are key elements that ensure the quality and safety of cell therapies. Growth factors and cytokines are important for the maintenance, growth, selection, and purification of cultures of cell therapy products. This chapter describes the accepted tests, procedures, and acceptance criteria for growth factors and cytokines that may be involved in the manufacturing of cell therapy products.

RECOMBINANT HUMAN INTERLEUKIN 4 (rhIL-4)

MHKCDITLQE	IIKTLNSLTE	QKTLCTELTV	TDIFAASKNT
TEKETFCRAA	TVLRQFYSHH	EKDTRCLGAT	AQQFHRHKQL
IRFLKRLDRN	LWGLAGLNSC	PVKEANQSTL	ENFLERLKTI
MREKYSKCSS			

$C_{658}H_{1071}N_{193}O_{197}S_8$ 15,096 Da

rhIL-4 is a single-chain polypeptide of 130 amino acid residues expressed in *Escherichia coli*. It is produced as a lyophilized powder and contains NLT 0.5×10^7 USP Units of IL-4/mg of total protein. Process specific host-cell DNA impurities in IL-4 with limits of less than 1 ng/mg are determined as described in *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)* ⟨1130⟩. Neither manufacturing license nor market approval is required for IL-4 intended for use as an ancillary material during manufacturing. Following are typical IL-4 quality attributes.

IDENTIFICATION

- **A.** Amino-terminal sequence analysis of at least eight amino acids is performed with an automated sequencer. Stepwise-released phenylthiohydantoin amino acids are identified with on-line reversed-phase high-performance liquid chromatography, on the basis of their elution times.
- **B.** Use the electrophoresis method followed by western blotting analysis to visualize the IL-4 protein. The method is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), described in the test for *Purity*.

Phosphate buffered saline; Laemmli sample buffer, reducing; and **Laemmli sample buffer, nonreducing:** Proceed as directed in the test for *Purity* in the *Assay*.
Standard stock solution: 50 µg/mL of reconstituted USP rHuman Interleukin 4 RS in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]
Standard solution: 20 µg/mL of IL-4, from *Standard stock solution*, in *Phosphate buffered saline*
Standard solution, reducing: Combine 20 µL of *Standard solution* and 5 µL of *Laemmli sample buffer, reducing*.
Standard solution, nonreducing: Combine 20 µL of *Standard solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Sample stock solution: 50 µg/mL of reconstituted IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]
Sample solution: 20 µg/mL of IL-4, from *Sample stock solution*, in *Phosphate buffered saline*
Sample solution, reducing: Combine 20 µL of *Sample solution* and 5 µL of *Laemmli sample buffer, reducing*.
Sample solution, nonreducing: Combine 20 µL of *Sample solution* and 5 µL of *Laemmli sample buffer, nonreducing*.
Analysis

Samples: *Standard solution, reducing; Standard solution, nonreducing; Sample solution, reducing;* and *Sample solution, nonreducing*

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Western blotting: After electrophoresis, the proteins are transferred onto a polyvinylidene fluoride (PVDF) membrane using standard procedures. Incubate the membrane for 1 h at room temperature with *Phosphate buffered saline* containing 0.1% Tween 20 and 5% skim milk powder. The membrane is then incubated with an anti-IL-4 antibody[1] (diluted appropriately in *Phosphate buffered saline*), followed by incubation with a secondary antibody at room temperature under gentle agitation for 1 h for each of the antibodies. The IL-4 protein band is identified by developing the membrane using a suitable detection system.[2]

Acceptance criteria: The developed Western blot should give a positive signal equivalent to the USP rHuman Interleukin 4 RS.

ASSAY

• **PURITY:** [NOTE—Purity is determined on the bulk material.] SDS-PAGE is performed as described under *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩ under reducing and nonreducing conditions.

Molecular weight marker: Use a suitable molecular weight marker containing protein bands between 10 and 200 kDa.

Phosphate buffered saline: 2.67 mM of potassium chloride, 1.47 mM of potassium phosphate (KH_2PO_4), 137.93 mM of sodium chloride, and 8.06 mM of dibasic sodium phosphate in water. Adjust to a pH of 7.0–7.3.

Laemmli sample buffer, nonreducing: 100 mM TRIS-HCl, pH 6.8, 50% glycerol, 0.25% bromophenol blue indicator, and 10% sodium lauryl sulfate in water

Laemmli sample buffer, reducing: Add 2.5 µL mercaptoethanol to 50 µL of *Laemmli sample buffer, nonreducing.*

Sample stock solution: 400 µg/mL of bulk IL-4 in *Phosphate buffered saline*

Sample solution 1: Combine 20 µL of *Sample stock solution* and 5 µL of *Laemmli sample buffer, nonreducing.*

Sample solution 2: Combine 20 µL of *Sample stock solution* and 5 µL of *Laemmli sample buffer, reducing.*

Control A stock solution: 4 µg/mL of IL-4, from *Sample stock solution,* in *Phosphate buffered saline.* [NOTE—*Control A solutions* are run in triplicates in both reducing and nonreducing conditions.]

Control A solution 1: Combine 20 µL of *Control A stock solution* and 5 µL of *Laemmli sample buffer, nonreducing.*

Control A solution 2: Combine 20 µL of *Control A stock solution* and 5 µL of *Laemmli sample buffer, reducing.*

Control B stock solution: 12 µg/mL of IL-4, from *Sample stock solution,* in *Phosphate buffered saline.* [NOTE—*Control B solutions* are run in duplicates in both reducing and nonreducing conditions.]

Control B solution 1: Combine 20 µL of *Control B stock solution* and 5 µL of *Laemmli sample buffer, nonreducing.*

Control B solution 2: Combine 20 µL of *Control B stock solution* and 5 µL of *Laemmli sample buffer, reducing.*

Electrophoretic conditions

(See *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.)

Mode: Discontinuous PAGE gel

Stacking gel: 4% acrylamide

Resolving gel: 12% acrylamide

Run conditions: 10 min at 100 V; then 30 min at 200 V

Protein detection: Silver stain

Analysis

Samples: *Sample solution 1, Sample solution 2, Control A solution 1, Control A solution 2, Control B solution 1,* and *Control B solution 2*

Incubate 25 µL of each *Sample solution* and *Control solution* under nonreducing conditions for 5 min at 60°, and load onto the gel. Incubate 20 µL of each *Sample solution* and *Control solution* under reducing conditions for 5 min at 60°, and load onto the gel. After silver staining and scanning the whole gel, determine the intensity of all detectable protein bands by densitometry, and calculate the percentage of each detectable protein band, in the *Sample solution,* twice by comparing the pixel intensity of each contaminating band with the mean value of *Control solutions A* and *B,* respectively, by the formulas:

$$\text{Result} = (A_{100}) \times 1/(A_1) \text{ and}$$

$$\text{Result} = (A_{100}) \times 3/(A_3)$$

A_{100} = intensity of one contaminating band of the *Sample solution*
A_1 = mean intensity of all detectable bands of *Control A solution*
A_3 = mean intensity of all detectable bands of *Control B solution*

IL-4 control solutions analysis should yield one detectable band with an apparent molecular weight of approximately 15 kDa. If values calculated by means of *Control A solution* are different from those revealed by comparison with *Control B solution,* the value corresponding to the highest amount of impurity should be taken. If the intensity of one of the contaminating bands is lower than the value of *Control A solution* (corresponding to 1%), the value of this contamination is set to 1%. The purity of the sample solution is then calculated:

$$\text{Result} = 100 - \Sigma C_n$$

C = percentage of each contamination given in rounded whole numbers
n = number of contaminants of the IL-4 *Sample solution*

Acceptance criteria: The purity of IL-4 is NLT 97%, as determined by SDS-PAGE.

• **PROTEIN CONTENT:** [NOTE—Protein content is determined on the basis of the packaged product.]

[1] A suitable anti-IL-4 antibody can be obtained from commercial sources (e.g., Dianova Inc.).
[2] A suitable detection system can be obtained from commercial sources (e.g., Pierce/Perbio Science).

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Phosphate buffered saline: Proceed as directed in the test for *Purity*.
Sample solution: 50 µg/mL of IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]
Blank: *Phosphate buffered saline*
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Pathlength: 1 cm
Analytical wavelength: 280 nm
Analysis
Samples: *Sample solution* and *Blank*
Calculate the protein concentration:

$$C = A_{280}/0.63$$

C = IL-4 concentration of the *Sample solution* (mg/mL)
A_{280} = absorbance at 280 nm

SPECIFIC TESTS

• **BIOIDENTITY:** [NOTE—The biological activity measurement is determined on the basis of the packaged product.]
RPMI 1640 medium with L-glutamine: Prepare a mixture of the ingredients in the quantities shown in sufficient water to obtain 1 L of medium, and sterilize by filtration:

Material	Quantity
Calcium nitrate ($Ca(NO_3) \cdot 4H_2O$)	100 mg
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	100 mg
Potassium chloride	400 mg
Sodium chloride	6000 mg
Sodium phosphate, dibasic anhydrous	800 mg
Sodium bicarbonate	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic acid	20 mg
L-Polyvinylidene fluoride L-cystine dihydrochloride	20 mg
L-Glutamic acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg
L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine disodium salt dihydrate	20 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline chloride	3 mg
D-Calcium pantothenate	0.25 mg
Folic acid	1 mg
i-Inositol	35 mg

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Material	Quantity
Niacinamide	1 mg
para-Aminobenzoic acid	1 mg
Pyridoxine hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine hydrochloride	1 mg
Vitamin B_{12}	0.005 mg
D-Glucose (dextrose)	2000 mg
Glutathione (reduced)	1 mg
Phenol red	5 mg

Growth medium: Using aseptic procedures, prepare the following tissue culture medium:

RPMI-1640 with L-glutamine	500 mL
Sodium pyruvate 100 mM	5 mL
Fetal bovine serum	50 mL
Human rGM-CSF[a]	3×10^4 International Units

[a] Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is added extemporaneously.

Sterilize by filtration, and store at between 2° to 8°. Use within 1 month. Add GM-CSF immediately before use.

Assay medium: Use *Growth medium* containing no GM-CSF.

Phosphate buffered saline: Proceed as directed in the test for *Purity* in the *Assay*.

Resazurin solution: 11 mg of resazurin in 100 mL of *Phosphate buffered saline*. [NOTE—Sterile filter and store solution protected from light at 4°. *Resazurin solution* is stable for at least 6 months if treated under sterile conditions.]

[NOTE—For all *Standard* and *Sample solutions*, IL-4 concentration is determined by photometry at 280 nm using an extinction coefficient (ε) of 0.63 $mg^{-1}cm^{-1}$.]

Standard stock solution: 50 µg/mL of USP rHuman Interleukin 4 RS in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]

Standard solutions: 36, 12, 4, 1.33, 0.44, 0.15, 0.05, 0.016, 0.006 ng/mL of IL-4, from *Standard stock solution* in *Assay medium*

Sample stock solution: 50 µg/mL of IL-4 in *Phosphate buffered saline*. [NOTE—Do not agitate while mixing; swirl gently.]

Sample solutions: 36, 12, 4, 1.33, 0.44, 0.15, 0.05, 0.016, 0.006 ng/mL of IL-4, from *Sample stock solution* in *Assay medium*

Control solution: Use the *Assay medium*.

Cell culture preparation: Prepare cell cultures of the human factor-dependent TF-1 cell line (ATCC No. CRL-2003), following the protocol described in the ATCC information sheet. Passage the cultures every 2–3 days, using 1:3 subcultures of the cells for up to 1 month. Seed density should be 0.5×10^6 cells/mL, and maximal density should be 3×10^6 cells/mL. Viability of the cells should be >90%. Maximal passage number is 24, and maximal cultivation time from thawing is 28 days. After 28 days, initiate a new culture. Cells are propagated using *Growth medium* at 37°, supplemented with air and 5% carbon dioxide.

Analysis

Samples: *Standard solutions*, *Sample solutions*, and *Control solution*

The activity of the *Sample solution* is determined in duplicate. Wash the cells three times in *Phosphate buffered saline*. Plate 2×10^4 TF-1 cells resuspended in 100 µL of *Assay medium* per well in 96-well, flat-bottom microplates. Incubate for 72 h at 37° and 5% CO_2 atmosphere in a humidified incubator in the presence or absence of various concentrations of *Standard solution*, *Sample solution*, or *Control solution* by adding 100 µL of the corresponding solution to each well. Add 30 µL of *Resazurin solution* to each well and incubate for another 24 h. Determine the fluorescence intensity per well by reading the plate with a microplate reader using 544 nm (excitation) and 590 nm (emission). Convert the fluorescence intensity in each well to a percentage of the maximum fluorescence intensity. For the *Sample solution* and *Standard solution*, plot the percentage of fluorescence intensity versus the concentration of the respective solution. By using the least squares method of regression analysis, compute the ED50 in ng/mL of the *Sample solution* and the *Standard solution*. The coefficient of determination for curve regression should be ≥ 0.98. Calculate the potency in USP Interleukin 4 Units/mg:

$$\text{Result} = A \times E_S/E_U$$

A = activity of USP rHuman Interleukin 4 RS (USP units/mg)
E_S = determined ED_{50} of *Standard solution* (ng/mL)
E_U = determined ED_{50} of *Sample solution* (ng/mL)

Acceptance criteria: NLT 0.5×10^7 USP IL-4 Units/mg

- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 50 USP Endotoxin Units/mg.

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ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at –80°.
- **LABELING:** Material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP rHuman Interleukin 4 RS

⟨111⟩ DESIGN AND ANALYSIS OF BIOLOGICAL ASSAYS

INTRODUCTION

The potency of several Pharmacopeial articles must be determined by bioassays. The aim of this chapter is to present a concise account of certain essential biometrical procedures for bioassays in chapters or monographs of *USP–NF*, namely outlier identification, confidence intervals for relative potency measurements, and combination of independent assays. For bioassays not in *USP–NF*, other methods may be appropriate. See general information chapter *Analysis of Biological Assays* ⟨1034⟩ which may be a helpful, but not mandatory, guidance.

REJECTION OF OUTLYING OR ABERRANT OBSERVATIONS

A response that is questionable because of failure to comply with the procedure during the course of an assay is rejected. Other aberrant values may be discovered only after the responses have been tabulated, but can then be traced to assay irregularities that justify their omission. The arbitrary rejection or retention of an apparently aberrant response can be a serious source of bias. In general, the rejection of observations solely on the basis of their relative magnitudes, without investigation as to cause, is a procedure to be used sparingly. Should it be understood, either following an investigation into cause or based on practical assay experience, that an observation's discordance is unlikely to arise from a reasonable expectation of response to assay treatments, then a suspected aberrant response or outlier may be tested against one of two criteria, both of which assume that the data have an approximately normal distribution (which may be satisfied only after a suitable transformation of the original responses). Alternative statistically sound approaches to outlier detection may be used. The conditions under which outlier testing will be conducted and the criterion to be used should be specified a priori in the lab's procedures if not specified in the monograph or chapter.

Criterion 1 (Dixon's Test)

The first criterion is based on the variation within a single group of supposedly equivalent responses, such as a group of animals given a common concentration of a sample. At a confidence level of 99%, a valid observation will be rejected once in 100 trials (when the suspected outlier can occur at only one end) or once in 50 trials (when the suspected outlier can occur at either end), provided that relatively few, if any, responses within the group are identical. Arrange the responses in order of magnitude from y_1 to y_N, where N is the number of observations in the group. Compute the relative gap by using the formulas in *Table 1* below.

Table 1

Sample Size (N)	Candidate Outlier is Smallest (y_1)	Candidate Outlier is Largest (y_N)
3–7	$G_1 = (y_2 - y_1)/(y_N - y_1)$	$G_1 = (y_N - y_{N-1})/(y_N - y_1)$
8–10	$G_2 = (y_2 - y_1)/(y_{N-1} - y_1)$	$G_2 = (y_N - y_{N-1})/(y_N - y_2)$
11–13	$G_3 = (y_3 - y_1)/(y_{N-1} - y_1)$	$G_3 = (y_N - y_{N-2})/(y_N - y_2)$

If G_1, G_2, or G_3, as appropriate, exceeds the critical value in *Table 2*, for the observed N, there is a statistical basis for identifying the discordant measurement as an outlier and considering its removal. For N larger than 13, use *Criterion 2*.

In samples from a normal population, at a confidence level of 99%, gaps equal to or larger than the following values of G_1, G_2, and G_3 occur with a probability $P = 0.01$, when outlier measurements can occur only at one end; or with $P = 0.02$, when they may occur at either end.

Table 2. Test for Outlier Measurements

N	3	4	5	6	7
G_1	0.988	0.889	0.780	0.698	0.637
N	8	9	10	—	—
G_2	0.683	0.635	0.597	—	—

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Table 2. Test for Outlier Measurements *(continued)*

N	11	12	13	—	—
G_3	0.679	0.642	0.615	—	—

Criterion 2 (Grubbs, Extreme Studentized Deviate Test)

The second criterion may be used to examine for outlying values in groups of supposedly equivalent responses and may also be used in examining the set of residuals from a fitted model (linear or nonlinear) where there is constant variance. The final model (which yields the residuals for outlier detection) should include all important design variables. (For further discussion of design variables, see general information chapter *Design and Development of Biological Assays* ⟨1032⟩, which may be a helpful, but not mandatory, resource.) (Note that for application to residuals, the following is an approximation. If the statistical software provides studentized residuals, those values should be used instead of those from the following equation.) For the value, R, that is furthest from the sample mean, compute the standardized deviation Z:

$$Z = (R - \bar{R})/S$$

where $\bar{R}$ and S are the mean and standard deviation, respectively, of the set of values. For residuals from a least squares fit, such as for a parallel line assay, $\bar{R} = 0$, and S is the square root of the residual mean square from the analysis. If $|Z|$ is greater than C as determined below, then the value R is identified as a statistical outlier at the 1% level.

$$C = \frac{(N-1)t_{df-1,p}}{\sqrt{N\left(N-2+t^2_{df-1,p}\right)}}$$

where N is the sample size, t is the one-sided $100p$ percentage point from the t distribution with df the degrees of freedom associated with S:

$$p = 1 - \frac{0.01}{2N}$$

Alternative outlier methods are available that are intended for use on data sets that may contain multiple outliers and for detection of outliers associated with the bioassay design or model. For further discussion of outliers, see general information chapter *Analytical Data—Interpretation and Treatment* ⟨1010⟩, which may be a helpful, but not mandatory, resource.

THE CONFIDENCE INTERVAL AND LIMITS OF POTENCY

The following method (Fieller's) is used to determine the confidence interval for an estimate of log relative potency from a parallel line assay or a slope ratio assay. Let $M = a/b$ be the ratio for which we need a confidence interval. For the estimates, a and b, we have their respective standard errors, SE_a and SE_b, and a covariance between them, denoted Cov. The confidence interval, (M_{Low}, M_{Up}), for the estimated log relative potency then is as follows:

$$(M_{Low}, M_{Up}) = \left\{ \frac{M - \frac{gCov}{SE_b^2} \pm \frac{t}{b}\sqrt{(1-g)\,SE_a^2 + M^2SE_b^2 - 2MCov + \frac{gCov^2}{SE_b^2}}}{1-g} \right\}$$

where:

$$g = \frac{t^2SE_b^2}{b^2}$$

and $t = t_{df,\alpha/2}$ is the upper $\alpha/2$ percentage point (or the two-sided α percentage point) with the residual degrees of freedom, df, from the statistical analysis and chosen confidence level, $100*(1-\alpha)$, (usually 95%). If $g \geq 1$, it means that the denominator, b, is not statistically significantly different from 0 and the use of the ratio is not sensible for those data. The length, L, of this confidence interval is $M_{Up} - M_{Low}$.

For those cases in which the estimates of a and b are statistically uncorrelated ($Cov = 0$), the confidence interval formula simplifies to the following:

$$(M_{Low}, M_{Up}) = \left\{ \frac{M \pm \frac{t}{b}\sqrt{(1-g)\,SE_a^2 + M^2SE_b^2}}{1-g} \right\}$$

For further discussion of confidence intervals for potency, see chapter ⟨1034⟩ which may be a helpful, but not mandatory, resource.

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COMBINATION OF INDEPENDENT ASSAYS

When the monograph or chapter permits, multiple independent assays may be performed until the combined results reduce the confidence interval width to within the limits specified in the pertinent monograph or chapter. Where two or more independent assays are required, each leading to a log-potency M, the M's are combined using one of the following two methods.

Method 1

Let M_i denote the logarithm of the relative potency of the ith assay of h assay results to be combined. To combine the h results, the mean, standard deviation, and standard error of the M_i are calculated in the usual way:

$$\text{Mean } \overline{M} = \sum_{i=1}^{h} M_i / h$$

$$\text{Standard Deviation } S = \sqrt{\frac{1}{h-1}\sum_{i=1}^{h}(M_i - \overline{M})^2}$$

$$\text{Standard Error } SE = S / \sqrt{h}$$

A 100(1 – α)% confidence interval is then found as:

$$\overline{M} \pm t_{h-1,\alpha/2} SE$$

where $t_{h-1,\alpha/2}$ is the upper α/2 percentage point (or the two-sided α percentage point) of a t-distribution with $h - 1$ degrees of freedom. The width, L, of this interval is $2t_{h-1,\alpha/2}SE$.

Method 2

It is assumed that the results of each of the h assays have been analyzed to give h values of log potency with associated confidence limits. For each assay, i, obtain the confidence interval for the log potency or log relative potency. Then compute value L_i by subtracting the ith lower confidence limit from the ith upper confidence limit. A weight w_i for each value of the log relative potency, M_i, is calculated as follows, where t_i has the same t-distribution value as that used in the calculation of confidence limits in the ith assay and is based on n_i degrees of freedom:

$$W_i = \frac{4t_i^2}{L_i^2}$$

The products w_iM_i are formed for each assay, and their sum is divided by the total weight (w) for all assays to give the weighted mean log relative potency and its standard error as follows:

$$\left.\begin{aligned} &\text{Mean, } \overline{M} = \sum_{i=1}^{h} w_i M_i / w \\ &\text{Approximate Standard Error, } SE = 1/\sqrt{w} \\ &\text{where } w = \sum_{i=1}^{h} w_i \end{aligned}\right\} (1)$$

Next compute an approximate chi-square:

$$\chi^2_M = \sum_{i=1}^{h} w_i (M_i - \overline{M})^2 = \sum_{i=1}^{h} w_i M_i^2 - w\overline{M}^2$$

If the value of the approximate χ^2_M is *well under* the 5% value shown in *Table 3*, compute the confidence interval using the mean and approximate standard error equations in (1) above; otherwise use *Alternate weights* as described below. Labs need to specify in their procedures how to quantify "well under". Absent such a specification, the 20% values of *Table 3* are suggested.

A 100(1 – α)% confidence interval in the log scale is then found as:

$$\overline{M} \pm L/2$$

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$$\text{where } L = \frac{2t_{df,\alpha/2}}{\sqrt{w}}\sqrt{1+\frac{4}{w^2}\times\sum_{i=1}^{h}\frac{w_i(w-w_i)}{n_i'}}$$

$$n_i' = n_i - 4\times\frac{h-2}{h-1}$$

$$\text{and } df = \frac{w^2}{\sum_{i=1}^{h} w_i^2 / n_i}$$

where $t_{N,\alpha/2}$ is the upper $\alpha/2$ percentage point (or the two-sided α percentage point) of a *t*-distribution with degrees of freedom, *df*. The width of this interval is *L*.

Table 3. Critical Values for Approximate Chi-Square Test

	Critical Values	
h	5%	20%
2	3.841	1.642
3	5.991	3.219
4	7.815	4.642
5	9.488	5.989
6	11.070	7.289
7	12.592	8.558
8	14.067	9.803
9	15.507	11.030
10	16.919	12.242

Alternate weights: The observed variation among the estimated log potencies or relative potencies can be divided into two components:

- intra-assay variation for assay *i*:
  $V_i = 1/w_i$
- inter-assay component of variation:

$$s_B^2 = \max\{0, \frac{1}{h-1}\sum_{i=1}^{h}(M_i-\bar{M})^2 - \frac{1}{h}\sum_{i=1}^{h}V_i\} \; s_B^2 = \max\{0, \frac{1}{h-1}\sum_{i=1}^{h}(M_i-\bar{M})^2 - \frac{1}{h}\sum_{i=1}^{h}V_i\}$$

For each assay, a weighting coefficient is then calculated as:

$$w_i' = \frac{1}{V_i + s_B^2}$$

The confidence interval is then found as:

$$\bar{M} \pm t \times SE',$$

$$\text{where } SE' = 1/\sqrt{w'} \text{ and } w' = \sum_{i=1}^{h} w_i'$$

and *t*, the *t-distribution value*, is often approximated by the value 2.

For further discussion of combination of assays, see ⟨1034⟩, which may be a helpful, but not mandatory, resource.

APPENDIX—KEY LITERATURE

Bliss, CI. Analysis of the biological assays in *USP XV. Drug Standards*, 24, 33–67, 1956.

Böhrer A. One-sided and two-sided critical values for Dixon's outlier test for sample sizes up to *n* = 30. *Economic Quality Control* 23, 5–13, 2008.

Cochran, WG. The combination of estimates from different experiments. *Biometrics* 10, 101–129, 1954.

Fieller, EC. Some problems in interval estimation. *Journal of the Royal Statistical Society, B* 16, 175–185, 1954.

Iglewicz, B and Hoaglin, DC. *How to Detect and Handle Outliers*. Quality Press, Milwaukee, 1993.

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⟨115⟩ DEXPANTHENOL ASSAY

INTRODUCTION

The following liquid chromatographic procedure is provided for the determination of dexpanthenol as a component in dietary supplements or pharmaceutical dosage forms.

While conducting this procedure, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably with the use of low-actinic glassware. Use the appropriate USP Reference Standards.

ASSAY

• **CHROMATOGRAPHIC METHODS, PROCEDURE 1**

This procedure can be used to determine dexpanthenol in:

- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Oral Solution*

Unless specified in the monograph, the *Standard solution*, *System suitability solution*, and *Sample solution* are prepared as follows.

Mobile phase: 0.2 M monobasic sodium phosphate and methanol (97:3). Adjust with 1.7 M phosphoric acid to a pH of 3.2 ± 0.1.

Standard solution: 80 µg/mL of USP Dexpanthenol RS in *Mobile phase*

System suitability solution: 80 µg/mL of USP Calcium Pantothenate RS in *Mobile phase*. Mix the resulting solution and *Standard solution* (1:1).

Sample solution: Equivalent to 80 µg/mL of dexpanthenol from Oral Solution in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between dexpanthenol and calcium pantothenate, *System suitability solution*

Tailing factor: NMT 2.0 for the dexpanthenol peak, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexpanthenol ($C_9H_{19}NO_4$) in the portion of the sample taken:

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

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

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

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

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

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Calcium Pantothenate RS

USP Dexpanthenol RS

⟨121⟩ INSULIN ASSAYS

INTRODUCTION

The most prominent manifestation of insulin activity, an abrupt decrease in blood glucose, was the basis for biologic assay from the time of its first clinical use. The assay, although relatively cumbersome, has the great merit of accurately reflecting the effect on the diabetic patient. The advent of practical yet sophisticated physicochemical methods (e.g., liquid chromatography) to measure insulin potency quantitatively has resulted in a more accurate and precise compendial test for insulin and insulin products. However, the bioidentity of insulin and insulin products cannot be assessed by these methods. Thus, a bioidentity test in rabbits is included in this chapter, and its use is called for in the appropriate monographs.

The *Rabbit Blood Sugar Method—Quantitative* is used to determine the potency of Insulin Reference Standards, for the validation of the stability of new insulin preparations, and to determine the specific activities of insulin analogs.

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ASSAY

Change to read:

• **RABBIT BLOOD SUGAR METHOD—QUANTITATIVE**

Diluent: Prepare an aqueous solution containing 0.1%–0.25% (w/v) of either cresol or phenol, 1.4%–1.8% (w/v) of glycerin, and sufficient hydrochloric acid to produce a pH between 2.5 and 3.5, unless otherwise directed in the individual monograph.

Standard stock solution: Prepare a solution containing 40 USP Insulin Units/mL of USP Insulin RS of the appropriate species in *Diluent* and having a pH between 2.5 and 3.5, unless otherwise directed in the individual monograph. ▲▲ (RB 1-May-2019) Store in a cold place, protected from freezing, and use within 6 months.

Standard solutions: Dilute portions of the *Standard stock solution* with *Diluent* to make two solutions, one to contain 1.0 USP Insulin Unit/mL (*Standard solution 1*), and the other to contain 2.0 USP Insulin Units/mL (*Standard solution 2*).

Sample stock solution: Proceed as directed in the *Standard stock solution*, except to use a suitable quantity of the preparation under test in place of USP Insulin RS of the appropriate species. The *Sample stock solution* contains about 40 USP Insulin Units/mL.

Sample solutions: Dilute portions of the *Sample stock solution* with *Diluent* to make two dilutions of the preparation under test, one of which may be expected, on the basis of the assumed potency, to contain 1.0 USP Insulin Unit/mL (*Sample solution 1*), and the other to contain 2.0 USP Insulin Units/mL (*Sample solution 2*). In the case of neutral insulin injection, adjust to a pH of 2.5–3.5 before making the dilutions.

Doses of the solutions to be injected: Select, on the basis of trial or experience, the dose of the dilutions to be injected, the volume of which usually will be between 0.30 and 0.50 mL. For each animal, the volume of the *Standard solution* is the same as that of the *Sample solution*.

Preparation of animal: Select suitable, healthy rabbits, each weighing NLT 1.8 kg. Keep the rabbits in the laboratory for NLT 1 week before use in the assay, maintaining them on an adequate uniform diet, with water available at all times.

Analysis: Divide the rabbits into four equal groups of preferably NLT six rabbits each. On the preceding day, approximately 20 h before the assay, provide each rabbit with an amount of food that will be consumed within 6 h. Follow the same feeding schedule before each test day. During the assay, withhold all food until after the final blood specimen is taken. Handle the rabbits with care to avoid undue excitement, and inject subcutaneously the doses indicated in the following design (see *Table 1*), the second injection being made on the day after the first injection, or NMT 1 week later. The time between the first and second injections is the same for all rabbits.

Table 1

Group	First Injection	Second Injection
1	*Standard solution 2*	*Sample solution 1*
2	*Standard solution 1*	*Sample solution 2*
3	*Sample solution 2*	*Standard solution 1*
4	*Sample solution 1*	*Standard solution 2*

Blood samples: At 1 h ± 5 min and 2.5 h ± 5 min after the time of injection, obtain from each rabbit a suitable blood specimen from a marginal ear vein. Blood can also be collected effectively from the central auricular artery.

Dextrose determination: Determine the dextrose content of the blood specimens by a suitable procedure that is adapted to automated analysis. The following procedure may be used.

Anticoagulant solution: Dissolve 1 g of edetate sodium and 200 mg of sodium fluoride in 1 L of water, and mix.

Dextrose standard preparations: Transfer known concentrations of USP Dextrose RS to suitable vessels, and dilute quantitatively and stepwise with *Anticoagulant solution* (1:9) to obtain a range of *Dextrose standard preparations* containing between 20 and 100 mg per 100 mL, having known concentrations similar to the concentrations in the rabbit blood samples.

Sample preparations: Pipet into separate, suitable vessels 0.1 mL of each *Blood sample* and 0.9 mL of *Anticoagulant solution*.

Analysis: Subject the *Sample preparations* to dialysis across a semipermeable membrane for a sufficient time so that the dextrose passes through the membrane into a saline TS solution containing glucose oxidase, horseradish peroxidase, 3-methyl-2-benzothiazolinone hydrazone hydrochloride TS, and *N,N*-dimethylaniline. The absorbances of the *Sample preparations* are determined at 600 nm in a recording colorimeter. The absorbances of the *Dextrose standard preparations* are similarly determined at the start and the end of each run.

Calculation: Calculate the response of each rabbit to each injection from the sum of the two blood sugar values, and subtract its response, disregarding the chronological order in which the responses were observed, to obtain the individual differences, *y*, as shown in *Table 2*.

When the data for one or more rabbits are missing in an assay, do not use the confidence interval formulas given here, but seek statistical help. The data can still be analyzed with proper analysis of variance.

When the number of rabbits, *f*, carried through the assay is the same in each group, total the *y*'s in each group and compute:

$$T_a = -T_1 + T_2 + T_3 - T_4$$

and

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$$T_b = T_1 + T_2 + T_3 + T_4$$

The logarithm of the relative potency of the test dilutions is $M' = 0.301\,T_a/T_b$. The potency of the injection in USP Units/mg equals the antilog (log $R + M'$), where:

$$R = v_S/v_U$$

v_S = number of USP Units/mL of the *Standard solution*
v_U = number of mg/mL of insulin of the corresponding *Sample solution*

Determine the 95% confidence interval for the log-relative potency using Fieller's Theorem (see *Appendix* and *Design and Analysis of Biological Assays* ⟨111⟩). If the confidence interval is more than 0.082, which corresponds at $P = 0.95$ to confidence limits of about ±10% of the computed potency, repeat the assay until the combined data of the two or more assays, redetermined as described in *Combination of Independent Assays* in ⟨111⟩, meet this acceptable limit.

Table 2

Group	Differences	Individual Response (y)	Total Response (T)	Standard Deviations of Differences (S)
1	*Standard solution 2 – Sample solution 1*	y_1	T_1	S_1
2	*Sample solution 2 – Standard solution 1*	y_2	T_2	S_2
3	*Sample solution 2 – Standard solution 1*	y_3	T_3	S_3
4	*Standard solution 2 – Sample solution 1*	y_4	T_4	S_4

Appendix: Fieller's Theorem for Determining the Confidence Interval for a Ratio

This version of Fieller's Theorem is for the case where the numerator and denominator are uncorrelated. The equation assumes that the numerator and denominator are normally distributed and that the groups of rabbits are of equal sizes. Then, the 95% confidence interval for the ratio is:

$$(L,U) = \frac{M' \pm \frac{t}{T_b}\sqrt{(1-g)s_N^2 + (M')^2 s_D^2}}{1-g}$$

where f (degrees of freedom in the standard errors) $= 4(k-1)$, where k is the number of rabbits in a group, t is the upper 97.5 percentile of the t-distribution with f degrees of freedom, and

$$g = \frac{t^2 s_D^2}{T_b^2}$$

If $g \geq 1$, the denominator is not significantly different from 0 and the formula does not work.

$$S_N = 0.301\sqrt{k}\sqrt{S_1^2 + S_2^2 + S_3^2 + S_4^2}$$
$$S_D = \sqrt{k}\sqrt{S_1^2 + S_2^2 + S_3^2 + S_4^2}$$

• **BIOIDENTITY TEST**

Proceed as directed in *Rabbit Blood Sugar Method—Quantitative* with the following modifications.

Procedure: Divide the rabbits into four equal groups of two rabbits each.

Calculation: Proceed as directed for *Calculation* in *Rabbit Blood Sugar Method—Quantitative*, but do not determine the confidence interval of the log-relative potency, M'.

Interpretation: If the potency value obtained is NLT 15 USP Units/mg, the *Bioidentity Test* requirement is met. If the potency value is less than 15 USP Units/mg, repeat the test using eight more rabbits. If the average potency of the two sets of tests is NLT 15 USP Units/mg, the requirement of the test is met.

ADDITIONAL REQUIREMENTS

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dextrose RS
USP Insulin Aspart RS
▲▲ (RB 1-May-2019)
USP Insulin Glargine RS
USP Insulin Human RS
USP Insulin Lispro RS
USP Insulin Pork RS

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⟨121.1⟩ PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS

INTRODUCTION

Several physicochemical procedures are used for the assessment of the quality attributes of Insulin Human, various insulin analogues, and animal derived insulins, hereafter called insulins. Of these procedures, peptide mapping and determination of high molecular weight proteins are similar for the various insulin drug substances and drug products. This chapter describes the tests for peptide mapping and for quantitative analysis of high molecular weight proteins that can be used for the various insulins.

Specific instructions that deviate from the general procedures outlined here are given in the respective drug substance or drug product monographs for the different insulins.

PEPTIDE MAPPING

Insulin digestion is performed using *Staphylococcus aureus* strain V8 (*S. aureus* V8) protease (also referred to as Endoproteinase Glu-C), a serine endoproteinase that cleaves at the C-terminal side of glutamyl and aspartyl residues in phosphate buffer at pH 7.8. This protease is specific for Glu-C digestion in ammonium bicarbonate or other non-phosphate-containing buffers at pH 7.8. The presence of proline on the carboxy side of the peptide bond inhibits cleavage. The buffer system used should cleave all glutamyl bonds in insulin without cleavage of the aspartyl bonds. In general, insulin is digested into four peptides in the buffer system described in the *Sample solution* below.

The following show the amino acid differences of the insulin analogues compared to Insulin Human and the *in silico* differences in the fragments obtained upon digestion with *S. aureus* V8 protease.

Figure 1 shows the four fragments of Insulin Human following digestion with *S. aureus* V8 protease.

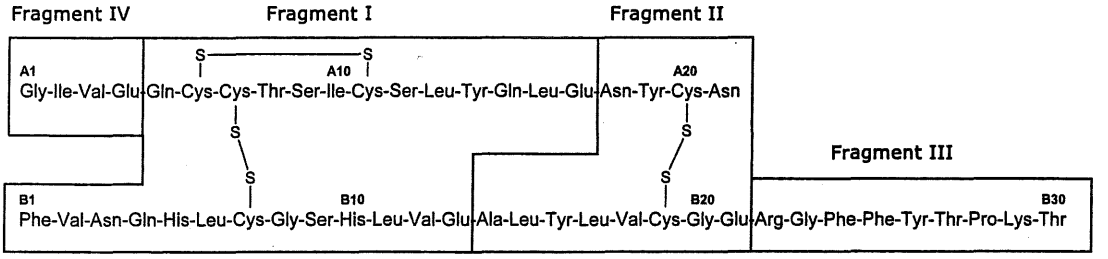


Figure 1. Insulin Human: *S. aureus* V8 protease digest fragments.

Table 1 shows the amino acid differences between insulin analogues, animal derived insulins, and Insulin Human.

Table 1. Amino Acid Differences between Insulin Analogues, Animal Derived Insulins, and Insulin Human

Insulin	Amino Acid Differences from Insulin Human
Insulin aspart	B28 Asp
Insulin glargine	A21 Gly, two Arg added to C-terminus of B chain
Insulin glulisine	B3 Lys, B29 Glu
Insulin lispro	B28 Lys, B29 Pro
Insulin porcine	B30 Ala
Insulin bovine	A8 Ala, A10 Val, B30 Ala

Table 2 shows specific *S. aureus* V8 protease digest fragments of insulin analogues and animal derived insulins. Amino acid differences compared to Insulin Human are highlighted.

Table 2. Amino Acid Differences: Insulin Human, Insulin Analogues, and Animal Derived Insulins (Differences with Insulin Human Are Shown in Boldface)

Insulin	Amino Acid Differences Compared to Insulin Human[a]	
Insulin aspart	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-**Asp**-Lys-Thr (B30)
Insulin glargine	Fragment II	(A18) Asn-Tyr-Cys-**Gly** (A21)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr-**Arg-Arg** (B32)
Insulin glulisine	Fragment I	(B1) Phe-Val-**Lys**-Gln-His-Leu-Cys-Gly-Ser-His-Leu (B11)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-**Glu**-Thr (B30)
Insulin lispro	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-**Lys-Pro**-Thr (B30)
Insulin porcine	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-**Ala** (B30)

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Table 2. Amino Acid Differences: Insulin Human, Insulin Analogues, and Animal Derived Insulins (Differences with Insulin Human Are Shown in Boldface) *(continued)*

Insulin	Amino Acid Differences Compared to Insulin Human[a]	
Insulin bovine	Fragment I	(A5) Gln-Cys-Cys-**Ala**-Ser-**Val**-Cys-Ser-Leu-Tyr-Gln-Leu-Glu (A17)
	Fragment III	(B22) Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-**Ala** (B30)

[a] A and B denote the A and B chains of insulin, respectively; numbers denote amino acid position in the chain.

- **PEPTIDE MAPPING PROCEDURE**

The following procedure is applicable for preparing peptide maps of Insulin Human, insulin analogues, and animal derived insulins.

HEPES buffer: Dissolve 2.38 g of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) in about 90 mL of water in a 100-mL volumetric flask. Adjust with 5 M sodium hydroxide to a pH of 7.5, dilute with water to volume, and mix.

Sulfate buffer: 2.0 M ammonium sulfate and 0.5 M sulfuric acid (1:1). Mix, and filter.

- **INSULIN DIGESTION**

The following procedure provides efficient cleavage of the glutamyl bonds of insulin. [NOTE—Volumes up to 20-fold higher can be used as long as the ratio of the solutions remains the same. If interfering autolysis byproducts are observed in the chromatogram when a digest of the enzyme alone is run, the enzyme-to-insulin ratio must be decreased and digestion time must be increased.]

Enzyme solution: Prepare a 1-mg/mL solution of *S. aureus* V8 protease in water (approximately 500 units/mg using casein as the substrate).

Sample solution: Prepare a 2.0-mg/mL solution of the insulin to be examined in 0.01 N hydrochloric acid. To a clean vial add 25 µL of the 2.0-mg/mL insulin solution, 100 µL of *HEPES buffer*, and 20 µL of *Enzyme solution* (final ratio is 25:100:20). Cap the vial, and incubate at 25° for 6 h. Stop the reaction by adding an equal volume of *Sulfate buffer*. Longer incubation times may be needed for analogues with poor solubility at pH 7.5.

Standard solution: Prepare at the same time and in the same manner a solution of the appropriate USP Insulin Reference Standard as directed in the *Sample solution*.

- **PEPTIDE FRAGMENT DETERMINATION**

Determine the peptide fragments using the following peptide mapping procedure (see *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩).

Solution A: Acetonitrile, water, and *Sulfate buffer* (100:700:200). Filter, and degas.

Solution B: Acetonitrile, water, and *Sulfate buffer* (400:400:200). Filter, and degas.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	95	5
30	41	59
35	20	80
40	95	5
50	95	5

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 50–100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Chromatogram comparability: In the chromatogram obtained from the *Standard solution*, identify the peaks due to digest fragments I, II, III, and IV. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with the appropriate USP Insulin Reference Standard.

Resolution: There should be complete separation of the peaks due to fragments II and III. The resolution is defined in the applicable insulin monograph.

Tailing factor: NMT 1.5 for digest fragments II and III

Analysis

Samples: *Sample solution* and *Standard solution*

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Condition the *Chromatographic system* by running at initial conditions, $t = 0$ min, for at least 15 min. Carry out a blank gradient program before injecting the digests. Separately inject equal volumes of the *Standard solution* and the *Sample solution*, and record the responses of each peak.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*.

LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS

• **PROCEDURE**

Solution A: 1 mg/mL of L-arginine in water

Mobile phase: *Solution A*, acetonitrile, and glacial acetic acid (65:20:15). Filter, and degas.

Resolution solution: Store a suitable amount of insulin drug substance at room temperature for a sufficient period of time (5–10 days, or as needed) to obtain insulin with more than 0.4% high molecular weight proteins. Prepare a 4-mg/mL solution in 0.01 N hydrochloric acid. Store the solution in a refrigerator, and use within 7 days. Alternatively, dissolve about 4 mg of USP High Molecular Weight Insulin Human RS in 1 mL of 0.01 N hydrochloric acid.

Sample solution: In a small vial, prepare a 4-mg/mL solution of insulin in 0.01 N hydrochloric acid, and mix to dissolve. Store in a refrigerator, and use within 7 days.

Chromatographic system

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

Mode: LC

Detector: UV 276 nm

Column: 7.8-mm × 30-cm; 5- to 10-µm packing L20

Temperatures

Autosampler: It is advisable to use a refrigerated autosampler.

Column: Ambient

Flow rate: 0.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Resolution solution*

Suitability requirements

Retention times: Between 13 and 17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and between 18 and 22 min for the insulin monomer, with salts eluting after the insulin monomer

Peak-to-valley ratio: The ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is NLT 2.0.

Analysis

Sample: *Sample solution*

Disregard any peaks with retention times greater than that of the insulin monomer.

Calculate the percentage of high molecular weight proteins in the portion of Insulin taken:

$$\text{Result} = \Sigma r_H/(\Sigma r_H + r_M) \times 100$$

Σr_H = sum of the responses of all peaks with retention times less than that of the insulin monomer

r_M = peak response of the insulin monomer

Acceptance criteria: As stated in the applicable insulin monograph

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP High Molecular Weight Insulin Human RS
(alternative, optional)

⟨123⟩ GLUCAGON BIOIDENTITY TESTS

INTRODUCTION

Change to read:

Glucagon is a ▲peptide▲ 1S (USP41) hormone that increases blood glucose levels via release of liver glycogen stores. ▲A robust and precise physicochemical chromatographic procedure is used in the glucagon assay to assign potency on a mass basis. Bioidentity is still required in *Glucagon for Injection*, and two procedure options are presented here: an in vivo procedure based on release of glucose from freshly prepared rat liver cells (hepatocytes) stimulated with glucagon ex vivo, or production of cyclic adenosine monophosphate (cAMP) in vitro in response to glucagon stimulation of the glucagon receptor cell line. To meet the acceptance criteria of the bioidentity test, only one of these bioidentity tests is required.▲ 1S (USP41)

PROCEDURE

Change to read:

• **▲A.▲ 1S (USP41) PRIMARY LIVER CELL ▲BIOIDENTITY TEST▲ 1S (USP41)**

[NOTE—All buffers are oxygenated, prepared with either *Sterile Water for Injection* or *Sterile Water for Irrigation*, warmed to 37°, and adjusted to a final pH of 7.4 unless otherwise indicated. At least two independent assays (replicates) must be performed utilizing two rat livers for each lot of glucagon. *Figure 1* demonstrates the process used to generate one

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replicate value. A minimum of two replicates are combined according to the *Calculations* section. The concentration range of the *Standard preparations* and *Assay preparations* may be modified to fall within the linear range of the *Assay*, and the calculations can be adjusted accordingly. Alternatively, full curve analysis using validated nonlinear statistical methods can be used, provided that similarity is demonstrated when analysts compare the responses of the *Standard preparations* and *Assay preparations*.]

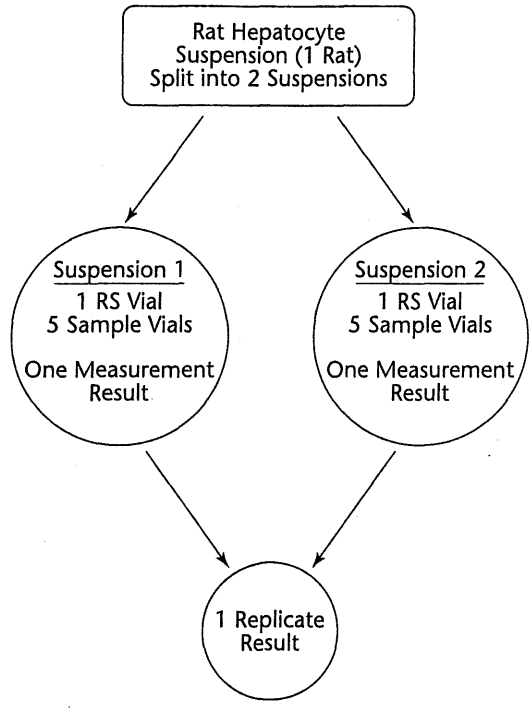


Figure 1. Rat hepatocyte assay method flow diagram (RS = Reference Standard).

Hepatocyte preparation

Calcium-free perfusion buffer with dextrose: Prepare a solution containing 7.92 g/L of sodium chloride, 0.35 g/L of potassium chloride, 1.80 g/L of dextrose, 0.19 g/L of edetic acid (EDTA), and 2.38 g/L of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Oxygenate before use.

Collagenase buffer: Prepare a solution containing 3.62 g/L of sodium chloride, 23.83 g/L of HEPES, 0.35 g/L of potassium chloride, 0.52 g/L of calcium chloride, and 1.8 g/L of dextrose. Adjust to a pH of 7.6. Immediately before perfusion, dissolve a quantity of collagenase in this solution to obtain a concentration of 0.02%–0.05%. The exact concentration of collagenase is determined empirically for each new lot of enzyme and is the amount that can consistently dissociate the tissue within 10 min of buffer entry and produce a viable cell concentration of NLT 3×10^6 cells/mL.

Wash buffer: Prepare a solution containing 7.92 g/L of sodium chloride, 0.35 g/L of potassium chloride, 0.19 g/L of EDTA, 2.38 g/L of HEPES, 0.11 g/L of calcium chloride, and 0.06 g/L of magnesium sulfate.

Incubation buffer: Prepare a solution containing 6.19 g/L of sodium chloride, 0.35 g/L of potassium chloride, 0.22 g/L of calcium chloride, 0.12 g/L of magnesium sulfate, 0.16 g/L of monobasic potassium phosphate, 11.915 g/L of HEPES, and 10 g/L of bovine serum albumin (1% BSA). Adjust to a pH of 7.5.

Test animals: Male Sprague-Dawley rats are maintained on a standard rat chow diet, given water ad libitum, and allowed to adjust to their new housing before testing. On the morning of the test, select a healthy rat weighing approximately 300–400 g, and administer 100 units of *Heparin Sodium* subcutaneously.

Procedure: [NOTE—Conduct this procedure in the morning to ensure that the rat has optimal glycogen in its liver and so that the procedure can be completed in 1 day.] Anesthetize the rat with an appropriate anesthetic. Open the abdominal cavity and isolate the portal vein. Insert an angiocatheter and tie into the portal vein at the general location of the lienal branch and then connect to a perfusion pump. Start the perfusion (25 mL/min) in situ with the previously warmed, oxygenated, *Calcium-free perfusion buffer with dextrose*. As the liver enlarges, cut the inferior vena cava to allow pressure equilibrium. [NOTE—About 300 mL of the perfusate is needed to clear the liver of red blood cells at a flow rate of 25–60 mL/min.] Then circulate *Collagenase buffer* at an appropriate flow rate so that the liver leaks perfusate out of the lobes in approximately 10 min (typically 25–60 mL/min). When the liver significantly increases in size, changes color and consistency, and starts to leak perfusate out of the lobes, change the system to the oxygenated prewarmed *Wash buffer*. About 100 mL of *Wash buffer* is needed to wash the liver of collagenase at a flow rate of 25 mL/min. Surgically remove the liver from the animal, and place in a prewarmed Petri dish containing a small amount of oxygenated *Wash buffer* (37°). Gently comb the liver with a stainless steel, fine-toothed comb to free the hepatocytes. Filter and wash the hepatocytes with *Wash buffer*, through prewetted cheesecloth (three layers thick, or through a 150-µm mesh polyethylene net) into a beaker. Transfer the cells to two centrifuge tubes and spin for about 1 min at

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600 rpm. Discard the supernatant fractions and resuspend the two pellets in *Incubation buffer*. Combine the two pellets in a suitable container and add sufficient *Incubation buffer* to make 150 mL.

System suitability of cell preparation: The cell yield may vary because of the collagenase activity and the viability of the hepatocytes. To check cell viability and to determine viable cell concentration, dilute a 100-µL aliquot of the cell suspension with 400 µL of *Wash buffer* and 500 µL of isotonic 0.4% trypan blue solution. Load aliquots of the cell suspension into both chambers of a hemocytometer and count all eight quadrants. To meet system suitability of the cell preparation method, a viable cell concentration of 3×10^6 cells/mL (acceptable range of 2.5×10^6 to 3.4×10^6 cells/mL) must be obtained to proceed with the bioassay. If the viable cell concentration exceeds the upper limit, additional *Incubation buffer* may be added to the cells to adjust the concentration to 3×10^6 cells/mL. In this case, the cells are counted again in a hemocytometer, as described above to verify the concentration. [NOTE—Viable cells are those cells that exclude the trypan blue.]

Glucose determination

Negative control solution: Prepare a solution containing 0.5% BSA using *Sterile Water for Injection* or *Sterile Water for Irrigation*.

Incubation flasks: Use specially prepared 25-mL conical flasks, the bottoms of which have been heated and pushed inward to form a conically raised center, or similar flasks that allow sufficient mixing when swirling. Place the *Incubation flasks* in an orbital shaker water bath at 35°.

Standard preparations: On the day of the assay, dissolve two vials of USP rGlucagon RS ▲▲1S (USP41) in 0.01 N hydrochloric acid or other suitable diluent (volume based on the potency of the Reference Standard lot) to obtain two solutions each containing 1 USP rGlucagon Unit/mL. All dilutions thereafter are made using *Negative control solution*. Accurately dilute measured volumes of each solution with *Negative control solution* to obtain an intermediate concentration of 400 µU/mL, and then dilute the intermediate to produce five concentrations: 200, 100, 50, 25, and 12.5 µU/mL (*Standard preparations*). Pipet 0.1 mL of each *Standard preparation* into separate *Incubation flasks*. Pipet 0.1 mL of *Negative control solution* into each of two flasks (*Negative control solutions 1* and *2*).

Assay preparations: Using accurately weighed quantities of glucagon samples, proceed as directed for *Standard preparations* or, if testing *Glucagon for Injection*, reconstitute 10 vials by slowly adding the contents of the accompanying prefilled syringes containing an appropriate glucagon diluent. Gently mix each vial until the glucagon is dissolved. Using the same syringes, withdraw the contents of five vials and place the solutions in a 25-mL volumetric flask. Repeat for the second five vials, transferring the contents to a second 25-mL volumetric flask. Dilute each flask with 0.01 N hydrochloric acid to volume. Dilute an accurate amount of each solution with 0.5% BSA to yield a concentration of 400 µU/mL, and dilute the intermediate to produce five *Assay preparation* concentrations: 200, 100, 50, 25, and 12.5 µU/mL. Then proceed as directed for the *Standard preparations*.

Reference stock solution: Dry USP Dextrose RS, and then transfer 1.0 g, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with saturated benzoic acid solution to volume.

Reference solutions: Transfer suitable quantities of *Reference stock solution* to four flasks, and dilute with saturated benzoic acid solution to obtain *Reference solutions* having concentrations of 100, 500, 1000, and 1500 mg/L.

Potassium ferrocyanide solution: Dissolve 1.25 g of trihydrate potassium ferrocyanide in 125 mL of *Sterile Water for Injection*, or use an appropriate commercial source.

System suitability: Analyze the *Potassium ferrocyanide solution*, the *Reference solutions*, and an additional 5 replicates of either the 500- or 1000-mg/L *Reference solution* in an appropriate glucose analyzer. [NOTE—*Potassium ferrocyanide solutions* are only appropriate standards for glucose analyzers that measure glucose oxidase activity. The procedure can also be performed using alternative platforms.] Prepare a standard curve using the *Reference solutions* as directed for the *Standard preparations*. The square root of the residual error mean square from the regression divided by the average of the response multiplied by 100% (line %RSD) must be NMT 2.0%. In addition, the response of the *Potassium ferrocyanide solution* must be NMT 30 mg/L, and the relative standard deviation must be NMT 2.0% for the replicate analyses of the middle *Reference solution*.

Procedure: Dispense 5 mL of *Hepatocyte preparation* into the *Incubation flasks* in sequence from high glucagon concentration to low glucagon concentration, alternating the *Standard preparations* with the *Assay preparations*. Swirl the flasks in an orbiting water bath at 125 rpm at 30°–35° for approximately 30 min. Following incubation, remove 1.0-mL aliquots from each *Incubation flask*, transfer to labeled microcentrifuge tubes, and centrifuge at 13,000 rpm for 15 s. Place each supernatant fraction in a labeled sampling tube for a glucose analyzer, and determine the glucose concentration (mg/L) of each *Standard preparation* and *Assay preparation*. Measure the background reading of *Negative control solutions 1* and *2*, and calculate the average of the two responses.

To conform to the linear range of the instrument being used, analysts may find it necessary to adjust by dilution each of the *Standard preparations* and *Assay preparations*. Use a glucose analyzer that has demonstrated appropriate specificity, accuracy, precision, and linear response over the range of concentrations being determined. Determine the increase in glucose concentration for each *Standard preparation* and *Assay preparation* compared to the average value of the *Negative control solution*.

Calculations

Calculate the relative potency of the glucagon samples using statistical methods for parallel-line assays, comparing the Reference Standard curve (from the *Standard preparations*) to the glucagon sample curve (from the *Assay preparations*). No dose–response reversals may occur within a run for the 25, 50, or 100 µU/mL *Standard preparations* and *Assay preparations*. [NOTE—Either the low- or high-dose level, but not both, may be excluded from the calculation in order to meet linearity requirements.] Because a minimum of two valid assays (rats) are required, the estimated potencies are combined using the procedures in *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*, and the width, *L*, of a 95% confidence interval for the estimated logarithm of the relative potency is calculated. If *L* is NMT 0.1938, the results are valid. If *L* is >0.1938, additional assays may be performed and combined until a valid *L* term results, and the relative potency is then calculated from all valid independent runs. Calculate the

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potency of the glucagon samples in USP rGlucagon Units/mg by multiplying the relative potency result by the potency of USP rGlucagon RS.

▲Acceptance criteria: NLT 0.80 USP rGlucagon Units/mg▲ 1S (USP41)

Add the following:

▲• B. IN VITRO CELL-BASED BIOIDENTITY TEST

Medium A: Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 mg/mL of D-glucose and sodium pyruvate, 4 mM L-alanine-L-glutamine[1] or L-glutamine, 10% (v/v) fetal bovine serum,[2] and 0.5 mg/mL G418 [3]

Buffer A: Hank's balanced salt solution[4] containing 5.3 mM potassium chloride, 0.4 mM potassium phosphate monobasic, 4.2 mM sodium bicarbonate, 137.9 mM sodium chloride, 0.3 mM sodium phosphate dibasic anhydrous, and 5.6 mM dextrose

Cell culture preparation: Remove glucagon receptor cell line[5] from cryostorage and immediately thaw at 37° until the cell suspension has just thawed. Aseptically transfer the cell suspension from the cryotube into a sterile test tube containing 10 mL of warmed *Medium A*. Mix and then pellet the cells by centrifugation for 5 min at 125 × g. Remove the supernate and resuspend the cells in fresh, warmed *Medium A*. Measure the quantity of cells contained in an aliquot of the suspension by suitable methods and adjust the cell concentration with *Medium A* such that the cell suspension is 0.5–5 × 10^3 cells/cm^2 of tissue culture flask surface. Inoculate tissue culture flasks[6] and store in a humidified incubator at 37° containing 5% carbon dioxide. Cells should be passaged 2–3 times/week when they are NMT 90% confluent but never trypsinized 2 days in a row. If cells are not ready for passaging after 3 days of culture, the medium should be replaced with fresh *Medium A*. Cells are passaged by first removing the medium from the cell flasks, followed by adding sufficient *Buffer A* prewarmed to room temperature to cover the surface of the flasks. The flasks are gently rocked to wash the cells. This wash fluid is discarded and then sufficient trypsin[7] is added to the cell flasks to cover the surface, followed by gentle rocking and placement back in the incubator. After 3–5 min, the trypsinized cells are aseptically collected from the flasks and transferred to a sterile centrifuge tube, then a volume of *Medium A* is added that is a minimum of 2 times the volume of trypsinized cells, and an aliquot is counted. The cell suspension is further diluted with fresh *Medium A* to a final cell concentration of 0.5–5 × 10^3 cells/cm^2 of tissue culture flask surface. After a minimum of 8 passages post-thaw but NMT 25 passages, the cells can be used in the assay. The day before an assay, follow the cell passaging instructions above but resuspend the cells in fresh, warmed *Medium A* to a final cell concentration of 4–5 × 10^4 cells/mL. [NOTE—Three identical, independent 96-well white plates[8] are needed for measurement of two test samples ("A" and "B") analyzed on each. All wells must be loaded with cells within 40 min of resuspension following trypsinization and counting in order to keep a homogeneous cell suspension.] Using constant, gentle mixing without foaming, each well is loaded with 0.1 mL of cell suspension and incubated overnight at 37° and 5% carbon dioxide (approximately 24 h ± 4 h) prior to starting the *Procedure*. [NOTE—All remaining solutions should be prepared on the day of the *Procedure*.]

Medium B: Krebs' salt solution containing 0.3% (v/v) human serum ▲albumin▲ (ERR 1-Aug-2018), 25 mM HEPES, 1.7 mM 3-isobutyl-1-methyl-xanthine, 0.2 mg/mL of glucose, 650 KIU/mL of aprotinin, and 0.0003% (v/v) polysorbate 80, pH 7.4

Medium C: Krebs' salt solution containing 0.3% (v/v) human serum ▲albumin▲ (ERR 1-Aug-2018) and 25 mM HEPES, pH 7.4

Standard stock solution: Reconstitute USP rGlucagon RS in *Water for Injection* or another suitable diluent to a concentration of 4 mg/mL by gently mixing on a rotator for 10 min or until completely clear. Dilute this material 1:1000 with *Medium B* to a concentration of 0.4 µg/mL and then 1:200 with *Medium B* to 20 ng/mL.

Standard solutions: Within an hour of use, dilute the 20 ng/mL *Standard stock solution* with *Medium B* to make a 1:5 concentration series of 4 ng/mL (R8), 0.8 ng/mL (R7), 160 pg/mL (R6), 32 pg/mL (R5), 6.4 pg/mL (R4), and 1.3 pg/mL (R3). A final dilution is made from R3 in *Medium B* to make 65 fg/mL (R2). [NOTE—Alternative suitable concentrations can be similarly prepared if necessary and validated.] R1 is a blank solution of *Medium B*. [NOTE—For these *Standard solutions*, as well as the *Sample solutions* and *cAMP standard solutions* described below, it may be helpful to prepare these dilutions in a dilution microplate that mimics the assay plate layout to easily and quickly transfer the materials to the wells of the assay plate.]

Sample solutions: Reconstitute two independent preparations of glucagon from the same lot of material in *Water for Injection* or the same suitable diluent used to prepare the *Standard stock solution* to a concentration of 4 mg/mL. Prepare by diluting this material further with *Medium B* to prepare a concentration series similar to that suggested for the *Standard solutions*. One preparation series is *Sample solution* A8–A2 and the other preparation series is *Sample solution* B8–B2.

cAMP standard solutions: Prepare a 5 mM cAMP[9] solution in water, and then prepare further solutions by dilution with *Medium B* as shown in *Table 1*. [NOTE—To conform to the linear range of the instrument being used, analysts may find it necessary to adjust by dilution each of the *Standard solutions*, *cAMP standard solutions*, and *Assay solutions*. Alternative suitable concentrations can be similarly prepared if necessary and validated.]

[1] Invitrogen catalog #31966-021 or suitable equivalent.
[2] Gibco catalog #10082-147 or suitable equivalent.
[3] Calbiochem catalog #345812 or suitable equivalent.
[4] Invitrogen catalog #14175 or suitable equivalent.
[5] Available from ATCC.
[6] Corning catalog #3151 or suitable equivalent.
[7] Gibco catalog #12563-011 or suitable equivalent.
[8] PerkinElmer catalog #6005680 or suitable equivalent.
[9] PerkinElmer catalog #6760635D or suitable equivalent.

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Table 1. Preparation of cAMP Standard Solutions

Starting cAMP Solution	Fold Dilution with Medium B	Final cAMP Solution	Final cAMP Standard Solution Name
5 mM	1:10	0.5 mM	—
0.5 mM	1:20	25 µM	C1
25 µM	1:3.3	7.5 µM	C2
25 µM	1:10	2.5 µM	C3
7.5 µM	1:10	0.75 µM	C4
2.5 µM	1:10	250 nM	C5
0.75 µM	1:10	75 nM	C6
250 nM	1:10	25 nM	C7
75 nM	1:10	7.5 nM	C8
Medium B only	—	No cAMP	C9

Donor biotin-cAMP beads: Dissolve 10 nmol biotinylated cAMP in 0.5 mL of phosphate-buffered saline, pH 7.4. Add 80 µL of this biotinylated cAMP solution to 33.8 mL of a 5 mM HEPES lysis buffer containing 0.1% (w/v) BSA and 0.3% (v/v) polysorbate 20, pH 7.4.[9] Add 270 µL of donor beads and 2.4 mL of 120 mM $MgCl_2$ solution.

Procedure: Remove the plates seeded with cells the day before, and discard the *Medium A* in each well. Wash the cells with 350 µL/well of *Medium C*, then discard the wash solution. Rapidly add 20 µL/well of diluted 1× anti-cAMP-acceptor beads. Add 30 µL/well of each *Standard solution*, *Sample solution*, *Medium B*, or *cAMP standard solution* as indicated in the plate layouts shown in *Tables 2* and *3*. [NOTE—The white plate for the *cAMP standard solutions* does not contain cells.] Cover the plate,[10] protecting it from light and evaporation, and incubate for 35–60 min in an incubator at 37° with gentle shaking. Next, dispense 60 µL of *Donor biotin-cAMP beads* per well, mix well, then cover[9] the plate again and wrap it with foil. [NOTE—The beads are light sensitive so plates should be kept dark or under green light conditions.] Gently rotate the plate on a shaker for at least 30 min at room temperature. Keep the plate in the dark, at room temperature, without shaking for 16–30 h before measuring the top luminescence from the plate wells on a suitable plate reader[11] and detection mode (excitation wavelength of about 680 nm; emission of 520–620 nm).

Table 2. Schematic Representation of the Standard and Sample Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
B	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
C	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
D	R1	R2	A2	B2	R3	A3	B3	R4	A4	B4	R8	—
E	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C9
F	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C6
G	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C4
H	R5	A5	B5	R6	A6	B6	R7	A7	B7	A8	B8	C1

LEGEND:
A2–A8 = Dilution series of *Sample solution* A (same solution is used on three plates, with 4 ng/mL as starting concentration).
R1–R8 = Dilution series of *Standard solution* for each test plate, with 4 ng/mL as starting concentration; as a result, each *Sample solution* is only compared to the *Standard solution* on that plate.
B2–B8 = Dilution series of *Sample solution* B (same solution is used on three plates, with 4 ng/mL as starting concentration).
— = No glucagon (contains 30 µL *Medium B* instead), but cells are present.
C = cAMP standard controls, no glucagon. C1 contains 25 µM cAMP, C4 contains 0.75 µM cAMP, C6 contains 75 nM cAMP, and C9 contains no cAMP.

Table 3. Schematic Representation of the cAMP Standards Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	—	—	—	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—	—	—	—
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—
D	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—
E	C1	C2	C3	C4	C5	C6	C7	C8	C9	—	—	—

[10] PerkinElmer catalog #6050195 or suitable equivalent.
[11] PerkinElmer catalog #2300-0000 or suitable equivalent.

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Table 3. Schematic Representation of the cAMP Standards Assay Plate *(continued)*

	1	2	3	4	5	6	7	8	9	10	11	12
F	C1	C2	C3	C4	C5	C6	C7	C8	C9	C1	C2	C3
G	C1	C2	C3	C4	C5	C6	C7	C8	C9	C1	C2	C3
H	C1	C2	C3	C4	C5	C6	C7	C8	C9	C1	C2	C3

C1–C9 = Dilution series of cAMP standard controls starting with C1, which contains 25 µM cAMP, through C9, which does not contain cAMP.

System suitability criteria: The lower asymptote of the 4-parameter logistic curves generated from *Sample solutions* A2–A8 and B2–B8 and *Standard solutions* R1–R8 must be above the lower limit (defined as C3) of the *cAMP standard solutions*. NLT 3-fold difference between the signals obtained in the wells of cells treated without glucagon (R1) versus those with the maximal amount of glucagon (R8). NMT 4 technical outliers may be omitted per standard curve. Any plate that fails one or more of these criteria is rejected and must be repeated. If either *Sample solutions* A or B fail one of these criteria, then only the passing series can be used for the calculations. [NOTE—The upper asymptotes of the 4-parameter logistic standard curves generated from *Sample solutions* A2–A8 and B2–B8 and *Standard solutions* R1–R8 should be approximately equal to or less than the C8 response of the *cAMP standard solutions* and within the linear range of the instrument used.]

Calculations: A series of two independent *Sample solution* preparations must be used for each test sample across three plates (assays). Outliers identified by Grubbs' test (see ⟨111⟩; but NMT 4/curve and NMT 1 data point from a replicate set) are omitted, and then the same number of *Standard solution* and *Sample solution* dose responses, including the 50% response (EC_{50}) of the standard/test sample within this range, are used to calculate the relative potency of the glucagon sample using statistical methods for parallel-curve analysis with a 4-parameter logistic fit using all replicate values. For each individual *Sample solution* compared to the *Standard solution*, the statistical tests for slope and parallelism must pass at the 95% level. Calculate the relative potency of the glucagon samples by comparing the Reference Standard curve (from the *Standard solutions*) to the glucagon sample curve (from the *Sample solutions*). Because a minimum of three valid assays are required, the estimated potencies are combined using the procedures in *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*, and the confidence interval is calculated using suitable statistical methods. If the confidence limits are between 64% and 156%, the results are valid. If not, up to two more assay plates may be performed and combined until a valid confidence interval results, and the relative potency is then calculated from all valid independent runs. Calculate the potency of the glucagon samples in USP rGlucagon Units/mg by multiplying the relative potency result by the potency of USP rGlucagon RS.

Acceptance criteria: NLT 0.80 USP rGlucagon Units/mg ▲1S (USP41)

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextrose RS
  USP rGlucagon RS

⟨124⟩ ERYTHROPOIETIN BIOASSAYS

INTRODUCTION

This chapter provides guidance about the use of bioassays to measure the biological activity of erythropoietin (EPO). Based on a relative potency approach described in general chapter *Design and Development of Biological Assays* ⟨1032⟩, the biological activity of an unknown EPO preparation typically is determined by comparison to the biological activity of a Reference Standard (RS). For this purpose, a USP Erythropoietin for Bioassays RS has been developed. The assays described in the chapter are not applicable to forms of EPO that have been engineered to prolong their half-life.

The USP Erythropoietin for Bioassays RS has been calibrated against the World Health Organization (WHO) International Standard for EPO using both the normocythemic mouse bioassay and the exhypoxic polycythemic mouse bioassay. Accordingly, the USP Erythropoietin for Bioassays RS has an assigned unitage in terms of International Units (IU) of EPO as defined by WHO. Of these two in vivo bioassays, the normocythemic mouse bioassay is less cumbersome and is more widely used for the determination of EPO potency. The assay is based on the measurement of EPO-stimulated reticulocyte maturation. Because the USP Erythropoietin for Bioassays RS has been calibrated against the WHO International Standard for EPO by in vivo bioassay, it can be used directly in the normocythemic mouse bioassay for the calibration of any process-specific EPO preparation.

However, if the intent is to use a validated in vitro bioassay to transfer the unitage from the USP Erythropoietin for Bioassays RS to a process-specific EPO preparation, then it is necessary to demonstrate that the USP Erythropoietin for Bioassays RS and the process-specific EPO preparation exhibit an equivalent ratio of in vitro to in vivo potency. This is because the biological activity of EPO shows a complex relationship between structure and function. In particular, the ratio of in vitro to in vivo activity is inversely correlated with type and degree of terminal sialylation. Highly sialylated products have a higher level of in vivo activity; thus, they have a relatively lower ratio of in vitro to in vivo activity. Because of differences in the manufacturing processes used in the preparation of EPO, the type and degree of terminal sialylation can be variable from one EPO preparation to another, including EPO preparations used as Reference Standards. As a result, the use of an in vitro assay to measure the biological activity of an EPO preparation requires a full understanding of the relationship between the EPO's in vivo and in vitro activity. This understanding is best achieved by comparing the test EPO preparation with the USP Erythropoietin for Bioassays RS in both the in vivo assay and in a specific in vitro assay.

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The USP Erythropoietin for Bioassays RS is assigned a unitage that represents its activity in both in vivo assays and in vitro assays. If the ratios of in vitro to in vivo potency for the material being tested and the USP Erythropoietin for Bioassays RS are equivalent, then the USP Erythropoietin for Bioassays RS can be used directly in the in vitro assay to calibrate the material being tested. However, if the ratios are not equivalent, then the material being tested and the USP Erythropoietin for Bioassays RS have a different ratio of in vitro to in vivo potency, and the standard cannot be used with its assigned potency in the in vitro assay. Instead, this ratio determined for the material being tested should be used to assign a process-specific in vitro assay unitage to the USP Erythropoietin for Bioassays RS. The USP Erythropoietin for Bioassays RS, with its adjusted in vitro assay unitage, then can be used in the in vitro assay to transfer the unitage from the USP Erythropoietin for Bioassays RS to the material being tested.

ASSAY

• **Normocythemic Mice Assay**

Solution A: Dissolve 10.75 g of disodium hydrogen phosphate dodecahydrate, 7.6 g of sodium chloride, and 10 g of bovine albumin in 1000 mL of water. Immediately before use adjust with sodium hydroxide solution or phosphoric acid to a pH of 7.2.

Standard solution 1: Dissolve USP Erythropoietin for Bioassays RS in *Solution A* to a concentration of 80 IU/mL.

Standard solution 2: Mix equal volumes of *Solution A* and *Standard solution 1*.

Standard solution 3: Mix equal volumes of *Solution A* and *Standard solution 2*.

Sample solution 1: Prepare the sample to be tested in *Solution A* to a concentration of 80 IU/mL.

Sample solution 2: Mix equal volumes of *Solution A* and *Sample solution 1*.

Sample solution 3: Mix equal volumes of *Solution A* and *Sample solution 2*.

The exact concentrations of each *Standard solution* and *Sample solution* may require adjustment based on the response range in the animals used.

Preparation of animals: At the beginning of the assay procedure, randomly distribute mice of a suitable age and strain (8-week-old B6D2F1 mice are suitable) into six cages. A minimum of eight mice per cage is recommended. Inject each animal subcutaneously with 0.5 mL of the appropriate treatment (one solution per cage), and put the animal in a new cage. Combine the mice in such a way that each cage housing the treated mice contains one mouse out of the six different treatments (each *Standard solution* and each *Sample solution*; six mice per cage).

Analysis: Four days after the injections, collect blood samples from the animals, and determine the number of reticulocytes by using a suitable procedure. The following method can be employed.

The volume of blood, dilution procedure, and fluorescent reagent may require modification to ensure maximum development and stability of fluorescence.

For concentrated colorant solution, use a solution of thiazole orange suitable for the determination of reticulocytes. Prepare at a concentration twice that necessary for the analysis.

Proceed with the following dilution steps. Dilute whole blood 500-fold in the buffer used to prepare the colorant solution. Dilute this solution two-fold in the concentrated colorant solution. After staining for 3–10 min, determine the reticulocyte count microfluorometrically in a flow cytometer. The percentage of reticulocytes is determined using a biparametric histogram: number of cells/red fluorescence (620 nm).

Calculate the potency by the usual statistical methods for a parallel-line assay. For additional information, see general chapter *Analysis of Biological Assays* ⟨1034⟩.

Acceptance criteria: The activity of each *Sample solution* is compared with that of the USP Erythropoietin for Bioassays RS and is expressed in IU. The confidence limits of the estimated potency ($P = 0.95$) are NLT 64% and NMT 156% of potency as determined by the method given above. The estimated potency is NLT 80% and NMT 125% of the stated potency.

ADDITIONAL REQUIREMENTS

• **USP Reference Standards** ⟨11⟩

USP Erythropoietin for Bioassays RS

⟨126⟩ SOMATROPIN BIOIDENTITY TESTS

Somatropin is a protein hormone that contains the same amino acid sequence as the human growth hormone produced by the pituitary gland. A robust and precise physicochemical chromatographic procedure is used in the assay to assign potency on a mass basis. Bioidentity is still required, and two procedure options are presented here: an in vivo bioassay procedure based on somatropin-induced weight gain in rats and a more precise rat cell line-based approach that measures production of ATP as a direct indicator of cell growth. [Note—The bioidentity test may be performed either on the somatropin bulk drug substance or on the drug product.]

PROCEDURE

• **In Vivo Bioidentity Test**

Buffer solution: 0.1 M ammonium bicarbonate. Adjust with sodium hydroxide to a pH of 8.0.

Standard solutions: 10–100 µg/mL of USP Somatropin RS in *Buffer solution*

Sample solutions: 10–100 µg/mL of somatropin in *Buffer solution*. [Note—Do not agitate while mixing; swirl gently.]

Control: *Buffer solution*

Test animals: Select an appropriate number of only female or only male Sprague Dawley rats hypophysectomized at 25–30 days of age. After hypophysectomization, feed the rats rat chow and 5% dextrose water for at least 72 h. After 72 h, feed the rats rat chow and filtered and deionized water adjusted with 1 N hydrochloric acid to a pH of 3.0 ± 0.25. Weigh the rats when they are 37–44 days old, and retain only healthy rats. Reweigh the remaining rats 7 days later, and use only those rats that are in good health and have not gained or lost >10% of their body weight in the previous 7-day period.

Analysis: Randomly divide the rats into standard, test, and control groups, each group containing approximately 10 rats. Each day for 10 days inject subcutaneously 0.1 mL of the *Standard solutions*, *Sample solutions*, and *Control*, to the standard,

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test, and control groups, respectively. Record the body weight of each animal at the start of the test and at approximately 18 h after the tenth injection.

Calculations: Determine the change in body weight for each rat during the 10-day period, and compute the potency of the *Sample solution* relative to that of the *Standard solution* using appropriate statistical analysis. Calculate the mean potency in USP Somatropin Units/mg and, using appropriate statistical methods, calculate the width, *L*, of a 95% confidence interval for the estimated base 10 logarithm of the relative potency. If *L* is >0.40, repeat the test until the results from two or more tests, combined by appropriate statistical methods, produce an *L* of NMT 0.40, corresponding to confidence limits of 63%–158% of the calculated potency.

Acceptance criteria: NLT 2 USP Somatropin Units/mg when *L* is NMT 0.40

• **IN VITRO CELL-BASED BIOIDENTITY TEST**

Medium A: Fischer's medium[1] containing 10% heat-inactivated fetal bovine serum,[2] 10% heat-inactivated horse serum,[3] 0.075% sodium bicarbonate, and 0.05 mM 2-mercaptoethanol. Filter sterilize. [NOTE—Store for up to 2 weeks at 2°–8°.]

Medium B: Fischer's medium containing 1% horse serum, 0.075% sodium bicarbonate, and 0.05 mM 2-mercaptoethanol. Filter sterilize. [NOTE—Store for up to 2 weeks at 2°–8°.]

Phosphate buffered saline:[4] Calcium- and magnesium-free phosphate buffered saline containing 1.5 mM monobasic potassium phosphate, 155 mM sodium chloride, and 3 mM dibasic sodium phosphate, pH 7.2–7.4

Cell culture preparation: Prepare cell suspension cultures of Nb2-11[5] cells in *Medium A* in a humidified incubator at 37° and containing 5% carbon dioxide. Cells should be passaged twice per week and reseeded at a density of 1×10^5 cells/mL for 2 days, 2×10^4 cells/mL for 3 days, and 1×10^4 cells/mL for 4 days in *Medium A*. [NOTE—Seeding densities may need adaptation when analysts qualify new lots of fetal bovine serum and horse serum.] On the day of an assay, cells are harvested from flasks and are pelleted by centrifugation for 7 min at about 218 × g. The supernatant is discarded, and the cells are washed twice with *Phosphate buffered saline* followed by centrifugation. The cells are resuspended in *Medium B* and are counted, and the cell concentration is adjusted to 1×10^5 cells/mL with *Medium B*. Except for the wells of the first column, transfer 50 µL of cell suspension into each well of a 96-well black tissue culture plate with a clear bottom.[6] Pipet 50 µL of *Medium B* into each well of the first column of the plate. [NOTE—Cover the plate, and incubate at 37° for up to 1 h while preparing the *Standard solutions* and *Test solutions*.]

Standard solutions: Reconstitute USP Somatropin RS in 1 mL of *Phosphate buffered saline*. From the reconstituted USP Somatropin RS, the *Standard solutions* and the *Positive control solution* are prepared for use in the *Procedure*. For the *Standard solutions*, the reconstituted USP Somatropin RS is further diluted with *Medium B* to a concentration of 2.0 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. For each plate approximately 1 mL of this solution is needed. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.]

Test solutions: For each test solution two results must be obtained by two independent preparations of somatropin. Reconstitute two independent preparations of somatropin in a suitable diluent (e.g., *Phosphate buffered saline* or *Water for Injection*) to a concentration of NMT 5 mg/mL. Prepare *Test solutions* by diluting this material further with *Medium B* to a concentration of 2.0 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. For each plate approximately 1 mL of this solution is needed. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.] One preparation is *Test solution A*, and the other preparation is *Test solution B*.

Positive control solution: Starting with the reconstituted stock preparation of USP Somatropin RS described in *Standard solutions*, dilute with *Medium B* to a concentration of 20 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.]

Procedure: Dispense 100 µL of *Medium B* into each well of a U-bottom, clear 96-well plate,[7] except those wells in column 12 (wells A12–H12 = positive control) and wells A2–A10. [NOTE—For each black plate seeded with cells as described in *Cell culture preparation,* one separate U-bottom plate is used for preparation of the *Standard solutions* and *Test solutions* dilutions.] Next, dispense 200 µL of 2.0-ng/mL *Standard solutions* into wells A3, A6, and A9 of the dilution plate. Dispense 200 µL of 2.0-ng/mL *Test solution A* into wells A2, A5, and A8 of the dilution plate. Dispense 200 µL of 2.0-ng/mL *Test solution B* into wells A4, A7, and A10 of the dilution plate. Dispense 100 µL of 20-ng/mL *Positive control solution* into the last plate column (wells A12–H12) of the dilution plate. Using a 12-channel pipet, perform serial two-fold dilutions on the plate by aspirating 100 µL from the first row (A2–A10), transfer to the second row, and mix three times. Then aspirate 100 µL from the second row, transfer to the third row, and mix three times, etc. Repeat this procedure across the whole plate up to row H. Discard the 100 µL aspirated from the last row. [NOTE—The wells of column 11 (wells A11–H11) are negative controls containing 100 µL of *Medium B*; the wells of column 1 (wells A1–H1) are blanks.]

Starting with the lowest concentration and using a 12-channel pipet, transfer 50 µL of each solution in the dilution plate to the respective well of the black plate containing the cells. [NOTE—During the transfer, immerse the pipet tips into the cell solution. This transfer yields an additional 1:2 dilution of somatropin, or a final concentration of 1.0 ng/mL for the highest dose added to the cells and 10 ng/mL for the *Positive control solution*. A plate layout is shown in *Table 1*.] Following all transfers, gently agitate the black plate for 30 s, and then incubate it in a humidified 37° incubator containing 5% carbon dioxide for 30 ± 2 h. Next, add 100 µL of reconstituted luminescent substrate solution[8] to all wells. Incubate the plates for 15 min at room temperature while gently agitating the plates on an appropriate plate shaker, protected from light. Incubate the plates for an additional 15 min at room temperature without shaking,

[1] Quality Biological, catalog #112-032-101, or equivalent.
[2] Gibco, catalog #10082-147, or equivalent.
[3] Gibco, catalog #26050-088, or equivalent.
[4] Amimed catalog, #8-05F29I; Gibco catalog, #20012-019, or equivalent.
[5] HPA Culture Collections or Sigma-Aldrich, catalog #97041101.
[6] Costar, catalog #3904, or equivalent.
[7] Greiner #650 160 plate or equivalent.
[8] CellTiter-Glo Luminescent Kit, e.g., Promega #G7573, or equivalent.

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protected from light. Read the luminescence from the plate wells in a suitable plate reader with a luminescence detection mode.

Table 1. Schematic Representation of the Final Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	A,#1	St,#1	B,#1	A,#1	St,#1	B,#1	A,#1	St,#1	B,#1	NEG	POS
B	BL	A,#2	St,#2	B,#2	A,#2	St,#2	B,#2	A,#2	St,#2	B,#2	NEG	POS
C	BL	A,#3	St,#3	B,#3	A,#3	St,#3	B,#3	A,#3	St,#3	B,#3	NEG	POS
D	BL	A,#4	St,#4	B,#4	A,#4	St,#4	B,#4	A,#4	St,#4	B,#4	NEG	POS
E	BL	A,#5	St,#5	B,#5	A,#5	St,#5	B,#5	A,#5	St,#5	B,#5	NEG	POS
F	BL	A,#6	St,#6	B,#6	A,#6	St,#6	B,#6	A,#6	St,#6	B,#6	NEG	POS
G	BL	A,#7	St,#7	B,#7	A,#7	St,#7	B,#7	A,#7	St,#7	B,#7	NEG	POS
H	BL	A,#8	St,#8	B,#8	A,#8	St,#8	B,#8	A,#8	St,#8	B,#8	NEG	POS

LEGEND:
BL = Blank: no somatropin, no cells.
A = Dilution series of *Test solution A* (with 1.0 ng/mL as starting concentration).
St = Dilution series of *Standard solutions* (with 1.0 ng/mL as starting concentration).
B = Dilution series of *Test solution B* (with 1.0 ng/mL as starting concentration).
NEG = Negative control: no somatropin, but cells are present.
POS = *Positive control solution:* maximum somatropin concentration (10 ng/mL).

Calculations: A minimum of two independent *Test solutions* preparations must be used for each test sample. The *Test solutions* and *Standard solutions* are normalized by protein content before calculation of the relative potency. [NOTE—1 mg of anhydrous somatropin is equivalent to 3.0 USP Somatropin Units.] Technical outliers (but NMT four per curve) are omitted, and then the same number of *Standard solutions* and *Test solutions* dose responses, including the 50% response (EC_{50}) of the standard per test sample within this range, are used to calculate the relative potency of each somatropin sample using statistical methods for parallel-line analysis. For each of the individual *Test solutions* compared to the *Standard solutions*, the statistical tests for linearity, slope, and parallelism must pass at the 95% level. The confidence limit for each calculated relative potency must be within 75%–133%. The relative potency of each of the *Test solutions* must be within the validated assay range (50%–200% relative potency) of the *Standard solutions*. The unweighted mean log relative potency is calculated from valid individual samples, and the potency is then calculated in USP Units/mg relative to the USP Somatropin RS.

System suitability criteria: The signal-to-noise ratio of the mean chemiluminescence signal of the *Positive control solution* to the mean chemiluminescence signal of the negative control wells must be NLT 3. NMT four technical outliers may be omitted per standard curve. Any plate that fails one or more of these criteria is rejected and must be repeated.

Acceptance criteria: NLT 2 USP Somatropin Units/mg

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Somatropin RS

⟨127⟩ FLOW CYTOMETRIC ENUMERATION OF CD34+ CELLS

INTRODUCTION

The CD34 antigen is expressed on the surface of almost all human hematopoietic stem and progenitor cells. The absolute number of CD34+ hematopoietic stem cells (HSCs) has been shown to correlate with in vitro colony-forming unit (CFU) assay activity and with clinical engraftment in hematopoietic grafts prepared from peripheral blood, bone marrow, and cord blood sources. The single-platform, flow cytometric CD34+ cell enumeration method described here is based on a clinical laboratory protocol,[1] established by the International Society of Hematotherapy and Graft Engineering (ISHAGE), now known as the International Society for Cellular Therapy (ISCT). This protocol can be used to enumerate CD34+ cells in samples of peripheral blood, leukapheresis products, bone marrow, and cord blood. CD34+ cell enumeration by flow cytometry is a rare event analysis, which requires specific gating instructions that are provided in this chapter. Furthermore, the USP CD34+ Cell Enumeration System Suitability RS has been developed to assess the reagents and ensure the correct gating during data acquisition and analysis.

IDENTIFICATION OF CD34+ HEMATOPOIETIC STEM CELLS

CD34+ cell enumeration by flow cytometry is a rare event analysis. For the analysis of CD34+ HSCs, cell samples are stained with fluorescently labeled antibodies against both the HSC antigen CD34 and the pan-leukocyte antigen CD45. Five parameters

[1] Sutherland DR, Keeney M, Gratama JW. Enumeration of CD34+ hematopoietic stem and progenitor cells. *Curr Protoc Cytom.* 2003;6:6.4.1–6.4.24.

General Chapters

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—forward light scatter (FSC), side light scatter (SSC), CD34 staining, CD45 staining, and viability dye staining are combined in a sequential, or Boolean, gating strategy to identify viable CD34+ cells.

CD34+ HSCs have FSC and SSC characteristics similar to lymphocytes; expressing both CD45 and CD34, and exhibiting dim CD45 expression and low SSC characteristics. Viability dye does not stain live cells, allowing the exclusion of dead cells from the analysis of viable cell preparations. For analysis of nonviable, fixed (preserved) cell preparations such as the USP CD34+ Cell Enumeration System Suitability Reference Standard (RS), either the flow cytometer viable cell analysis gate is fully opened to include all cells, or the *Viability dye* is omitted from the analysis.

ENUMERATION CONSIDERATIONS

The CD34+ cell enumeration method described here relies on the use of synthetic fluorescent microspheres (counting beads) as internal enumeration controls. Homogeneous counting beads are added to the cell sample at a known concentration and volume, or the counting beads may be procured, pre-aliquoted, and lyophilized in special sample tubes, in which all subsequent cell staining and processing steps are conducted. To avoid the loss of counting beads, the wash steps are omitted, and protein-containing sample buffers are used. An ammonium chloride-based, red blood cell (RBC)-lysing protocol is used for fresh cell preparations; no lysing is necessary for frozen, thawed, or fixed cell preparations.

After the cells are stained and processed, the counting beads and cells are simultaneously analyzed on a flow cytometer. The number of CD34+ cells/µL in the cell sample can be directly calculated by comparing the absolute number of target CD34+ cells and the number of counting beads detected in the same data file. The USP CD34+ Cell Enumeration System Suitability RS is used to verify that the correct reagents and flow cytometer gating parameters were used.

EQUIPMENT SPECIFICATIONS

The following equipment is needed:

- A pipettor capable of accurately reverse-pipetting microliter volumes
- A flow cytometer with the following minimum specifications (see *Flow Cytometry* ⟨1027⟩):
  - Detection capabilities for FSC; SSC; "green" fluorescence emission (range, 510–550 nm); "yellow" fluorescence emission (range, 560–590 nm); and "red" fluorescence emission (>600 nm)
  - Light-scatter and fluorescence measurements that are correlated to time
  - Light-scatter resolution allowing for the identification of lymphocytes, monocytes, granulocytes, and fluorescent counting beads
  - Detection and data acquisition rates of at least 5000 cells/s
  - A 488-nm laser excitation source
  - Fluorescence intensity sensitivity allowing for the detection of cellular autofluorescence
  - Analysis software, including a data file structure in the Flow Cytometry Standard (FCS) or equivalent, with spectral overlap correction capability

FLOW CYTOMETER INSTRUMENT SETUP AND CONSIDERATIONS

[NOTE—For general information, see ⟨1027⟩ and the flow cytometer manufacture's recommendations.]

Ensure that the sheath fluid receptacle is filled, the waste fluid receptacle is empty, and all caps are tightly closed. Verify that the laser power, system vacuum, and pressure are properly set.

Using fluorochrome-conjugated calibration microspheres, verify proper system alignment by measuring the average detection rate, mean fluorescence, mean FSC, mean SSC, and the respective calculated coefficient of variance (CV) values as measured by each detector. All values should fall within the ranges recommended by the manufacturer of the flow cytometer instrument. Adjust the detector threshold to ensure that all counting bead events are included.

Use either antibody-binding microspheres or preserved cells, such as the USP CD34+ Cell Enumeration System Suitability RS, to establish a matrix of detector values while adjusting for spectral overlap (compensation). Note that although either microspheres or cells can be stained with anti-CD34 phycoerythrin (PE) and anti-CD45 fluorescein isothiocyanate (FITC) antibodies, only fresh cells can be stained with *Viability dye*.

REAGENTS

Phosphate-buffered saline (PBS): 138 mM sodium chloride, 2.7 mM potassium chloride, 8 mM dibasic sodium phosphate, and 1.47 mM monobasic potassium phosphate. [NOTE—pH 7.0–7.4. If needed, adjust pH with hydrochloric acid.]

Dilution buffer: 1% (w/v) bovine serum albumin or human serum albumin in *PBS*

10X Stock ammonium chloride RBC lysis buffer: 1.5 M ammonium chloride, 0.01 M sodium bicarbonate, and 0.01 M ethylenediaminetetraacetic acid (EDTA)[2]

Diluted (1X) RBC lysis buffer: *10X Stock ammonium chloride RBC lysis buffer* diluted 1:10 with water

[2] A suitable fixative-free RBC lysis reagent can be obtained from BD Biosciences: BD Pharm Lyse™ Lysing Buffer Catalog No. 555899, BD Ammonium Chloride Lysing Solution (10X) Catalog No 344563, or equivalent.

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Anti-CD34 PE: PE-conjugated mouse IgG1 anti-human CD34 antibody (clones QBEnd10, 8G12, 581, Birma K3, or equivalent class II or class III PE-conjugated anti-CD34 antibodies), appropriately titrated

Anti-CD45 FITC: FITC-conjugated mouse IgG1 anti-human CD45 antibody (clones J33, T29/33, or HLE-1 or equivalent FITC-conjugated anti-CD45 antibodies), appropriately titrated

Viability dye: 1 µg/mL of 7-aminoactinomycin D (7-AAD) or equivalent, freshly diluted from a stock solution of 100 µg/mL[3]

Instrument calibration beads: Fluorochrome-conjugated instrument calibration microspheres[4]

Suspended counting beads: Fluorochrome-conjugated counting beads, formulated in liquid suspension[5]

Lyophilized counting bead tube: Fluorochrome-conjugated counting beads, formulated and lyophilized in a counting tube[6]

System suitability standard USP CD34+ Cell Enumeration System Suitability RS: Reconstitute the entire vial of USP CD34+ Cell Enumeration System Suitability RS with 0.5 mL of distilled water.

Cell samples: Fresh, fixed, or cryopreserved and thawed cell suspensions containing CD34+ cells, with a minimum of 100,000 nucleated cells/sample. Cell samples may include peripheral blood, leukapheresis product, cord blood, or bone marrow.

SAMPLE PREPARATION

Use reverse-pipetting techniques for all sample dilutions and transfers. Dilute *Cell samples* with *Dilution buffer* to obtain a total nucleated cell concentration of 10–30 × 10^6 cells/mL. Add *Anti-CD45 FITC* and *Anti-CD34 PE* to a 12-mm × 75-mm polystyrene tube (or a *Lyophilized counting bead tube*), followed by *Viability dye* to a final concentration of 1 µg/mL. Add 100 µL of well-mixed *Cell samples* to the bottom of the tube, and mix. Incubate for 20 min protected from light. Add 2 mL of *Diluted (1X) RBC lysis buffer* to fresh samples or add 2 mL of *Dilution buffer* to a fixed or frozen and thawed sample; vortex to mix. For fresh *Cell samples*, incubate for 10 min protected from light; for fixed or frozen then thawed *Cell samples*, no incubation time is needed. Place samples on ice. Samples should be analyzed within 1 h of lysis.

Immediately before acquiring data on the flow cytometer, pipet 100 µL of well-mixed *Suspended counting beads* (if not using *Lyophilized counting bead tubes*) to the prepared *Cell samples* described above. Cap the tube, and gently mix by inversion. Do not add counting beads to *Cell samples* intended to be used for adjustment of the instrumentation compensation matrix.

Immediately proceed to flow acquisition to collect a minimum of 75,000 CD45+ events and a minimum of 100 CD34+ cells. Acquire and analyze data by creating regions and logical gates (manually, or by using software appropriate for the flow cytometer), as described in *Table 1*.

System Suitability Requirements

Use the USP CD34+ Cell Enumeration System Suitability RS to verify that the gating strategy described below allows the detection and quantitation of CD34+ HSCs. Note that for fixed *Cell samples*, the viability gate (*R1* in *Table 1*) must be opened to include all events; alternatively, the *Viability dye* can be omitted. Calculate the number of CD34+ HSCs/µL in USP CD34+ Cell Enumeration System Suitability RS.

Acceptance Criteria

The results should fall within the range provided in the USP CD34+ Cell Enumeration System Suitability RS certificate.

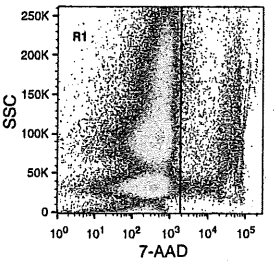
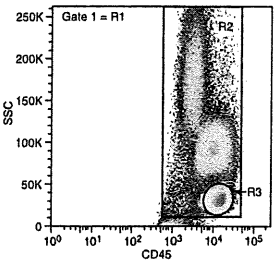
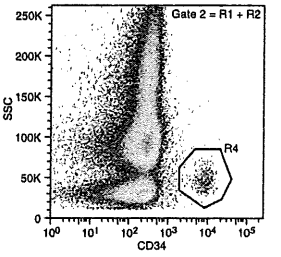
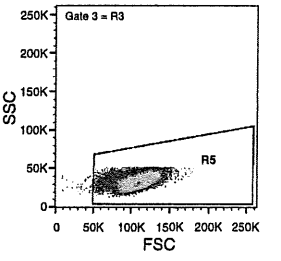
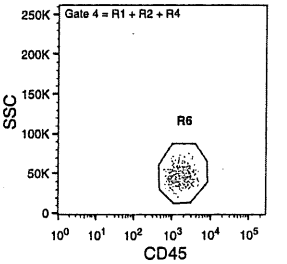
DATA ACQUISITION AND ANALYSIS

For flow acquisition, collect a minimum of 75,000 CD45+ events and a minimum of 100 CD34+ cells. Acquire and analyze data by creating regions and logical gates (manually, or using software appropriate for the flow cytometer) as described in *Table 1*. The best results are obtained when cell and bead data (events) are displayed as bivariate dot plots. *Table 1* contains representative dot plots and gating strategies for fresh cell samples. Dot plot displays may vary depending on the cell sample, flow cytometer, and software used. Troubleshooting guidelines can be found in *Table 2*.

[3] Suitable reagents can be obtained from BD Biosciences as part of a kit, Catalog No. 344563, or BD Biosciences Catalog No. 555899, or equivalent.
[4] A suitable reagent can be obtained from BD Biosciences: Catalog No. 641319, or equivalent.
[5] Suitable reagents can be obtained from Dako: Catalog No. K2370 (in a kit) or Dako Catalog No. S2366, or equivalent.
[6] A suitable reagent can be obtained from BD Biosciences: Catalog No. 340334.

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Table 1. Dot Plot Descriptions and Gating Instructions

Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
1	SSC (linear) vs. *Viability dye* fluorescence (log)	Define viable cells	• Display all unselected events. Create region *R1* around events with little-to-no fluorescence. • Included in the region *R1*: viable cells, debris. • Excluded from the region *R1*: dead and dying cells, counting beads.	
2	SSC (linear) vs. CD45 fluorescence (log)	Define viable CD45+ leukocytes and lymphocytes	• Display *R1*-selected events. Create region *R2* around events with CD45+ fluorescence, excluding debris (i.e., events with very low SSC). Within *R2*, create region *R3* around events with high CD45+ fluorescence and low SSC. • Included in the region *R2*: viable CD45+ leukocytes. • Included in the region *R3*: viable lymphocytes. • Excluded from the region *R2*: cell debris, platelets, and unlysed RBCs. • Excluded from the region *R3*: granulocytes, monocytes, and other non-lymphocytes.	
3	SSC (linear) vs. CD34 fluorescence (log)	Define viable CD34+ cells	• Display *R2*-selected events. Create region *R4* around events with CD34+ fluorescence and low SSC. • Included in the region *R4*: viable CD34+ cells. • Excluded from the region *R4*: CD34- cells.	
4	SSC (linear) vs. FSC (linear)	Define lymphocytes and blasts	• Display *R3*-selected events. Create region *R5* to exclude debris (low SSC and low FSC). • Included in the region *R5*: viable lymphocytes and blasts. • Excluded from the region *R5*: small debris and events with high SSC values.	
5	SSC (linear) vs. CD45 fluorescence (log)	Define CD45 dim CD34+ cells	• Display *R4*-selected events. Create region *R6* around events with low-to-intermediate SSC and intermediate CD45 expression. • Included in the region *R6*: viable CD34+ HSCs. • Excluded from the region *R6*: cells that express high levels of CD45.	

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Table 1. Dot Plot Descriptions and Gating Instructions *(continued)*

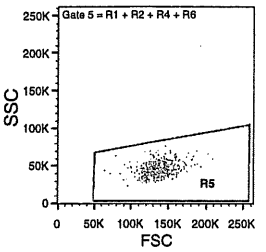
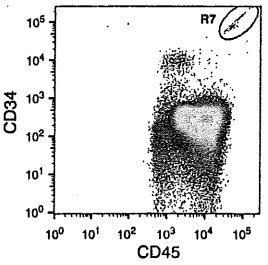
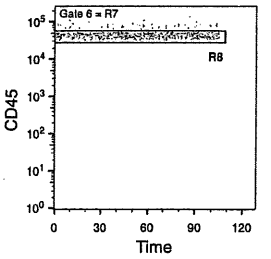
Step	Format	Purpose	Gating Instructions	Representative Dot Plots and Gating Strategies
6	SSC (linear) vs. FSC (linear)	Confirm CD34+ HSCs	• Display *R6*-selected events, and paste a copy of the *R5* gate parameters into the plot. Events in *R5* have light-scatter characteristics similar to lymphocytes and blasts, confirming the identity of the events as CD34+ HSCs. • Included in the region *R5*: confirmed viable CD34+ HSCs. • Excluded from the region *R5*: debris and other irrelevant events. • For counting bead enumeration, proceed to either Step 7 or Step 8 according to the recommendations of the bead manufacturer.	
7	CD34 fluorescence (log) vs. CD45 fluorescence (log)	Quantify total counting beads	• Display all ungated events. Create region *R7* around events with both high CD34+ and high CD45+ fluorescence. • Included in the region *R7*: total counting beads. • Excluded from the region *R7*: cells and debris.	
8	CD45 fluorescence (log) vs. time (linear)	Quantify "singlet" (e.g., unaggregated) counting beads	• When recommended by the counting bead manufacturer, display *R7*-selected events. Create *R8* around events with lower fluorescence intensity. • Included in the region *R8*: singlet counting beads. • Excluded from the region *R8*: counting bead aggregates.	

Table 2. Troubleshooting Guidelines (see also ⟨1027⟩)

Problems	Possible Reasons	Solutions	Comments
Unable to clearly define the bead gate (*R7*) in *Table 1*, graph 7 due to fluorescent debris	Degraded fresh cell sample; fixed sample; too many platelets; incomplete RBC lysis; nucleated RBCs (e.g., in cord blood)	Use a far-red fluorescence channel (>600 nm), instead of FITC, to gate counting beads.	Counting beads are highly fluorescent in all channels. Autofluorescence interference decreases dramatically at longer wavelengths (>600 nm).
Unable to find CD34+ population in *Table 1*, graph 3	Incorrect compensation settings for PE and *Viability dye*	Repeat the spectral compensation setup procedure, and adjust settings accordingly.	Make sure that counting beads are not in the compensation matrix sample, because they can interfere with compensation settings.
Unsure of gating for singlet or total beads	High levels of counting bead aggregates	Follow the counting bead manufacturer's recommendation for including or excluding aggregates.	Counting bead concentration calculations will vary by manufacturer.
Want to use a different CD34 antibody clone	Multi-parametric flow cytometry considerations	Alternative antibody clones must be carefully validated against the specified CD34 antibodies. Choose a class II or class III antibody that detects all CD34 isoforms and glycoforms.	Class I antibodies do not detect all CD34 glycoforms.
Want to use a different CD45 antibody clone	Multi-parametric flow cytometry considerations	Alternative antibody clones must be carefully validated against the specified CD45 antibodies. Choose an antibody that detects all isoforms and all glycoforms of CD45.	—

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CALCULATION OF ABSOLUTE CD34 NUMBER

The absolute number of viable CD34+ cells/µL is calculated as follows:

$$\text{Viable CD34+ cells/µL} = (\text{number of CD34+ cells})/(\text{number of counting beads}) \times (\text{bead concentration} \times DF)$$

DF = sample dilution factor

The number of CD34+ cells is determined from the viable CD34+ cells (region *R5* from *Table 1*, Step 6). Depending on the recommendations of the counting bead manufacturer, the number of counting beads is determined from either the total counting beads (region *R7* from *Table 1*, graph 7) or the singlet counting beads (region *R8* from *Table 1*, graph 8). The bead concentration is specified by the counting bead manufacturer.

ADDITIONAL REQUIREMENTS

USP Reference Standards ⟨11⟩

USP CD34+ Cell Enumeration System Suitability RS

⟨129⟩ ANALYTICAL PROCEDURES FOR RECOMBINANT THERAPEUTIC MONOCLONAL ANTIBODIES

INTRODUCTION

This chapter provides analytical procedures for murine, chimeric, and humanized IgG isotype monoclonal antibodies and subtypes (e.g., IgG1 and IgG2). Subclasses show differences in amino acid sequence and in the number of disulfide bonds, and in some cases they require subclass-specific analysis. This chapter applies to monoclonal antibodies for therapeutic and prophylactic use and for use as in vivo diagnostics. It does not apply to monoclonal antibodies used as reagents in the manufacture of medicinal products, for which applicable requirements are determined by the appropriate regulatory agency.

Naturally occurring polyclonal antibodies in serum or plasma can bind to many different targets as part of the immune system. In contrast, therapeutic monoclonal antibodies for human use are preparations of an immunoglobulin (or a fragment of an immunoglobulin) that have specificity for a target and are derived from a single clone of cells. Therapeutic monoclonal antibodies can bind to and neutralize soluble antigens, and their mechanism of action (MOA) often involves blocking of the ligand from binding to its cognate receptor. Alternatively, therapeutic monoclonal antibodies can recognize and bind to cell-bound antigens and in these cases may also engage the immune system through Fc (crystallizable fragment) mediated effector functions as part of their MOA. Monoclonal antibodies that have a single defined binding specificity are monospecific, whereas recombinantly engineered bispecific monoclonal antibodies can bind to two different targets (e.g., antigens). IgG-type monoclonal antibodies have a molecular weight of approximately 150 kD. Each molecule consists of two heavy and two light polypeptide chains that have a molecular weight of approximately 50 and 25 kD, respectively. Antibodies can be schematically represented as a Y joined by disulfide bridges, where each arm of the Y is called the Fab domain, and the stem of the Y is called the Fc domain. Monoclonal antibodies are glycoproteins that have a glycosylation site in the Fc portion located on each of the heavy chains and have possible additional glycosylation sites in the Fab domain, depending on the molecule.

The specificity of a monoclonal antibody is based on its antigen binding site, which is located in the Fab part of the molecule. The Fc domain contains receptor binding sites that are associated with antibody effector functions that have subclass-specific applications such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Pharmacokinetic effects via binding to the neonatal Fc receptor also are present in this domain.

Monoclonal antibodies are produced in cell culture using a seed lot system with master and working cell banks. After harvest, purification steps ensure that product- and process-related impurities are reduced to an acceptable level.

Currently licensed monoclonal antibody therapeutics include those involved in the activation of effector cells, cell killing, cross-linked induced apoptosis, antagonism against several targets, and agonist antibodies.

This chapter includes purity assessments by size-exclusion chromatography (SE-HPLC), capillary electrophoresis, and analysis of oligosaccharides, and it provides validated procedures for each of these.

Although this chapter is focused on IgG-type monoclonal antibodies, the principles of the tests included can apply to other related molecules, such as IgM or other isotype antibodies, antibody fragments, and Fc-fusion proteins. When the active substance is a conjugated antibody, such tests can be performed on the purified antibody before modification or conjugation.

Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in *Validation of Compendial Procedures* ⟨1225⟩ and must be shown to give equivalent or better results.

• **SIZE-EXCLUSION CHROMATOGRAPHY**

Although SE-HPLC is robust for measuring monomer and high-molecular-weight species (HMWS, aggregates), the quantitation of low-molecular-weight species (LMWS, fragments) can be highly variable depending on the mAb studied. The measurement of LMWS is better quantitated by capillary electrophoresis sodium dodecyl sulfate (CE-SDS).

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Mobile phase: Prepare by mixing 10.5 g of dibasic potassium phosphate, 19.1 g of monobasic potassium phosphate, and 18.6 g of potassium chloride per L of water. Verify that the pH is 6.2 ± 0.1. Pass through a membrane filter of ≤ 0.45-µm or smaller pore size.

System suitability solution: Prepare a 10-mg/mL USP Monoclonal IgG System Suitability RS solution in *Mobile phase* by reconstituting the contents of one vial with 200 µL of *Mobile phase*. Reconstituted *System suitability solution* should be used within 24 h after reconstitution, and should be stored at 2°–8° if not used immediately.

System suitability blank: Use *Mobile phase*.

Sample solution: Dilute the sample to 10 mg/mL in *Mobile phase* if dilution is required. Similarly, a blank should be prepared using an equivalent dilution of formulation buffer in *Mobile phase*.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 7.8-mm × 30-cm; 5-µm packing L59

Temperatures

Column: Ambient

Autosampler: Maintain at 2°–8°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Run time: 30 min

System suitability

Sample: *System suitability solution*

[NOTE—Approximate retention times for polymer, dimer, monomer, and LMWS are 12, 15, 18, and 22 min, respectively.]

Suitability requirements

Chromatogram: The chromatogram of the *System suitability solution* is consistent with the typical chromatogram as illustrated in the USP Certificate for USP Monoclonal IgG System Suitability RS.

Chromatogram similarity: The chromatographic profiles of *System suitability solution* injections that bracket injections of *Sample solutions* in a sample set are consistent with each other and with the typical chromatographic profile as illustrated in the USP Certificate for USP Monoclonal IgG System Suitability RS. All named peaks in the typical chromatogram must be present, be well-resolved, and in the same elution order as that in the *System suitability solution* chromatograms.

Quantitative criteria: Bracketing *System suitability solution* injections must meet the following quantitative criteria:
The percent peak area of the HMWS in the *System suitability solution* must be 0.4%–0.67%.
The percent peak area of the main peak in the *System suitability solution* must be 99.1%–99.6%.
The percent peak area of the LMWS in the *System suitability solution* must be NMT 0.2%.

Analysis

Samples: *Blank*, *System suitability solution*, and *Sample solution*

Injections of the *Sample solution* should be bracketed minimally with single injections of the *System suitability solution*. An injection of a *Blank* should be included as the final injection before the final *System suitability solution* injection.

Calculations: Using a suitable electronic integrator or computer system, analyze the bracketing *System suitability solution* injections. Exclude from integration any peaks that are present in the *Blank*. Protein-related peaks that elute before the main peak are classified as HMWS; those that elute after the main peak are classified as LMWS. Report the percentage of peak areas for the HMWS, main peak, and LMWS.

• **CAPILLARY SDS ELECTROPHORESIS (REDUCED AND NONREDUCED)**

Capillary sodium dodecyl sulfate (CE-SDS) electrophoresis is a sensitive, analytical method for quantitative estimation of product-related impurities such as nonglycosylated molecules, half antibodies, and fragments, and thus is also useful as a stability-indicating assay. After denaturation with SDS, the method allows the analysis of the complete antibody under nonreducing conditions, and the analysis of light and heavy chains (LC and HC, respectively) under reducing conditions.

[NOTE—Slight variations in sample preparation may be necessary depending on the stability of the individual antibody. If 15 min incubation at 70° leads to fragmentation or cleavage of disulfide bonds for a particular antibody sample, adjust the incubation time accordingly.]

SDS sample buffer: Prepare a solution containing 100 mM tris-hydrochloride (tris-HCl), a pH of 8.95 ± 0.05, and 1% (w/w) SDS.

SDS gel buffer: Buffer at a pH of 8.0 containing 0.2% (w/w) SDS and an appropriate hydrophilic polymer acting as a molecular sieve[1]

Alkylation solution: 250 mM iodoacetamide in water prepared immediately before use

System suitability solution: Reconstitute 1 vial of USP Monoclonal IgG System Suitability RS with 0.5 mL of *Water for Injection* (WFI) to a final concentration of 4 mg/mL.

Internal standard solution: 5 mg/mL of 10 kDa internal standard[2] in water containing 0.5% (w/w) SDS and 0.2% (w/v) sodium azide

Nonreduced system suitability solution: Mix 25 µL of the *System suitability solution* with 4 µL of *Internal standard solution*. Mix 66 µL of *SDS sample buffer* with 5 µL of the *Alkylation solution*. Add the *SDS sample buffer* and *Alkylation solution* mixture to the *System suitability solution* and *Internal standard solution* mixture. Mix thoroughly, and heat the mixture at 70° for 15 min. Allow to cool for 3 min at room temperature, centrifuge at 300 × g for 1 min, and transfer 100 µL into autosampler vials.

[1] Available from Beckman Coulter as a component of test kit, catalog number A10663, or a suitable equivalent.
[2] Available from Beckman Coulter as a component of test kit, catalog number A10663, or a suitable equivalent.

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Reduced system suitability solution: Mix 25 µL of the *System suitability solution* with 66 µL of *SDS sample buffer*. Add 4 µL of *Internal standard solution* and 5 µL of neat β-mercaptoethanol. Mix thoroughly, and heat the mixture at 70° for 15 min. Allow to cool for 3 min at room temperature, centrifuge at 300 × g for 1 min, and transfer 100 µL into autosampler vials.

Blank solution: Mix 45 µL of water with 50 µL of *SDS sample buffer*. Add 4 µL of *Internal standard solution* and 5 µL of neat β-mercaptoethanol for the reduced conditions or 5 µL of *Alkylation solution* for the nonreducing conditions. Mix thoroughly, and heat the mixture at 70° for 15 min. Allow to cool for 3 min at room temperature, centrifuge at 300 × g for 1 min, and transfer 100 µL into autosampler vials.

Nonreduced sample solution: Dilute 100 µg of the sample to be tested with 50–95 µL of *SDS sample buffer* to give a final volume of 95 µL. Add 4 µL of *Internal standard solution* and 5 µL of the *Alkylation solution*. Mix thoroughly, and heat the mixture at 70° for 15 min. Allow to cool for 3 min at room temperature, centrifuge at 300 × g for 1 min, and transfer 100 µL into autosampler vials.

Reduced sample solution: Dilute 100 µg of the sample to be tested with 50–95 µL of *SDS sample buffer* to give a final volume of 95 µL. Add 4 µL of *Internal standard solution* and 5 µL of neat β-mercaptoethanol. Mix thoroughly, and heat the mixture at 70° for 15 min. Allow to cool for 3 min at room temperature, centrifuge at 300 × g for 1 min, and transfer 100 µL into autosampler vials.

Instrumental conditions

Mode: CE-SDS

Detector: UV or diode array 220 nm

Capillary: Fused-silica capillary (i.d. 50 µm) cut to a total length of 30 cm with an effective length of 20 cm.[3] The following preconditioning and prefilling steps are required between each run.

Preconditioning of the capillary: Rinse for 3 min at 70 psi with 0.1 N sodium hydroxide followed by 0.1 N hydrochloric acid for 1 min at 70 psi, and water for 1 min at 70 psi.

Prefilling of capillary: Rinse for 10 min at 70 psi with *SDS gel buffer*.

Sample injection: Electrokinetic injection, 5.0 kV reversed polarity for 20 s

Voltage separation

Nonreduced conditions: Run time 40 min at 15 kV, reversed polarity

Reduced conditions: Run time 40 min at 15 kV, reversed polarity

Temperatures

Sample storage: Approximately 25°

Capillary: Approximately 25°

System suitability

Suitability requirements for reducing conditions

Sample: *Reduced system suitability solution*

Electropherogram: The electropherogram of the *Reduced system suitability solution* is consistent with the typical electropherogram illustrated in the USP Certificate for USP Monoclonal IgG System Suitability RS.

Resolution: The main peak of the heavy chain and the peak of the nonglycosylated heavy chain can be clearly identified. The resolution between the nonglycosylated heavy chain and the intact heavy chain is NLT 1.2.

Ratio of nonglycosylated to total heavy chain: Use time-corrected peak areas (see *Capillary Electrophoresis* ⟨1053⟩, *Quantification*, which may be a helpful, but not mandatory resource) for calculation. Calculate the ratio of nonglycosylated to total heavy chain by dividing the relative amount of nonglycosylated heavy chain by the sum of all heavy chains, and multiply by 100. The ratio of nonglycosylated to total heavy chain in the *System suitability solution* should be within the limits of 0.75%–1.34%.

Suitability requirements for nonreducing conditions

Sample: *Nonreduced system suitability solution*

Electropherogram: The electropherogram of the *Nonreduced system suitability solution* is consistent with the typical electropherogram illustrated in the USP Certificate for USP Monoclonal IgG System Suitability RS.

Resolution: The IgG main peak can be clearly identified. The resolution between the IgG main peak and Fragment 1 (F1) is NLT 1.3.

Amount of main peak: Calculate the relative amount of the main peak by dividing the sum of the time-corrected peak area of the main IgG peaks by the sum of all time-corrected peak areas excluding internal standard peaks and all system peaks, and multiply by 100. The relative amount of the main IgG peak of the *System suitability solution* should be within the limits of 61.4%–86.4%.

Relative standard deviation: NMT 2% for the migration of the internal standard in the bracketing *Blank solution*. No other peaks migrating after the internal standard are detectable.

Analysis

Samples: *Blank solution*, *System suitability solution*, and *Sample solution*

[NOTE—Based on instrument requirements, the field applied across the capillary and the conditions for the sample injection may be adjusted to achieve *System suitability*.] Apply a field strength of 500 V/cm (reversed polarity) for 40 min, using the *SDS gel buffer* as the electrolyte in both buffer reservoirs. Electrophorese the samples, using a 20-s reversed polarity and electrokinetic injection at 5.0 kV, into the anodic end of the capillary, and record the electropherograms at 220 nm. Rinse for 3 min at 70 psi with 0.1 N sodium hydroxide followed by 0.1 N hydrochloric acid for 1 min at 70 psi, and water for 1 min at 70 psi. Inject at least in duplicate.

Calculate the time-corrected peak areas of all peaks migrating after the internal standard peak.

Reduced conditions: Calculate the relative amount of product peak by dividing the sum of the time-corrected peak areas of heavy chain, nonglycosylated heavy chain, and light chain by the sum of all time-corrected peak areas for peaks appearing after the internal standard, and multiply by 100.

[3] Beckman Coulter catalog number 338472 or equivalent.

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Nonreduced conditions: Calculate the relative amount of product peak by dividing the sum of the time-corrected peak area of the main IgG peak by the sum of all time-corrected peak areas for peaks appearing after the internal standard, and multiply by 100.

• **OLIGOSACCHARIDE ANALYSIS—ANALYSIS OF *N*-LINKED OLIGOSACCHARIDES OF MONOCLONAL ANTIBODIES**

The inclusion of oligosaccharide analysis in a specification for a monoclonal antibody drug substance should be assessed using science- and risk-based approaches and should be defined on a product-specific basis. If applicable, one or more of the following analytical approaches can be employed for oligosaccharide profiling or quantitation of individual structures. Other validated methods that are suitable for determination of *N*-linked glycosylation utilizing different modes of separation, labeling, or detection can be used. [NOTE—For monoclonal antibodies with both Fc and Fab *N*-linked oligosaccharides, additional sample preparation procedures (e.g., treatment with the enzyme papain) can be employed to separate the domains before analyses so that information at individual glycosylation sites can be obtained. All additional sample preparation steps for the method require validation.]

[NOTE—Numerical acceptance criteria may be defined for individual glycan structures. If applicable, acceptance criteria must be derived from product-specific information taking into consideration a suitable range of historical batch data.]

Method A: Capillary electrophoresis with laser-induced fluorescence detection

Run buffer: Prepare 100 mM triethanolamine (TEA) and 10% glycerol at pH 7.5 by dissolving 1.492 g of TEA and 10 g of glycerol in 80 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.5, and dilute with water to a final volume of 100 mL.

Sample buffer: Dilute 1 mL of *Run buffer* with 9 mL of water.

Enzyme reaction buffer: Prepare 50 mM sodium phosphate, pH 7.5.

APTS labeling reagent: Dissolve 5 mg of 8-aminopyrene-1,3,6-trisulfonate (APTS) in 48 µL of 15% acetic acid, and mix.

Sample solution: Add 2 µL of peptide *N*-glycosidase F (PNGase F) (5 units/mL) to 50 µg of sample, and adjust with *Enzyme reaction buffer* to a volume of 50 µL. [NOTE—The unit definition for PNGase F may differ among commercial sources. It may be necessary to experimentally determine the appropriate quantity of PNGase F required for the complete release of *N*-linked oligosaccharides in the sample.] Incubate at 37° for 18 h. Separate released oligosaccharides by centrifugation using a 10,000 molecular-weight-cut-off (MWCO) centrifugal filter. Dry the released oligosaccharides in a centrifugal vacuum evaporator to dryness. Add 2 µL of *APTS labeling reagent* and 2 µL of 1 M sodium cyanoborohydride to the dried oligosaccharides. Incubate at 55° for 90 min. Add 46 µL of water to quench the reaction and mixture. Add 5 µL of APTS-labeled sample to 95 µL of *Sample buffer*.

System suitability solution: Resuspend each of the suitable glycan standards (10 µg) in 50 µL of water, and mix on a vortex mixer. Add 5 µL of a suitable glycan standard (1 µg) to a microcentrifuge tube. Dry the glycan standard using centrifugal vacuum evaporation at ambient temperature. Add 2 µL of sodium cyanoborohydride to each standard. Add 2 µL of the *APTS labeling reagent* to each standard. Mix the standards on a vortex mixer, and centrifuge briefly to settle the liquid before incubation at 55° for 90 min. Quench the reaction by the addition of 46 µL of water to each standard. Standards can be stored at –20° up to 5 weeks.

Instrumental conditions

Mode: CE

Detector: Laser-induced fluorescence detector (488-nm excitation wavelength; 520-nm emission wavelength)

Capillary: 50-µm inner diameter bare fused-silica capillary with 50-cm total length and 40-cm separation length

Sample injection: 10-s hydrodynamic injection

Separation voltage: 22kV for 50 min

Samples: *Sample solution* and *System suitability solution*

Analysis: Integrate peaks in the resultant electropherogram, and report relative percentage time-corrected peak areas of major glycan structures relevant to the product. Comparison to suitable product-specific reference standard can be performed.

Method B: Liquid chromatography with fluorescence detection

Enzyme reaction buffer: Prepare 100 mM sodium phosphate, a pH of 7.5.

Labeling buffer: Prepare 8% (w/v) sodium acetate trihydrate and 4% (w/v) boric acid solutions in methanol by dissolving 800 mg of sodium acetate trihydrate and 400 mg of boric acid in 10 mL of methanol.

Labeling reagent solution: Prepare a 100-mg/mL stock solution of anthranilic acid (2-AA) in *Labeling buffer*. Mix with an equal volume of 1 M sodium cyanoborohydride in methanol for a final *Labeling reagent solution* containing 50 mg/mL of 2-AA and 0.5 M sodium cyanoborohydride. [NOTE—Keep the labeling reagent protected from light.]

Solution A: Prepare 50 mM ammonium formate, pH 4.4.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	32.5	67.5
48.0	42.5	57.5
49.0	100	0
53.0	100	0
54.0	32.5	67.5
60.0	32.5	67.5

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Sample preparation: Transfer 80 µg of test sample to a microcentrifuge tube. Add 3 µL of PNGase F (2.5 units/mL). [NOTE—The unit definition for PNGase F may differ among commercial sources. It may be necessary to experimentally determine the appropriate quantity of PNGase F required for complete release of *N*-linked oligosaccharides in the sample.] Add 14 µL of *Enzyme reaction buffer.* Add water to 70 µL of total volume, and incubate at 37° for 18 h. Add 70 µL of freshly prepared *Labeling reagent solution*, and incubate at 70° for 2 h in a fume hood. Allow to cool, and add 60 µL of 50% methanol to each sample. Mix, then centrifuge. Remove 140 µL of the supernatant, and transfer to a microcentrifuge tube. Add 500 µL of acetonitrile to the supernatant-containing tube. Load the sample onto a hydrophilic-interaction solid-phase extraction cartridge to remove excess labeling reagent. Wash the cartridge with 95% acetonitrile (approximately 10 mL). Elute the labeled glycans from the cartridge with 0.5 mL of 20% acetonitrile. Dilute the eluate 1:1 with acetonitrile.

Chromatographic system

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

Mode: LC

Column: 4.6-mm × 15-cm; 3-µm packing L68

Detector: Fluorescence (360-nm excitation wavelength; 420-nm emission wavelength)

Column temperature: Maintain column at 30°.

Flow rate: 0.8 mL/min

Injection volume: 50 µL

Analysis

Sample: *Sample preparation*

Integrate peaks in the resulting chromatogram, and report relative percentage peak areas of major glycan structures relevant to the product. Comparison to a suitable product-specific reference standard can be performed.

• **OLIGOSACCHARIDE ANALYSIS—SIALIC ACID ANALYSIS**

[NOTE—The range of the standard curve or the mass of test sample may require modification depending on the sialic acid content of the monoclonal antibody.]

Solution A: Prepare 100 mM sodium hydroxide by diluting 10.4 mL of a 50% (w/w) sodium hydroxide solution in 2 L of water. [NOTE—Use high-quality water of high resistivity (18 MΩ-cm or better) that contains as little dissolved oxygen as possible.]

Solution B: Prepare a solution containing 100 mM sodium hydroxide and 1 M sodium acetate by adding 82.0 g of sodium acetate to 800 mL of water. Add 5.2 mL of 50% (w/w) sodium hydroxide, and dilute with water to a final volume of 1000 mL.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	93	7
10.0	70	30
11.0	70	30
12.0	93	7
15.0	93	7

Standard stock solution: Prepare 0.2 mM solutions of USP *N*-Acetylneuraminic Acid RS (NeuAc) and USP *N*-Glycolylneuraminic Acid RS (NeuGc) in 20 mM sodium acetate buffer, pH 5.2. Dilute an appropriate volume of this solution with 20 mM sodium acetate buffer, pH 5.2, to obtain a 0.02 mM *Standard stock solution.*

Internal standard stock solution: Prepare a 0.1 mM solution of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) in 20 mM sodium acetate buffer, adjusted to a pH of 5.2.

Standard solutions: Prepare as indicated in *Table 3*.

Table 3

Standard	Concentration (µM)	Volume of Standard Stock Solution (µL)	Volume of 20 mM Sodium Acetate Buffer, pH 5.2 (µL)	Volume of Internal Standard Stock Solution (µL)
1	10	250	235	15
2	5	125	360	15
3	4	100	385	15
4	2	50	435	15
5	1	25	460	15
6	0.4	10	475	15

System suitability solution: Prepare 3 µM solutions of NeuAc, NeuGc, and KDN in 20 mM sodium acetate buffer, adjusted to a pH of 5.2. Store the *System suitability solution* at −70°.

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Sample solution: Pipet a volume of test sample equivalent to 0.5 mg into a microcentrifuge tube. Dilute with 20 mM sodium acetate buffer, pH 5.2, to a total volume of 475 µL. Confirm the pH with a test strip, and add 10 µL of 10 milliunit/µL of neuraminidase. Incubate for 5 h at 37°. Add 15 µL of *Internal standard stock solution*. Mix on a vortex mixer, and transfer the sample to an autosampler vial. [NOTE—A slight adjustment in sample preparation may be necessary depending on the test sample and the quality of the enzyme. Adjust the incubation time accordingly.]

Chromatographic system

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

Mode: LC

Detector: Integrated amperometric detector with gold electrode

Column: 4-mm × 25-cm; 10-µm packing L46

Flow rate: 1 mL/min

Injection volume: 50 µL

Use the following waveform for the electrochemical detector (see *Table 4*).

Table 4

Time (s)	Potential (V)	Integration
0.00	+0.10	—
0.20	+0.10	Begin
0.40	+0.10	End
0.41	−2.00	—
0.42	−2.00	—
0.43	+0.60	—
0.44	−0.10	—
0.50	−0.10	—

Analysis

Samples: *Standard solutions*, *System suitability solution*, and *Sample solution*

Integrate the NeuAc, NeuGc, and *Internal standard stock solution* peaks in each chromatogram. For the *Standard solutions*, evaluate the NeuAc and NeuGc peak areas relative to the peak area of the *Internal standard stock solution* as follows.

Area of NeuAc peak/area of *Internal standard stock solution* peak × 100

Area of NeuGc peak/area of *Internal standard stock solution* peak × 100

Generate NeuAc and NeuGc standard curves. Quantify NeuAc and NeuGc in the *Sample solution* by comparison of the relative percent of NeuAc of the *Internal standard stock solution* and the relative percent of NeuGc of the *Internal standard stock solution*, using the standard curves.

Divide the determined NeuAc and NeuGc concentrations by the test sample concentration, and report as molar ratio (i.e., numbers of NeuAc and NeuGc per molecule of test sample).

⟨130⟩ PROTEIN A QUALITY ATTRIBUTES

INTRODUCTION

Protein A is coupled to a resin support in order to create protein A affinity chromatography media commonly used in the manufacturing of recombinant therapeutic monoclonal antibodies. Natural protein A is derived from *Staphylococcus aureus* and contains five homologous antibody binding regions and a C-terminal region for cell wall attachment. In addition to naturally derived protein A, recombinant material manufactured in *Escherichia coli*, as well as several engineered versions of the protein, also manufactured recombinantly, have entered the market place. When immobilized on a column, protein A provides a highly efficient and robust purification method for purifying antibodies at various scales. However, protein A ligand from the column can co-elute with the antibody during purification, an effect which is often referred to as protein A leaching. This tendency increases as the chromatography medium ages. Engineered versions of protein A may improve the pH tolerance of the medium, but do not eliminate leaching. It is the current regulatory expectation that leached protein A should be cleared during the purification of antibodies for human use, and manufacturing processes should be validated accordingly. Enzyme-Linked Immunosorbent Assay (ELISA)-based residuals testing is generally employed during process development and validation to assure the efficient removal of residual protein A during process steps following protein A affinity chromatography. In addition, the manufacturer should have a clear understanding and documentation of resin and ligand quality through raw materials qualification and column lifetime studies.

General Chapter ⟨130⟩ describes quality attributes of protein A ligands that are used in chromatography media for the manufacture of therapeutic monoclonal antibodies: Protein A; rProtein A; rProtein A, C-Cys; rProtein A, B4, C-Cys.

• **PROTEIN A**

$C_{1995}H_{3163}N_{597}O_{697}S_3$

46,760

N-terminal Sequence AQHDEA

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C-terminal Sequence IAADNK

Protein A is derived from *Staphylococcus aureus*. The structure is composed of a single polypeptide chain containing four IgG binding domains. With the exception of IgG_3, all other human IgGs bind to protein A. Each molecule of Protein A is capable of binding two IgG molecules. It is manufactured as a bulk solution at a concentration of greater than 20 mg protein A per mL with an IgG-binding potency of greater than 95%. Because Protein A is used as an ancillary material in the manufacture of recombinant therapeutic drugs, regulatory requirements differ from those for therapeutic drug products.

- **PACKAGING AND STORAGE:** Store in closed containers at the temperature indicated on the label.
- **LABELING:** Preserve in sealed containers, and store at a temperature of –20° or below.
- **USP REFERENCE STANDARDS** ⟨11⟩

  USP Endotoxin RS

  USP Protein A RS
- **IDENTIFICATION**

  A. SDS-PAGE: It meets the requirements of *Identification* test *A* under *rProtein A* using USP Protein A RS.

  B. IgG Binding: It meets the requirements of *Identification* test *B* under *rProtein A* using USP Protein A RS.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu per mL, and the total yeasts and molds count does not exceed 10 cfu per mL.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains not more than 1 USP Endotoxin Unit per mg of total protein. [NOTE—The *Bacterial Endotoxins Test* for Protein A is used to describe the quality of this ancillary material. This test does not define the acceptable level of bacterial endotoxin in the preparation of injectable dosage forms in which Protein A is used.]
- **TOTAL PROTEIN**

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

  Prepare triplicate samples for analysis by diluting Protein A to 3.0 mg per mL in *Water for Injection*. Measure the absorbance of each sample at 275 nm after correcting for the absorbance using *Water for Injection* as the blank. Determine the protein concentration using the equation:

$$\text{Protein concentration (mg per mL)} = (A275/0.149)$$

  in which A is the absorbance of Protein A at the wavelength of 275 nm and 0.149 is the molar absorptivity. Average the triplicate results, and determine a coefficient of variance (CV): the CV is ≤5%.
- **LIMIT OF COMMON CONTAMINANT PROTEIN AND CORRESPONDING ASSAY**

  Enterotoxin B: Enterotoxin B is determined using a commercially available microstrip enzyme-immunoassay kit.[1] Wells of the microstrips are coated with sheep antibodies to enterotoxin B. Standard curves are made using the ELISA kit control. The negative controls are wells coated with serum from nonimmunized sheep. The level of enterotoxin is determined from the standard curve. The specification for the enterotoxin B level is ≤1 ng per mg of total protein.
- **CHROMATOGRAPHIC PURITY**

[NOTE—The size-exclusion chromatographic purity test resolves Protein A from high molecular weight contaminants.]

Mobile phase: Prepare a solution of 50 mM sodium dihydrogen phosphate, pH 6.5 in the following manner. Add 6.9 ± 0.1 g of sodium dihydrogen phosphate into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 5 M sodium hydroxide to a pH of 6.50 ± 0.05. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.22-µm membrane filter.

Column regeneration solution: Prepare a solution of 0.1 M sodium dihydrogen phosphate, pH 3.0 in the following manner. Add 13.8 ± 0.1 g of sodium dihydrogen phosphate into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with hydrochloric acid to a pH of 3.0 ± 0.1. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.22-µm membrane filter.

Column storage solution: Mix 100 mL of methanol with 900 mL of water.

Test solution: Dilute Protein A to approximately 1 mg per mL with *Mobile phase*.

Calibration standards: Using *Mobile phase,* prepare separate 1 mg per mL solutions of each of the following: thyroglobulin (670 kD), IgG (150 kD), beta lactoglobulin (36 kD), and lysozyme (14 kD).

Standard solution: Prepare a solution containing 1 mg per mL of USP Protein A RS in *Mobile phase.*

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The liquid chromatograph is equipped with a 280-nm detector and a 7.8-mm × 30-cm column that contains packing L33. Equilibrate the column for approximately 30 minutes at 0.5 mL of *Mobile phase* per minute or until a stable baseline is achieved.

Procedure: Separately inject 100 µL of each sample, and run the samples in the following sequence: *Calibration standards,* thyroglobulin, IgG, beta lactoglobulin, and lysozyme; the *Standard solution;* and the *Test solution.* Run the sequence three times isocratically using *Mobile phase* at 0.5 mL per minute for 30 minutes. Absorbance is detected at 280 nm. Analyze the 280-nm peak data, and pick the retention time (RT) with the largest peak area. Using the data from the *Calibration standards,* plot the mean RT versus the log molecular weight to produce the standard curve. The purity should be ≥95% in the main peak. Use the formula from the standard curve to give the log molecular weights of the *Test solutions.* Convert the log molecular weights of the *Test solutions* and the *Standard solutions* to actual molecular weights. The apparent molecular weight of protein A from the *Standard solution* is between 156 and 205 kDa; and the Protein A from the *Test solution* is within the same range.

Column cleaning and storage: Rinse the column with 100 mL of *Column regeneration solution,* and store by flushing with 100 mL of *Column storage solution.*

rPROTEIN A, C-CYS

$C_{1478}H_{2320}N_{432}O_{503}S_4$

[1] A suitable enzyme immunoassay kit is available from TECRA International Pty Ltd., Australia (No. SETVIA96).

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34317.5 Da
N-terminal Sequence AQHDEAQQNA
rProtein A, C-Cys is a recombinant Protein A lacking the C-terminal membrane binding part; instead, a C-terminal cysteine has been introduced for directed immobilization purposes. It has five homologous IgG binding domains identical to the native Protein A and is produced using *Escherichia coli* as the host cell followed by purification with conventional chromatography. rProtein A, C-Cys is manufactured as a bulk solution with an IgG-binding potency of greater than 95%. Because rProtein A, C-Cys is used as an ancillary material in the manufacture of recombinant therapeutic drugs, regulatory requirements differ from those for therapeutic drug products.

- **PACKAGING AND STORAGE:** Store in closed containers at the temperature indicated on the label.
- **LABELING:** Preserve in sealed containers, and store at a temperature of –20° or below.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP rProtein A, C-Cys RS
- **IDENTIFICATION**
  A. SDS-PAGE: It meets the requirements of *Identification* test *A* under *rProtein A* using USP rProtein A, C-Cys RS.
  B. IgG Binding: It meets the requirements of *Identification* test *B* under *rProtein A* using USP rProtein A, C-Cys RS.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu per mL, and the total yeasts and molds count does not exceed 10 cfu per mL.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains not more than 1 USP Endotoxin Unit per mg of total protein. [NOTE—The *Bacterial Endotoxins Test* for rProtein A, C-Cys is used to describe the quality of this ancillary material. This test does not define the acceptable level of bacterial endotoxin in the preparation of injectable dosage forms in which rProtein A, C-Cys is used.]
- **TOTAL PROTEIN**
  (See ⟨857⟩.)
  Prepare triplicate samples for analysis by diluting the rProtein A, C-Cys to 3.0 mg per mL in *Water for Injection*. Measure the absorbance of each sample at 275 nm after correcting for the absorbance using *Water for Injection* as the blank. Determine the protein concentration using the equation:

$$\text{Protein concentration (mg per mL)} = (A275/0.22)$$

  in which A is the absorbance of rProtein A, C-Cys, at the wavelength of 275 nm and 0.22 is the molar absorptivity. Average the triplicate results, and determine a coefficient of variance (CV): the CV is ≤2.5%.
- **CHROMATOGRAPHIC PURITY**
  [NOTE—The size-exclusion chromatographic purity test resolves rProtein A, C-Cys from high molecular weight contaminants and low molecular weight contaminants.]
  Mobile phase: Prepare a solution of 0.02 M sodium phosphate, pH 7.2 containing 0.15 M sodium chloride in the following manner. Add 0.96 ± 0.02 g of monobasic sodium phosphate hydrate, 2.32 ± 0.02 g of dibasic sodium phosphate dihydrate, and 8.76 g ± 0.02 g of sodium chloride into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 1 M sodium hydroxide to a pH of 7.2 ± 0.05. Transfer this solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter.
  EDTA solution: Prepare a 20 mM ethylenediaminetetraacetic acid (EDTA) solution by dissolving 0.74 ± 0.02 g of EDTA in 100 mL of *Mobile phase.*
  DTT solution: Prepare a 100 mM DL-dithiothreitol (DTT) solution by dissolving 1.54 ± 0.02 g of DTT in 100 mL of *Mobile phase.*
  Pretreatment solution: Prepare a solution containing a mixture of *EDTA solution* and *DTT solution* (1:1, v/v). [NOTE—Prepare fresh just before use.]
  Test solution: Dilute rProtein A, C-Cys 1 to 5 in *Pretreatment solution,* and mix gently. Incubate the sample at 40° for 60 minutes.
  Chromatographic system
  (See *Chromatography* ⟨621⟩.)
  The liquid chromatograph is equipped with a 214-nm detector and a 10-mm × 30-cm column that contains packing L54. Equilibrate the column with at least two column volumes of *Mobile phase* at a flow rate of 0.4 mL per minute.
  Procedure: Inject 100 µL of *Pretreatment solution,* and allow the chromatography to continue for at least two column volumes. Repeat this twice before injecting 100 µL of the *Test solution.* Absorbance is detected at 214 nm. Integrate the main peak from the *Test solution* run and all other peaks not present in the *Pretreatment solution* runs. Calculate the percentage of impurities in the portion of the rProtein A, C-Cys taken by the formula:

$$100(r_i/r_s)$$

  in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: the sum of all impurities is not more than 5%; and the *Test solution* shows a major peak at approximately 35 minutes.

rPROTEIN A
$C_{1917}H_{3039}N_{565}O_{658}S_3$
44,618
N-terminal Sequence FLRPVE
Protein A is a component of the cell wall of *Staphylococcus aureus.* Recombinant Protein A (rProtein A) consists of five homologous immunoglobulin (IgG) binding domains (E, D, A, B, C) followed by a partial X domain sequence. It is expressed in *Escherichia coli* and purified via a column chromatography process. IgG columns are not used in the purification process. It is manufactured as a bulk solution with an IgG-binding potency greater than 95%. Release testing methods and specifications

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are described below. Because rProtein A is used as an ancillary material in the manufacture of recombinant therapeutic drugs, regulatory requirements differ from those of therapeutic drug products.

- **PACKAGING AND STORAGE:** Store in closed containers at the temperature indicated on the label.
- **LABELING:** The labeling states that the material is of recombinant DNA origin along with the lot number, storage conditions, and the statement "Formulated in *Water for Injection.*"
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP rProtein A RS
- **IDENTIFICATION**
  A. SDS-PAGE
  Molecular weight marker: Use a suitable molecular weight marker (MWM) containing protein bands between 20 and 200 kD.
  PBS solution: Prepare a solution that contains 8065.0 mg and 200.0 mg of sodium chloride and potassium chloride, respectively, per L of 0.01 M sodium phosphate buffer, pH 7.4.
  4X Sample buffer:[2] Dissolve 0.666 g of tris-hydrochloride, 0.682 g of tris base, 0.800 g of lithium dodecyl sulfate (LDS), 0.006 g of ethylenedinitrilotetraacetic acid (EDTA), and 4 g of glycerol in 8 mL of water; add 0.75 mL of 1% Coomassie brilliant blue G-250 (see Coomassie Blue G-250 in *Reagents* under *Reagents, Indicators, and Solutions*) solution and 0.25 mL of 1% phenol red solution. Mix well, and adjust the volume with water to 10 mL.
  2X Sample buffer: Prepare a mixture of *4X Sample buffer* and water (1:1).
  1X Sample buffer: Prepare a mixture of *2X Sample buffer* and water (1:1).
  1 M Dithiothreitol solution: Dissolve 0.154 g of DL-dithiothreitol (DTT) in 1 mL of water.
  2X Reducing sample buffer: Mix 180 µL of *2X Sample buffer* and 20 µL of *1 M Dithiothreitol solution.*
  20X Running buffer:[3] Dissolve 104.6 g of 3-(*N*-morpholino)propanesulfonic acid (MOPS), 60.6 g of tris base, 10 g of sodium dodecyl sulfate (SDS), and 3.0 g of EDTA in 400 mL of water. Mix well, and adjust with water to 500 mL.
  1X Running buffer: Prepare a solution of water and *20X Running buffer* (19:1).
  Gel staining solution: Prepare a solution of Coomassie brilliant blue R-250 (see Coomassie Brilliant Blue R-250 in *Reagents* under *Reagents, Indicators, and Solutions*) having a concentration of 0.5 g per L in a mixture of water, isopropanol, and acetic acid (6.5: 2.5: 1.0). Filter, and store at room temperature. Silver staining is not recommended.
  Destaining solution: Mix 100 mL of acetic acid with 900 mL of water.
  Standard preparation: Dilute USP rProtein A RS to 0.4 mg per mL with *PBS solution.* Further dilute this solution 1:1 with *2X Reducing sample buffer,* and incubate in a closed tube for 5 minutes at 90°. Mix, and quick spin prior to loading.
  Test preparation: Dilute rProtein A with *PBS solution* to 0.4 mg per mL. Proceed as directed under *Standard preparation* beginning with "Further dilute."
  Comix solution: Dilute rProtein A and USP rProtein A RS with *PBS solution* to 0.8 mg per mL. This solution contains 0.4 mg per mL of each protein. Proceed as directed under *Standard preparation* beginning with "Further dilute."
  SDS-PAGE gel and apparatus set-up: Assemble gel apparatus following the manufacturer's instructions. Lock the gel tension wedge in place, and fill approximately 200 mL of *1X Running buffer* into the inside chamber. If there are no leaks, pour 600 mL of *1X Running buffer* into the outer chamber. Gently pull the comb out of the cassette to immerse the wells in *1X Running buffer.* Load 10 µL of each preparation as directed below under *Gel loading* onto a 10% Bis-Tris SDS-PAGE gel.[4]
  Gel loading: Use the following gel loading scheme when running one *Test preparation* (see *Table 1*). Each *Test preparation* is run by itself and as part of the *Comix solution* that contains the rProtein A and USP rProtein A RS.

Table 1

Lane	Sample	Load Volume (µL)	Load Amount (µg)
1	*1X Sample buffer*	10	N/A
2	MWM	20	N/A
3	*Test preparation* #1	10	2
4	*Comix solution* #1	10	4 (total)
5	*Test preparation* #1	10	2
6	*1X Sample buffer*	10	N/A
7	*Standard preparation*	10	2
8	MWM	20	N/A
9	—	—	—
10	—	—	—

Running the gel: Set the voltage to 125 volts, and run at a constant voltage. Run the gels until the bromophenol blue band is approximately 5 mm from the bottom of the gel (approximately 120 to 140 minutes).

[2] 4X NuPAGE LDS sample buffer is available from Invitogen (No. NP0007).
[3] 20X NuPAGE MOPS SDS Running Buffer is available from Invitrogen (No. NP0001).
[4] 10% Bis-Tris SDS-PAGE gel is available from Invitrogen (No. NP0301).

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Gel staining: Pour approximately 100 mL of *Gel staining solution* into the staining container. Place the gel into the staining container, and allow the stain to completely cover the gel. Cook the gel and container in a microwave for 30 seconds. Place the staining container on an orbital shaker, and stain the gel for 1 hour with gentle shaking.

Destaining: Drain the *Gel staining solution,* and add enough *Destaining solution* to the container to cover the gel. Place the container on an orbital shaker, and shake at low speed. Change the *Destaining solution* as necessary until a clear background is obtained. After destaining, rinse the gel thoroughly with water, and leave the gel in water for 10 minutes before scanning.

Gel scanning: Apply some water to the glass plate of the scanner, and place the gels on a wetted glass plate. Eliminate any bubbles. Using appropriate settings, scan the gels.

Data analysis: Choose a band between the 20 kD and 30 kD bands of the MWM to calculate the percentage of the retention factor. Draw a line in one lane (lane containing *1X Sample buffer*) from the well to the apex (region of greatest intensity) of the chosen band.

The length of this line is denoted as the total distance (D_T). For the lanes containing samples draw a line from the well to the apex of each band. For each band the length of this distance is the migration distance (D_M) in mm. Record the D_T and D_M on the report sheet for each peak or band. The total distance should be the same for each lane on a gel. Calculate the percentage of the retention factor (R_F) of each major peak or band, and document on the report sheet using the following equation:

$$\%R_F = D_M/D_T \times 100$$

Also for each gel, record the number of bands and approximate molecular weight of each band in each sample.

System suitability: All bands between 20 kD and 70 kD are present. The lane containing *1X Sample buffer* does not contain any bands.

Specificity: The rProtein A has one major band and a similar molecular weight that corresponds to those of the USP rProtein A RS. The *Comix solution* also shows a single major band.

B. IgG Binding: [NOTE—The IgG binding assay is a functional method for determining the percentage of rProtein A capable of binding to immobilized human polyclonal immunoglobulin. Since the percent of functional rProtein A in each lot is not less than 95%, the assay measures unbound protein versus total protein injected. This is done by comparing the absorbance in the flow-through to absorbance from an injection bypassing the column.]

Sample pretreatment (desalting): In order to remove any buffer components that may contribute to absorbance in the "unbound" IgG column fraction, samples are desalted with *Solution A.* Desalting may be performed using a suitable desalting column[5] depending on the volumes required.

IgG column: A 1-mL Sepharose column[6] with immobilized human polyclonal IgG (hIgG) is required to perform this assay. [NOTE—The IgG column requires washing when it is new, when it has performed several analysis cycles, or after system suitability failure. Column washing procedure is not required for each sample injection.]

Column washing solution A: Prepare a solution of 0.5 M acetic acid, pH 3.4 by adding 28.6 mL of acetic acid into a 1000-mL beaker, diluting to 900 mL with water, and adjusting with ammonium acetate to a pH of 3.4. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter.

Column washing solution B: Prepare a solution of 50 mM Tris, pH 7.6, 150 mM sodium chloride, and 0.05% Tween 20 by the following procedure. Add 6.06 ± 0.01 g of Tris and 8.77 ± 0.01 g of sodium chloride into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 0.5 M sodium hydroxide to a pH of 7.60 ± 0.05. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter (buffer solution). Add 0.5 mL of Tween 20 into 1 L of the buffer solution and mix thoroughly.

Solution A: Prepare a solution of 20 mM monobasic sodium phosphate and 150 mM sodium chloride, pH 7.6 by the following procedure. Add 2.76 ± 0.01 g monobasic sodium phosphate hydrate and 8.77 ± 0.01 g sodium chloride into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 5 M sodium hydroxide to a pH of 7.60 ± 0.05. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter.

Solution B: Prepare a solution of 100 mM phosphoric acid pH 2.8 by the following procedure. Add 6.8 mL of phosphoric acid into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 2 M potassium hydroxide to a pH of 2.80 ± 0.05. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume.

Mobile phase: Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation: Thaw USP rProtein A RS, and use directly.

Test preparation: Prepare a 4.0 to 6.0 mg per mL rProtein A solution in *Solution A.*

Chromatographic system: The liquid chromatograph is equipped with a 280-nm detector and a 1-mL column with immobilized hIgG. The chromatograph is equipped with a bypass valve to allow flow to be diverted from the column. Each analysis consists of a series of two injections, one where the sample is injected onto the column and one where the sample bypasses the column and flows directly into the detector. Perform three replicate analyses. The chromatograph is programmed as follows (see *Table 2*).

[5] Zeba columns are available from Pierce; Nap-10 columns are available from GE Healthcare.
[6] HiTrap IgG Sepharose 6 FF column is available from GE Healthcare (No. 90-1003-97).

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Table 2

Flow Rate (mL per minute)	Time (minutes)	Solution A (%)	Solution B (%)	Valve Position	Elution
1.0	0–6	100	0	Column	Re-equilibration
1.0	6–12	100→0	0→100	Column	Re-equilibration
1.0	12–22	100	0	Column	Equilibration
0.4	22–25	100	0	Column	Equilibration
0.4 (sample injected)	25–35	100	0	Column	Isocratic
1.0	35–49	100→0	0→100	Column	Regeneration
1.0	49–63	0→100	100→0	Column	Re-equilibration
1	63–65	100	0	Bypass	Equilibration
0.4	65–68	100	0	Bypass	Equilibration
0.4 (sample injected)	68–75	100	0	Bypass	Isocratic

Chromatograph the *Standard preparation,* record the peak responses, and calculate the percentage of hIgG binding as directed for *Procedure:* the percentage of hIgG binding ≥ 95% and the relative standard deviation for replicate analysis is not more than 1%.

Procedure: Inject a volume (about 100 µL) of the *Test preparation.* Record the chromatogram, and measure the peak responses. Calculate the percentage of hIgG binding activity by the following formula:

$$100 - 100(r_C/r_B)$$

in which r_C is the unbound material peak response from the column injection and r_B is the bypass peak response from the bypass injection. Each replicate analysis of the *Test preparation* is not less than 95% of hIgG binding. Report the average value from three replicate analyses.

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu per mL, and the total yeasts and molds count does not exceed 10 cfu per mL.
- **Bacterial Endotoxins Test** ⟨85⟩: It contains not more than 0.5 USP Endotoxin Unit per mg of total protein. [Note—The *Bacterial Endotoxins Test* for rProtein A is used to describe the quality of this ancillary material. This test does not define the acceptable level of bacterial endotoxin in the preparation of injectable dosage forms in which rProtein A is used.]
- **Total Protein**

(See ⟨857⟩.)

Prepare triplicate samples for analysis by diluting the rProtein A to 3.0 mg per mL in *Water for Injection.* Measure the absorbance of each sample at 275 nm after correcting for the absorbance using *Water for Injection* as the blank. Determine the protein concentration using the equation:

$$\text{Protein concentration (mg per mL)} = (A_{275}/0.165)$$

in which A is the absorbance of rProtein A at the wavelength of 275 nm and 0.165 is the molar absorptivity. Average the triplicate results, and determine a coefficient of variance (CV): the CV is ≤5%.

- **UV Spectral Analysis:** Dilute rProtein A to 1 mg per mL in *Water for Injection.* Using a scanning UV spectrophotometer and *Water for Injection* as the blank, obtain spectral scans over the range of 240 to 360 nm. From the resulting data, calculate the absorbance value at 270 nm and the ratio of absorbance at 270 to 250 nm (i.e., E270/E250): the absorbance at 270 nm of a 1 mg per mL solution of rProtein A in *Water for Injection* is within the range 0.14–0.20.
- **Chromatographic Purity**

[Note—The size-exclusion chromatographic purity test resolves rProtein A from high molecular weight contaminants.]

Mobile phase: A solution of 0.3 M sodium phosphate, pH 7 is prepared by mixing monobasic and dibasic phosphate solutions in the following manner. Weigh 21.3 ± 0.1 g of dibasic anhydrous sodium phosphate, and dissolve in 500 mL of water to obtain a 0.3 M dibasic sodium phosphate solution *(Solution 1).* Into a separate container, weigh 18.0 ± 0.1 g monobasic anhydrous sodium phosphate, and dissolve in 500 mL of water to obtain a 0.3 M monobasic sodium phosphate solution *(Solution 2).* Calibrate a pH meter using pH calibrators at a pH of 7 and 10. Add 400 mL of *Solution 1* to a 1-L beaker. Transfer the pH probe to the beaker. Slowly add *Solution 2* to the solution until the pH is 7.0 ± 0.1. Pass the solution through a 0.45-µm membrane filter.

Standard solution: Dilute USP rProtein A RS to 1 mg per mL in *Mobile phase.*

Test solution: Dilute rProtein A to 1 mg per mL in *Mobile phase.*

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The liquid chromatograph is equipped with a 214-nm and 280-nm detector and a 9.4-mm × 25-cm column that contains packing L35. The flow rate is 1 mL per minute. Chromatograph the *Standard solution* as directed for *Procedure:* rProtein A shows a single major peak at approximately 9 minutes and the area percentage is ≥98% at 214 nm and ≥95% at 280 nm.

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Procedure: Inject 100 µL of the *Test solution* into the chromatograph, run isocratically for 15 minutes, and record the chromatogram. The values for the rProtein A from the *Test solution* correspond to the specifications of the USP rProtein A RS from the *Standard solution.*

- **ISOFORMS**

Standard solution: Thaw USP rProtein A RS, and use directly.
Test solution: Dilute rProtein A to 4 mg per mL in *Water for Injection.*
pI Markers: Use a suitable marker set containing markers between 3 and 10.[7]
IEF gel: Use a suitable gel with the range of between 3 and 10 and a size of 100 × 125 mm.[8]
Procedure: Apply 5-µL aliquots of the *pI Markers, Test solution,* and the *Standard solution* to the *IEF gel,* and run under 1W of power for approximately 10 minutes. Remove the sample mask, and apply power with concurrent cooling between 5° to 10° of the focusing chamber for 40 minutes at a setting of 1000V, 20 mA, 25W. Fix the *IEF gel* for 1 hour in 20% trichloroacetic acid, then stain using a suitable stain for IEF gels.[9] Finally, wash and dry the gel: the correlation coefficient of the best fit line for the *pI Markers* versus their migration in cm is ≥0.990, and the rProtein A from the *Standard solution* shows a single major band within the pI range of 4.6 to 5.2. A single band is seen in the *Test solution* that corresponds to the pI range of the *Standard solution.*

- **LIMIT OF TRITON X-100**

Mobile phase: Prepare a filtered and degassed mixture of water and acetonitrile (60:40).
Test solution: Dilute rProtein A to 5 mg per mL in *Water for Injection.*
Triton X-100 spike solution: Combine 5 mg per mL of USP rProtein A RS and 0.15% Triton X-100 (9:1) to obtain a solution having known concentrations of rProtein A and 0.015% Triton X-100.
Chromatographic system
(See *Chromatography* ⟨621⟩.)
The liquid chromatograph is equipped with a 214-nm and a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L11. The flow rate is 1 mL per minute. Chromatograph the *Triton X-100 spike solution* as directed for *Procedure:* Triton X-100 has a single major peak at approximately 9 minutes, and the rProtein A shows a smaller or undetectable peak at the same retention time.
Procedure: Inject about 100 µL of the *Test solution* into the chromatograph, run isocratically for 35 minutes, and record the chromatogram. The absorbance is detected at 223 nm: the Triton X-100 peak is not more than 0.015% (equivalent to the *Triton X-100 spike solution*).

rPROTEIN A, B_4, C-CYS

$C_{1177}H_{1854}N_{326}O_{384}S_1$
26747.6 Da
N-terminal Sequence AQGTVDAKFD

rProtein A, B_4, C-Cys is a recombinant protein derived from the B-domain of Protein A. The Protein A domain has been alkali-stabilized by site-specific mutagenesis and multimerized to a tetramer with a C-terminal cysteine for directed immobilization purposes. rProtein A, B_4, C-Cys is produced using *Escherichia coli* as the host cell followed by purification with conventional chromatography. rProtein A, B_4, C-Cys is manufactured as a bulk solution with an IgG-binding potency of greater than 95%. Because rProtein A, B_4, C-Cys is used as an ancillary material in the manufacture of recombinant therapeutic drugs, regulatory requirements differ from those for therapeutic drug products.

- **PACKAGING AND STORAGE:** Store in closed containers at the temperature indicated on the label.
- **LABELING:** Preserve in sealed containers, and store at a temperature of –20° or below.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Endotoxin RS
USP rProtein A, B4, C-Cys RS

- **IDENTIFICATION**

A. SDS-PAGE: It meets the requirements of *Identification* test *A* under *rProtein A* using USP rProtein A, B4, C-Cys RS.
B. IgG Binding: It meets the requirements of *Identification* test *B* under *rProtein A* using USP rProtein A, B4, C-Cys RS.

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu per mL, and the total yeasts and molds count does not exceed 10 cfu per mL.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains not more than 1 USP Endotoxin Unit per mg of total protein. [NOTE—The *Bacterial Endotoxins Test* for rProtein A, B_4, C-Cys is used to describe the quality of this ancillary material. This test does not define the acceptable level of bacterial endotoxin in the preparation of injectable dosage forms in which rProtein A, B_4, C-Cys is used.]
- **TOTAL PROTEIN**

(See ⟨857⟩.)
Formulation buffer solution: Prepare a solution of 0.02 M potassium phosphate, pH 7.0, containing 0.15 M potassium chloride and 2 mM of ethylenediaminetetraacetic acid (EDTA) in the following manner. Add 2.72 ± 0.01 g of monobasic potassium phosphate anhydrous, 11.18 g ± 0.22 g of potassium chloride, and 0.744 ± 0.02 g of EDTA into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 1 M sodium hydroxide to a pH of 7.00 ± 0.05. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter.
Test preparation: Dilute the rProtein A, B_4, C-Cys to 3.0 mg per mL with *Formulation buffer solution.*
Procedure: Prepare triplicate samples for analysis. Measure the absorbance of each *Test preparation* at 275 nm after correcting for the absorbance using the *Formulation buffer solution* as the blank. Determine the protein concentration using the equation:

[7] pI markers in the 3–10 range are available from BioRad (No. 161-0310).
[8] IEF gels in the 3–10 range are available from Cambrex (No. 56015).
[9] ISS Pro-Blue is available from Integrated Separation Systems.

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Protein concentration (mg per mL) = (A275/0.22)

in which A is the absorbance of rProtein A, B_4, C-Cys, at the wavelength of 275 nm and 0.22 is the molar absorptivity. Average the triplicate results, and determine a coefficient of variance (CV): the CV ≤2.5%.

• **CHROMATOGRAPHIC PURITY**

[NOTE—The size-exclusion chromatographic purity test resolves rProtein A, B_4, C-Cys from high molecular weight contaminants and low molecular weight contaminants.]

Mobile phase: Prepare a solution of 0.02 M sodium phosphate, pH 7.2 containing 0.15 M sodium chloride in the following manner. Add 0.96 ± 0.02 g of monobasic sodium phosphate hydrate, 2.32 ± 0.02 g of dibasic sodium phosphate dihydrate, and 8.76 g ± 0.02 g of sodium chloride into a 1000-mL beaker. Dilute with water to 900 mL, and adjust with 1 M sodium hydroxide to a pH of 7.2 ± 0.05. Transfer this solution into a 1000-mL volumetric flask, and dilute with water to volume. Pass the solution through a 0.45-µm membrane filter.

EDTA solution: Prepare a 20 mM EDTA solution by dissolving 0.74 ± 0.02 g of EDTA in 100 mL of *Mobile phase.*

DTT solution: Prepare a 100 mM DL-dithiothreitol (DTT) solution by dissolving 1.54 ± 0.02 g of DTT in 100 mL of *Mobile phase.*

Pretreatment solution: Prepare a solution containing a mixture of *EDTA solution* and *DTT solution* (1:1, v/v). [NOTE—Prepare fresh just before use.]

Test solution: Dilute rProtein A, B_4, C-Cys 1 to 5 in *Pretreatment solution,* and mix gently. Incubate the sample at 40° for 60 minutes.

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The liquid chromatograph is equipped with a 214-nm detector and a 10-mm × 30-cm column that contains packing L54. Equilibrate the column with at least two column volumes of *Mobile phase* at a flow rate of 0.4 mL per minute.

Procedure: Inject 100 µL of *Pretreatment solution,* and allow the chromatography to continue for at least two column volumes. Repeat this twice before injecting 100 µL of the *Test solution.* Absorbance is detected at 214 nm. Integrate the main peak from the *Test solution* run and all other peaks not present in the *Pretreatment solution* runs. Calculate the percentage of impurities in the portion of rProtein A, B_4, C-Cys taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of all the responses of all the peaks: the sum of all impurities is not more than 5%; and the *Test solution* shows a major peak at approximately 37 minutes.

⟨151⟩ PYROGEN TEST

INTRODUCTION

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL/kg injected intravenously within a period of NMT 10 min. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations (see *Biologics* ⟨1041⟩). A validated, equivalent in vitro pyrogen or bacterial endotoxin test may be used in place of the in vivo rabbit pyrogen test,[1] where appropriate.

APPARATUS AND DILUENTS

Render the syringes, needles, and glassware free from pyrogens by heating at 250° for NLT 30 min or by any other suitable method. Treat all diluents and solutions for washing and rinsing of devices or parenteral injection assemblies in a manner that will assure that they are sterile and pyrogen-free. Periodically perform control pyrogen tests on representative portions of the diluents and solutions for washing or rinsing of the apparatus. Where *Sodium Chloride Injection* is specified as a diluent, use Injection containing 0.9% of sodium chloride (NaCl).

TEMPERATURE RECORDING

Use an accurate temperature-sensing device such as a clinical thermometer, or thermistor probes or similar probes that have been calibrated to assure an accuracy of ±0.1° and have been tested to determine that a maximum reading is reached in less than 5 min. Insert the temperature-sensing probe into the rectum of the test rabbit to a depth of NLT 7.5 cm, and, after a period of time NLT that previously determined as sufficient, record the rabbit's body temperature.

[1] United States Food and Drug Administration. Guidance for industry. Pyrogen and endotoxins testing: questions and answers. Rockville, MD: Food and Drug Administration; June 2012. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm310098.pdf.

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TEST ANIMALS

Use healthy, mature rabbits. House the rabbits individually in an area of uniform temperature between 20° and 23° and free from disturbances likely to excite them. The temperature varies NMT ±3° from the selected temperature. Before using a rabbit for the first time in a pyrogen test, condition it NMT 7 days before use by a sham test that includes all of the steps as directed in *Procedure* except injection. Do not use a rabbit for pyrogen testing more frequently than once every 48 h, nor prior to 2 weeks following a maximum rise of its temperature of 0.6° or more while being subjected to the pyrogen test, or following its having been given a test specimen that was adjudged pyrogenic.

PROCEDURE

Perform the test in a separate area designated solely for pyrogen testing and under environmental conditions similar to those under which the animals are housed and free from disturbances likely to excite them. Withhold all food from the rabbits used during the period of the test. Access to water is allowed at all times, but may be restricted during the test. If rectal temperature-measuring probes remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture. NMT 30 min prior to the injection of the test dose, determine the "control temperature" of each rabbit: this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one group of test rabbits, use only those rabbits whose control temperatures do not vary by more than 1° from each other, and do not use any rabbit with a temperature exceeding 39.8°.

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 mL of the test solution per kg of body weight, completing each injection within 10 min after start of administration. The test solution is *either* the product, constituted if necessary as directed in the labeling, *or* the material under test treated as directed in the individual monograph and injected in the dose specified therein. For pyrogen testing of devices or injection assemblies, use washings or rinsings of the surfaces that come in contact with the parenterally administered material or with the injection site or internal tissues of the patient. Assure that all test solutions are protected from contamination. Perform the injection after warming the test solution to a temperature of 37 ± 2°. Record the temperature at 30-min intervals between 1 and 3 h subsequent to the injection.

TEST INTERPRETATION AND CONTINUATION

Consider any temperature decreases as zero rise. If no rabbit shows an individual rise in temperature of 0.5° or more above its respective control temperature, the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.5° or more, continue the test using five other rabbits. If NMT three of the eight rabbits show individual rises in temperature of 0.5° or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3°, the material under examination meets the requirements for the absence of pyrogens.

RADIOACTIVE PHARMACEUTICALS

Test Dose for Preformulated, Ready-to-Use Products Labeled with Radioactivity

AGGREGATED ALBUMIN AND OTHER PARTICLE-CONTAINING PRODUCTS

For the rabbit pyrogen test, dilute the product with *Sodium Chloride Injection* to NLT 100 µCi/mL, and inject a dose of 3 mL/kg of body weight into each rabbit.

OTHER PRODUCTS

Where physical half-life of radionuclide is greater than 1 day: Calculate the maximum volume of the product that might be injected into a human subject. This calculation takes into account the maximum recommended radioactive dose of the product, in µCi, and the radioactive assay, in µCi/mL, of the product at its expiration date or time. Using this information, calculate the maximum volume dose per kg to a 70-kg human subject.

For the rabbit pyrogen test, inject a minimum of 10 times this dose per kg of body weight into each rabbit. If necessary, dilute with *Sodium Chloride Injection*. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.

Where physical half-life of radionuclide is less than 1 day: For products labeled with radionuclides with a half-life of less than 1 day, the dosage calculations are identical to those described in the first paragraph in *Other Products*. These products may be released for distribution prior to completion of the rabbit pyrogen test, but such test must be initiated at NMT 36 h after release.

Test Dose for Pharmaceutical Constituents or Reagents to Be Labeled

The following test dose requirements pertain to reagents that are to be labeled or constituted prior to use by the direct addition of radioactive solutions such as *Sodium Pertechnetate Tc 99m Injection*, i.e., "cold kits".

Assume that the entire contents of the vial of nonradioactive reagent will be injected into a 70-kg human subject, or that $^1/_{70}$ of the total contents per kg will be injected. If the contents are dry, constitute with a measured volume of *Sodium Chloride Injection*.

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For the rabbit pyrogen test, inject ($^1/_7$) of the vial contents per kg of body weight into each rabbit. The maximum dose per rabbit is the entire contents of a single vial. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.

⟨161⟩ MEDICAL DEVICES—BACTERIAL ENDOTOXIN AND PYROGEN TESTS

INTRODUCTION

The methods and requirements in this chapter apply to assemblies or devices labeled sterile and nonpyrogenic that are in contact directly or indirectly with the cardiovascular system, lymphatic system, or cerebrospinal fluid. This includes, but may not be limited to, the following:

- Fluid pathways of catheters and administration sets such as solution administration sets, extension sets, transfer sets, blood administration sets, intravenous catheters, implants, extracorporeal oxygenator tubing, dialysis tubing, intramuscular drug delivery catheters, and transfusion and infusion assemblies
- Liquid medical devices such as dialysate
- Implantable medical devices such as heart valves and vascular grafts, and other medical devices with a nonpyrogenic claim that may come into contact with blood or cerebrospinal fluid
- Gels with a nonpyrogenic claim including demineralized bone matrices and drug delivery systems

BACTERIAL ENDOTOXINS

For the bacterial endotoxins test (BET) assay parameters, procedures, standards, and controls for the gel-clot and kinetic and endpoint assays, proceed as directed in *Bacterial Endotoxins Test* ⟨85⟩, substituting the medical device eluate for the product sample.

DEFINITIONS

Endotoxin limit

- The endotoxin limit for the finished device is NMT 20 USP Endotoxin Units per device and NMT 2.15 USP Endotoxin Units for devices in contact with cerebrospinal fluid.
- For devices that directly or indirectly contact the intraocular environment, a lower endotoxin limit may apply (*1*).
- The endotoxin limit for the device extract in Endotoxin Units/mL is calculated as:

$$\text{Result} = (K \times N)/V$$

K = limit for each device
N = number of devices tested
V = total volume of the extract

Kit: A kit is defined as a collection of individual devices in its primary package, or a variety of related devices. Each individual type of device may have its own product endotoxin limit and should be tested and evaluated on an individual basis; see *Table 1* [refer to ANSI/AAMI ST72 (*2*)].

Table 1. Selection of Product Units for Testing

Product Unit	Item for Testing
Individual medical device in a primary package where each medical device is used individually in clinical practice	Individual medical device
Set of components in a primary package where components are assembled as a product and are used together in clinical practice	Combination of components
A number of identical medical devices in one primary package where each medical device is used independently in clinical practice	Single medical device taken from the primary package
Kit of procedure-related medical devices where each medical device is used independently in clinical practice and where each medical device may have a different endotoxin limit	Each type of medical device that has a nonpyrogenic claim, or all items together

Lambda (λ): λ is the test method sensitivity for gel-clot methods. For turbidometric or chromogenic technique, λ is equal to the lowest endotoxin concentration on the referenced standard curve.

Product families: Product families for the purposes of BET may be defined on the basis of common materials of construction and/or common components. The choice of family members should be justified and documented. If a product

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family contains devices of many sizes, the device with the largest surface area claimed to be nonpyrogenic is chosen as the representative member of the family to be used in suitability testing.

Extracting or rinsing solution: *Water for BET* [see *Bacterial Endotoxins Test* ⟨85⟩, *Reagents and Test Solutions, Water for Bacterial Endotoxins Test (BET)*] or other suitable solvent demonstrated to be noninterfering with the BET assay is used to extract or rinse the medical device.

Sampling

1. *Sample*: A sample for end-product testing must be in its final configuration and packaging, including all component parts that make up the final medical device.
2. *Representative sampling*: A representative sample is a sample plan that is created and justified based on the assumption that the manufacturing process is validated and in a state of control.
3. *Sample size*: The number of devices chosen for routine testing is dependent on the size of the lot, level of control, statistical considerations, and historical performance. In most cases, each lot of product must be tested using an appropriate number of samples, NMT 10, taken at random to represent the quality of the lot. Alternate sampling plans that utilize small sample sizes or do not test each lot of product must be clearly defined and must be supported/justified by a robust risk assessment.
4. *Pre-post sterilization samples*: When qualifying a BET assay for pre-sterilization samples, equivalency in test results must be shown with post-sterilization samples to ensure that sterilization and post-sterilization handling have no adverse impact on the accuracy of the test result. Pre-sterilization testing is inappropriate for products that support microbial growth.

Suitability testing (also known as test for interfering factors): Suitability studies of device extracts are designed to ensure that the device extract under test does not adversely impact the accuracy of the test result by either a) causing the assay to underestimate endotoxin (inhibition) or b) contributing to overestimation of endotoxin (enhancement).

REAGENTS AND TEST SOLUTIONS

See ⟨85⟩.

PREREQUISITES TO TESTING

Apparatus

All equipment used in the extraction or testing process must be properly qualified and/or calibrated as appropriate. This equipment includes but is not limited to water baths, heat blocks, mechanical pipetters (fixed, adjustable, and repeating pipetters), thermometers, and plate/tube/cartridge readers.

Depyrogenate all glassware and other heat-stable materials in a hot air oven using a validated process. If employing plastic apparatus such as microplates and pipet tips for automatic pipetters, use apparatus that is shown to be free of detectable endotoxin and does not interfere with the test.

Analysts must be properly trained to perform the assay.

The assay sensitivity must be confirmed as described in *Bacterial Endotoxins Test* ⟨85⟩, *Gel-Clot Technique, Preparatory Testing, Test for Confirmation of Labeled Lysate Sensitivity* for gel-clot reagents and *Bacterial Endotoxins Test* ⟨85⟩, *Photometric Quantitative Techniques, Preparatory Testing, Assurance of Criteria for the Standard Curve* for quantitative methods. Assay sensitivity confirmation must be carried out when a new batch of reagent (lysate or endotoxin) is used or when there is any change in the test conditions that could affect the outcome of the test.

PREPARATION OF DEVICES: STANDARD METHODS

Endotoxin Limit

The endotoxin limit for the extracting or rinsing solution is:

$$\text{Result} = (K \times N)/V$$

K = amount of endotoxin allowed per device (20 Endotoxin Units per device; 2.15 for intrathecal devices)
N = number of devices tested
V = total volume of the extract or rinse

[NOTE—The volume of the rinse or extract may be adjusted for the size and configuration of the device.]

The standard extracting fluid for extracting, rinsing, or soaking medical devices is *Water for BET*. If necessary, other solvents may be used after it has been demonstrated that they do not interfere with the performance of the assay. Extraction is not required for liquid medical devices. Either the entire device if claimed to be nonpyrogenic or all components of the device that are claimed to be nonpyrogenic, including fluid pathways, must come in contact with the extraction fluid for the entire course of the extraction.

The standard extraction method is to soak or immerse the device or flush the fluid pathway with extracting fluid that has been heated to 37 ± 1.0°, keeping the extracting fluid in contact with the relevant surface(s) for NLT 1 h at controlled room

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temperature. Alternate extraction or rinsing methods may be used, but must be demonstrated to be equivalent to or better than the standard method. Extracts from individual medical devices are typically pooled for testing.

Suitability

Suitability of the BET assay must be demonstrated for each product or product family. The number of batches of product chosen for the suitability study is reflective of the level of control of the process and must be justified.

For the suitability test, medical device extracts or liquid medical devices are considered to be the sample solutions under test and are tested as outlined for the chosen technique in *Bacterial Endotoxins Test* ⟨85⟩, *Gel-Clot Technique, Preparatory Testing, Test for Interfering Factors*.

If necessary, adjust the pH of the solution to be examined (or dilution thereof) so that the pH of the mixture of the lysate and sample solution falls within the pH range specified by the lysate manufacturer, usually pH 6.0–8.0. The pH may be adjusted by use of an acid, base, or suitable buffer as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with *Water for BET* in containers free of detectable endotoxin. Buffers must be shown to be free of detectable endotoxin and interfering factors.

If the undiluted rinsing or extracting solution or liquid medical device is determined to be unsuitable for ⟨85⟩, the test for interfering factors may be repeated utilizing dilution or a method that will neutralize or eliminate the interfering substance.

If glucan interference is suspected, the use of a glucan-blocking agent or endotoxin-specific lysate is permissible.

Dilution in *Water for BET* or other validated solvent by a factor not to exceed the maximum valid dilution (MVD) is permissible. The MVD for medical devices is the following:

$$\text{MVD} = \text{Endotoxin limit (of the rinsing, extracting, or diluting solution in Endotoxin Units/mL)}/\lambda$$

λ = sensitivity of the test method in Endotoxin Units/mL

Interference may be overcome by suitable treatment of the device extract. Methods for overcoming interference and the qualification of such treatments are described in ⟨85⟩. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described in ⟨85⟩ using the preparation to be examined to which standard endotoxin has been added and which has then been submitted to the chosen treatment.

DEMONSTRATION OF CONTINUED SUITABILITY

The continued suitability of the assay should be re-assessed when significant changes are made to the product, process, raw material supply, manufacturing and/or testing site, BET reagent source, or any other change that could adversely affect the accuracy of the BET result. The results of this re-assessment should be documented, and suitability studies re-executed as necessary.

Changes to extraction techniques require a new suitability study. Because of possible changes in interference patterns between standard BET methods, a change in BET methodology (e.g., from gel-clot limits test to kinetic chromogenic) requires a re-execution of the suitability study.

Routine Testing

Routine testing of the finished medical devices must utilize the same extraction techniques that were documented in the successful suitability study.

Routine testing is to be performed as outlined in ⟨85⟩, following instructions for incubation and controls listed under the chosen assay technique.

Interpretation of Test Results

See ⟨85⟩. The preparation under test complies with the test if the mean endotoxin concentration of the replicates of *Solution A*, after correction for dilution and concentration, is less than 20 Endotoxin Units per medical device (2.15 Endotoxin Units per medical device for those devices that come in contact with the cerebrospinal fluid.) For liquid medical devices, the limit meets the requirements set forth in *Bacterial Endotoxins Test* ⟨85⟩, *Determination of Maximum Valid Dilution (MVD), Endotoxin Limit, footnote 2*.

If a device fails to meet this requirement, an investigation may be initiated per documented procedures. A failed, valid BET assay may not be repeated using *Pyrogen Test* ⟨151⟩ in its place.

PYROGENS

For samples that cannot be tested by BET because of nonremovable inhibition or enhancement of the test, ⟨151⟩ is applied. Select an appropriate number of devices, NMT 10, and obtain a pooled extract utilizing preparation methods appropriate to the device as directed for bacterial endotoxins, but with volumes of rinse or extraction fluid not to exceed 40 mL of sterile saline test solution per device. The requirements of ⟨151⟩ must be met.

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REFERENCES

1. U.S. Food and Drug Administration. Guidance for industry. Pyrogen and endotoxins testing: Questions and answers. Rockville, MD: Food and Drug Administration; June 2012. www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm314718.htm. Accessed 12 July 2016.
2. ANSI/AAMI ST72. Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing. Arlington, VA: Association for the Advancement of Medical Instrumentation; 2011.

⟨162⟩ DIPHTHERIA ANTITOXIN POTENCY TESTING FOR HUMAN IMMUNE GLOBULINS

An in vitro method is provided that is suitable for determining the potency of diphtheria antitoxin (antibodies against the diphtheria toxin) in preparations of plasma-derived human immune globulins. Diphtheria toxin is produced by *Corynebacterium diphtheriae* and has the ability to produce a cytopathogenic effect on susceptible epithelial cell lines. The test is based on the ability of diphtheria antitoxin to neutralize the diphtheria toxin, decreasing its cytotoxic effect. Specifically, the test determines the potency of the diphtheria antitoxin based on its ability to inhibit the cytotoxic effect of diphtheria toxin on cultured Vero cells (African green monkey kidney epithelial cells) relative to a reference standard. The mitochondrial dehydrogenases of live Vero cells can reduce the dye 3-4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) to a blue/black product that is then measured by absorbance at 540 nm. If no or little diphtheria antitoxin is present, then diphtheria toxin induces cell death and the inability of cells to reduce MTT, resulting in the presence of white or colorless wells. Acceptance criteria are defined by the appropriate regulatory agencies.

ASSAY

• **PROCEDURE**

DMEM-5 solution: Dulbecco's Modified Eagle's Medium[1], supplemented to contain 5% fetal bovine serum (FBS), 2 mM L-glutamine, 50 µg/mL of gentamicin, and 2.5 µg/mL of fungizone. [NOTE—Alternatively, a combination of 0.1 mg/mL of kanamycin sulfate, 0.1 units/mL of penicillin, and 0.1 mg/mL of streptomycin can be used in place of gentamicin.]

DMEM-2 solution: Dulbecco's Modified Eagle's Medium[1], supplemented to contain 2% fetal bovine serum (FBS), 2 mM L-glutamine, 50 µg/mL of gentamicin, and 2.5 µg/mL of fungizone. [NOTE—Alternatively, a combination of 0.1 mg/mL of kanamycin sulfate, 0.1 units/mL of penicillin, and 0.1 mg/mL of streptomycin can be used in place of gentamicin.]

MTT solution: 5 mg/mL of MTT in phosphate-buffered saline.[2] Prepare just before use and minimize exposure to room light.

Extraction solution: 0.4 N hydrochloric acid in isopropanol

Toxin test solution: Obtain a liquid preparation of toxin from a culture of *C. diphtheriae*.[3] It may have been rendered sterile by filtration, and sterility may be maintained by the inclusion of a suitable antimicrobial preservative. Store the filtrate in the dark at 2°–8° for several weeks until the activity is considered to be constant, as determined by testing. Dilute the toxin in *DMEM-5 solution* to obtain a suitable *Toxin test solution* that provides a working equivalence with the *Standard solution*. [NOTE—An appropriate *Toxin test solution* may be empirically determined as follows. Titrate the toxin against a fixed concentration of antitoxin (e.g., 0.125 units/mL) using volumes and media as defined below. Determine the lowest concentration of toxin that causes Vero cell toxicity in the presence of the antitoxin concentration chosen. This can be defined as LCD/20 (where "LCD" stands for limit of cytotoxic dose, and "/20" refers to 1/20 units/mL = 0.05 units/mL). This is a minimum concentration that should be used in the *Assay*.]

Trypsin–EDTA solution: Dissolve 0.4 g of trypsin, 0.2 g of ethylenediaminetetraacetic acid (EDTA), and 0.85 g of sodium chloride in 100 mL of sterile water. Use cell culture grade and sterile reagents to prepare. Alternatively, a premade sterile preparation may be used.

Cell culture preparation: Prepare Vero cells[4] by culturing in 75-cm² tissue culture flasks in *DMEM-5 solution* at 36 ± 1°, 5% carbon dioxide (CO_2), and a humidified environment. [NOTE—Alternative flask sizes may be used by adjusting the volumes used below.] After 2–3 days of growth, the medium may be replaced by *DMEM-2 solution*, with growth allowed to continue. When the cells have reached a confluent monolayer, discard the culture media. Wash the cell layer by pipetting 5 mL of *Trypsin–EDTA solution* into the flask and gently rocking the flask back and forth for approximately 30 s. Remove and discard the *Trypsin–EDTA solution*. Add an additional 5 mL of *Trypsin–EDTA solution*, and gently rock back and forth for 1 min. Remove and discard all but 1 mL. Incubate at 36 ± 1°, 5% carbon dioxide (CO_2), in a humidified environment for approximately 10 min or until the cell sheet begins to slough from the flask, and tap the flask to release the cells. Add 10 mL of *DMEM-5 solution* to the trypsinized cells, count the cells, and adjust the cell suspension to 1×10^5 cells/mL in the same medium.

Standard solutions: In sterile tubes, prepare four concentrations of the standard by diluting U.S. Standard Diphtheria Antitoxin[5] with *DMEM-2 solution* to 1 unit/mL, followed by serial dilutions in the same media that result in the additional concentrations of 0.5, 0.25, and 0.1 units/mL.

[1] 11885-092 Life Technologies, or equivalent.
[2] 14190-250 Life Technologies, or equivalent.
[3] Diphtheria toxin code 12/282 from NIBSC or suitable alternative.
[4] American Type Culture Collection CCL-81.
[5] Use appropriate U.S. Standard Diphtheria Antitoxin as designated by the Center for Biologics Evaluation and Research, Food and Drug Administration.

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Sample solutions: In sterile tubes, dilute each human immune globulin test sample to 1.25% protein with *DMEM-2 solution*. Using the diluted sample, further dilute the samples in *DMEM-2 solution* to achieve an expected diphtheria antitoxin concentration that is within the range of the *Standard solutions* (i.e., 1 to 0.1 units/mL).

Analysis

Samples: *Toxin test solution*, *Standard solutions*, and *Sample solutions*

Test each *Standard solution* or *Sample solution* in duplicate. Label 96 well culture plates by marking off the 8-well columns in groups of two; all four *Standard solutions* and one *Sample solution* can be tested per plate. As described below, columns 11 and 12 can be used for the untreated cell control and the toxin control, respectively. [NOTE—Alternative plate layouts maintaining the dilutions can be used.] Add 112.5 µL of *DMEM-2 solution* to each well in row A, columns 1–10 only (these are standard- or sample-nonspecific toxicity controls). Add 75 µL of *DMEM-2 solution* to each well in rows B–H, columns 1–10 only.

Add 37.5 µL of a *Standard solution* dilution or a *Sample solution* to wells A1–A2, and add 75 µL of the same solution to wells B1–B2. Add 37.5 µL of a second *Standard solution* dilution or *Sample solution* to wells A3–A4, and add 75 µL of the same solution to wells B3–B4. Continue to make similar additions of *Standard solution* dilutions or *Sample solutions* to each group of two columns in columns 1–10. Make two-fold serial dilutions by transferring 75 µL from each well in row B to each well in row C of the same column. Mix and then continue transferring 75 µL in the same manner down each column. Discard the last 75 µL after mixing row H. Add 75 µL of the *Toxin test solution* to rows B–H in columns 1–10. Add 150 µL of *DMEM-2 solution* to all wells in column 11 (cell control), and add 75 µL of *DMEM-2 solution* plus 75 µL of *Toxin test solution* to all wells in column 12 (toxin control).

Cover the plates, and incubate them for 1 h at 36 ± 1°, at 5% carbon dioxide (CO_2), and in a humidified environment. Add 150 µL of cell suspension to all wells, cover the plates, and incubate them at 36 ± 1°, at 5% carbon dioxide (CO_2), and in a humidified environment. Incubate for 4–5 days, checking periodically for microbial contamination by microscopic examination. Once toxicity control wells in row A containing the lowest concentration of a *Standard solution* are nearly confluent, add 15 µL of *MTT solution* to each well. Cover the plates, and incubate them in the carbon dioxide (CO_2) incubator at 37° for 3 h. Discard the medium, and add 150 µL of *Extraction solution* to each well. Cover the plates, and place aluminum foil over them to minimize light exposure. Shake gently to solubilize the blue formazan formed by viable cells. Using a suitable plate reader, read the absorbance of each well at a wavelength of 540 nm.

Calculation: The cutoff dilution is the highest dilution in which there are still viable cells in the well but beyond which there are no viable cells. Cutoff dilution is defined by a corresponding absorbance (Abs). [NOTE—The average absorbance value can be calculated for the cell control wells in column 11 and divided by 2 to obtain a 50% control Abs value. This value can be used to determine cutoff dilutions. The cutoff dilution for each *Standard solution* and *Sample solution* can therefore be defined as the highest dilution where the Abs value is greater than the 50% control Abs value.] Determine the cutoff dilutions for each *Standard solution* and *Sample solution*. Plot the cutoff dilutions obtained for all *Standard solutions* (expressed as the reciprocal of the cutoff dilution) versus U.S. Standard Diphtheria Antitoxin units. Calculate a linear regression line from the data. Calculate the geometric mean for the four cutoff dilutions associated with each *Sample solution*. Compare the mean cutoff dilution to the linear regression of the *Standard solution* data to determine a titer, in units/mL, for each *Sample solution*. To obtain the final potency value for the *Sample solution*, multiply the titer by any dilution that was made to the *Sample solution* prior to the *Assay*.

System suitability: The correlation coefficient of the standard curve must be >0.995. The slope of the standard curve must be between 26 and 38. The test is valid if the cells in wells of row A, columns 1–10, appear normal and similar to the cell control wells in column 11. In addition, the cutoff dilution values for *Standard solution* and *Sample solution* replicates should be within one serial dilution of each other, and the cutoff dilution must be within the range of the *Sample solution* serial dilutions.

⟨165⟩ PREKALLIKREIN ACTIVATOR

DEFINITION

Prekallikrein activator (PKA), also known as Factor XIIa, activates prekallikrein to kallikrein. Activated kallikrein cleaves kininogens into kinins (e.g., bradykinin) that can result in a loss of blood pressure. PKA is assayed by its ability to activate prekallikrein, which once activated cleaves a synthetic peptide substrate and releases a chromophore that can be measured spectrophotometrically. The amount of PKA is determined by comparison with reference standard material. The procedure presented in this chapter traditionally has been used to determine the amount of PKA in human plasma derivatives such as albumin and immunoglobulin preparations.

Some immunoglobulin preparations may contain kallikrein or kallikrein-like activity in the absence of the added prekallikrein substrate, resulting in turnover of the synthetic peptide substrate. If present, this kallikrein or kallikrein-like activity requires consideration when analysts verify the assay for suitability under the conditions of use. Some questions to consider when verifying the assay for suitability under the conditions of use include:

- Are sample matrix effects observed?
- Is the appropriate statistical model used for data analysis?
- Is an endpoint analysis sufficient, or is a kinetic analysis needed?

(For informational purposes only, refer to sections 2, 4, and 5 of *Design and Development of Biological Assays* ⟨1032⟩ when considering assay fitness for use.)

ASSAY

• **PREKALLIKREIN ASSAY**

Buffer A: Prepare a solution of 0.05 M tris(hydroxymethyl)aminomethane and 0.15 M sodium chloride. Adjust with 2 N hydrochloric acid to a pH of 7.95–8.05.

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Buffer B: Prepare a solution of 1.0 M tris(hydroxymethyl)aminomethane. Adjust with 2 N hydrochloric acid to a pH of 7.95–8.05.
Solution A: Prepare a solution of 0.33 N hydrochloric acid.
Solution B: Prepare a solution of 0.33 N sodium hydroxide.
Solution C: Prepare a solution of 50% acetic acid.
Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test specific for kallikrein in *Buffer A* to obtain a solution having a concentration of about 1.2 mM.
Prekallikrein substrate: Use a suitable *Prekallikrein substrate*. Test the *Prekallikrein substrate* for the absence of kallikrein activity by mixing 1 part with 20 parts of the *Chromogenic substrate solution* prewarmed at 37°, and incubate at 37° for 2 min. The substrate is suitable if the increase in absorbance at 405 nm is less than 0.001/min. [NOTE—To remove proteinase inhibitor contamination from the *Prekallikrein substrate*, acid treatment of the prekallikrein should be performed individually on each sample before use.] If frozen, thaw *Prekallikrein substrate* at 35°–39°. Add 2.0 mL of *Solution A* to 4 mL of *Prekallikrein substrate*, and incubate for 15 min at 23°–25°. Bring pH back to neutrality with 2.0 mL of *Solution B*. Add 200 µL of *Buffer B*, and store at room temperature for NMT 4 h.
Standard solutions: Dissolve the contents of a vial of USP Prekallikrein Activator RS in 0.5 mL of water. Dilute USP Prekallikrein Activator RS with *Buffer A* (1:5). Make five evenly spaced *Standard solutions* between 0 and 8.5 IU/mL.
Sample solution: Dilute sample appropriately to fall within the range of the standard dilutions with *Buffer A* performing at minimum (1:1).
System suitability
Samples: *Buffer A* and *Standard solutions*
Suitability requirements
Assay range: If the absorbance value of a sample is higher than the highest value of the standard curve, dilute the sample with *Buffer A* and repeat the *Assay*. If the absorbance of a sample is lower than the *Assay* limit of detection, the activity should be reported as less than the limit of detection.
Correlation coefficient: NLT 0.99
Analysis
Samples: *Buffer A*, *Standard solutions*, and *Sample solution*
[NOTE—This procedure also can be performed using alternative platforms. Care must be taken to minimize the time between the addition of *Prekallikrein substrate* to the *Standard solutions* and the *Sample solution* because some degradation of the *Prekallikrein substrate* can occur with time when thawed.]
To four separate individual wells of a suitable microtiter plate transfer 25 µL of either the *Standard solutions* or the *Sample solution*. Incubate the microtiter plate for 2–3 min at 35°–39°. Start to prewarm *Buffer A*, the *Prekallikrein substrate*, and the *Chromogenic substrate solution* at 35°–39°. For each *Standard solution* and *Sample solution*, in two of the four wells add 100-µL *Buffer A* and to the other two wells add 100-µL prewarmed *Prekallikrein substrate*; incubate at 35°–39° for 10 min. Add 125 µL of prewarmed *Chromogenic substrate solution* to all wells in the same order as the *Buffer A* and *Prekallikrein substrate* additions, and incubate at 35°–39° for 20 min. Stop the reactions with 50 µL of *Solution C*, and mix well. Read absorbance of each well of the plate at 405 nm within 2 h. Subtract the average A_{405} of the two wells containing *Buffer A* for each *Standard solution* and *Sample solution* to give the corrected A_{405} values. Using the least-squares method of linear regression, generate a standard curve from the activities of the *Standard solutions* (IU/mL) and their corresponding corrected absorbance values. Calculate prekallikrein activities of samples from the calibration curve of the standards. To obtain the corrected activity in IU/mL, multiply each activity by the sample's dilution factor.

ADDITIONAL REQUIREMENTS
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Prekallikrein Activator RS

⟨171⟩ VITAMIN B_{12} ACTIVITY ASSAY

ASSAY
• **PROCEDURE**
Assay preparation: Place a suitable quantity of the material to be assayed, previously reduced to a fine powder if necessary and accurately measured or weighed, in an appropriate vessel containing, for each g or mL of material taken, 25 mL of an aqueous extracting solution prepared just prior to use to contain, in each 100 mL, 1.29 g of disodium phosphate, 1.1 g of anhydrous citric acid, and 1.0 g of sodium metabisulfite. Autoclave the mixture at 121° for 10 minutes. Allow any undissolved particles of the extract to settle, and filter or centrifuge, if necessary. Dilute an aliquot of the clear solution with water so that the final test solution contains vitamin B_{12} activity approximately equivalent to that of the *Standard cyanocobalamin solution* which is added to the assay tubes.
Standard cyanocobalamin stock solution: To a suitable quantity of USP Cyanocobalamin (Crystalline) RS, accurately weighed, add sufficient 25 percent alcohol to make a solution having a known concentration of 1.0 µg of cyanocobalamin per mL. Store in a refrigerator.
Standard cyanocobalamin solution: Dilute a suitable volume of *Standard cyanocobalamin stock solution* with water to a measured volume such that after the incubation period as described for *Procedure*, the difference in transmittance between the inoculated blank and the 5.0-mL level of the *Standard cyanocobalamin solution* is not less than that which corresponds to a difference of 1.25 mg in dried cell weight. This concentration usually falls between 0.01 ng and 0.04 ng per mL of *Standard cyanocobalamin solution*. Prepare a fresh standard solution for each assay.

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Basal medium stock solution: Prepare the medium according to the following formula and directions. A dehydrated mixture containing the same ingredients may be used provided that, when constituted as directed in the labeling, it yields a medium comparable to that obtained from the formula given herein.

Add the ingredients in the order listed, carefully dissolving the cystine and tryptophane in the hydrochloric acid before adding the next eight solutions in the resulting solution. Add 100 mL of water, mix, and dissolve the dextrose, sodium acetate, and ascorbic acid. Filter, if necessary, add the polysorbate 80 solution, adjust the solution to a pH between 5.5 and 6.0 with 1 N sodium hydroxide, and add purified water to make 250 mL.

L-Cystine	0.1 g
L-Tryptophane	0.05 g
1 *N* Hydrochloric Acid	10 mL
Adenine–Guanine–Uracil Solution	5 mL
Xanthine Solution	5 mL
Vitamin Solution I	10 mL
Vitamin Solution II	10 mL
Salt Solution A	5 mL
Salt Solution B	5 mL
Asparagine Solution	5 mL
Acid-hydrolyzed Casein Solution	25 mL
Dextrose, Anhydrous	10 g
Sodium Acetate, Anhydrous	5 g
Ascorbic Acid	1 g
Polysorbate 80 Solution	5 mL

Acid-hydrolyzed casein solution: Prepare as directed under *Calcium Pantothenate Assay* ⟨91⟩.

Asparagine solution: Dissolve 2.0 g of L-asparagine in water to make 200 mL. Store under toluene in a refrigerator.

Adenine–guanine–uracil solution: Prepare as directed under *Calcium Pantothenate Assay* ⟨91⟩.

Xanthine solution: Suspend 0.20 g of xanthine in 30 mL to 40 mL of water, heat to about 70°, add 6.0 mL of 6 N ammonium hydroxide, and stir until the solid is dissolved. Cool, and add water to make 200 mL. Store under toluene in a refrigerator.

Salt solution A: Dissolve 10 g of monobasic potassium phosphate and 10 g of dibasic potassium phosphate in water to make 200 mL. Add 2 drops of hydrochloric acid, and store under toluene.

Salt solution B: Dissolve 4.0 g of magnesium sulfate, 0.20 g of sodium chloride, 0.20 g of ferrous sulfate, and 0.20 g of manganese sulfate in water to make 200 mL. Add 2 drops of hydrochloric acid, and store under toluene.

Polysorbate 80 solution: Dissolve 20 g of polysorbate 80 in alcohol to make 200 mL. Store in a refrigerator.

Vitamin solution I: Dissolve 10 mg of riboflavin, 10 mg of thiamine hydrochloride, 100 µg of biotin, and 20 mg of niacin in 0.02 N glacial acetic acid to make 400 mL. Store, protected from light, under toluene in a refrigerator.

Vitamin solution II: Dissolve 20 mg of para-aminobenzoic acid, 10 mg of calcium pantothenate, 40 mg of pyridoxine hydrochloride, 40 mg of pyridoxal hydrochloride, 8 mg of pyridoxamine dihydrochloride, and 2 mg of folic acid in dilute neutralized alcohol (1 in 4) to make 400 mL. Store, protected from light, in a refrigerator.

Tomato juice preparation: Centrifuge commercially canned tomato juice so that most of the pulp is removed. Suspend about 5 g per L of analytical filter-aid in the supernatant, and filter, with the aid of reduced pressure, through a layer of the filter-aid. Repeat, if necessary, until a clear, straw-colored filtrate is obtained. Store under toluene in a refrigerator.

Culture medium: [NOTE—A dehydrated mixture containing the same ingredients may be used provided that, when constituted as directed in the labeling, it yields a medium equivalent to that obtained from the formula given herein.] Dissolve 0.75 g of water-soluble yeast extract, 0.75 g of dried peptone, 1.0 g of anhydrous dextrose, and 0.20 g of potassium biphosphate in 60 mL to 70 mL of water. Add 10 mL of *Tomato juice preparation* and 1 mL of *Polysorbate 80 solution*. Adjust the solution with 1 N sodium hydroxide to a pH of 6.8, and add water to make 100 mL. Place 10-mL portions of the solution in test tubes, and plug with cotton. Sterilize the tubes and contents in an autoclave at 121° for 15 minutes. Cool as rapidly as possible to avoid color formation resulting from overheating the medium.

Suspension medium: Dilute a measured volume of *Basal medium stock solution* with an equal volume of water. Place 10-mL portions of the diluted medium in test tubes. Sterilize, and cool as directed above for the *Culture medium*.

Stock culture of *Lactobacillus leichmannii*: To 100 mL of *Culture medium* add 1.0 g to 1.5 g of agar, and heat the mixture, with stirring, on a steam bath, until the agar dissolves. Place approximately 10-mL portions of the hot solution in test tubes, cover the tubes suitably, sterilize at 121° for 15 minutes in an autoclave (exhaust line temperature), and allow the tubes to cool in an upright position. Inoculate three or more of the tubes, by stab transfer of a pure culture of *Lactobacillus leichmannii*.* (Before first using a fresh culture in this assay, make not fewer than 10 successive transfers of the culture in a 2-week period.) Incubate 16 to 24 hours at any selected temperature between 30° and 40° but held constant to within ±0.5°, and finally store in a refrigerator.

* Pure cultures of *Lactobacillus leichmannii* may be obtained as No. 7830 from the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

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Prepare fresh stab cultures at least three times each week, and do not use them for preparing the inoculum if more than 4 days old. The activity of the microorganism can be increased by daily or twice-daily transfer of the stab culture, to the point where definite turbidity in the liquid inoculum can be observed 2 to 4 hours after inoculation. A slow-growing culture seldom gives a suitable response curve, and may lead to erratic results.

Inoculum: [NOTE—A frozen suspension of *Lactobacillus leichmannii* may be used as the stock culture, provided it yields an inoculum comparable to a fresh culture.] Make a transfer of cells from the *Stock culture of Lactobacillus leichmannii* to 2 sterile tubes containing 10 mL of the *Culture medium* each. Incubate these cultures for 16 to 24 hours at any selected temperature between 30° and 40° but held constant to within ±0.5°. Under aseptic conditions, centrifuge the cultures, and decant the supernatant. Suspend the cells from the culture in 5 mL of sterile *Suspension medium*, and combine. Using sterile *Suspension medium*, adjust the volume so that a 1 in 20 dilution in saline TS produces 70% transmittance when read on a suitable spectrophotometer that has been set at a wavelength of 530 nm, equipped with a 10-mm cell, and read against saline TS set at 100% transmittance. Prepare a 1 in 400 dilution of the adjusted suspension using *Basal medium stock solution*, and use it for the test inoculum. (This dilution may be altered, when necessary, to obtain the desired test response.)

Calibration of spectrophotometer: Check the wavelength of the spectrophotometer periodically, using a standard wavelength cell or other suitable device. Before reading any tests, calibrate the spectrophotometer for 0% and 100% transmittance, using water and with the wavelength set at 530 nm.

Procedure: Cleanse meticulously by suitable means, followed preferably by heating at 250° for 2 hours, hard-glass test tubes, about 20 mm × 150 mm in size, and other necessary glassware because of the high sensitivity of the test organism to minute amounts of vitamin B_{12} activity and to traces of many cleansing agents.

To test tubes add, in duplicate, 1.0 mL, 1.5 mL, 2.0 mL, 3.0 mL, 4.0 mL, and 5.0 mL, respectively, of the *Standard cyanocobalamin solution*. To each of these tubes and to four similar empty tubes add 5.0 mL of *Basal medium stock solution* and water to make 10 mL.

To similar test tubes add, in duplicate, respectively, 1.0 mL, 1.5 mL, 2.0 mL, 3.0 mL, and 4.0 mL of the *Assay preparation*. To each tube add 5.0 mL of *Basal medium stock solution* and water to make 10 mL. Place one complete set of standard and assay tubes together in one tube rack and the duplicate set in a second rack or section of a rack, preferably in random order.

Cover the tubes suitably to prevent bacterial contamination, and sterilize the tubes and contents in an autoclave at 121° for 5 minutes, arranging to reach this temperature in not more than 10 minutes by preheating the autoclave, if necessary. Cool as rapidly as practicable to avoid color formation resulting from overheating the medium. Take precautions to maintain uniformity of sterilizing and cooling conditions throughout the assay, since packing tubes too closely in the autoclave, or overloading it, may cause variation in the heating rate.

Aseptically add 0.5 mL of *Inoculum* to each tube so prepared, except two of the four containing no *Standard cyanocobalamin solution* (the uninoculated blanks). Incubate the tubes at a temperature between 30° and 40° held constant to within ±0.5°, for 16 to 24 hours.

Terminate growth by heating to a temperature not lower than 80° for 5 minutes. Cool to room temperature. After agitating its contents, place the container in a spectrophotometer that has been set at a wavelength of 530 nm, and read the transmittance when a steady state is reached. This steady state is observed a few seconds after agitation when the reading remains constant for 30 seconds or more. Allow approximately the same time interval for the reading on each tube.

With the transmittance set at 100% for the uninoculated blank, read the transmittance of the inoculated blank. If the difference is greater than 5% or if there is evidence of contamination with a foreign microorganism, disregard the results of the assay.

With the transmittance set at 100% for the uninoculated blank, read the transmittance of each of the remaining tubes. Disregard the results of the assay if the slope of the standard curve indicates a problem with sensitivity.

Calculation: Prepare a standard concentration-response curve by the following procedure. Test for and replace any aberrant individual transmittances. For each level of the standard, calculate the response from the sum of the duplicate values of the transmittances (Σ) as the difference, $y = 2.00 - \Sigma$. Plot this response on the ordinate of cross-section paper against the logarithm of the mL of *Standard cyanocobalamin solution* per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, y, adding together the two transmittances for each level of the *Assay preparation*. Read from the standard curve the logarithm of the volume of the *Standard preparation* corresponding to each of those values of y that falls within the range of the lowest and highest points plotted for the standard. Subtract from each logarithm so obtained the logarithm of the volume, in mL, of the *Assay preparation* to obtain the difference, x, for each dosage level. Average the values of x for each of three or more dosage levels to obtain $\bar{x} = M'$, the log-relative potency of the *Assay preparation*. Determine the quantity, in µg, of USP Cyanocobalamin (Crystalline) RS corresponding to the cyanocobalamin in the portion of material taken for assay by the equation:

$$\text{antilog } M = \text{antilog } (M' + \log R)$$

R = number of µg of cyanocobalamin that was assumed to be present in each mg (or capsule or tablet) of the material taken for assay

Replication: Repeat the entire determination at least once, using separately prepared *Assay preparations*. If the difference between the two log potencies M is not greater than 0.08, their mean, $\bar{M}$, is the assayed log-potency of the test material (see *Vitamin B_{12} Activity Assay* under *Design and Analysis of Biological Assays* ⟨111⟩). If the two determinations differ by more than 0.08, conduct one or more additional determinations. From the mean of two or more values of *M* that do not differ by more than 0.15, compute the mean potency of the preparation under assay.

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ADDITIONAL REQUIREMENTS
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cyanocobalamin (Crystalline) RS

Chemical Tests and Assays

IDENTIFICATION TESTS

⟨181⟩ IDENTIFICATION—ORGANIC NITROGENOUS BASES

INTRODUCTION
The purpose of this test is the identification of tertiary amine compounds. This spectroscopic test has a limited degree of specificity and, therefore, the conformance with all additional identification tests listed in a particular monograph is necessary to ensure the identity of the specimen under examination.

ASSAY
- **PROCEDURE**

Standard solution: In a separator dissolve 50 mg of the corresponding USP Reference Standard in 25 mL of 0.01 N hydrochloric acid.

Sample solution: Depending upon the nature of the sample, dissolve 50 mg of the bulk substance under test in 25 mL of 0.01 N hydrochloric acid, or shake a quantity of powdered tablets or the contents of capsules, equivalent to 50 mg of the substance, with 25 mL of 0.01 N hydrochloric acid for 10 min. Transfer the liquid to a separator, filtering if necessary, and washing the filter and the residue with several small portions of water.

Instrumental conditions
(See *Mid-Infrared Spectroscopy* ⟨854⟩.)
Mode: IR
Wavelength range: 7–15 µm (1430 cm^{-1} to 650 cm^{-1})
Cell: 1-mm
Blank: Carbon disulfide

Analysis
Samples: *Standard solution* and *Sample solution*
Treat each solution as follows: Add 2 mL of 1 N sodium hydroxide and 4 mL of carbon disulfide, and shake for 2 min. Centrifuge if necessary to clarify the lower phase, and pass it through a dry filter, collecting the filtrate in a small flask provided with a glass stopper. Determine the absorption spectra of the filtered *Standard solution* and *Sample solution* without delay.

Acceptance criteria: The spectrum of the *Sample solution* must show all of the significant absorption bands present in the spectrum of the *Standard solution*.

⟨191⟩ IDENTIFICATION TESTS—GENERAL

INTRODUCTION
Procedures in this chapter are referenced in monographs for the identification of official articles and their components. Any acids, bases, or other reagents used in these procedures should not interfere with the results. Volumes may be scaled proportionally unless otherwise indicated. All of the tests include approximate amounts, except where specified.

Instrumental techniques described in this chapter may be used in lieu of chemical identification tests. Those instrumental techniques are not exhaustive and other techniques, such as nuclear magnetic resonance, ion-selective electrodes, and near-infrared, may be used in lieu of a chemical identification test provided that they are suitable and validated.

Unless otherwise specified in the monograph, if a chemical identification test is selected for an ion, then all chemical test procedures listed for the ion shall be met. If an instrumental identification test is selected, then only one instrumental technique is required for the ion(s).

CHEMICAL IDENTIFICATION TESTS

Acetate
- **A.** Dissolve about 30 mg of the substance to be examined in 3 mL of water, or use 3 mL of the prescribed solution. Adjust the pH of the solution with sodium hydroxide to be slightly alkaline. Add 0.25 mL of lanthanum nitrate test solution (TS). If a white precipitate is formed, filter the solution. Add successively 0.1 mL of iodine and potassium iodide TS 3, and 0.1 mL of ammonia TS 2 to the solution. If no blue color is observed, heat carefully to boiling. In the presence of acetates, a dark color develops or a blue precipitate is formed.
- **B.** With neutral solutions of acetates, ferric chloride TS produces a red color that is destroyed by the addition of mineral acids.

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Aluminum
- **A.** By using 6 N ammonium hydroxide, solutions of aluminum salts yield a gelatinous, white precipitate that is insoluble in an excess of 6 N ammonium hydroxide.
- **B.** 1 N sodium hydroxide or sodium sulfide TS with solutions of aluminum salts produces a similar gelatinous, white precipitate, which dissolves in an excess of either of the same reagents.

Ammonium
- **A.** Add 0.2 g of magnesium oxide to the solution under test. Pass a current of air through the mixture, and direct the gas that escapes to just beneath the surface of the indicator solution prepared previously by mixing 1 mL of 0.1 M hydrochloric acid and 0.05 mL of methyl red TS 2. In the presence of ammonium, the color of the indicator solution is changed to yellow. After directing the gas into the indicator solution for a sufficient period of time, add 1 mL of freshly prepared sodium cobaltinitrite TS to the indicator solution. Upon the addition of the sodium cobaltinitrite TS, a yellow precipitate is formed when ammonium is present.

Antimony
- **A.** With hydrogen sulfide, solutions of antimony(III) compounds, strongly acidified with hydrochloric acid, yield an orange precipitate of antimony sulfide that is insoluble in 6 N ammonium hydroxide but is soluble in ammonium sulfide TS.

Barium
- **A.** Solutions of barium salts yield a white precipitate with the addition of 2 N sulfuric acid. This precipitate is insoluble in hydrochloric acid and in nitric acid.

Benzoate
- **A.** In neutral solutions, benzoates yield a salmon-colored precipitate with ferric chloride TS.
- **B.** In moderately concentrated solutions, benzoates yield a precipitate of benzoic acid upon acidification when 2 N sulfuric acid is added. This precipitate is readily soluble in ethyl ether.

Bicarbonate
- **A.** Bicarbonates effervesce with acids, evolving a colorless gas that, when passed into calcium hydroxide TS, produces a white precipitate immediately.
- **B.** A cold solution (1:20) of a soluble bicarbonate or the solution prescribed in the specific monograph remains unchanged or is only slightly colored by phenolphthalein TS.

Bismuth
- **A.** When dissolved in a slight excess of nitric acid or hydrochloric acid, bismuth salts yield a white precipitate upon dilution with water. This precipitate is colored brown by hydrogen sulfide, and the resulting compound dissolves in a warm mixture of equal parts of nitric acid and water.

Bisulfite See *Sulfite*.

Borate
- **A.** To 1 mL of a borate solution acidified with hydrochloric acid, add 3 or 4 drops of iodine TS and 3 or 4 drops of polyvinyl alcohol solution (1:50): an intense blue color is produced.

Bromide
- **A.** Solutions of bromides, upon the addition of chlorine TS dropwise, liberate bromine, which is dissolved by shaking with chloroform, coloring the chloroform red-to-reddish brown.
- **B.** Silver nitrate TS produces, in solutions of bromides, a yellowish-white precipitate that is insoluble in nitric acid and is slightly soluble when 6 N ammonium hydroxide is added.

Calcium
- **A.** Solutions of calcium salts form insoluble oxalates when treated as follows. To a solution of the calcium salt (1:20) or the solution prescribed in the specific monograph, add 2 drops of methyl red TS and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid dropwise, until the solution is acid to the indicator. Upon the addition of ammonium oxalate TS, a white precipitate is formed. This precipitate is insoluble when 6 N acetic acid is added but dissolves in hydrochloric acid.

Carbonate
- **A.** Carbonates effervesce with acids, evolving a colorless gas that, when passed into calcium hydroxide TS, produces a white precipitate immediately.
- **B.** A cold solution (1:20) of a soluble carbonate or the solution prescribed in the specific monograph is colored red by phenolphthalein TS, whereas a similar solution of a bicarbonate remains unchanged or is only slightly colored.

Chlorate
- **A.** Solutions of chlorates yield no precipitate with silver nitrate TS. The addition of sulfurous acid to this mixture produces a white precipitate that is insoluble in nitric acid but is soluble in 6 N ammonium hydroxide.
- **B.** Upon ignition, chlorates yield chlorides, recognizable by appropriate tests.
- **C.** When sulfuric acid is added to a dry chlorate, decrepitation occurs and a greenish-yellow gas is evolved. [**CAUTION**—Use only a small amount of chlorate for this test, and exercise extreme caution in performing it.]

Chloride
- **A.** With silver nitrate TS, solutions of chlorides yield a white, curdy precipitate that is insoluble in nitric acid but is soluble in a slight excess of 6 N ammonium hydroxide.
- **B.** When testing amine (including alkaloidal) hydrochlorides that do not respond to the above test, add 1 drop of diluted nitric acid and 0.5 mL of silver nitrate TS to a solution of the substance being examined containing, unless otherwise directed in the monograph, about 2 mg of chloride ion in 2 mL: a white, curdy precipitate is formed. Centrifuge the mixture without delay, and decant the supernatant layer. Wash the precipitate with three 1-mL portions of nitric acid solution (1:100), and discard the washings. Add ammonia TS dropwise to this precipitate. It dissolves readily.
- **C.** When a monograph specifies that an article responds to the test for dry chlorides, mix the solid to be tested with an equal weight of manganese dioxide, moisten with sulfuric acid, and gently heat the mixture: chlorine, which is recognizable by the production of a blue color with moistened starch iodide paper, is evolved.

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Citrate
- **A.** To 15 mL of pyridine add a solution or suspension of a few mg of a citrate salt in 1 mL of water or the solution prescribed in the specific monograph, and shake. To this mixture add 5 mL of acetic anhydride, and shake: a light red color is produced.

Cobalt
- **A.** Solutions of cobalt salts (1:20) in 3 N hydrochloric acid or the solution prescribed in the specific monograph yield a red precipitate when heated on a steam bath with an equal volume of a hot, freshly prepared solution of 1-nitroso-2-naphthol (1:10) in 9 N acetic acid.
- **B.** Solutions of cobalt salts, when saturated with potassium chloride and treated with potassium nitrite and acetic acid, yield a yellow precipitate.

Copper
- **A.** Cupric compounds, acidified with hydrochloric acid, deposit a red film of metallic copper upon a bright, untarnished surface of metallic iron.
- **B.** An excess of 6 N ammonium hydroxide, when added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue-colored solution.
- **C.** With potassium ferrocyanide TS, solutions of cupric salts yield a reddish-brown precipitate, insoluble in diluted acids.

Hypophosphite
- **A.** Hypophosphites in solution yield a white precipitate with mercuric chloride TS. This precipitate becomes gray when an excess of hypophosphite is present.
- **B.** Solutions of hypophosphites, acidified with sulfuric acid and warmed with cupric sulfate TS, yield a red precipitate.

Iodide
- **A.** Solutions of iodides, upon the addition of chlorine TS dropwise, liberate iodine, which colors the solution from yellow to red. When the solution is shaken with chloroform, the solution is colored violet. The iodine thus liberated gives a blue color with starch TS.
- **B.** Silver nitrate TS produces, in solutions of iodides, a yellow, curdy precipitate that is insoluble in nitric acid and insoluble in 6 N ammonium hydroxide.

Iron
- **A.** Ferrous and ferric compounds in solution yield a black precipitate with ammonium sulfide TS. This precipitate dissolves in cold, 3 N hydrochloric acid and evolves hydrogen sulfide.

  Ferric salts
  - **A.** Acid solutions of ferric salts yield a dark blue precipitate with potassium ferrocyanide TS.
  - **B.** With an excess of 1 N sodium hydroxide added to the ferric salts solutions, a reddish-brown precipitate is formed.
  - **C.** With ammonium thiocyanate TS, solutions of ferric salts produce a deep red color that is not destroyed by dilute mineral acids.

  Ferrous salts
  - **A.** Solutions of ferrous salts yield a dark blue precipitate with potassium ferricyanide TS. This precipitate is insoluble in 3 N hydrochloric acid but is decomposed by 1 N sodium hydroxide.
  - **B.** With 1 N sodium hydroxide, solutions of ferrous salts yield a greenish-white precipitate; when shaken, the color changes rapidly to green and then to brown.

Lactate
- **A.** When solutions of lactates are acidified with sulfuric acid, potassium permanganate TS is added; the mixture is heated, and acetaldehyde evolves. Acetaldehyde can be detected by allowing the vapor to come into contact with a filter paper that has been moistened with a freshly prepared mixture of equal volumes of 20% aqueous morpholine and sodium nitroferricyanide TS: a blue color is produced.

Lead
- **A.** With 2 N sulfuric acid, solutions of lead salts yield a white precipitate that is insoluble in 3 N hydrochloric or 2 N nitric acid but is soluble in warm 1 N sodium hydroxide and in ammonium acetate TS.
- **B.** With potassium chromate TS, solutions of lead salts, free or nearly free from mineral acids, yield a yellow precipitate that is insoluble in 6 N acetic acid but is soluble in 1 N sodium hydroxide.

Lithium
- **A.** With sodium carbonate TS, moderately concentrated solutions of lithium salts, made alkaline with sodium hydroxide, yield a white precipitate on boiling. The precipitate is soluble in ammonium chloride TS.
- **B.** Solutions of lithium salts are not precipitated by 2 N sulfuric acid or soluble sulfates (distinction from strontium).

Magnesium
- **A.** Solutions of magnesium salts in the presence of ammonium chloride yield not more than a slightly hazy precipitate when neutralized with ammonium carbonate TS, but on the subsequent addition of dibasic sodium phosphate TS, a white, crystalline precipitate, which is insoluble in 6 N ammonium hydroxide, is formed.

Manganese
- **A.** With ammonium sulfide TS, solutions of manganous salts yield a salmon-colored precipitate that dissolves in acetic acid.

Mercury
- **A.** When applied to bright copper foil, solutions of mercury salts, free from an excess of nitric acid, yield a deposit that, upon rubbing, becomes bright and silvery in appearance.
- **B.** With hydrogen sulfide, solutions of mercury compounds yield a black precipitate that is insoluble in ammonium sulfide TS and in boiling 2 N nitric acid.

  Mercuric salts
  - **A.** Solutions of mercuric salts yield a yellow precipitate with 1 N sodium hydroxide.
  - **B.** Solutions of mercuric salts yield, in neutral solutions with potassium iodide TS, a scarlet precipitate that is very soluble in an excess of the reagent.

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Mercurous salts

- **A.** Mercurous compounds are decomposed by 1 N sodium hydroxide, producing a black color.
- **B.** With hydrochloric acid, solutions of mercurous salts yield a white precipitate that is blackened by the addition of 6 N ammonium hydroxide.
- **C.** With potassium iodide TS, a yellow precipitate, which may become green upon standing, is formed.

Nitrate

- **A.** When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture is cooled, and a solution of ferrous sulfate is superimposed; a brown color is produced at the junction of the two liquids.
- **B.** When a nitrate is heated with sulfuric acid and metallic copper, brownish-red fumes evolve.
- **C.** Nitrates do not decolorize acidified potassium permanganate TS (distinction from nitrites).

Nitrite

- **A.** When treated with dilute mineral acids or with 6 N acetic acid, nitrites evolve brownish-red fumes. The solution colors starch–iodide paper blue.

Oxalate

- **A.** Neutral and alkaline solutions of oxalates yield a white precipitate with calcium chloride TS. This precipitate is insoluble in 6 N acetic acid but is dissolved by hydrochloric acid.
- **B.** Hot acidified solutions of oxalates decolorize potassium permanganate TS.

Permanganate

- **A.** Solutions of permanganates acidified with sulfuric acid are decolorized by hydrogen peroxide TS and by sodium bisulfite TS, in the cold, and by oxalic acid TS in a hot solution.

Peroxide

- **A.** Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color upon the addition of potassium dichromate TS. On shaking the mixture with an equal volume of ethyl ether and allowing the liquids to separate, the blue color is found in the ethyl ether layer.

Phosphate

[NOTE—Where the monograph specifies the identification test for *Phosphate*, use the tests for orthophosphates, unless the instructions specify the use of the pyrophosphate tests or indicate that the product is to be ignited before performing the test.]

Orthophosphates

- **A.** With silver nitrate TS, neutral solutions of orthophosphates yield a yellow precipitate that is soluble in 2 N nitric acid and in 6 N ammonium hydroxide.
- **B.** With ammonium molybdate TS, acidified solutions of orthophosphates yield a yellow precipitate that is soluble in 6 N ammonium hydroxide. This precipitate may be slow to form.

Pyrophosphates

- **A.** With silver nitrate TS, pyrophosphates obtained by ignition yield a white precipitate that is soluble in 2 N nitric acid and soluble in 6 N ammonium hydroxide.
- **B.** With ammonium molybdate TS, pyrophosphates obtained by ignition yield a yellow precipitate that is soluble in 6 N ammonium hydroxide.

Potassium

- **A.** In neutral, concentrated, or moderately concentrated solutions of potassium salts (depending upon the solubility and the potassium content), sodium bitartrate TS produces a white crystalline precipitate that is soluble in 6 N ammonium hydroxide and in solutions of alkali hydroxides and carbonates. The formation of the precipitate, which is usually slow, is accelerated by stirring or rubbing the inside of the test tube with a glass rod. The addition of a small amount of glacial acetic acid or alcohol also promotes the precipitation.

Salicylate

- **A.** In moderately concentrated solutions of salicylates, ferric chloride TS produces a violet color.
- **B.** Addition of acids to moderately concentrated solutions of salicylates produces a white, crystalline precipitate of salicylic acid that melts between 158° and 161°.

Silver

- **A.** With hydrochloric acid, solutions of silver salts yield a white, curdy precipitate that is insoluble in nitric acid but is readily soluble in 6 N ammonium hydroxide.
- **B.** A solution of a silver salt, to which 6 N ammonium hydroxide and a small quantity of formaldehyde TS are added, deposits, upon warming, a mirror of metallic silver upon the sides of the container.

Sodium

- **A.** Unless otherwise specified in an individual monograph, prepare a solution to contain 0.1 g of the sodium compound in 2 mL of water. Add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate TS, and heat to boiling. Allow to cool in ice water and, if necessary, rub the inside of the test tube with a glass rod. A dense precipitate is formed.

Sulfate

- **A.** With barium chloride TS, solutions of sulfates yield a white precipitate that is insoluble in hydrochloric acid and in nitric acid.
- **B.** With lead acetate TS, neutral solutions of sulfates yield a white precipitate that is soluble in ammonium acetate TS.
- **C.** Hydrochloric acid produces no precipitate when added to solutions of sulfates (distinction from thiosulfates).

Sulfite

- **A.** When treated with 3 N hydrochloric acid, sulfites and bisulfites yield sulfur dioxide, which blackens filter paper moistened with mercurous nitrate TS.

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Tartrate

• **A.** Dissolve a few mg of a tartrate salt in 2 drops of sodium metaperiodate solution (1:20). Add 1 drop of 1 N sulfuric acid and after 5 min add a few drops of sulfurous acid, followed by a few drops of fuchsin-sulfurous acid TS: a reddish-pink color is produced within 15 min.

Thiocyanate

• **A.** With ferric chloride TS, solutions of thiocyanates yield a red color that is not destroyed by moderately concentrated mineral acids.

Thiosulfate

• **A.** With hydrochloric acid, solutions of thiosulfates yield a white precipitate that soon turns yellow, and evolve sulfur dioxide, which blackens filter paper moistened with mercurous nitrate TS.

• **B.** The addition of ferric chloride TS to solutions of thiosulfates produces a dark violet color that disappears quickly.

Zinc

• **A.** In the presence of sodium acetate, solutions of zinc salts yield a white precipitate with hydrogen sulfide. This precipitate is insoluble in acetic acid but is dissolved by 3 N hydrochloric acid.

• **B.** Ammonium sulfide TS produces a similar precipitate in neutral and in alkaline solutions.

• **C.** With potassium ferrocyanide TS, zinc salts in solution yield a white precipitate that is insoluble in 3 N hydrochloric acid.

INSTRUMENTAL IDENTIFICATION TESTS

Instrumental techniques described in this section may be used in lieu of procedures described in *Chemical Identification Tests*. Instrumental techniques provide flexibility in the choice of identification tests. All instrumental techniques shall follow method validation procedures for identification tests (see *Validation of Compendial Procedures* ⟨1225⟩, *Validation, Data Elements Required for Validation, Category IV*). *Instrumental Identification Tests* must demonstrate specificity. In addition, other suitable, validated instrumental techniques may be used.

The selection of the appropriate sample preparation depends on the material under test and must be appropriate for the technique being used. The analyst may use any of the following preparation procedures, with the appropriate verification. An electronic library spectrum of the Reference Standard may be used in comparison to the test sample provided adequate specificity is maintained. When using solvents, the solvent must be free of interfering species. Use USP Reference Standards where available (see *General Notices, 5.80 USP Reference Standards*).

• IDENTIFICATION USING X-RAY FLUORESCENCE SPECTROMETRY

X-ray fluorescence spectrometry (XRF) can typically be used for identification of elements with atomic numbers from magnesium through uranium. The actual range of elements for which the instrument is capable is dependent on the instrument design. Guidance regarding the use of XRF may be found in *X-Ray Fluorescence Spectrometry* ⟨735⟩.

Sample preparation

Powders/solids: Powders and solids can be used without further manipulation, or material can be made into pellets as described in ⟨735⟩.

Neat liquids: Liquids can be used without further manipulation, provided the liquid is a single phase, is compatible with the sample holder, and has sufficiently low volatility.

Samples in solution: Samples may be dissolved in an appropriate solvent.

Procedure

Blank: For *Powders/solids* and *Neat liquids*, prepare a blank sample holder. For *Samples in solution*, use the solvent as a blank.

Reference standard and **Sample:** Prepare each *Reference standard* and *Sample* by using sufficient material as required for the specific manufacturer's instrumentation.

Analysis: Analyze the *Blank, Reference standard,* and *Sample* according to the manufacturer's suggestions for the specific instrument. If there is any interference, use the *Blank* to make the correction. The spectrum of the *Reference standard* exhibits radiation characteristic of the element(s) under investigation. The energy bands of the *Sample* exhibit radiation characteristic of the element(s) under investigation and compare qualitatively with that of the *Reference standard*.

• IDENTIFICATION USING ATOMIC SPECTROSCOPY TECHNIQUES: ATOMIC ABSORPTION SPECTROSCOPY, INDUCTIVELY COUPLED PLASMA–OPTICAL EMISSION SPECTROSCOPY, INDUCTIVELY COUPLED PLASMA–MASS SPECTROMETRY

Atomic absorption (AA) spectroscopy techniques can be used for the identification of many elements. Guidance regarding the use of AA spectroscopy may be found in *Atomic Absorption Spectroscopy* ⟨852⟩ . Guidance regarding the use of inductively coupled plasma–optical emission spectroscopy (ICP–OES) or inductively coupled plasma–mass spectrometry (ICP–MS) may be found in *Plasma Spectrochemistry* ⟨730⟩.

Sample preparation: Samples should be dissolved in an appropriate solvent. If dissolving the sample is not possible, digestion may be necessary.

Procedure

Blank: Prepare an appropriate solution that does not contain the analyte(s) of interest and is compatible with the technique being used.

Standard solution: Standards must contain the analyte of interest. All solutions should be matrix-matched where possible.

Sample solution: Prepare the sample in the same solvent as the standard.

Analysis: Analyze the *Blank, Standard solution,* and *Sample solution* according to the manufacturer's suggestions for the specific instrument. When a wavelength-based technique is used, wherever possible select at least two wavelengths characteristic of the analyte(s) of interest. When a mass-based technique is used, wherever possible select at least two isotopes characteristic of the analyte(s) of interest. If two isotopes are unavailable, or if an element is monoisotopic, it may be possible to monitor the oxide of the analyte(s) ($m + 16$), if one is formed. If no oxide is formed, or if multiple isotopes cannot be evaluated because of interferences or because of a limited number of isotopes, then a different technique may be required. If interfering elements are present in the sample solution, wavelengths or masses examined must be selected to unequivocally identify the analyte(s) of interest.

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• **IDENTIFICATION USING ION CHROMATOGRAPHY**

Ion chromatography (IC) can be used to identify a number of anions and cations found in drug substances (for additional information, see *Ion Chromatography* ⟨1065⟩).

Apparatus: Analyte detection will typically utilize suppressed conductivity, although other detection methods are possible, depending on the analyte (see ⟨1065⟩). The ion-exchange column must be capable of separating the analyte from any other ions of the same charge known to be in the sample at a concentration ≥5% of the analyte.

Procedure

Blank: Use the sample solvent as a blank.

Standard and **Sample solutions:** Dissolve or dilute the sample in water. Other solvents can be used if compatible with the IC column.

Analysis: Analyze an equal volume of the *Blank*, Reference Standard, and sample (according to the manufacturer's suggestions for the specific instrument and column dimensions). The counterion is identified if a peak in the sample has the same retention time as the peak in the Reference Standard, and there is not a peak of ≥5% of its size at the same retention time in the *Blank*.

• **IDENTIFICATION BY OTHER LIQUID CHROMATOGRAPHY TECHNIQUES IN CHROMATOGRAPHY ⟨621⟩**

Some of the ions suitable for IC identification can also be identified by other forms of liquid chromatography. Sample and Reference Standard concentrations, as well as injection volumes, need to be adjusted, depending on the detection technique used. All high-concentration components in the test substance should be analyzed to judge whether there is interference with the analyte of interest.

• **IDENTIFICATION USING RAMAN SPECTROSCOPY**

Raman spectroscopy can be used for identification of counterions. Guidance regarding the use of Raman spectroscopy may be found in *Raman Spectroscopy* ⟨1120⟩.

Apparatus: Prepare the spectrometer for operation according to the instrument instruction manual and the instrument manufacturer's recommendations. An instrument performance check and the quality of spectra collected should be evaluated at time of use or following manufacturer's instructions.

Procedure

Reference standard and **Sample:** All reference material and sample spectra should be collected using identical instrumental parameters. These instrumental parameters may be determined by the analyst based on the nature of the sample and the type of analysis and should be selected based on the quality of spectra needed. Use the appropriate sample container and/or sample holding apparatus, depending on the type of reference material and sample (powder, liquid, paste, film, or other) being analyzed. Transfer the sample or reference material into the appropriate sample container and/or holder, as needed, and acquire the spectrum for each.

Analysis: Qualitatively compare the Raman spectra obtained from the reference material and the *Sample*. The *Sample* complies with the identity test if the spectrum exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard, where available.

Change to read:

• **IDENTIFICATION USING MID-INFRARED SPECTROSCOPY**

Mid-infrared spectroscopy techniques can be used for identification of counterions. Guidance regarding the use of mid-infrared spectroscopy may be found in *Mid-Infrared Spectroscopy* ⟨854⟩ and ▲*Spectroscopic Identification Tests* ⟨197⟩▲ (CN 1-May-2020). If the monograph contains an identification test by IR (e.g., ⟨197⟩) and a reference to ⟨191⟩, then mid-infrared cannot be used as an instrumental replacement for the chemical identification tests prescribed in this chapter.

Procedure

Standard and **Sample solutions:** Sample preparation may be conducted using any of the procedures described in ⟨854⟩ that are appropriate for the sample of concern. The standard preparation should be conducted in the same way but using the USP Reference Standard of the substance under test, where available.

Analysis: Record the spectra of the test specimen and the corresponding Reference Standard over the range from about 3800 to 650 cm^{-1} (2.6–15 µm). The IR absorption spectrum of the preparation of the test specimen, previously dried under conditions specified for the corresponding Reference Standard, unless the Reference Standard is to be used without drying, exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard, where available.

⟨193⟩ IDENTIFICATION—TETRACYCLINES

The following chromatographic procedures are provided to confirm the identity of Pharmacopeial drug substances that are of the tetracycline type, such as doxycycline, oxytetracycline, and tetracycline, and to confirm the identity of such compounds in their respective Pharmacopeial dosage forms. Two procedures are provided, one based on paper chromatography (*Method I*) and the other on thin-layer chromatography (*Method II*). *Method I* is to be used unless otherwise directed in the individual monograph.

PROCEDURES

Standard solution: Unless otherwise directed in the individual monograph, dissolve the USP Reference Standard for the drug substance being identified in the same solvent and at the same concentration as for the *Test solution*.

Test solution: Prepare as directed in the individual monograph.

• **METHOD I**

pH 3.5 Buffer: Dissolve 13.4 g of anhydrous citric acid and 16.3 g of dibasic sodium phosphate in 1000 mL of water, and mix.

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Developing solvent: On the day of use, mix 10 volumes of chloroform, 20 volumes of nitromethane, and 3 volumes of pyridine.

Mixed test solution: Mix equal volumes of the *Standard solution* and the *Test solution*.

Chromatographic sheet: Draw a spotting line 2.5 cm from one edge of a 20-cm × 20-cm sheet of filter paper (Whatman No. 1, or equivalent). Impregnate the sheet with *pH 3.5 Buffer* by passing it through a trough filled with *pH 3.5 Buffer*, and remove the excess solvent by firmly pressing the sheet between nonfluorescent blotting papers.

Procedure: To a suitable chromatographic chamber, prepared for ascending chromatography (see *Chromatography* ⟨621⟩) add *Developing solvent* to a depth of 0.6 cm. Apply at 1.5-cm intervals 2 µL each of the *Standard solution*, the *Test solution*, and the *Mixed test solution* to the spotting line of the *Chromatographic sheet*. Allow the sheet to dry partially, and while still damp place it in the chromatographic chamber with the bottom edge touching the *Developing solvent*. When the solvent front has risen about 10 cm, remove the sheet from the chamber, and expose the sheet to ammonia vapor. Examine the chromatogram under long-wavelength UV light. Record the positions of the major yellow fluorescent spots: the R_F value of the principal spot obtained from the *Test solution* and from the *Mixed test solution* corresponds to that obtained from the *Standard solution*.

• **METHOD II**

Resolution solution: Unless otherwise directed in the individual monograph, prepare a solution in methanol containing 0.5 mg each of USP Chlortetracycline Hydrochloride RS, USP Doxycycline Hyclate RS, USP Oxytetracycline RS, and USP Tetracycline Hydrochloride RS per mL.

Developing solvent: Prepare a mixture of 0.5 M oxalic acid, previously adjusted with ammonium hydroxide to a pH of 2.0, acetonitrile, and methanol (80:20:20).

Chromatographic plate: Use a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of octylsilanized chromatographic silica gel mixture. Activate the plate by heating it at 130° for 20 minutes, allow to cool, and use while still warm.

Procedure: Separately apply 1 µL each of the *Standard solution*, the *Test solution*, and the *Resolution solution* to the *Chromatographic plate*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Expose the plate to ammonia vapors for 5 minutes, and promptly locate the spots on the plate by viewing under long-wavelength UV light: the chromatogram of the *Resolution solution* shows clearly separated spots, and the principal spot obtained from the *Test solution* corresponds in R_F value, intensity, and appearance to that obtained from the *Standard solution*.

ADDITIONAL REQUIREMENTS

USP Reference Standards ⟨11⟩

USP Doxycycline Hyclate RS
USP Oxytetracycline RS
USP Tetracycline Hydrochloride RS

⟨197⟩ SPECTROSCOPIC IDENTIFICATION TESTS

(Use of the chapter title *Spectrophotometric Identification Tests* is permitted until May 1, 2020.)

Change to read:

INTRODUCTION AND SCOPE

This chapter provides several tests and procedures that are used to confirm the chemical identity of a specific material in its respective monograph. When one of the tests listed in this chapter is referenced in a monograph, it must be used to confirm the identity of the material. Alternative identification procedures may be used, provided they are demonstrated to be equivalent to or better than the specified procedure and if they meet the requirements specified in the *Equivalent/Alternative Tests* section.

Spectroscopic tests contribute meaningfully toward the identification of many official articles. The test procedures that follow are applicable to substances that absorb, transmit, reflect, or scatter electromagnetic radiation in the near-infrared (NIR), infrared (IR), visible or near-infrared (Raman), ultraviolet (UV), visible (Vis), or X-ray ranges (see *Mid-Infrared Spectroscopy* ⟨854⟩, *Ultraviolet-Visible Spectroscopy* ⟨857⟩, *X-Ray Powder Diffraction* ⟨941⟩, ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), and *Raman Spectroscopy* ⟨1120⟩. The NIR, IR, and Raman spectra, or X-ray diffraction pattern of a substance, compared with the spectrum or diffraction pattern obtained with the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. The UV or Vis absorption spectrum of a substance, on the other hand, does not exhibit a high degree of specificity in most cases. To provide unambiguous confirmation of the identity of a substance, it may be necessary to execute two (or more) identity tests, as specified in a large proportion of compendial monographs.

IDENTIFICATION METHODOLOGY

Suitable identification methodology must be used for the chemical identification of materials through comparison with the appropriate compendial standards. Where alternative techniques are used, it must be demonstrated that the alternative identification methodology is suitable for the intended application (see *Validation of Compendial Procedures* ⟨1225⟩). Identification procedures should be able to discriminate between materials similar in molecular structure. The lack of specificity

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of a single technique may be compensated by other supporting analytical procedure(s) or an application of an additional identification technique.

The IR spectrum of a substance, compared with that obtained using equivalent instruments and conditions for the corresponding USP Reference Standard, is the most widely used methodology for chemical identification in compendial monographs. In general practice, the analysis of the sample and the USP Reference Standard are completed at the same time; however, if the USP Reference Standard spectrum was obtained previously using equivalent instruments and conditions, it is appropriate to compare the sample spectrum with the stored USP Reference Standard spectrum.

Under conditions where the IR spectrum lacks specificity for definitive chemical identification, additional spectroscopic information can be used to supplement chemical identification. For example, conformance with both IR and UV test specifications, as specified in a large proportion of compendial monographs, provides complementary information for the definitive identity of the sample under examination. In these instances, the combined spectroscopic information enables discrimination between compounds similar in structure that would not be possible from either IR spectrum or UV spectrum alone.

For preparing the standards used in the applications of this chapter, unless the directions for preparing the USP Reference Standard are explicitly specified in the monograph procedure, the USP Reference Standard must be used in accordance with instructions on its label. For preparation of the test sample, the sample must be prepared in accordance with the directions in the individual monograph procedure. Where no specific instructions are provided for sample preparation, handle the sample in the same manner as described by the USP Reference Standard label. For example, dry the sample as per drying conditions on the label of the corresponding USP Reference Standard.

INFRARED SPECTROSCOPY

(Use of the section title *Infrared Absorption* is permitted until May 1, 2020.)

Several methods are indicated for the preparation of test samples and USP Reference Standards for analysis by infrared spectroscopy (see *Table 1* and ⟨854⟩). The approaches for the techniques used in IR identity testing are summarized in *Table 1*.

Table 1. Infrared Spectroscopy Sample Preparation Techniques[a]

Method Reference	Sample Preparation
⟨197A⟩	The substance under examination is intimately in contact with an internal reflection element for attenuated total reflection (ATR) analysis.
⟨197D⟩	The substance under examination is mixed intimately with potassium bromide and transferred to a sample container for diffuse reflection (DR) analysis.
⟨197E⟩	The substance under examination is pressed as a thin sample against a suitable plate for IR microscopic analysis.
⟨197F⟩	The substance under examination is a thin film of a neat liquid or semisolid between suitable (e.g., sodium chloride or potassium bromide) plates, or a thin microcrystalline or glassy film deposited from a solution or cooled from a melt.
⟨197K⟩	The substance under examination is mixed intimately with potassium bromide and compressed into a transparent pellet.
⟨197M⟩	The substance under examination (10–20 mg) is finely ground and dispersed in a drop of mulling agent (unless otherwise directed in the monograph, mineral oil is to be used).
⟨197S⟩	A solution of designated concentration is prepared in the solvent specified in the individual monograph. The solution is examined in 0.1-mm cells, unless a different cell path length is specified.

[a] Each of the techniques in the table can be used as alternative methods when any other technique from the table is required in the monograph (see *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*).

In each instance, infrared spectra of both the sample and corresponding USP Reference Standard are obtained using the same sample preparation technique and measurement parameters. Record and compare the spectra of the sample and the corresponding USP Reference Standard over the range from 3800 to 650 cm^{-1}, unless otherwise specified in the individual monograph. The comparison must establish that the IR spectrum of the preparation of the sample exhibits maxima only at the same wavenumbers as that of the appropriately prepared corresponding USP Reference Standard. If there are differences between the spectra, and the sample spectrum was compared with a previously obtained and electronically stored spectrum of the USP Reference Standard, the comparison must be repeated concomitantly with a freshly prepared USP Reference Standard.

Differences between the USP Reference Standard spectrum and sample spectrum that may be observed are sometimes attributable to differences in the solid-state form of the materials, if a solid-state technique is used (e.g., ⟨197A⟩, ⟨197K⟩, or ⟨197M⟩). If a specific crystal form is not specified in the monograph, where spectral differences between the sample and USP Reference Standard are observed, recrystallize both the sample and USP Reference Standard under identical conditions to produce the same solid-state form, unless specific procedures are provided in the individual monographs. Dissolve equal portions of the sample and the USP Reference Standard in equal volumes of a suitable solvent, evaporate the solutions to dryness in similar containers under identical conditions, and repeat the identification test on the residues. Other techniques for recrystallizing the sample and USP Reference Standard based on known scientific principles may be used with appropriate scientific justification.

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Change to read:

NEAR-INFRARED AND RAMAN SPECTROSCOPY

The reference ⟨197NIR⟩ in a monograph signifies that a test sample and a standard sample are examined by the NIR spectroscopic technique (see ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020)), and the reference ⟨197R⟩ in a monograph signifies that a test sample and a standard sample are examined by the Raman spectroscopic technique (see ⟨1120⟩). NIR and Raman spectroscopic procedures may be used to confirm the identity of materials. The approach is similar to that of IR identity testing but is often augmented through the use of spectral libraries and chemometrics. A sample can thus be analyzed by NIR or Raman techniques, with the resulting spectrum compared with stored spectra in the spectral library through the use of multivariate analysis. In general, a visual comparison or simple overlay of the spectra alone may not be sufficient and additional evaluation may be needed.

In both techniques, samples can be directly interrogated with minimal or no sample preparation. Measurement is nondestructive and noninvasive, and data collection can often be made through glass or plastic containers.

A description of these techniques, and the strategy for procedure and chemometrics model development and validation can be found in the appropriate associated chapters when available (see *Chemometrics* ⟨1039⟩, ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), and ⟨1120⟩, and the following chapters to be published at a later date: *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩ and *Raman Spectroscopy—Theory and Practice* ⟨1858⟩).

ULTRAVIOLET-VISIBLE SPECTROSCOPY

(Use of the section title *Ultraviolet Absorption* is permitted until May 1, 2020.)

The reference ⟨197U⟩ in a monograph signifies that a sample solution and a Standard solution are examined spectroscopically, in 1-cm cells, over the spectral range from 200 to 400 nm, unless otherwise specified in the individual monograph (see ⟨857⟩). Dissolve a portion of the substance under examination in the designated medium to obtain a sample solution having the concentration specified in the monograph. Record and compare the spectra obtained for the sample solution and the Standard solution. Review or calculate the absorptivities and/or absorbance ratios and compare the results, and where appropriate, compare to criteria specified in an individual monograph.

The comparison must establish that the UV spectrum of the preparation of the sample exhibits absorption maxima and minima only at the same wavelengths as those of the appropriately prepared corresponding USP Reference Standard, and that the absorptivities and/or absorbance ratios are within the specified limits. If there are differences in the spectra, and the sample spectrum was compared with a previously obtained and electronically stored spectrum of the USP Reference Standard, the comparison must be concomitantly repeated with a freshly prepared USP Reference Standard.

Unless otherwise specified in the monograph, absorbances indicated for the calculations of the absorptivities and/or absorbance ratios are those measured at the maximum absorbance wavelength (within ±2 nm) specified in the individual monograph. Where the absorbance is to be measured at about the specified wavelength other than that of maximum absorbance, the abbreviations for minimum (min) and shoulder (sh) are used, respectively, in an absorption spectrum.

The reference ⟨197U-LC⟩ in a monograph signifies that, when a diode array detector is used in tandem with an LC procedure test in the monograph, the spectra of the major chromatographic peak(s) of the sample solution and Standard solution are examined spectroscopically.

The requirements are met if the UV spectra of the sample solution and of the Standard solution exhibit maxima and minima at the same wavelengths, and, if applicable, the absorptivities and/or absorbance ratios are within specified limits.

X-RAY POWDER DIFFRACTION

The reference ⟨197XR⟩ in a monograph signifies that a test sample and a standard sample are examined according to ⟨941⟩.

Prepare and mount the specimen as directed in ⟨941⟩. Unless otherwise indicated in the monograph, record the diffraction pattern in a 2θ-range from as near to 0° as possible to at least 32°. Unless otherwise specified in the monograph, the requirements are met if the X-ray diffraction pattern of the test specimen conforms to that of the corresponding USP Reference Standard obtained using equivalent instruments and conditions. Differences in the diffraction line intensities (but not line positions) between the sample and the Standard are acceptable.

The comparison must establish that the diffraction pattern of the preparation of the test specimen conforms to the diffraction pattern of the corresponding USP Reference Standard. If there are differences in the diffraction pattern and the sample diffraction pattern was compared with a previously obtained and electronically stored diffraction pattern of the USP Reference Standard, the comparison must be repeated concomitantly with a freshly prepared USP Reference Standard.

Change to read:

EQUIVALENT/ALTERNATIVE TESTS

In addition to IR, NIR, Raman, X-ray, and UV absorption, several other spectroscopic methodologies can be utilized for the identification of the specimen under examination. The methods cited in this chapter may be used for identification of materials as an alternative method to the method referenced in the monograph, provided that the alternative technique has been determined to be suitable for identification. Suitable identification tests must be able to discriminate between compounds similar in molecular structure that are likely to be present. The choice of such potentially interfering materials must be based

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on sound scientific judgment, with consideration of interferences that could occur. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination).

For information regarding sample preparation and measurement parameters associated with an alternative identification method, refer to the appropriate general chapter (see *Mass Spectrometry* ⟨736⟩, *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩, ⟨854⟩, ⟨857⟩, ⟨941⟩, ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), and ⟨1120⟩.

Add the following:

▲⟨198⟩ NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IDENTITY TESTING OF BACTERIAL POLYSACCHARIDES USED IN VACCINE MANUFACTURE

1. INTRODUCTION AND SCOPE

This chapter describes the application of nuclear magnetic resonance (NMR) spectroscopy to the identity testing of bacterial polysaccharides used in vaccine manufacture. The identity of the saccharide component in polysaccharide and glycoconjugate vaccines should be confirmed at various stages of the manufacturing process, including bulk monovalent polysaccharide, blended polysaccharide bulk, activated polysaccharide (if isolated), bulk monovalent conjugate, blended conjugate bulks, and final fills. NMR is an appropriate method to confirm the identity of polysaccharides, although it is most useful for bulk monovalent polysaccharides and activated polysaccharides (if isolated). This chapter describes the use of NMR spectroscopy for this purpose, but alternative validated approaches may also be suitable.

For additional information on NMR, including its general principles and applications, see *Applications of Nuclear Magnetic Resonance Spectroscopy* ⟨1761⟩. More specific information on instrument qualification, procedure validation, and reference standards are included in *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.

1.1 NMR Spectra

NMR spectra for identity testing are typically collected for samples dissolved in deuterated water (D_2O) or a combination of deuterated water and small amounts of one or more internal standards for chemical shift calibration, quantification, or other purposes. The chemical shift of the resonance from residual water in deuterated solvent (HOD) is more temperature sensitive than other solvent resonances. The spectral separation and resolution of the HOD resonance may be optimized by the choice of temperature at which the spectrum is obtained.

1.2 *O*-Acetylated Polysaccharides

For those polysaccharides that are *O*-acetylated, the consistency of the degree of *O*-acetylation is considered part of the identity test. This could be evaluated through the use of the spectrum of a reference polysaccharide with an acceptable degree of *O*-acetylation, quantification of resonances characteristic of the *O*-acetylation in the spectrum of the intact polysaccharide, or chemical de-*O*-acetylation and relative quantification of the resulting acetate anion compared to the polysaccharide (see *2.4 Experimental Procedures*). The implementation of any given approach is likely to be product specific.

For some polysaccharides, a quantitative specification for the minimum degree of *O*-acetylation may exist; the approaches described in this chapter, appropriately validated, can be used to perform that assay. Requirements for the *O*-acetyl content of polysaccharides are typically quoted as a minimum quantity of "*O*-acetyl residues" (measured by a Hestrin assay) in millimoles per gram of dry weight of polysaccharide. This NMR assay method described in this chapter reports a value based on the average number of *O*-acetyl groups per repeat unit. A method to calibrate between the two approaches should be developed during method validation.

Change to read:

2. PROCEDURE

2.1 Equipment Requirements

A description of a typical NMR spectrometer is given in ⟨1761⟩, and approaches to installation, performance, and operational qualification are given in ⟨761⟩. This section, and the rest of the chapter, refers to specific requirements for the identity testing of bacterial polysaccharides.

NMR SPECTROMETER

An NMR spectrometer with a minimal nominal field strength corresponding to a proton resonance frequency of NLT 400 MHz and with control of the sample temperature should be used. Temperature calibration within ±3° of the desired temperature is sufficient, provided a consistent sample temperature between the test and reference samples is achieved (or demonstrated in

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method validation), with temperature stability within a run of NMT 0.5°. NMR probes optimized for proton detection and suitable for 5-mm (outside diameter) NMR tubes should be used. Other probe options are possible but should be proven suitable before use.

PROCESSING PARAMETERS

A weighting function may be applied to the free induction decay (FID) before Fourier transform: a suitable line broadening function may be applied, and in some cases 0.3 Hz has been found suitable. After transform of the FID, the phase is adjusted to pure ▲absorption▲ (ERR 1-Jul-2018) phase. Baseline correction should be applied consistently.

NMR TUBES

These should be of a quality suitable for use in high-field spectrometers and qualified for use in the assay.

2.2 Reagents for Vaccine Polysaccharide Sample Solutions

SOLVENTS

Deuterated solvents subject to proton exchange with the polysaccharide [e.g., deuterated water (D_2O)] must be selected with the highest deuterium proportion (e.g., >99.9 atom % D).

CHEMICAL SHIFT REFERENCE COMPOUNDS

Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS; preferred), sodium trimethylsilylpropionate (TSP), or a deuterated analogue (TSP-d_4) are classically used and set to 0 ppm for the methyl signals. The reference material is usually added at low amounts [0.1%–0.01% (w/v) has been found to be appropriate] to the deuterated water used to dissolve the final sample.
[NOTE—Deuterated water >99.96% ^2H containing 0.01% DSS (w/v) is commercially available.]

SODIUM DEUTEROXIDE

Forty percent w/v (10 M) sodium deuteroxide (NaOD) in deuterated water (>99 atom % D) is commonly used to induce the de-*O*-acetylation of polysaccharides.

2.3 Sample Requirements

An aliquot of bulk purified polysaccharide or appropriate process intermediates in solid or liquid state containing 0.5–20 mg of saccharide content is suitable for preparation of NMR analytical samples. To obtain a solid aliquot, the relevant amount of material in liquid solution is dried under vacuum (using a freeze-drier, lyophilizer, or other solvent evaporator).

REFERENCE SPECTRA FOR ANTICIPATED IMPURITIES

Reference spectra for anticipated process-related impurities (e.g., ethanol, antifoaming agents, phenol, cetyl trimethylammonium bromide) should be obtained under the same experimental conditions as those used to analyze the test sample and at concentrations found similar to those in routine test samples. These spectra provide evidence for those resonances that may be excluded from further consideration when assigning the identity of the samples.

REFERENCE SPECTRA FOR POLYSACCHARIDES

Reference spectra for the polysaccharides samples being tested should be obtained by collecting spectra of authentic samples under identical sample preparation and spectrometer acquisition and data processing conditions to those used for test samples. Comparison of the spectra of test samples with these reference spectra allows the identity of the test samples to be confirmed.

2.4 Experimental Procedures

Two experimental protocols are described below. The first protocol is suitable for samples in which a single one-dimensional ^1H NMR spectrum will produce all of the required information. The second protocol includes in situ base-catalyzed de-*O*-acetylation of the polysaccharide to release *O*-acetyl groups as acetate anions, both simplifying the spectrum of the polysaccharide and allowing quantification of the degree of *O*-acetylation. A second one-dimensional ^1H NMR spectrum is collected after the de-*O*-acetylation step. The choice of which protocol to adopt will depend upon the product being tested and the information required; many stages in these two protocols are similar (e.g., sample preparation).

SAMPLE PREPARATION

NMR analytical samples of polysaccharide and its derivatives can be prepared as either solid- or liquid-state aliquots. NMR analytical samples are usually prepared by dissolving the solid aliquot in about 0.7 mL of deuterated solvent or, more rarely, by adding at least 10% (v/v) of deuterated solvent to the liquid aliquot. The solution is mixed to obtain a uniform concentration and is subsequently transferred to a 5-mm NMR tube.

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[NOTE—NMR instrument design may allow or require the use of smaller volumes; users should confirm this for their instrument configuration.]

DEUTERIUM EXCHANGE

With suitable method validation, lyophilization from deuterium oxide ("deuterium exchange") may be used to reduce the intensity of the resonance from residual water (H_2O)/HOD.

pH

Under some circumstances, control of the pH of the sample may be appropriate.

2.5 Procedure 1a

This procedure uses visual comparison with a reference spectra and/or comparison of test chemical shifts with reference chemical shift values.

SCOPE

This approach is compatible with polysaccharides that lack *O*-acetylation, such as *Haemophilus influenzae* type b or many pneumococcal polysaccharides, or with *O*-acetylated polysaccharides, such as *Neisseria meningitidis* or many pneumococcal polysaccharides where the product and spectral consistency allow identity to be established through direct comparison of test and reference spectra.

NMR SPECTRUM OF THE TEST SAMPLE

The NMR spectrum of the test sample is acquired under the same instrument operational and data processing parameters to those used to obtain the reference spectrum. Confirmation of identity can be based on visual comparison of the test and product-specific reference spectra, comparing resonance position, line width, relative intensity, and multiplicity. The complete spectrum, distinctive regions of the spectrum, or a series of at least five resonances (or three distinctive resonances in polysaccharides with monosaccharide or disaccharide repeat units) can be used in this comparison (as long as these are shown to be diagnostic).

RESONANCES

In a well-understood assay, resonances assigned as deriving from process-related impurities that are known to be removed at a later stage in the manufacturing process or otherwise proven not to impact product quality can be excluded from determination of identity.

2.6 Procedure 1b

This procedure uses analytical samples in deuterated water (D_2O) and a comparison with a reference spectrum by calculation of a correlation coefficient.

SCOPE

This approach is compatible with *Streptococcus pneumoniae* polysaccharides where the consistency of the product and the spectrum allow identity to be established through direct mathematical comparison of test and reference spectra.

With mathematical comparisons, a distinctive region of each spectrum (e.g., 4.64–5.89 ppm) is compared to spectra generated for designated reference samples for each polysaccharide of interest. The similarity of two spectral profiles for a test and reference sample acquired under identical conditions and in the same matrix is evaluated using a correlation coefficient (ρ). A positive identification of the sample polysaccharide is achieved when ρ is NLT 0.95 (or another validated value) between the sample and reference spectra.

2.7 Procedure 2

The procedure is in-tube base-catalyzed de-*O*-acetylation and quantification of the acetate content.

SCOPE

In some cases, the degree of *O*-acetylation and their position, accompanied by spontaneous *O*-acetyl migration in solution, can complicate the NMR profile. Base-catalyzed de-*O*-acetylation of the polysaccharide in the NMR tube has proven useful for the *Salmonella typhi* Vi and the meningococcal Groups A, C, W135, and Y polysaccharides. Adding a base may also reduce line widths.

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SAMPLE HANDLING

The sample is prepared as in *Sample Preparation* in *2.4 Experimental Procedures* and a one-dimensional ^{1}H NMR spectrum is acquired as in *2.5 Procedure 1a*. This spectrum provides information on the presence/amount of acetate anion in the native sample. Sodium deuteroxide (NaOD) in deuterated water (D_2O) is added to the sample in the NMR tube to a final concentration of approximately 200 mM [equivalent to adding 14 µL of 10 M sodium deuteroxide (NaOD) in deuterated water (D_2O) to a 0.7-mL sample]. De-*O*-acetylation is very rapid for almost all polysaccharides, but appropriate conditions should be evaluated in method development and validation. The NMR spectrum of the sample is reacquired after de-*O*-acetylation using acquisition conditions appropriate for quantitative spectroscopy. The spectrum of the de-*O*-acetylated material is characteristic of the polysaccharide backbone, and the degree of *O*-acetylation in the original sample is calculated from the integrals of the acetate anion and an appropriate resonance arising from the saccharide backbone. If the amount of acetate anion in the native sample is low, for example <5%, correction of the intensity of the acetate anion resonance in the de-*O*-acetylated sample is not necessary.

2.8 Assay Criteria

TEMPERATURE

The temperature of the sample should be the same, within limits established during method validation, as that of the reference standard, and the sample temperature can be determined by the chemical shift difference between the HOD resonance and the internal chemical shift reference standard. For example, when using TSP-d_4 as the reference standard, calculate the actual temperature using the following equation based on the chemical shift of the HOD signal:

$$\delta = 5.051 - 0.0111(T)$$

where δ is the chemical shift of the HOD peak, and T is the temperature in Celsius (°).

Other equations apply when different chemical shift reference standards are used. Consistency in the chemical shift difference between the HOD and chemical shift reference standard is a surrogate for sample temperature.

CHEMICAL SHIFT REFERENCE STANDARD

The full width half-height (*fwhh*) line width of a defined resonance from a small molecule (e.g., the chemical shift reference standard) in the test sample should be within limits set during method validation to indicate both acceptable shimming of the magnet and sample temperature stability during the analysis. The signal-to-noise ratio of a defined signal within the spectrum of the polysaccharide material should exceed the specification established during method validation.

2.9 System Suitability

SYSTEM SUITABILITY SOLUTION

Dissolve 1 vial of USP PS NMR System Suitability RS in deuterated water (>99.9% D, 1 mL) containing 0.01% DSS with brief mixing.

[NOTE—For example, for 10 s using a rotary mixer.]

Transfer 0.7 mL of this solution into an NMR tube (5-mm outside diameter).

[NOTE—NMR instrument design may allow or require the use of smaller volumes; users should confirm this for their instrument configuration.]

SYSTEM SUITABILITY: PROCEDURE

Using an NMR spectrometer operating at NLT 400 MHz nominal frequency for ^{1}H, acquire an FID using NLT 64 scans with a 90° pulse, a spectral width of 12 ppm, a recycle delay of NLT 30 s, a number of data points NLT 64,000, and with the spectral window centered at 4 ppm. Record the ^{1}H NMR spectra of the *System suitability solution* at a stable temperature of 25 ± 3°. Obtain the spectrum using the above parameters. Transform the data after exponential multiplication (0.3 Hz line broadening). The DSS methyl signal should be set to 0.00 ppm.

ACCEPTANCE CRITERIA

- The chemical shifts for the *N*-acetyl, GlcA H-2, and GlcNAc H-1 anomeric resonances in the *System suitability solution* should be observed at 2.01 ± 0.05, 3.33 ± 0.05, and 5.15 ± 0.05 ppm, respectively.
- The line width (*fwhh*) of the *N*-acetyl resonance at 2.01 ppm should be NMT 3.5 Hz.

2.10 USP Reference Standards ⟨11⟩

USP PS NMR System Suitability RS

▲ 1S (USP41)

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⟨201⟩ THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

PROCEDURES

• **GENERAL PROCEDURE**

The following procedure is applicable as an aid in verifying the identities of many compendial drug substances as such and in their respective dosage forms.

Prepare a test solution as directed in the individual monograph. On a line parallel to and about 2 cm from the edge of a suitable thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩) apply 10 µL of this solution and 10 µL of a *Standard solution* prepared from the USP Reference Standard for the drug substance being identified, in the same solvent and at the same concentration as the test solution, unless otherwise directed in the individual monograph. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and water (180:15:1), unless otherwise directed in the individual monograph, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Unless otherwise directed in the individual monograph, locate the spots on the plate by examination under short-wavelength UV light. The R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

• **PROCEDURE FOR BACITRACIN, NEOMYCIN, AND POLYMYXIN B**

The following thin-layer chromatographic procedure is applicable as an aid in verifying the identities of bacitracin, neomycin, and polymyxin B active ingredients and in dosage forms when present singly and in two- and three-component mixtures. The reference ⟨201BNP⟩ in a monograph signifies that this procedure is intended.

Prepare a *Test solution* as follows, unless otherwise directed in the individual monograph.

Test solution

For drug substances: Dissolve a portion of Bacitracin, Bacitracin Zinc, Neomycin Sulfate, or Polymyxin B Sulfate in 0.1 N hydrochloric acid to obtain a solution containing about 500 USP Bacitracin Units per mL, 3.5 mg of neomycin (base) per mL, or 10,000 USP Polymyxin B Units per mL.

For solutions: Where the solution contains neomycin and polymyxin B, dilute a portion of it with 0.1 N hydrochloric acid to obtain a solution containing the equivalent of about 3.5 mg of neomycin (base) per mL. Where the Solution contains polymyxin B but not neomycin, dilute a portion of it with 0.1 N hydrochloric acid to obtain a solution containing about 10,000 USP Polymyxin B Units per mL.

For creams, lotions, and ointments: Where the Cream, Lotion, or Ointment contains Bacitracin or Bacitracin Zinc, transfer a portion of it equivalent to about 500 USP Bacitracin Units, to a 15-mL centrifuge tube. Where the Cream, Lotion, or Ointment contains neomycin, but not Bacitracin or Bacitracin Zinc, transfer a portion of it equivalent to about 3.5 mg of neomycin (base) per mL to a 15-mL centrifuge tube. Add 4 mL of chloroform to the centrifuge tube, and shake well to disperse the Cream, Lotion, or Ointment. Add 1 mL of 0.1 N hydrochloric acid, vortex for 4 minutes, centrifuge, and use the clear supernatant.

[NOTE—The *Modified test solution* as described below in the *Modified procedure* may be used in lieu of the *Test solution*.]

Standard bacitracin solution: Dissolve a portion of USP Bacitracin Zinc RS in 0.1 N hydrochloric acid to obtain a solution containing 500 USP Bacitracin Units per mL.

Standard neomycin solution: Dissolve a portion of USP Neomycin Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing the equivalent of 3.5 mg of neomycin (base) per mL.

Standard polymyxin B solution: Dissolve a portion of USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing 10,000 USP Polymyxin B Units per mL. Where the article under test also contains Bacitracin or Bacitracin Zinc, dissolve a portion of USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing 500*J* USP Polymyxin B Units per mL, *J* being the ratio of the labeled amount of USP Polymyxin B Units to the labeled amount of USP Bacitracin Units in each g of Cream, Lotion, or Ointment.

Developing solvent solution: Prepare a mixture of methanol, isopropyl alcohol, methylene chloride, ammonium hydroxide, and water (4: 2: 2: 2: 1.5).

Procedure: Apply 10 µL of the *Test solution* and each of the relevant *Standard solutions* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a presaturated chromatographic chamber, and develop the chromatogram with the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 105° for 10 minutes. Spray the plate with a 0.2% solution of ninhydrin in butyl alcohol, and heat at 105° for 5 minutes. The R_F value of each principal spot in the chromatogram of the *Test solution* corresponds to that of the principal spot in the chromatogram obtained from each relevant *Standard solution* as appropriate for the labeled active ingredient or ingredients specified on the label. If the chromatogram of the *Test solution* yields excessive streaking, proceed as directed for *Modified procedure*.

Modified procedure: Transfer the *Test solution* to a 15-mL centrifuge tube, add 10 mL of saturated aqueous picric acid solution (1.2%, w/v), vortex for 1 minute, centrifuge for 10 minutes, and discard the supernatant. Wash the residue with 1-mL portions of water until no yellow color is observed in the washing. Discard the washings, and dry the residue under a stream of nitrogen at 50°. Dissolve the residue in 1 mL of acetone, add 1 mL of a freshly prepared solution of sulfuric acid in acetone (1 in 100), shake, centrifuge for 5 minutes, and discard the supernatant. Rinse the residue with 1 mL of acetone, centrifuge briefly, and discard the washing. Repeat the washing until no yellow color is observed. Dry the residue under a stream of nitrogen at 50°. Dissolve the residue in 0.5 mL of 0.1 N hydrochloric acid (*Modified test solution*). Repeat the *Procedure* using this *Modified test solution* instead of the *Test solution*. The R_F value of each principal spot in the chromatogram of the *Modified test solution* corresponds to that of the principal spot in the chromatogram obtained from each relevant *Standard solution* as appropriate for the active ingredient or ingredients specified on the label.

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USP Reference Standards ⟨11⟩
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

⟨202⟩ IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY

INTRODUCTION

The following procedure for the USP *Identification* test is used to identify fixed oils using high-performance thin-layer chromatography (HPTLC), with a suitable octadecylsilyl silica gel as the coating substance.

IDENTIFICATION

• METHOD I

Mobile phase 1: Ethyl ether
Mobile phase 2: Methylene chloride, glacial acetic acid, and acetone (20:40:50)
System suitability solution 1: Dissolve about 20 mg (1 drop) of USP Corn Oil RS in 3 mL of methylene chloride.
System suitability solution 2: Dissolve about 20 mg (1 drop) of USP Olive Oil RS in 3 mL of methylene chloride.
Standard solution: Dissolve about 20 mg (1 drop) of the appropriate USP Reference Standard in 3 mL of methylene chloride.
Sample solution: Dissolve about 20 mg (1 drop) of a fixed oil sample in 3 mL of methylene chloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: HPTLC
Plate: 20 cm × 10 cm, silica gel 60 RP-18, 0.15–0.2 mm layer, 4–8 µm particle size[1]
Application volume: 1 µL, manually spot
Spray reagent: 100 mg/mL of phosphomolybdic acid in 96% alcohol
System suitability
Samples: *System suitability solution 1* and *System suitability solution 2*
Suitability requirements
Resolution: The four principal spots from corn oil are clearly identified and separated, and the two principal spots from olive oil are clearly identified and separated.
[NOTE—Retardation factors (R_F) are provided for informational purposes only to aid in spot identification. The R_F values for the four principal spots for USP Corn Oil RS are 0.39, 0.45, 0.51, and 0.56, and the R_F values for the two principal spots for USP Olive Oil RS are 0.39 and 0.45.]
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Samples* in separate bands to the previously marked starting point on an HPTLC plate, and develop the plate in the following order:
1. Ensure that the spots are at least 3 mm above the surface of the mobile phase. Develop two times over a path of 0.5 cm using *Mobile phase 1*. Remove the plate from the chamber after each run, and allow the plate to dry in air.
2. Develop two times over a path of 8 cm using *Mobile phase 2*. Allow the plate to dry for about 5 min after each development and before spraying with a spray reagent.

Spray the plate with the *Spray reagent*.
Heat the plate at 120° for about 1 min, and examine in daylight.
Acceptance criteria: The R_F values of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

• METHOD II

Mobile phase: Methylene chloride, glacial acetic acid, and acetone (20:40:50)
System suitability solution 1: Dissolve 25 µL of USP Corn Oil RS in 3 mL of methylene chloride.
System suitability solution 2: Dissolve 25 µL of USP Olive Oil RS in 3 mL of methylene chloride.
Standard solution: Dissolve 25 µL of the appropriate USP Reference Standard in 3 mL of methylene chloride.
Sample solution: Dissolve 25 µL of a fixed oil sample in 3 mL of methylene chloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: HPTLC
Plate: 20 cm × 10 cm, silica gel 60 RP-18 (or RP-18 F_{254}), 0.15–0.2 mm layer, 4–8 µm particle size
Conditioning of plate: Condition the plate to a relative humidity of about 33%.
Application volume: 2 µL as bands of 8 mm. A suitable automated apparatus is used.
Spray reagent: 25 mg/mL of phosphomolybdic acid in 96% alcohol
System suitability
Samples: *System suitability solution 1* and *System suitability solution 2*
Suitability requirements
Resolution: The four principal bands from corn oil are clearly identified and separated, and the two principal bands from olive oil are clearly identified and separated.

[1] HPTLC Silica Gel 60 RP-18 plate from Merck EMD, or equivalent.

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[NOTE—Retardation factors (R_F) are provided for informational purposes only to aid in band identification. The R_F values for the four principal bands for USP Corn Oil RS are 0.20, 0.26, 0.30, and 0.34, and the R_F values for the two principal bands for USP Olive Oil RS are 0.20 and 0.26.]

Analysis

Samples: *Standard solution* and *Sample solution*

Predevelop the plate with methylene chloride to the upper edge. Dry the plate at 120° for 10 min.

Apply the *Samples* in separate bands to the previously marked starting point on an HPTLC plate.

Ensure that the bands are at least 3 mm above the surface of the mobile phase.

Develop over a path of 7 cm using *Mobile phase*. Allow the plate to dry in air.

Treat the plate with the *Spray reagent*.

Heat the plate at 120° for 3 min, and examine in daylight.

Acceptance criteria: The R_F values of the principal bands of the *Sample solution* correspond to those of the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Almond Oil RS
USP Borage Seed Oil RS
USP Canola Oil RS
USP Corn Oil RS
USP Cottonseed Oil RS
USP Evening Primrose Oil RS
USP Flax Seed Oil RS
USP Olive Oil RS
USP Palm Oil RS
USP Peanut Oil RS
USP Safflower Oil RS
USP Sesame Oil RS
USP Soybean Oil RS
USP Sunflower Oil RS

⟨203⟩ HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY PROCEDURE FOR IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN

Change to read:

INTRODUCTION

This chapter describes a procedure for use in a USP Identification test that ▲employs▲ (USP 1-May-2020) the technique of high-performance thin-layer chromatography (HPTLC). ▲Precise▲ (USP 1-May-2020) control of the ▲experimental▲ (USP 1-May-2020) variables ▲is essential for reproducible analysis. [NOTE—For additional information,▲ (USP 1-May-2020) see *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure* ⟨1064⟩.]

Change to read:

▲▲ (USP 1-MAY-2020)EQUIPMENT

The equipment used for HPTLC ▲analysis typically includes:▲ (USP 1-May-2020)

- Plates: Unless otherwise specified in the individual monograph, use 20 × 10 cm plates coated with a uniform 200-µm layer of porous (60-Å pore size) silica gel having irregular particles of 2–10 µm and an average particle size of 5 µm, a polymeric binder, and a fluorescence indicator (F_{254}). [NOTE—▲▲ (USP 1-May-2020)Glass plates are preferred over aluminum-backed sheets.▲▲ (USP 1-May-2020)]
- A device suitable for ▲application of indicated volumes as bands of▲ (USP 1-May-2020) specified length at the ▲designated▲ (USP 1-May-2020) positions.
- A device suitable for controlling the activity of the stationary phase ▲by preconditioning the plate to a prescribed▲ (USP 1-May-2020) relative humidity.
- A suitable chromatographic chamber ▲to maintain reproducible vapor phase composition and to allow for control of developing distance▲ (USP 1-May-2020) (for example, a twin-trough chamber).
- A device suitable for reproducible drying of the developed plate.
- A device suitable for treatment of the plate with derivatization reagent, if required.
- A device suitable for ▲controlled plate▲ (USP 1-May-2020) heating as part of the derivatization procedure, if required.

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- A ▲device▲ (USP 1-May-2020) suitable for ▲illumination under prescribed observation conditions, typically, short-wave UV (nominally 254 nm), long-wave UV (nominally 365 or 366 nm),▲ (USP 1-May-2020) and white light. ▲ [NOTE—Light sources used for chromatogram visualization typically produce broad-band emission; therefore, the maxima quoted above carry only nominal significance.]
- A photographic system for documentation of chromatograms under the prescribed visualization conditions.▲ (USP 1-May-2020)

Change to read:

PROCEDURE

Preparation of the Test Solution

Unless otherwise stated in an individual monograph, 100 mg of a powdered botanical ingredient, 10 mg of a dry extract, ▲▲ (USP 1-May-2020) or the amount of a dosage form containing the equivalent of the aforementioned quantities of the botanical ingredient is sonicated for 15 min with 1 mL of methanol. After ▲filtration or▲ (USP 1-May-2020) centrifugation, the filtrate or supernatant is used as the *Sample solution*. ▲For essential oils, 50 µL/mL solution in toluene is▲ (USP 1-May-2020) used as the *Sample solution*.

Preparation of the Standard Solutions

Unless otherwise stated in an individual monograph, Reference Standards of individual marker compounds are dissolved ▲in methanol▲ (USP 1-May-2020) at a concentration of 1 mg/mL. The Reference Standard extracts are shaken and sonicated in methanol at a concentration of 10 mg/mL or, for essential oils, ▲in toluene at 50 µL/mL.▲ (USP 1-May-2020)

Sample Application and Plate Layout

Samples are applied as narrow bands of ▲8-mm▲ (USP 1-May-2020) length at a distance of ▲8 mm▲ (USP 1-May-2020) from the lower edge of the plate ▲ [NOTE—Refer to ⟨1064⟩ for additional information.]▲ (USP 1-May-2020) The system suitability standards are applied to the ▲track▲ (USP 1-May-2020) nearest to the ▲lateral▲ (USP 1-May-2020) edge at NLT 20 mm from the ▲lateral▲ (USP 1-May-2020) edge of the plate. The distance between tracks, center-to-center, is NLT ▲11 mm.▲ (USP 1-May-2020) All application volumes are specified in the individual monographs, ▲and▲ (USP 1-May-2020) usually range from 2–10 µL. The developing distance is marked with a pencil close to one of the ▲lateral▲ (USP 1-May-2020) edges of the plate before the development; ▲alternatively,▲ (USP 1-May-2020) an electronic solvent front detection device ▲is used.▲ (USP 1-May-2020)

Preconditioning of the Plate

Following sample application and unless otherwise stated in an individual monograph, the plate is conditioned at a relative humidity of 33% ▲by exposure to the atmosphere generated by vapors in equilibrium with saturated magnesium chloride solution in a sealed chamber▲ (USP 1-May-2020) for a minimum of 10 min. ▲Other frequently used saturated salt solutions of potassium thiocyanate and sodium chloride establish equilibrium relative humidity of 47% and 75%, respectively, at 25°.▲ (USP 1-May-2020)

Preparation of the Developing Chamber and Development of the Plate

Where a twin-trough chamber is used, the rear trough is fitted with filter paper. The chamber is charged with a sufficient volume of developing solvent to wet the filter paper completely and achieve a level of developing solvent of exactly 5 mm in both troughs. With the lid closed, the chamber is ▲allowed to achieve saturation for▲ (USP 1-May-2020) 20 min. ▲▲ (USP 1-May-2020) The plate is introduced ▲vertically▲ (USP 1-May-2020) into the front trough of the chamber ▲with the chromatographic layer facing▲ (USP 1-May-2020) the filter paper. ▲Once the solvent front▲ (USP 1-May-2020) has ▲ascended 6 cm from the application line,▲ (USP 1-May-2020) the plate is removed from the chamber and dried in a vertical position in a current of cold air that does not affect the integrity of the separated zones. ▲Alternative▲ (USP 1-May-2020) chamber configurations and developing distances may be specified in an individual monograph. [NOTE—Other development chambers may be employed if the results obtained fulfill all of the system suitability criteria.]

Derivatization Procedure

Where derivatization reagents are used, ▲appropriate▲ (USP 1-May-2020) volumes of reagents ▲▲ (USP 1-May-2020) (typically 1–2 mL) are ▲uniformly▲ (USP 1-May-2020) sprayed onto the plate or the plate is immersed into the reagent solution at a defined speed and for a defined dwell time. [NOTE—Immersion speed of 50 mm/s and the dwell time of 1 s ▲are recommended▲ (USP 1-May-2020) for most nonaqueous reagents.]

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Visualization

Chromatograms on the plate are visualized as directed in an individual monograph, ▲typically under short-wave UV, long-wave UV,▲ (USP 1-May-2020) or white light prior to and after derivatization.

System Suitability

▲A general system suitability requirement is that the solvent front and the chromatographic bands are strictly parallel to the horizontal sides of the chromatographic plate. Specific suitability parameters are detailed in the monographs. Typically, to assess▲ (USP 1-May-2020) suitability of the chromatographic system for resolution, position, and color of the bands, ▲▲ (USP 1-May-2020) two or more reference substances are selected that have similar but just separable R_F values under the chromatographic conditions to be used; for example, chlorogenic acid (blue) and hyperoside (yellow-orange) ▲are commonly▲ (USP 1-May-2020) used for flavonoids ▲methods. System suitability is required every time the analysis is performed; it is conducted on the same plate alongside the test articles. Failure to meet the system suitability requirements invalidates the analysis. In the event of failure, its cause needs to be identified and corrected (see ⟨1064⟩ for potential sources of failure), and the analysis repeated until the suitability requirements have been met. Individual▲ (USP 1-May-2020) Reference Standards, ▲or their▲ (USP 1-May-2020) mixtures, ▲are typically employed▲ (USP 1-May-2020) for system suitability ▲monitoring, and the use of▲ (USP 1-May-2020) the USP Reference Standard extracts ▲is common. The▲ (USP 1-May-2020) resolution, position, and colors of the key bands of the ▲Reference Standard chromatogram▲ (USP 1-May-2020) should match the description in the monograph within a specified tolerance. ▲▲ (USP 1-May-2020)

Evaluation and Acceptance Criteria

Chromatograms of the *Sample solution* and *Standard solution* are compared against the descriptions in the *Acceptance criteria* section of the monograph with respect to ▲band▲ (USP 1-May-2020) position, ▲▲ (USP 1-May-2020)separation, color, and relative intensity.

Documentation

▲Test results are required to be documented▲ (USP 1-May-2020) in an auditable manner to comply with the current good manufacturing practices. Proper documentation tools should be employed; for example, a ▲digital▲ (USP 1-May-2020) camera ▲capable of capturing images▲ (USP 1-May-2020) under ▲prescribed illumination, or a scanning densitometer along with the▲ (USP 1-May-2020) imaging software suitable for archival, retrieval, and analysis of the results.

▲Standard Parameters

Unless otherwise indicated in an individual monograph, the following standard parameters, as specified in *Table 1*, are to be employed in every procedure referencing this chapter.

Table 1

Parameter	Standard
Chromatographic plate, glass, 20 × 10 cm	200-µm silica layer, 5-µm average particle size, with fluorescence indicator F_{254}
Application position, from plate lower edge	8 mm
Band application length	8 mm
Band spacing, center-to-center	NLT 11 mm
Relative humidity	33%
Plate conditioning time	10 min
Chamber saturation time	20 min
Developing distance	6 cm
Temperature	22 ± 5°▲ (USP 1-May-2020)

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LIMIT TESTS

⟨206⟩ ALUMINUM

This procedure is provided to demonstrate that the content of aluminum (Al) does not exceed the limit given in the individual monograph of a substance labeled as intended for use in hemodialysis. [NOTE—The *Standard Preparations* and the *Test Preparation* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

NITRIC ACID DILUENT

Transfer 40 mL of nitric acid to a 1000-mL volumetric flask, and dilute with water to volume.

STANDARD PREPARATIONS

Treat some aluminum wire with 6 N hydrochloric acid at 80° for a few minutes. Dissolve about 100 mg of the treated wire, accurately weighed, in a mixture of 10 mL of hydrochloric acid and 2 mL of nitric acid by heating at about 80° for approximately 30 minutes. Continue heating until the volume is reduced to about 4 mL. Cool to room temperature, and add 4 mL of water. Evaporate to about 2 mL by heating. Cool, and transfer this solution, with the aid of water, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a third 100-mL volumetric flask, dilute with water to volume, and mix. The concentration of aluminum in this *Standard Preparation* is about 1.0 µg per mL. If a more diluted *Standard Preparation* is required, transfer 1.0-, 2.0-, and 4.0-mL portions of this solution to separate 100-mL volumetric flasks, dilute with *Nitric Acid Diluent* to volume, and mix. These solutions contain 0.01, 0.02, and 0.04 µg of Al per mL, respectively.

TEST PREPARATION

Unless otherwise directed in the monograph, transfer an accurately weighed amount (in g) of the test substance, as specified in the monograph, to a 100-mL plastic volumetric flask, add 50 mL of water, and sonicate for 30 minutes. Add 4 mL of nitric acid, dilute with water to volume, and mix.

PROCEDURE

Determine the absorbances of the *Standard Preparations* and the *Test Preparation* at the aluminum emission line at 309.3 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an aluminum hollow-cathode lamp and a flameless electrically heated furnace, using the *Nitric Acid Diluent* as the blank. Plot the absorbances of the *Standard Preparations* versus the content of Al, in µg per mL, drawing a straight line best fitting the three points. From the graph so obtained, determine the quantity, in µg, of Al in each mL of the *Test Preparation*. Calculate the amount of Al in the specimen taken, in µg per g, by multiplying this value by 100/W, where W is the weight, in g, of the substance taken to prepare the *Test Preparation*.

⟨207⟩ TEST FOR 1,6-ANHYDRO DERIVATIVE FOR ENOXAPARIN SODIUM

The following procedure is used to determine the levels of 1, 6-anhydro forms in enoxaparin sodium. [NOTE—The test for the 1,6-anhydro derivative is conducted only where specified in the individual monograph.]

INTRODUCTION

The disaccharides specified in this general chapter are listed by name and structure in *Appendix 1*; the oligosaccharides are listed in *Appendix 2*.

Depolymerization of heparin into enoxaparin sodium produces a partial but characteristic conversion of glucosamines at the reducing termini of oligosaccharide chains with terminal glucosamine 6-*O* sulfate, yielding 1,6-anhydro derivatives (see *Figure 1*).

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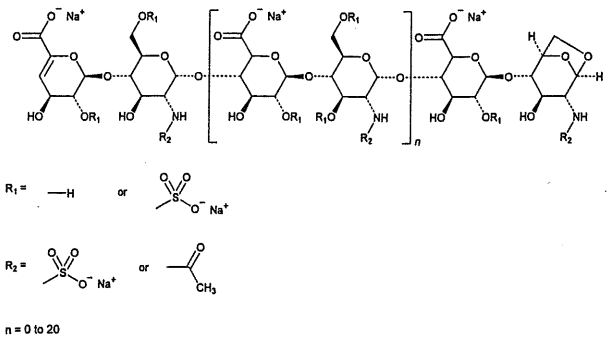


Figure 1. Structure of enoxaparin sodium containing a 1,6-anhydro derivative on the reducing end of the chain.

The percentage of oligosaccharide chains that are cyclized in a 1,6-anhydro ring is a characteristic of enoxaparin sodium.

PROCEDURES

• **Depolymerization of Enoxaparin Sodium by Heparinases and Resulting Oligosaccharides**

The assay involves HPLC analysis of a depolymerized enoxaparin sodium solution by a mixture of heparinases. After enzymatic depolymerization, the main 1,6-anhydro residues of enoxaparin sodium observed are 1,6-anhydro ΔIIS and 1,6-anhydro ΔIIS^{epi}, and 1,6-anhydro ΔIS and 1,6-anhydro $\Delta IS–IS^{epi}$ (see *Appendix 2.*).

The 1,6-anhydro $\Delta IS–IS^{epi}$ tetrasaccharide (2-*O*-sulfated mannosamine form) is not completely cleaved by the heparinases. The two disaccharides (1,6-anhydro ΔIIS and 1,6-anhydro ΔIIS^{epi}), which generally co-elute, are poorly resolved with respect to ΔIIA (see *Appendix 1*), especially because the latter occurs as two anomers: α and β. To allow quantitation of 1,6-anhydro ΔIIS and 1,6-anhydro ΔIIS^{epi}, the enoxaparin sodium sample already depolymerized by heparinases is then reduced by sodium borohydride (see *Figure 2*).

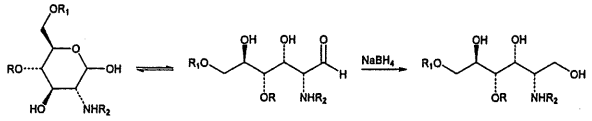


Figure 2. Reduction of oligosaccharides by sodium borohydride

The sodium borohydride reduction eliminates the $\alpha \leftrightarrow \beta$ anomeric effect by opening the terminal oligosaccharide ring. The four 1,6-anhydro derivatives (see *Appendix 2.*) are not reduced by sodium borohydride because the ring opening is blocked by the 1,6-anhydro bridge. The reduction of the oligosaccharides decreases their retention time, whereas the retention time of the 1,6-anhydro derivatives remains unchanged. Thus, it is possible to separate the two compounds—1,6-anhydro ΔIIS and 1,6-anhydro ΔIIS^{epi}—from the reduced ΔIIA disaccharide peak.

[NOTE—1,6-Anhydro ΔIIS and 1,6-anhydro ΔIIS^{epi} are eluted as two nonresolved peaks and are quantitated together as a single compound, 1,6-anhydro ΔIIS. Therefore, for the purpose of simplification, the epimeric form is not referred to in the remaining text.]

Change to read:

• **Procedure**

Solutions

Solution A: Dissolve 0.280 g of monobasic sodium phosphate in 950 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Solution B: Dissolve 140 g of sodium perchlorate in 950 mL of *Solution A,* adjust with phosphoric acid to a pH of 3.0, and dilute with *Solution A* to 1000 mL.

Mobile phase: Use variable mixtures of filtered and degassed *Solution A* and *Solution B* as directed in *Chromatographic system.*

Sodium/Calcium acetate pH 7.0 solution: Dissolve 10 mg of bovine serum albumin and 32 mg of calcium acetate in 60 mL of water. Add 580 µL of glacial acetic acid, and adjust with 2 M sodium hydroxide to a pH of 7.0. Transfer to a 100-mL volumetric flask, and dilute with water to volume. Pass the solution through a filter having a porosity of 0.45 or 0.22 µm.

Potassium phosphate pH 7.0 buffer: Dissolve 68 mg of monobasic potassium phosphate and 10 mg of bovine serum albumin in 30 mL of water in a 50-mL volumetric flask. Adjust with potassium hydroxide, if necessary, to a pH of 7.0, and dilute with water to volume. Pass the solution through a filter having a porosity of 0.45 or 0.22 µm.

Sodium borohydride solution: Dissolve 12 mg of sodium borohydride in 400 µL of water, and mix on a vortex mixer. [NOTE—Prepare fresh *immediately* before use.]

Heparinase 1 solution: Dissolve heparinase 1 (see *Reagent Specifications* under *Reagents, Indicators,* and *Solutions*) [reference: heparin lyase I, EC 4.2.2.7] in *Potassium phosphate pH 7.0 buffer* to obtain a solution having an activity of 0.4 IU per mL. Store the solution at –20° until ready to use. [NOTE—Heparinase solutions can be stored for 3 months at –20°.]

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Heparinase 2 solution: Dissolve heparinase 2 (see *Reagent Specifications* under *Reagents, Indicators,* and *Solutions* [no EC number] in *Potassium phosphate pH 7.0 buffer* to obtain a solution having an activity of 0.4 IU per mL. Store the solution at –20° until ready to use.

Heparinase 3 solution: Dissolve heparinase 3 (see *Reagent Specifications* under *Reagents, Indicators,* and *Solutions* [reference: heparitinase I, EC 4.2.2.8] in *Potassium phosphate pH 7.0 buffer* to obtain a solution having an activity of 0.4 IU per mL. Store the solution at –20° until ready to use.

Heparinases 1, 2, 3, solution: Prepare a 1:1:1 (v:v:v) mixture of *Heparinase 1 solution, Heparinase 2 solution,* and *Heparinase 3 solution.*

Peak identification solutions

[NOTE—The depolymerized test solutions and Standard solutions must be prepared at the same time. Depolymerized test solutions are stable for 1 month at –20°. Also, the reduced test solutions and Standard solutions must be prepared at the same time. Reduced solutions are also stable for 1 month at –20°.]

Disaccharide solutions: Separately prepare a 0.25 mg per mL solution of each disaccharide[1] ΔIA, ΔIIA, ΔIIIA, ΔIVA, ΔIS, ΔIIS, ΔIIIS, ΔIVS (see *Appendix 1*). Chromatograph each disaccharide solution, and record the peak responses.

Reduced disaccharide solutions: To 60 µL of each *Disaccharide solution,* add 10 µL of freshly prepared *Sodium borohydride solution.* Mix on a vortex mixer, and allow to stand at room temperature for at least 4 hours. Chromatograph each solution, and record the peak response.

Blank solution: Prepare a mixture of 20 µL of water, 70 µL of *Sodium/Calcium acetate pH 7.0 solution,* and 100 µL of the *Heparinases 1, 2, 3 solution.* Mix gently by inversion, and allow to stand for at least 48 hours in a 25° water bath. Prepare a mixture of 60 µL of this depolymerized solution with 10 µL of freshly prepared *Sodium borohydride solution.* Homogenize, and allow to stand at room temperature for at least 4 hours. Chromatograph the resulting solution, and record the peak responses.

Test solution 1: Prepare two solutions, each containing 20 mg of enoxaparin sodium in 1 mL of water.

Standard solution 1: Prepare one solution containing 20 mg of USP Enoxaparin Sodium RS in 1 mL of water.

Test solution 2: For each solution, prepare a mixture of 20 µL of *Test solution 1,* 70 µL of *Sodium/Calcium acetate pH 7 solution,* and 100 µL of *Heparinases 1, 2, 3 solution.* Mix gently by inversion, and allow to stand for at least 48 hours in a 25° water bath. After 48 hours of depolymerization, chromatograph the solution, and record the peak responses.

Standard solution 2: Prepare a mixture of 20 µL of *Standard solution 1,* 70 µL of *Sodium/Calcium acetate pH 7 solution,* and 100 µL of *Heparinases 1, 2, 3 solution.* Mix gently, and allow to stand for at least 48 hours in a 25° water bath. After 48 hours of depolymerization, chromatograph the solution, and record the peak responses.

Test solution 3: For each depolymerized test solution, prepare a mixture of 60 µL of *Test solution 2* and 10 µL of freshly prepared *Sodium borohydride solution.* Homogenize, and allow to stand loosely capped at room temperature for at least 4 hours before injecting into the chromatograph. *Test Solution 3* is stable for 48 hours at room temperature.

Standard solution 3: Prepare a mixture of 60 µL of *Standard solution 2* and 10 µL of freshly prepared *Sodium borohydride solution.* Homogenize and mix on a vortex mixer, and allow to stand loosely capped at room temperature for at least 4 hours before injecting into the chromatograph. *Standard solution 3* is stable for 48 hours at room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The liquid chromatograph is equipped with a 234-nm detector and a 3-mm × 25-cm column that contains 5-µm packing L14. A guard column packed with the same material should also be used. The flow rate is 0.45 mL per minute, the column temperature is maintained at 50°, and the injection volume is 10 µL. The chromatograph is programmed as follows.

Time (min)	Solution A (%)	Solution B (%)	Elution
0–20	97→65	3→35	Linear gradient
20–50	65→0	35→100	Linear gradient
50–60	0	100	Isocratic
60–61	0→97	100→3	Linear gradient for re-equilibration
61–79	97	3	Isocratic for re-equilibration

Chromatograph the reduced *Test solution 3* and the reduced *Standard solution 3,* and record the peak responses as directed for *Procedure.*

Depolymerization suitability test: The ratio of the peak area of 1,6-anhydro-ΔIS-IS to that of 1,6-anhydro ΔIS is not more than 1.15 for the depolymerized *Standard solution 2.*

Column performance suitability test: Identify the peaks corresponding to reduced ΔIA and 1,6-anhydro-ΔIS for the *Standard solution 3:* the retention time of reduced ΔIS is between 27 and 33 minutes for the depolymerized and reduced *Standard solution 3;* and the resolution, *R,* between reduced ΔIA and 1,6-anhydro-ΔIS is not less than 1.5.

Reduction suitability test: The ratio of the peak area of ΔIS disaccharide to that of reduced ΔIS in the depolymerized and reduced *Standard solution 3* and *Test solution 3* is not more than ▲0.02.▲ (ERR 1-Oct-2018)

Procedure/Calculation: Separately inject equal volumes of the reduced *Test solutions 3* and the reduced *Standard solution 3* into the chromatograph. Use the normalized area percentage method for calculation. Each peak is integrated from the dwell volume peak to the last detected peak. Measure the area of each analyte peak after excluding solvent peaks

[1] Suitable disaccharides are available from Grampian Enzymes (GE-H1001, GE-G1002, GE-H1003, GE-H1004, GE-H1005, GE-H1006, GE-H1007, GE-H1008), Nisthouse, Harray, Orkney, KW17 2LQ, United Kingdom, Tel: 01856 771771, Scottish Local Authority: Orkney Islands.

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at the beginning of the chromatogram and in the *Blank solution*. Using the previously obtained chromatograms of the *Reduced disaccharide solutions*, identify peaks belonging to the eight reduced disaccharides in the chromatograms for *Test solution 3* and *Standard solution 3*. The peaks belonging to 1,6-Anhydro ΔIS, 1,6-Anhydro ΔIIS, and 1,6-Anhydro ΔIS-IS^{epi} are identified from the relative retention times provided in *Table 1* and the Reference chromatogram provided with USP Enoxaparin Sodium RS. Once the peaks have been identified, use the values in *Table 1* to calculate the (w/w) percentage of the three main 1,6-anhydro derivatives obtained after depolymerization of enoxaparin sodium using the following formula:

$$\% \text{ 1,6-anhydro i (w/w)} = (100 \times MW_i \times A_i)/\ \Sigma(MW_x \times A_x)$$

MW_i = molecular weight of the 1,6-anhydro peak i
A_i = area of the 1,6-anhydro peak i
MW_x = molecular weight of either the peak X or the zone X specified by its retention time
A_x = area of either the peak X or the zone X specified by its retention time

[NOTE—Once the method is established, the peaks belonging to the different di- and tetrasaccharides can be easily identified using the USP Enoxaparin Sodium RS chromatogram. Thus, the use of the disaccharide Standards is only needed during the method-implementation stage.]

Calculate the molar percentage of components containing a 1,6-anhydro structure at the reducing end of their chain in the enoxaparin sodium test sample according to the following formula:

$$\%1.6anhydro = 100 \times \frac{MW}{\sum MW_x \times Area_x} \times$$
$$(Area\Delta Is1.6anydro + Area\Delta IIs1.6anhydro + Area\Delta Is - Is1.6anhydro)$$

in which MW is the mass-average molecular mass (see *Identification* test *D* under *Enoxaparin Sodium*); MW_x and $Area_x$ are the molecular weight and the area, respectively, of either the peak X or the range X specified by its retention time. The molar percentage of components having a 1,6-anhydro structure at the reducing end of their chain is between 15% and 25%. Typical retention times and molecular masses attributed to different oligosaccharide structures are provided in *Table 1*.

Table 1. Typical Relative Retention Times (t_{RR}) and Molecular Masses Attributed to Different Compounds

Compound	t_{RR}	Molecular Mass (Daltons)
—	< 0.25	741
Reduced ΔIVA	0.25	401
—	0.25 < t_{RR} < 0.51	741
Reduced ΔIVS	0.51	461
—	0.51 < t_{RR} < 0.55	483
Reduced ΔIIA	0.55	503
—	0.55 < t_{RR} < 0.59	503
1,6-Anhydro ΔIIS	0.59	443
—	0.59 < t_{RR} < 0.64	503
Reduced ΔIIIA	0.64	503
—	0.64 < t_{RR} < 0.72	533
Reduced ΔIIS	0.72	563
—	0.72 < t_{RR} < 0.80	563
Reduced ΔIIIS	0.80	563
—	0.80 < t_{RR} < 0.88	583
Reduced ΔIA	0.88	605
—	0.88 < t_{RR} < 0.90	635
1,6-Anhydro ΔIS	0.90	545
—	0.90 < t_{RR} <0.98	635
Reduced ΔIIA-IVSglu	0.98	1066
—	0.98< t_{RR} <1.00	635
Reduced ΔIS	1.00	665
—	1.00< t_{RR} <1.04	665

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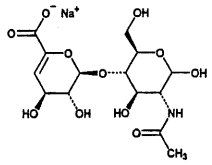
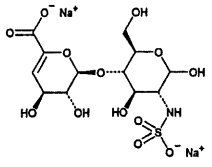
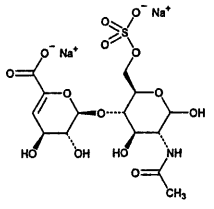
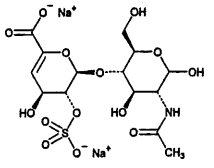
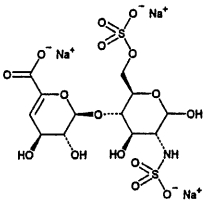
Table 1. Typical Relative Retention Times (t_{RR}) and Molecular Masses Attributed to Different Compounds* *(continued)*

Compound	t_{RR}	Molecular Mass (Daltons)
ΔIS	1.04	665
—	$1.04 < t_{RR} < 1.10$	1228
Reduced ΔIIA-IISglu	1.10	1168
—	$1.10 < t_{RR} < 1.27$	1228
1,6-Anhydro ΔIS-IS	1.27	1210
—	$t_{RR} > 1.27$	1228

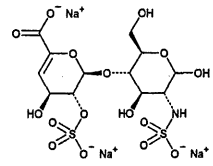
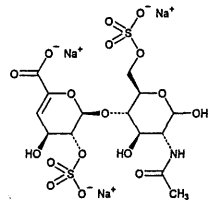
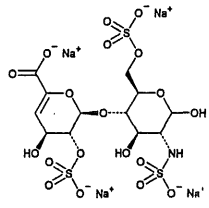
* Relative retention times were obtained with a depolymerized and reduced batch of enoxaparin sodium. They are expressed relative to the retention time of the main peak corresponding to reduced ΔIS. Note that according to the quality of the column, relative retention times can change slightly.

ADDITIONAL REQUIREMENTS

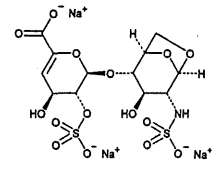
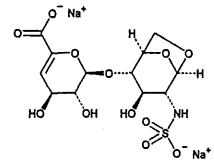
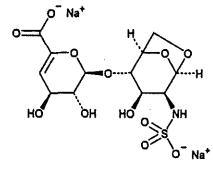
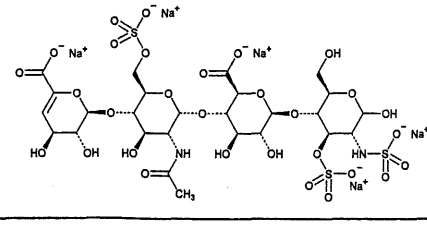
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Enoxaparin Sodium RS
- **APPENDIX 1: STANDARD DISACCHARIDE STRUCTURES**

ΔIVA		ΔUA-(1→4)α-GlcNAc
ΔIVS		ΔUA-(1→4)α-GlcN(NS)
ΔIIA		ΔUA-(1→4)α-GlcNAc(6S)
ΔIIIA		ΔUA-2S-(1→4)α-GlcNAc
ΔIIS		ΔUA-(1→4)α-GlcN (NS,6S)

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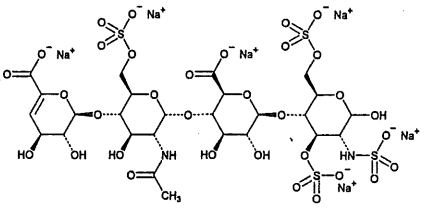
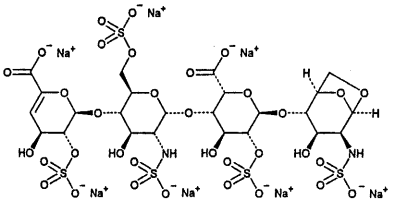
ΔIIIS		ΔUA-2S-(1→4)α-GlcN (NS)
ΔIA		ΔUA-2S-(1→4)α-GlcNAc(6S)
ΔIS		UA-2S-(1→4)α-GlcN (NS,6S)

- **APPENDIX 2: OLIGOSACCHARIDE STRUCTURES**

1,6-Anhydro ΔIS or 1,6-Anhydro ΔIS glucose	
1,6-Anhydro ΔIIS or 1,6-Anhydro ΔIIS glucose	
1,6-Anhydro ΔIIS epi or 1,6-Anhydro ΔIIS mannose	
ΔIIA-IVSglu	

General Chapters

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ΔIIA-IISglu	
1,6-Anhydro ΔIS-IS epi or 1,6-Anhydro ΔIS-IS mannose	

⟨208⟩ ANTI-FACTOR Xa AND ANTI-FACTOR IIa ASSAYS FOR UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS

This chapter provides information and procedures to determine factor Xa inhibitory activity and factor IIa (thrombin) inhibitory activity for unfractionated heparin (UFH) and low molecular weight heparins (LMWH).

INTRODUCTION

Unfractionated heparin and LMWH exert their anticoagulant effect by potentiating the activity of plasma coagulation inhibitors. Of all the commonly known glycosaminoglycans, only UFH, LMWH, and heparan sulfate (hereafter referred to generally as heparin) contain a specific pentasaccharide sequence that can bind to the plasma coagulation inhibitor, antithrombin (AT). AT-dependent assays, therefore, are developed to ensure the specificity of the methods for measuring the anticoagulant activity of heparin. Binding of heparin to AT induces a conformational change, thereby increasing AT's binding to and subsequent inactivation of activated blood coagulation factors. The major coagulation targets for the AT–heparin complex are factor IIa (thrombin) and coagulation factor Xa. Once AT is activated by the pentasaccharide sequence of heparin, it interacts with factors Xa and IIa via its reactive center loop. For efficient inhibition of thrombin, the heparin molecule also must bind to both AT and IIa. This interaction requires an extra length of approximately 13 monosaccharides attached at the nonreducing end of the AT-binding heparin pentasaccharide sequence. This minimum heparin motif for AT inhibition of thrombin, known as the C-domain, has an approximate molecular weight of 5400 Da. Although the potentiation of AT inactivation of factor Xa also depends on molecular weight, the additional saccharide units of the C-domain are not essential, and heparin with a molecular weight less than 5400 Da can potentiate AT to inactivate factor Xa. By convention, the potency ratio of anti-factor Xa to anti-factor IIa for UFH is 1. Unfractionated heparin is heterogenous and polydisperse but contains little or no material of molecular weight less than 5400 Da. The mean molecular weights of LMWH products are lower than those of UFH, and they contain a higher proportion of material that weighs less than 5400 Da. The potency ratio of anti-factor Xa to anti-factor IIa for LMWH products is greater than 1.5. This chapter describes assay procedures for the measurement of anti-factor Xa and anti-factor IIa activity of LMWH heparin in the presence of AT.

In the test system, heparin is bound to AT, and factor IIa or factor Xa added to the mixture binds to the heparin–AT complex. The residual factor IIa or factor Xa not inhibited by the heparin–AT complex is quantified by a chromogenic substrate that is specific for either factor IIa or factor Xa and is added in the final step. Analysts note an inverse relationship wherein more color is produced by more residual enzyme, which equates to less heparin activity.

As for any enzymatic assay, temperature and timing of the reaction, proper handling of the reagents, and the order in which the reagents are added are critical to the optimal performance of the assay.

Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated Heparin

- ANTI-FACTOR Xa ACTIVITY FOR UFH

The following procedure is used where specified in the individual monographs. This assay can be performed manually in plastic tubes utilizing heated block stations or water bath. Microtiter plate equipment with a reader and automated coagulometer can improve reproducibility and throughput.

pH 8.4 buffer: Dissolve amounts of tris(hydroxymethyl)aminomethane, edetic acid or edetate sodium, and sodium chloride in water containing 0.1% of polyethylene glycol 6000 to obtain solutions having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust, if necessary, with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) as directed by the manufacturer, and further dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 1.0 Antithrombin IU/mL.

Factor Xa solution: Reconstitute bovine factor Xa as directed by the manufacturer (see *Factor Xa* in *Reagents, Indicators, and Solutions—Reagent Specifications*), and further dilute in *pH 8.4 buffer* to obtain a solution that gives an absorbance value

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of 0.65–1.25 at 405 nm when assayed as described below but using 30 µL of *pH 8.4 buffer* instead of 30 µL of the *Standard solutions* or the *Sample solutions*. [NOTE—*Factor Xa solution* contains about 3 nanokatalytic units/mL but can vary depending on the manufacturer of factor Xa or the substrate used.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) specific for factor Xa in water to obtain a concentration of 1 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least 5 dilutions in the concentration range of 0.03–0.375 USP Heparin Units/mL.

Sample solutions: Dissolve or dilute an accurately measured quantity of Heparin Sodium in *pH 8.4 buffer*, and dilute with the same buffer to obtain solutions having activities approximately equal to those of the *Standard solutions*.

Analysis: [NOTE—The procedure also can be performed using alternative platforms. Perform the test with each *Standard solution* and *Sample solution* in duplicate.]

To each of a series of suitable plastic tubes placed in a water bath set at 37°, transfer 120 µL of *pH 8.4 buffer*. Then separately transfer 30 µL of the different dilutions of the *Standard solutions* or the *Sample solutions* to the tubes. Add 150 µL of *Antithrombin solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Factor Xa solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300 µL of *Chromogenic substrate solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for exactly 2 min. Add 150 µL of *Stopping solution* to each tube, and mix. Prepare a blank for zeroing the spectrophotometer by adding the reagents in reverse order, starting with the *Stopping solution* and ending with the addition of 150 µL of *pH 8.4 buffer*, and excluding the *Standard solutions* or the *Sample solutions*. Record the absorbance at 405 nm against the blank. The volume of the reactants can be increased or decreased to suit the assay format provided that the proportions of the reference sample or the test sample and the reagents are kept the same.

Calculations: Plot the log of the absorbance values of the *Standard solutions* and the *Sample solutions* vs. heparin concentrations in USP Units. Calculate the activity of heparin sodium in USP Units/mg using statistical methods for slope ratio assays. Calculate the anti-factor Xa activity of heparin sodium:

$$\text{Result} = A \times (S_T/S_S)$$

A = the potency of USP Heparin Sodium for Assays RS
S_T = slope of the line for the *Sample solutions*
S_S = slope of the line for the *Standard solutions*

Express the anti-factor Xa activity of the *Sample solution* as USP Heparin Units/mg, calculated on the dried basis. Calculate the ratio of anti-factor Xa activity against anti-factor IIa potency (see *Assay* below):

anti-factor Xa activity/anti-factor IIa potency

Acceptance criteria: 0.9–1.1

• **ANTI-FACTOR IIa ACTIVITY FOR UNFRACTIONATED HEPARIN**

pH 8.4 buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, 0–10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range of 0.005–0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis: [NOTE—The procedure also can be performed using alternative platforms.] For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. The volume of the reactants can be increased or decreased to suit the assay format provided that the proportions of the reference sample or the test sample and the reagents are kept the same.

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Two different types of measurements can be recorded:

1. Endpoint Measurement: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution.* Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). The RSD over the blank readings is less than 10%.
2. Kinetic Measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see ⟨857⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions,* and calculate the potency of heparin sodium in USP Units/mL using statistical methods for slope ratio assays. Express the potency of heparin sodium/mg, calculated on the dried basis.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions,* and calculate the potency of heparin sodium in USP Units/mL using statistical methods for parallel-line assays. Express the potency of heparin sodium/mg, calculated on the dried basis.

Acceptance criteria: The potency of heparin sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins

• ANTI-FACTOR Xa ACTIVITY FOR LOW MOLECULAR WEIGHT HEPARIN

Acetic acid solution: Glacial acetic acid and water (42:58)

pH 7.4 polyethylene glycol 6000 buffer: Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000 or 10.0 g of bovine serum albumin, adjust with hydrochloric acid to pH 7.4, and dilute with water to 1000 mL.

pH 7.4 buffer: Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to pH 7.4, and dilute with water to 1000 mL.

pH 8.4 buffer: Dissolve 3.03 g of tris(hydroxymethyl)aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.

Human antithrombin solution: Reconstitute a vial of human antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin Units per mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Antithrombin Unit/mL.

Factor Xa solution: Reconstitute an accurately weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions —Reagent Specifications*) as directed by the manufacturer's instructions. Further dilute the stock solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min or 0.8 absorbance units after 4 min of incubation with the chromogenic substrate when assayed as described below but using as an appropriate volume, *V,* the volume in µL of *pH 7.4 buffer* instead of *V* µL of the standard or the sample solution.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for factor Xa in water to obtain a concentration of about 3 mM. Dilute with substrate *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM. [NOTE—Preheat reagents to 37 ± 1° 15 min before use.]

Standard solutions: Reconstitute and dilute the entire contents of an ampule of USP Low Molecular Weight Heparin for Bioassays RS with distilled water and then further dilute with *pH 7.4 buffer* to obtain at least four dilutions in the concentration range of 0.025–0.2 USP anti-factor Xa Units/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of LMWH similar to those obtained for the *Standard solutions.*

Analysis

Samples: *Standard solutions, Sample solutions, Human antithrombin solution, pH 7.4 buffer, Factor Xa solution, Chromogenic substrate solution,* and *Acetic acid solution*

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions;* and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions.* [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add 50 µL of *Human antithrombin solution* and an equal volume, *V,* of either the blank, *pH 7.4 buffer,* or an appropriate dilution of the *Sample solutions* or the *Standard solutions.* Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 min. Add to each tube 100 µL of *Factor Xa solution,* and incubate for 1.0 min. Add 250 µL of *Chromogenic substrate solution.* Stop the reaction after about 4.0 min with 250 µL of *Acetic acid solution.* Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see ⟨857⟩). Use quartz or disposable polystyrene cuvettes. The volume of the reactants can be increased or decreased to suit the assay format provided that the proportions of the reference sample or the test sample and the reagents are kept the same.

System suitability: The assay is valid if the following requirements are met:

1. A blank solution gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min (or 0.8 absorbance units in total) when assayed using an appropriate volume (50 µL) of *pH 7.4 buffer* instead of 50 µL of the *Standard solution* or the *Sample solution.*
2. The reading of the blank B2 is not more than ±0.05 absorbance units against blank B1.

Calculations: For this bioassay, parallel-line or slope ratio analysis can be applied.

Calculate the potency of the test sample in USP anti-factor Xa Unit/mL, using a statistical model for parallel assays, plotting absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions.* In some cases, log

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transformation of absorbance may be needed to obtain linearity for the dose–response curves. The assay is valid when the data fulfill the acceptance criteria for regression, linearity, and parallelism as required for parallel line assay. For slope ratio analysis, plot absorbance against concentrations of *Sample solutions* and of the *Standard solutions*. In some cases, log transformation of absorbance may be needed to obtain linearity for the dose–response curves. The assay is valid when the data fulfill the acceptance criteria for regression, linearity, and common intercept as required for slope ratio assay (see *Design and Analysis of Biological Assays* ⟨111⟩ and *Analysis of Biological Assays* ⟨1034⟩).

Express the anti-factor Xa activity of sample/mg, calculated on the dried basis:

Anti-factor Xa USP Unit/mg = [standard potency (USP Unit/mL) × potency ratio]/[sample concentration (mg/mL)]

• **ANTI-FACTOR IIa ACTIVITY FOR LOW MOLECULAR WEIGHT HEPARIN**

Acetic acid solution, pH 7.4 polyethylene glycol 6000 buffer, pH 7.4 buffer, pH 8.4 buffer, and **Human antithrombin solution:** Proceed as directed in the *Anti-Factor Xa Activity*, except that the concentration of the *Human antithrombin solution* is 0.5 Antithrombin Unit/mL.

Thrombin human solution: Reconstitute thrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL. Use the *Thrombin human solution* immediately after preparation.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for the amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Dilute immediately before use with *pH 8.4 buffer* to 0.5 mM.

Standard solutions: Reconstitute and dilute the entire contents of an ampule of USP Low Molecular Weight Heparin for Bioassays RS with distilled water, and further dilute with *pH 7.4 buffer* to obtain at least four dilutions having concentrations in the range of 0.015–0.075 USP Unit of anti-factor IIa activity/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of LMWH similar to those obtained for the *Standard solutions*.

Procedure: Proceed as directed under *Anti-Factor Xa activity*, but use *Thrombin human solution* instead of *Factor Xa solution* and use the *Human antithrombin solution* as described above.

System suitability: The assay is valid if the following requirement is met:

1. The reading of blank B2 is NMT ±0.05 absorbance units against blank B1.

Calculations: Proceed as directed for calculation of *Anti-Factor Xa Activity*.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Heparin Sodium for Assays RS
USP Low Molecular Weight Heparin for Bioassays RS

⟨209⟩ LOW MOLECULAR WEIGHT HEPARIN MOLECULAR WEIGHT DETERMINATIONS

This chapter provides procedures used to determine molecular weight (MW) distribution and weight-average molecular weight for low molecular weight heparins (LMWH).

INTRODUCTION

Low molecular weight heparins are prepared from Heparin Sodium, USP, by partial depolymerization. LMWHs are polydisperse, i.e., they are made up of polysaccharide chains with a range of molecular weights. The MW distribution is a defining characteristic for each LMWH product. The *USP* contains specific monographs for LMWH products, including limits on molecular weight distribution parameters.

Most techniques for determination of the MW distribution of LMWHs make use of gel permeation chromatography (GPC), sometimes referred to as size exclusion chromatography (SEC). In order to derive molecular weight distribution from a chromatogram, it is necessary to know the relationship between retention time and MW. This chapter describes a GPC method that is calibrated using the Low Molecular Weight Heparin Molecular Weight Calibrant Reference Standard (RS). This preparation is a single polydisperse Standard for which the distribution of molecular weights is described in the form of a Broad Standard Table (see the USP Certificate for USP Low Molecular Weight Heparin Molecular Weight Calibrant RS) that lists the log of the molecular weight (log MW) of a number of reference points and the matching percent fractions by weight of the calibrant above or below these reference points. The Broad Standard Table is used together with a chromatogram of the calibrant to fit a suitable function, in this case a third degree polynomial, to the relationship between log MW and retention time, thus generating a calibration curve for the chromatographic system. With this calibration curve, the analyst calculates the weight-average molecular weight and distribution parameters from the chromatogram of a sample of LMWH.

All the calculations described in this chapter may be performed using a spreadsheet program, but this method is not recommended as it is laborious and open to human error. Proprietary software capable of automating the calibration and molecular weight calculations can be obtained from a number of chromatography systems manufacturers.

PROCEDURE

• **MOLECULAR WEIGHT MEASUREMENTS OF LOW MOLECULAR WEIGHT HEPARINS BY GEL PERMEATION CHROMATOGRAPHY**

The following procedure, with any necessary variations, is used where specified in the individual monographs.

Ammonium acetate stock solution: 1 M ammonium acetate in water

Sodium azide solution: 1% (w/v) sodium azide in water

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Mobile phase: Transfer 100 mL of *Ammonium acetate stock solution* to a 1-L volumetric flask, add 20 mL of *Sodium azide solution*, and dilute with water to volume. Filter using a nylon membrane of 0.45-µm pore size prior to use.
Calibration solution: Add 2 mL of *Mobile phase* to a vial containing USP Low Molecular Weight Heparin Molecular Weight Calibrant RS. Filter using a nylon membrane of 0.45-µm pore size prior to use.
Sample solution: 5 mg/mL of low molecular weight heparin sample in *Mobile phase*. Filter using a nylon membrane of 0.45-µm pore size.
System suitability solution: 5 mg/mL of USP Dalteparin Sodium RS in *Mobile phase*. Filter using a nylon membrane of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—The temperature of the refractive index detector must be set at the same temperature as that of the *Column temperature*.]
Mode: LC
Detector: Refractive index
Columns
Analytical: 7.8-mm × 30-cm; 5-µm packing L59 in series with a 7.8-mm × 30-cm; 5-µm packing L59[1]
Guard: 6-mm × 4-cm; 7-µm packing L59
Column temperature: 30°
Flow rate: 0.5 mL/min
Column equilibration: 0.5 mL/min for at least 2 h
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: There is baseline resolution between the last peak of the USP Low Molecular Weight Heparin Molecular Weight Calibrant RS and the salt peak, or negative exchange peaks.
Calibration curve: The coefficient of determination of the calibration curve fitted to the Broad Standard Table values must be NLT 0.990, using a third-order polynomial equation.
Weight-average molecular weight (M_w): Take the mean of the calculated M_w from the duplicate injections of the *System suitability solution*, and round to the nearest 50 Da. The chromatographic system is suitable if the M_w of the USP Dalteparin Sodium RS is within 150 Da of the labeled value as stated in the USP Certificate for USP Dalteparin Sodium RS.
Analysis
Samples: Inject 20 µL of the *Calibration solution* (single injection), *System suitability solution* (duplicate injections), and *Sample solution* (duplicate injections), and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks (about 50 min).
[NOTE—The calibrant, Standard, or sample of low molecular weight heparin will give a broad peak between about 25 and 45 min, followed by a later eluting narrow salt peak, as illustrated in the USP Certificate for USP Low Molecular Weight Heparin Molecular Weight Calibrant RS.]
Calculations: Calculate the total area under the low molecular weight heparin peak from the *Calibration solution* and the cumulative area at each point under the peak as a percent of the total. Do not include the salt peak. Using the Broad Standard Table provided in the USP Certificate for USP Low Molecular Weight Heparin Molecular Weight Calibrant RS, identify those points in the chromatogram for which the percent cumulative area is closest to the percent fractions listed in the Broad Standard Table, and assign the MW in this Table to the corresponding retention time (RT) in the chromatogram. For the set of retention times and molecular weights identified, fit log(MW) vs. RT to a third-order polynomial function using suitable gel permeation chromatography (GPC) software, or find values of a, b, c, and d such that $\log MW = a + (b \times RT) + [c \times (RT)^2] + [d \times (RT)^3]$.
Using the same GPC software, for each of the duplicate chromatograms of the *System suitability solution and the Sample solution*, with the calibration function derived as described above, calculate M_w:

$$M_w = \Sigma(RI_i \times M_i)/\Sigma RI_i$$

RI_i = detector response at each point
M_i = MW at each point

Round the mean value of M_w to the nearest 50 Da. Using the same GPC software, determine for each of the duplicate *Sample solution* chromatograms the percentage of product with molecular weight as indicated in the product monograph.
Acceptance criteria: As indicated in the product monograph

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dalteparin Sodium RS
USP Low Molecular Weight Heparin Molecular Weight Calibrant RS

[1] The method was validated using a guard column TSK SWXL 6-mm × 4-mm; 7-µm in series with two analytical columns: TSK G3000 SWXL 7.8-mm × 30-cm; 5-µm in series with a TSK G2000 SWXL 7.8-mm × 30-cm; 5-µm.

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⟨210⟩ MONOSACCHARIDE ANALYSIS

INTRODUCTION

The glycan composition of therapeutic glycoproteins may impact the biological activity, stability, pharmacokinetics, pharmacodynamics, efficacy, and immunogenicity of the molecule. The glycosylation pattern, or specific aspects of it such as the degree of sialylation, may form part of the product characterization and release specifications. One approach to ensuring the consistency of glycosylation and compliance of the product with specifications is to quantify specific monosaccharides present in the product. Monosaccharides are released by degradation of the product and quantified by chromatographic approaches. The most commonly determined sugars are *N*-acetylneuraminic acid (Neu5Ac) and its variant *N*-glycolylneuraminic acid (Neu5Gc). These monosaccharides can be selectively released from the protein either by dilute acid hydrolysis or by enzymatic methods, whereas increasingly aggressive hydrolysis conditions release neutral monosaccharides including mannose, galactose, and fucose, and then glucosamine and galactosamine. Separation and quantification of released monosaccharides by high-performance liquid chromatography (HPLC) can be achieved with or without fluorophore labeling.

SAMPLE HANDLING PRIOR TO MONOSACCHARIDE RELEASE

The glycoprotein sample should be as free of salts, excipients, and other carrier materials as possible. This is typically achieved by: 1) dialysis against water or a volatile buffer using an appropriate membrane, or ultrafiltration through an appropriate membrane; 2) HPLC on an appropriate gel-permeation column eluted with water or a volatile buffer, monitored by UV absorbance or refractive index; 3) sample trapping on a solid-phase extraction (SPE) cartridge, followed by washing away salts and excipients and elution of the required sample. Where residual components are known to be present, their lack of interference in saccharide analysis should be confirmed during validation to verify either no bias or an acceptable level of bias due to matrix components. Sample concentration may be required.

SIALIC ACID QUANTIFICATION

Sialic acids can be analyzed and quantified through the use of high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) without modification, or by the use of 1,2-diamino-4,5-methylenoxybenzene (DMB) labeling and reversed-phase HPLC (RP-HPLC) analysis equipped with a fluorescence detector. The combination of DMB labeling and RP-HPLC with fluorescence detection is significantly more sensitive than HPAEC-PAD analysis.

[NOTE—Under some circumstances, the presence of large amounts of protein may diminish the PAD response. For analysis by HPAEC-PAD, the use of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN), as an internal standard, can be an option to compensate for this effect. An example is to prepare an internal standard solution: 0.1 mM of USP KDN RS in water. In the final dilution step, add 15 µL of the internal standard solution into each standard or sample solution, and reduce the amount of water added by 15 µL to keep the final volume of solution unchanged. Integrate the Neu5Ac, Neu5Gc, and KDN peaks in each chromatogram, and use the ratio of the Neu5Ac or Neu5Gc peak areas relative to the peak area of KDN for system suitability and sample analysis calculation.]

Sialic acids may be *O*-acetylated, and depending on the protein sample and the analytical procedure selected, it may be necessary to de-*O*-acetylate before sialic acid is released. Normally, dilute acid hydrolysis and anion exchange chromatography analysis lead to de-*O*-acetylation. Enzymatic methods of release can preserve *O*-acetyl groups, and the combined use of DMB labeling and RP-HPLC analysis (as in *Procedure 3*) retains the *O*-acetyl groups during the analytical steps.

A typical de-*O*-acetylation can be achieved by adding 10% of the sample's volume of 1 M sodium hydroxide to the sample, incubating at room temperature for 30 min, and then adding 10% of the sample's volume of 1 M hydrochloric acid to neutralize the reaction (e.g., to 20 µL of sample add 2 µL of 1 M sodium hydroxide, incubate at room temperature for 30 min, and then add 2 µL of 1 M hydrochloric acid to neutralize).

PROCEDURES

- **PROCEDURE 1: ENZYMATIC RELEASE AND ANALYSIS BY HPAEC-PAD OF UNLABELED SIALIC ACIDS**

Sample buffer: 20 mM sodium acetate prepared as follows. Dissolve 164 mg of sodium acetate anhydrous in 80 mL of water. Adjust with glacial acetic acid to a pH of 5.2, and dilute with water to 100 mL.

Standard stock solution: 50 µM of USP *N*-Acetylneuraminic Acid RS and 2 µM of USP *N*-Glycolylneuraminic Acid RS in water

Standard solutions: See *Table 1* for typical five-level calibration *Standard solutions*.

Table 1

Standard Solutions	Standard Stock Solution (µL)	Sample Buffer (µL)	Water (µL)	Concentration of Neu5Ac (µM)	Concentration of Neu5Gc (µM)
Blank	0	200	300	0	0
1	10	200	290	1	0.04
2	20	200	280	2	0.08
3	30	200	270	3	0.12
4	40	200	260	4	0.16
5	50	200	250	5	0.2

[NOTE—1 µM = 1 nmol/mL = 1 × 10^{-3} nmol/µL = 1 pmol/µL.]

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Sample solution: To 50 µL of 6–60 µg/mL of protein sample (equivalent to about 1.5 nmol of sialic acid) in *Sample buffer* add 4 µL of 0.5 mU/µL (2 mU) of neuraminidase[1] in water and make up to a total volume of 200 µL with *Sample buffer*. Incubate for 5 h at 37°. [NOTE—Or use validated sample preparation and time/temperature ranges.] Add 300 µL of water, and mix.

HPAEC analysis

[NOTE—Use either *Chromatographic system 1* or *Chromatographic system 2*.]

Solution A: 0.1 M sodium hydroxide prepared as follows. Dilute 5.28 mL of 50%–52% sodium hydroxide solution with water to 1000 mL. [NOTE—The solution must be sealed well to avoid absorbing carbon dioxide.]

Solution B: 1 M sodium acetate in 0.1 M sodium hydroxide prepared as follows. Dissolve 82.0 g of anhydrous sodium acetate in 800 mL of water. Pass through a nylon filter of 0.2-µm pore size. Add 5.28 mL of 50%–52% sodium hydroxide solution, and dilute with water to 1000 mL.

Chromatographic system 1

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

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2	98	2
9.5	82	18
11	82	18
11.5	98	2
17	98	2

Mode: LC

Detector: Integrated amperometric detector with gold electrode (for an example of a waveform, see *Table 4*)

Columns

Guard: 3-mm × 3-cm; packing L69

Analytical: 3-mm × 15-cm; packing L69

Flow rate: 0.5 mL/min

Injection volume: 50 µL

[NOTE—For the system described, typical retention times for Neu5Ac and Neu5Gc are about 8 and 12 min, respectively.]

Chromatographic system 2

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

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	93	7
10	70	30
11	70	30
12	93	7
15	93	7

Mode: LC

Detector: Integrated amperometric detector with gold electrode (for an example of a waveform, see *Table 4*)

Columns

Guard: 4-mm × 5-cm; 10-µm packing L46[2]

Analytical: 4-mm × 25-cm; 10-µm packing L46[2]

Flow rate: 0.8 mL/min

Injection volume: 50 µL

[NOTE—For the system described, typical retention times for Neu5Ac and Neu5Gc are about 6 and 10 min, respectively.]

[1] Neuraminidase: recombinant *Arthrobacter ureafaciens* neuraminidase produced in *Escherichia coli* (*E. coli*), or similar enzyme, that releases both α2-3 and α2-6-linked sialic acid. One unit is defined as the amount of enzyme that will hydrolyze 1 µmole of 2′-*O*-substituted α-D-*N*-acetylneuraminic acid per minute at the specified pH at 37°.

[2] Ethylvinylbenzene/divinylbenzene substrate available from Thermo as Carbopac PA10, or a suitable equivalent.

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Table 4

Time (s)	Potential (V)	Gain Region	Ramp	Integration
0	0.1	Off	Ramp	Off
0.2	0.1	On	Ramp	On
0.4	0.1	Off	Ramp	Off
0.41	−2	Off	Ramp	Off
0.42	−2	Off	Ramp	Off
0.43	0.6	Off	Ramp	Off
0.44	−0.1	Off	Ramp	Off
0.5	−0.1	Off	Ramp	Off

System suitability
Samples: *Blank* and *Standard solutions*
Suitability requirements
Chromatogram of the blank: No peaks at the same retention time as Neu5Ac or Neu5Gc, *Blank*
Correlation coefficient (*R*) calculated for the standard curve: NLT 0.99, *Standard solutions*
Relative standard deviation: NMT 5%, *Standard solution 3*

Analysis
Samples: *Standard solutions* and *Sample solution*
For the *Standard solutions*, generate a Neu5Ac and Neu5Gc standard curve of peak response versus concentrations using least square regression. From the standard curve, determine the concentration of Neu5Ac and Neu5Gc in the *Sample solution*. Divide the determined Neu5Ac and Neu5Gc concentrations by the protein concentration in the *Sample solution*, and report as molar ratio (nmol:nmol). [NOTE—Convert the protein concentration from mg/mL to μM (1 μM = 1 nmol/mL).]

• **PROCEDURE 2: ACID HYDROLYSIS AND ANALYSIS BY HPAEC-PAD OF UNLABELED SIALIC ACIDS**
Sample buffer, HPAEC analysis, System suitability, and **Analysis:** Proceed as directed in *Procedure 1*.
[NOTE—*Standard solutions* and *Sample solution* are to be prepared at the same time and subjected to the same hydrolysis and drying procedures. Using vacuum centrifuge for drying is described in the procedures; other suitable drying methods may also be used.]
[NOTE—Sialic standards subjected to the hydrolysis procedure may degrade to a different extent than sialic acids released from a glycoprotein during the procedure. A validated method in which the standard is not subjected to the hydrolysis conditions may be used.]
Standard stock solution: 50 μM of USP *N*-Acetylneuraminic Acid RS and 2 μM of USP *N*-Glycolylneuraminic Acid RS in water
Standard solutions: Add the amount of *Standard stock solution* to each microcentrifuge tube as indicated in *Table 5*.

Table 5

Standard Solutions	Standard Stock Solution (μL)	Neu5Ac (nmol)	Neu5Gc (nmol)
Blank	0	0	0
1	10	0.5	0.02
2	20	1	0.04
3	30	1.5	0.06
4	40	2	0.08
5	50	2.5	0.1

Dry each standard in a vacuum centrifuge without heat. Add 100 μL of 2 M acetic acid to each microcentrifuge tube, and hydrolyze all the standards series at approximately 80° for 2 h ± 15 min in a heating block. [NOTE—Or use validated time/temperature ranges.] Allow the tubes to cool to room temperature for approximately 10 min, and briefly centrifuge to ensure the entire sample is in the well of the tube. Dry the solutions in a vacuum centrifuge without heat, and resuspend in 300 μL of water. Before analysis, add 200 μL of *Sample buffer*. The final concentrations are summarized in *Table 6*.

Table 6

Standard Solutions	Concentration of Neu5Ac (μM)	Concentration of Neu5Gc (μM)
Blank	0	0
1	1	0.04
2	2	0.08

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Table 6 *(continued)*

Standard Solutions	Concentration of Neu5Ac (µM)	Concentration of Neu5Gc (µM)
3	3	0.12
4	4	0.16
5	5	0.2

Sample solution: Add desalted protein, approximately 6–60 µg (equivalent to about 1.5 nmol of sialic acid) into a microcentrifuge tube. Dry in a vacuum centrifuge without heat. Add 100 µL of 2 M acetic acid, and hydrolyze at approximately 80° for 2 h ± 15 min in a heating block. [NOTE—Or use validated sample preparation and time/temperature ranges.] Allow the tube to cool to room temperature for approximately 10 min and briefly centrifuge to ensure the entire sample is in the well of the tube. Dry the sample in a vacuum centrifuge without heat, and resuspend the sample in 300 µL of water. Before analysis add 200 µL of *Sample buffer*.

Change to read:

- **PROCEDURE 3: ENZYMATIC HYDROLYSIS AND ANALYSIS BY RP-HPLC OF DMB-LABELED SIALIC ACIDS**

Labeling reagent: Aqueous solution of DMB, acetic acid, 2-mercaptoethanol, and sodium hydrosulfite[3]

Standard stock solution: 200 µM of USP *N*-Acetylneuraminic Acid RS and 10 µM of USP *N*-Glycolylneuraminic Acid RS in water

Standard solutions: See *Table 7* for the typical dilutions. Then label each solution as directed by *Labeling*.

Table 7

Standard Solutions	Standard Stock Solution (µL)	Water (µL)	Concentration of Neu5Ac before Labeling (µM)	Concentration of Neu5Gc before Labeling (µM)
Blank	0	400	0	0
1	4	396	2	0.1
2	8	392	4	0.2
3	20	380	10	0.5
4	40	360	20	1
5	80	320	40	2

The concentrations of the resulting labeled *Standard solutions* are listed in *Table 8*.

Table 8

Standard Solutions	Concentration of Neu5Ac after Labeling (µM)	Concentration of Neu5Gc after Labeling (µM)
Blank	0	0
1	0.02	0.001
2	0.04	0.002
3	0.1	0.005
4	0.2	0.01
5	0.4	0.02

Labeling: Transfer 5 µL of the to-be-labeled *Standard solution* or *Sample solution* into a microcentrifuge tube, add 20 µL of *Labeling reagent*, cap the tube, mix by vortexing, and incubate for 3 h at 50° in the dark. Terminate the reaction by adding 475 µL of water, cap, and vortex thoroughly. This solution should be kept at 2°–8° and analyzed within 24 h. [NOTE—The *Standard solution* and *Sample solution* are to be labeled simultaneously.]

Sample solution: Transfer approximately 0.5–50 µg of protein (equivalent to about 5 pmol of sialic acid) into a 1.5-mL microcentrifuge tube. Perform de-*O*-acetylation if needed. Adjust to a pH of 6.0 ± 0.3. [NOTE—The pH needs to be optimized based on the enzyme used.] Add 2 µL of 5 U/mL (10 mU) of neuraminidase,[1] adjust to a total volume of 50 µL, and incubate for 16–24 h at 37°. [NOTE—Or use validated sample preparation and time/temperature ranges.] Label the solution as directed in *Labeling* before analysis.

RP-HPLC analysis

[NOTE—Use either *Chromatographic system 3* or *Chromatographic system 4*.]

[3] Available from Ludger as LudgerTag Sialic Acid DMB Labeling Kit, catalog number LT-KDMB-A1, or a suitable equivalent. [NOTE—For example, prepare the DMB reagent in the order that follows. Add 1.5 mL of water to a glass vial. To this solution add 172 µL of glacial acetic acid, and mix well. Add 112 µL of 2-mercaptoethanol, and mix well. Add 4.9 mg of sodium hydrosulfite to the solution, and mix well. The solution may then become cloudy in appearance. Lastly, add 3.5 mg of DMB hydrochloride and 200 µL of water, and mix the solution well. This solution is aliquoted into 200-µL aliquots and can be stored in the dark at –20° for up to 12 months.]

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Mobile phase: Acetonitrile, methanol, and water (9:7:84)
Chromatographic system 3
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence detector with an excitation wavelength of 373 nm and an emission wavelength of 448 nm
Column: 2.1-mm × 10-cm; 1.9- to 2.2-µm packing L1
Flow rate: 0.25 mL/min
Injection volume: 5 µL
Temperatures
Column: 30°
Autosampler: 2°–8°
Run time: 10 min (isocratic)
[NOTE—For the system described, typical retention times for Neu5Ac and Neu5Gc are about 2.5 and 2 min, respectively.]
Chromatographic system 4
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence detector with an excitation wavelength of 373 nm and an emission wavelength of 448 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 5 µL
Temperatures
Column: 30°
Autosampler: 2°–8°
Run time: 30 min (isocratic)
[NOTE—For the system described, typical retention times for Neu5Ac and Neu5Gc are about 10 and 8 min, respectively.]
System suitability
Samples: *Blank* and *Standard solutions*
Suitability requirements
Chromatogram of the blank: No peaks at the same retention time as Neu5Ac or Neu5Gc, *Blank*
Correlation coefficient (*R*) calculated for the standard curve: NLT 0.99, *Standard solutions*
Relative standard deviation: NMT 5%, *Standard solution 3*
Analysis
Samples: *Standard solutions* and *Sample solution*
For the *Standard solutions*, generate a Neu5Ac and Neu5Gc standard curve of peak response versus concentrations using least square regression. From the standard curve, determine the concentration of Neu5Ac and Neu5Gc in the *Sample solution*. Divide the determined Neu5Ac and Neu5Gc concentrations by the protein concentration in the *Sample solution*, and report as molar ratio (nmol:nmol). [NOTE—Convert the protein concentration from mg/mL to ▲µM▲ (ERR 1-Jun-2018) (1 µM = 1 nmol/mL).]

• **PROCEDURE 4: ACID HYDROLYSIS AND ANALYSIS BY RP-HPLC OF DMB-LABELED SIALIC ACIDS**
Labeling reagent, Blank, Labeling, RP-HPLC analysis, System suitability, and **Analysis:** Proceed as directed in *Procedure 3*.
[NOTE—*Standard solutions* and *Sample solution* are to be prepared at the same time and subjected to the same hydrolysis and drying procedures. Using vacuum centrifuge for drying is described in the procedures; other suitable drying methods may be used.]
[NOTE—Sialic standards subjected to the hydrolysis procedure may degrade to a different extent than sialic acids released from a glycoprotein during the procedure. A validated method in which the standard is not subjected to the hydrolysis conditions may be used.]
Standard stock solution: 50 µM of USP *N*-Acetylneuraminic Acid RS and 2.5 µM of USP *N*-Glycolylneuraminic Acid RS in water
Standard solutions: Add the amount of *Standard stock solution* as indicated in *Table 9* to each microcentrifuge tube. Dry in vacuum centrifuge without heating.

Table 9

Standard Solution	Standard Stock Solution (µL)	Neu5Ac (pmol)	Neu5Gc (pmol)
Blank	0	0	0
1	1	50	2.5
2	2	100	5
3	5	250	12.5
4	10	500	25
5	20	1000	50

Add 25 µL of 2 M acetic acid into the tube and briefly centrifuge to ensure the sample is entirely in the well of the tube. Incubate at 80° for 2 h ± 15 min. [NOTE—Or use validated time/temperature ranges.] Allow the tube to cool to room

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temperature for approximately 10 min, and then vortex and centrifuge. Label the solution as directed in *Labeling* before analysis. The concentrations of the resulting labeled *Standard solutions* are listed in *Table 10*.

Table 10

Standard Solution	Concentration of Neu5Ac after Labeling (µM)	Concentration of Neu5Gc after Labeling (µM)
Blank	0	0
1	0.02	0.001
2	0.04	0.002
3	0.1	0.005
4	0.2	0.01
5	0.4	0.02

Sample solution: Transfer a desalted sample containing approximately 0.5–50 µg of protein (equivalent to about 5 pmol of sialic acid) into a 0.5-mL microcentrifuge tube. Dry in a vacuum centrifuge without heating. Add 25 µL of 2 M acetic acid into the tube and briefly centrifuge to ensure all of the sample is in the well of the tube. Incubate at 80° for 2 h ± 15 min. [NOTE—Or use validated sample preparation and time/temperature ranges.] Allow the tube to cool to room temperature for approximately 10 min. Then vortex and centrifuge. Label the solution as directed in *Labeling* before analysis.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP *N*-Acetylneuraminic Acid RS
USP *N*-Glycolylneuraminic Acid RS
USP KDN RS

3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid.

⟨211⟩ ARSENIC

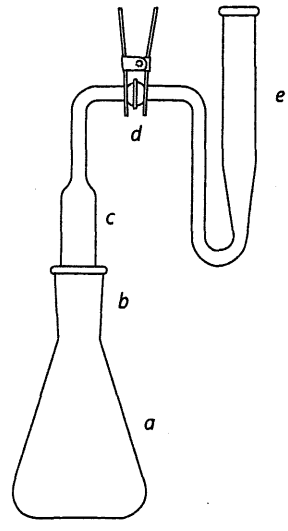
This procedure is designed to determine the presence of trace amounts of arsenic (As) by converting the arsenic in a substance under test to arsine, which is then passed through a solution of silver diethyldithiocarbamate to form a red complex. The red color so produced is compared, either visually or spectrophotometrically, to the color produced similarly in a control containing an amount of arsenic equivalent to the limit given in the individual monograph. Limits are stated in terms of arsenic (As). The content of arsenic does not exceed the limit given in the individual monograph.

Two methods are provided, the methods differing only in the preliminary treatment of the test substance and the standard. Generally, *Method I* is used for inorganic materials, while *Method II* is used for organic materials.

PROCEDURES

Apparatus

The apparatus (see *illustration*) consists of an arsine generator (*a*) fitted with a scrubber unit (*c*) and an absorber tube (*e*) with standard-taper or ground glass ball-and-socket joints (*b* and *d*) between the units. However, any other suitable apparatus, embodying the principle of the assembly described and illustrated, may be used.



Arsenic Test Apparatus

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Arsenic Trioxide Stock Solution: Dissolve 132.0 mg of arsenic trioxide, previously dried at 105° for 1 hour and accurately weighed, in 5 mL of sodium hydroxide solution (1 in 5) in a 1000-mL volumetric flask. Neutralize the solution with 2 N sulfuric acid, add 10 mL more of 2 N sulfuric acid, then add recently boiled and cooled water to volume, and mix.

Standard Arsenic Solution: Transfer 10.0 mL of *Arsenic Trioxide Stock Solution* to a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, then add recently boiled and cooled water to volume, and mix. Each mL of *Standard Arsenic Solution* contains the equivalent of 1 µg of arsenic (As). Keep this solution in an all-glass container, and use within 3 days.

Method I

Standard Preparation: Pipet 3.0 mL of *Standard Arsenic Solution* into a generator flask, and dilute with water to 35 mL.

Test Preparation: Unless otherwise directed in the individual monograph, transfer to the generator flask the quantity, in g, of the test substance calculated by the formula:

$$3.0/L$$

L = limit of arsenic (ppm)

Dissolve in water and dilute with water to 35 mL.

Procedure: Treat the *Standard Preparation* and the *Test Preparation* similarly as follows. Add 20 mL of 7 N sulfuric acid, 2 mL of potassium iodide TS, 0.5 mL of stronger acid stannous chloride TS, and 1 mL of isopropyl alcohol, and mix. Allow to stand at room temperature for 30 minutes. Pack the scrubber tube (*c*) with two pledgets of cotton that have been soaked in saturated lead acetate solution, freed from excess solution by expression, and dried in vacuum at room temperature, leaving a 2-mm space between the two pledgets. Lubricate the joints (*b* and *d*) with a suitable stopcock grease designed for use with organic solvents, and connect the scrubber unit to the absorber tube (*e*). Transfer 3.0 mL of silver diethyldithiocarbamate TS to the absorber tube. Add 3.0 g of granular zinc (No. 20 mesh) to the mixture in the flask, immediately connect the assembled scrubber unit, and allow the evolution of hydrogen and the color development to proceed at room temperature for 45 minutes, swirling the flask gently at 10-minute intervals. Disconnect the absorber tube from the generator and scrubber units, and transfer the absorbing solution to a 1-cm absorption cell. Any red color produced by the *Test Preparation* does not exceed that produced by the *Standard Preparation*. If necessary or desirable, determine the absorbance at the wavelength of maximum absorbance between 535 and 540 nm, with a suitable spectrophotometer or colorimeter, using silver diethyldithiocarbamate TS as the blank.

Interfering Chemicals: Metals or salts of metals, such as chromium, cobalt, copper, mercury, molybdenum, nickel, palladium, and silver, may interfere with the evolution of arsine. Antimony, which forms stibine, produces a positive interference in the color development with silver diethyldithiocarbamate TS; when the presence of antimony is suspected, the red colors produced in the two silver diethyldithiocarbamate solutions may be compared at the wavelength of maximum absorbance between 535 and 540 nm, with a suitable colorimeter, since at this wavelength the interference due to stibine is negligible.

Method II

[**CAUTION**—Some substances may react with explosive violence when digested with hydrogen peroxide. Exercise safety precautions at all times]

[NOTE—If halogen-containing compounds are present, use a lower temperature while heating the test specimen with sulfuric acid, avoid boiling the mixture, and add the hydrogen peroxide with caution, before charring begins, to prevent loss of trivalent arsenic.]

[NOTE—If the test substance reacts too rapidly and begins charring with 5 mL of sulfuric acid before heating, use instead 10 mL of cooled dilute sulfuric acid (1 in 2), and add a few drops of the hydrogen peroxide before heating.]

Standard Preparation: Pipet 3.0 mL of *Standard Arsenic Solution* into a generator flask, add 2 mL of sulfuric acid, mix, and add the total amount of 30 percent hydrogen peroxide used in preparing the *Test Preparation*. Heat the mixture to strong fuming, cool, add cautiously 10 mL of water, and again heat to strong fumes. Repeat this procedure with another 10 mL of water to remove any traces of hydrogen peroxide. Cool, and dilute with water to 35 mL.

Test Preparation: Unless otherwise directed in the individual monograph, transfer to a generator flask the quantity, in g, of the test substance calculated by the formula:

$$3.0/L$$

L = limit of arsenic (ppm)

Add 5 mL of sulfuric acid and a few glass beads, and digest in a fume hood, preferably on a hot plate and at a temperature not exceeding 120°, until charring begins. (Additional sulfuric acid may be necessary to wet some specimens completely, but the total volume added should not exceed 10 mL.) Cautiously add, dropwise, 30 percent hydrogen peroxide, allowing the reaction to subside and again heating between drops. Add the first few drops very slowly with sufficient mixing, in order to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the specimen from caking on glass exposed to the heating unit. *Maintain oxidizing conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens.* Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate until fumes of sulfur trioxide are copiously evolved, and the solution becomes colorless or retains only a light straw color. Cool, add cautiously 10 mL of water, mix, and again evaporate to strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 mL of water, wash the sides of the flask with a few mL of water, and dilute with water to 35 mL.

Procedure: Proceed as directed for *Procedure* under *Method I*.

Interfering Chemicals: See *Interfering Chemicals* under *Method I*.

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⟨212⟩ OLIGOSACCHARIDE ANALYSIS

INTRODUCTION

Analysis of the asparagine (Asn-) linked oligosaccharide (also known as the *N*-linked oligosaccharide or *N*-glycan) composition of selected recombinant therapeutic proteins has become necessary for product characterization or release specifications of these proteins. For information on the characterization and assessment of protein glycosylation, see *Glycoprotein and Glycan Analysis—General Considerations* ⟨1084⟩. Chapter ⟨1084⟩ covers general analytical strategies and also highlights criteria for selecting appropriate methods for specific analytical challenges, thereby providing the context for this chapter. This chapter focuses on the analysis of *N*-linked oligosaccharides that are released from glycosylated recombinant therapeutic proteins. Therefore, the chapter provides 1) a generic procedure for enzymatic release of *N*-glycans using peptide *N*-glycosidase F (PNGase F), 2) two different approaches for fluorophore labeling of the released *N*-linked oligosaccharides, and 3) five analytical procedures based on chromatographic and electrophoretic separation, as well as performance criteria for the separation. Alternative analytical strategies are discussed in ⟨1084⟩. Although the procedures in this chapter are validated, the procedures must be verified with the individual specific product (see *Verification of Compendial Procedures* ⟨1226⟩). Furthermore, validation is required when the procedure is optimized for a specific product [e.g., outside the allowed parameter limits defined in *Chromatography* ⟨621⟩, *System Suitability* (see *Validation of Compendial Procedures* ⟨1225⟩)]. Data analysis, quantitation, and lot release specifications, which are expected to be product specific, will be found in individual product monographs; therefore, these aspects are not covered in this chapter.

Labeling oligosaccharides with a fluorophore before analysis provides an important advantage of enhanced detection of oligosaccharides during separation, because oligosaccharides have no or low absorptivity of either ultraviolet (UV) or visible light and no significant fluorophore groups. This chapter describes two different methods for fluorophore labeling of oligosaccharides: derivatization of oligosaccharides using 2-aminobenzamide (2-AB) for liquid chromatographic separation, and labeling of oligosaccharides with 8-aminopyrene-1,3,6-trisulfonic acid (APTS) for capillary electrophoresis (CE) separation. Although electrochemical detection (pulsed amperometric detection or pulsed electrochemical detection) is also used in oligosaccharide analysis by using a strong base elution, the electrochemical detection method is less sensitive and has a higher noise-to-signal ratio. Therefore, this detection method is not addressed in this chapter.

Many different chromatography and electrophoresis methods have been developed to analyze oligosaccharides, and the procedures described in this chapter have been used successfully for this purpose. The choice of procedures discussed is meant to reflect the most commonly and broadly used approaches for supporting current recombinant glycosylated biological medicines in commerce. These analytical procedures include normal phase or hydrophilic interaction liquid chromatography (HILIC), high-performance anion exchange liquid chromatography (HPAEC), and CE. Several other chromatographic modes that separate oligosaccharides by size, shape, and polarity are also commonly used for oligosaccharide separation, but those other chromatographic modes are not discussed in this chapter. The general principles of chromatography are covered in ⟨621⟩, and therefore ⟨621⟩ should be considered within that context.

USP Reference Standards (RS) have been developed to assess system suitability for these analytical procedures:

- USP Oligosaccharide System Suitability Mixture A RS consists of a mixture of partially sialylated, partially galactosylated, and partially fucosylated biantennary *N*-linked oligosaccharides that were released from human polyclonal immunoglobulin G (IgG) by PNGase F.
- USP Oligosaccharide System Suitability Mixture B RS consists of a mixture of high-mannose *N*-linked oligosaccharides with traces of hybrid chains that were released from bovine ribonuclease B (RNase B) by PNGase F.
- USP Oligosaccharide System Suitability Mixture C RS is a mixture of bi-, tri-, and tetra-antennary *N*-linked oligosaccharides with variable sialylation with or without fucose (Fuc; also containing Le^x-type structures); the mixture was released from human α1 acid-glycoprotein by PNGase F.
- USP Oligosaccharide System Suitability Mixture D RS is a mixture of sialylated bi- and tri-antennary complex type structures with an additional sialic acid on one of the tri-antennary structures; the mixture was released from bovine fetuin by PNGase F.

ANALYTICAL PROCEDURES OVERVIEW

Table 1 outlines the applications and separation principles of the analytical procedures included in this chapter. The choice of the appropriate RS will depend on the expected glycan content of the product being analyzed.

Table 1

Analytical Procedures	Type of *N*-linked Oligosaccharides to Be Separated	Applicable USP Reference Standards
Normal phase liquid chromatography/HILIC procedure 1: • Column: 2.0-mm × 15-cm; 3-µm packing L68 • More oligosaccharide peaks compared to *HILIC procedure 2* • Longer mobile phase with shallow gradient compared to *HILIC procedure 2* • Enhanced sensitivity for smaller sample size	Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture A RS
	High-mannose chains, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture B RS

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Table 1 *(continued)*

Analytical Procedures	Type of *N*-linked Oligosaccharides to Be Separated	Applicable USP Reference Standards
Normal phase liquid chromatography/HILIC procedure 2: • Column: 4.6-mm × 25-cm; 5-µm packing L68 • Fewer oligosaccharide peaks observed compared to *HILIC procedure 1* • Shorter mobile phase with less shallow gradient compared to *HILIC procedure 1*	Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture A RS
	High-mannose chains, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture B RS
HPAEC procedure 1 • Guard column: 4.0-mm × 5-cm; 10-µm packing L46 • Column: 4.0-mm × 25-cm; 10-µm packing L46 • Mobile phase: 0–75 mM sodium acetate in isocratic 0.15 N sodium hydroxide • Selectivity is different from *HILIC procedures 1* and *2* with respect to the size and composition of oligosaccharide	Relatively simple biantennary chains with no or low levels of sialylated structures, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture A RS
	High-mannose chains, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture B RS
HPAEC procedure 2 • Guard column: 4.0-mm × 5-cm; 10-µm packing L46 • Column: 4.0-mm × 25-cm; 10-µm packing L46 • Mobile phase: 150–450 mM sodium acetate in isocratic 0.15 N sodium hydroxide • Suitable for separation of charged *N*-linked oligosaccharides	Bi-, tri-, and tetra-antennary *N*-linked oligosaccharides with variable sialylation, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture C RS
	Sialylated bi- and tri-antennary complex type structures with an additional sialic acid on one of the tri-antennary structure, after 2-AB labeling	USP Oligosaccharide System Suitability Mixture D RS
Capillary electrophoresis • Appropriate for APTS- oligosaccharides • Capillary: 50-µm inner diameter with 50 cm of total length and 40 cm of separation length	Relatively simple biantennary chains with no or low levels of sialylated structures, after APTS labeling	USP Oligosaccharide System Suitability Mixture A RS
	High-mannose chains, after APTS labeling	USP Oligosaccharide System Suitability Mixture B RS

SAMPLE PREPARATION

See *Appendix* (*Table 17*) for abbreviations and structures of glycans. The sample preparation is for all of the procedures that follow.

Ideally, the samples should be free of salts, excipients, and other carrier materials that may interfere with the analysis. This can be achieved by either 1) dialysis against water, a suitable buffer, or a volatile buffer using an appropriate membrane, 2) sample trapping on a solid-phase extraction (SPE) cartridge followed by washing away of salts and excipients and elution of the required sample, or 3) ultrafiltration using an appropriate membrane.

[NOTE—In addition to RS, a control sample with a known glycan profile should be included in the overall procedure to confirm correct performance of the analysis. A reaction blank control only containing the buffer matrix of the glycoprotein sample can also be included in the overall procedure.]

• **ENZYMATIC RELEASE OF *N*-GLYCANS**

[NOTE—The following is a generic method that should be optimized for individual products depending on the amount of glycoprotein to be digested and the glycan structures, especially on the basis of the protein-to-glycan ratio in the molecule and the accessibility of the sugars.]

Procedure 1 for chromatographic separation

Enzyme reaction buffer: 50 mM sodium phosphate pH 7.5 or 15 mM tris(hydroxymethyl)-aminomethane, and adjust with hydrochloric acid to a pH of 7.0.

Digestion with PNGase F: Transfer 0.1–2.25 mg of glycoprotein to a vial, and adjust with *Enzyme reaction buffer* to a final volume of 30–100 µL. Add PNGase F to the glycoprotein sample in a ratio of 0.5–15 units of PNGase F to 0.1 mg of glycoprotein. Incubate at 37° for 12–24 h. Cool at room temperature for about 5 min. Vortex gently and centrifuge briefly. [NOTE—One unit of PNGase F is defined as the amount of enzyme required to catalyze the release of *N*-linked oligosaccharides from 1.0 nmol of denatured ribonuclease B per min at a pH of 7.5 at 37°, and is equal to 1 IUB milliunit. Complete digestion by PNGase F can be assessed by observing a mobility shift of the deglycosylated proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by Coomassie Blue staining or on SDS-capillary gel electrophoresis (SDS-CGE), as shown by reduction in mass of approximately 2 kDa per glycan chain cleaved.]

Two methods are described below for performing the separation of released glycans from glycoprotein.

Method 1, using an ultrafiltration membrane with a molecular weight cutoff of 30 kDa:[1,2] [NOTE—Ultrafiltration membranes with a lower molecular weight cutoff may be used for proteins < 150 kDa.]

Remove trace amounts of glycerin by centrifuging at 11,000 × *g* and rinsing the membrane with 0.5 mL of water. Discard the permeate. Add 0.1 mL of water into the sample reservoir and then add the digested glycoprotein

[1] A suitable ultrafiltration membrane is YM-30 microcon centrifugal filter device from Millipore (catalog numbers 42422, 42409, and 42410) or an equivalent.

[2] A suitable ultrafiltration membrane is EMD Millipore Ultra-0.5 Centrifugal Filter Unit (catalog number UFC501096) or an equivalent.

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sample to the reservoir. Rinse the reaction vial with 0.1 mL of water and add to the sample reservoir. Centrifuge at 11,000 × *g* and collect the permeate. Rinse the sample reservoir twice using 0.1 mL of water for each wash and collect the permeate. Combine the permeates (approximately 0.5 mL) and bring the samples to complete dryness using a centrifugal evaporator without heat.

Method 2, using a reversed-phase SPE cartridge:[3] Add 2.0 mL of methanol into a syringe, attach the syringe to the cartridge, and use the syringe plunger to pass methanol through to waste. Add into a syringe 6.0 mL of 5% (v/v) acetic acid prepared in water. Attach the syringe to the cartridge and use the syringe plunger to pass the acetic acid solution through to waste. Gently apply the digested sample onto an individual cartridge. Into a 1-mL syringe add 0.5 mL of 5% (v/v) acetic acid, attach the syringe to the cartridge, and use the syringe plunger to pass the acetic acid through to waste. To a 2- to 3-mL syringe add 1.5 mL of 5% (v/v) acetic acid. Attach the syringe to the cartridge, and use the syringe plunger to pass the acetic acid solution through, collecting the eluting solution into a 1.5-mL tube. Bring the samples to complete dryness using a centrifugal evaporator without heat.

Procedure 2 for capillary electrophoresis separation

Enzyme reaction buffer: 50 mM sodium phosphate, pH 7.5

Digestion with PNGase F: Add 2 µL of PNGase F (5 units/µL) to 50 µg of glycoprotein sample, and adjust with *Enzyme reaction buffer* to a final volume of 50 µL. Incubate at 37° for 18 h. Separate released oligosaccharides by centrifugation using a centrifugal filter with a 10,000 molecular weight cut-off.[2] In a centrifugal vacuum evaporator, dry the released oligosaccharides to dryness.

[NOTE—Follow the guidelines listed in *Table 1* to select the appropriate USP RS and label.]

• **2-AB LABELING[4] FOR LIQUID CHROMATOGRAPHIC SEPARATION**

Solution A: Mix glacial acetic acid and dimethyl sulfoxide (3:7, v/v).

Solution B: Add 1.5 mL of *Solution A* to 75 mg of 2-AB. Mix well by gentle vortexing to completely dissolve 2-AB.

Labeling solution: Add 1 mL of *Solution B* to 63 mg of sodium cyanoborohydride. Mix well by gentle vortexing. Cap the mixture tightly and incubate at 70° for 1–2 min for complete dissolution. Cool at room temperature for 10 min. Use this *Labeling solution* within 1 h of its preparation, and protect the solution from light.

2-AB labeled standard solution: Add 5–15 µL of *Labeling solution* to 1 vial of USP Oligosaccharide System Suitability Mixture A RS, USP Oligosaccharide System Suitability Mixture B RS, USP Oligosaccharide System Suitability Mixture C RS, or USP Oligosaccharide System Suitability Mixture D RS, and mix well.

2-AB labeled sample solution: Add 5–15 µL of *Labeling solution* to the dried glycan sample after enzymatic release and separation steps, and mix well.

Procedure: Immediately incubate the *2-AB labeled standard solution* and the *2-AB labeled sample solution* at 37° for 16–18 h or at 65° for 2 h. Allow to cool at room temperature for 10 min. Centrifuge briefly.

Dried 2-AB labeled standard or Dried 2-AB labeled sample: Two methods for the removal of free 2-AB are described below.

Method 1, using a gel filtration spin column: Prepare suitable G-10 microcentrifuge spin columns[5] by tapping the column gently to ensure that the dry resin is settled at the bottom. Remove the caps, and place the column in a 2-mL collection tube. Add 0.5 mL of water to the resin, and let it swell for at least 15 min. A longer swelling time (up to 24 h) is acceptable if maintained at 2°–8°. Centrifuge the column at maximum speed for 5–10 s. Remove the water from the collection tube. Wash the resin by adding 0.5 mL of water, and centrifuge the column. Repeat the washing step one more time. After removing the water from the final wash step, spin at maximum speed for 10 s to remove residual water from the resin. Residual water is sufficiently removed if the resin appears white. Place the column in a new, labeled collection tube. Add 100 µL of water to the *2-AB labeled standard solution* and the *2-AB labeled sample solution,* and then apply the entire solution to the center of each washed G-10 column, respectively. Place the G-10 columns in the microcentrifuge and spin at approximately 200 × *g* for 1 min. Apply the flow-through (approximately 90–120 µL) to the center of the second unused but previously washed G-10 column and spin at approximately 200 × *g* for 1 min. Transfer the repeat flow-through (approximately 60–100 µL) to a 0.5-mL microcentrifuge tube. Dry the eluates by centrifugal evaporation with no heat.

Method 2, using an SPE cartridge: Prepare suitable SPE cartridges[6] by washing with 1.0 mL of water, followed by 5 × 1.0 mL of 30% (v/v) acetic acid, and then 1.0 mL of acetonitrile. Apply the *2-AB labeled standard solution* and the *2-AB labeled sample solution* to the center surface of the cartridge discs, and allow the solution to incubate on the disc for 15–20 min. Wash each disc with 1 mL of acetonitrile, followed by 6 × 1.0 mL of 96% (v/v) acetonitrile, allowing each aliquot to drain before the next is applied. Elute the *2-AB labeled standard solution* and the *2-AB labeled sample solution* with 3 × 0.5 mL of water, allowing each aliquot to drain before the next is applied. Dry the eluates by centrifugal evaporation with no heat. [NOTE—Very small hydrophobic glycans may be lost during this procedure. During method verification, it should be confirmed that no oligosaccharide species are lost during the 2-AB removal step by comparing the chromatographic profiles of an unextracted reaction blank, an unextracted sample, and an extracted sample.]

• **APTS LABELING FOR CAPILLARY ELECTROPHORESIS SEPARATION**

APTS labeling reagent: Dissolve 5 mg of trisodium APTS in 48 µL of 15% (v/v) acetic acid.

1 M sodium cyanoborohydride: 1 M sodium cyanoborohydride in tetrahydrofuran

Run buffer: Dissolve 1.492 g of triethanolamine (TEA) and 10 g of glycerol, accurately weighed, in 80 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.5, and dilute with water to a final volume of 100 mL.

Sample buffer: Dilute 1.0 mL of *Run buffer* with 9.0 mL of water.

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[3] A suitable SPE cartridge is Glyko GlycoClean R Cartridge from Prozyme (catalog number GKI-4756) or an equivalent.

[4] A suitable 2-AB labeling kit is Glyko Signal 2-AB Labeling Kit from Prozyme (catalog number GKK-404), LudgerTage 2-AB labeling kit from QA Bio (catalog number LT-KAB-A2), or an equivalent.

[5] A suitable prepacked Sephadex G-10 column is Macro Spin G-10 mini SEC column from Harvard Apparatus (catalog number 743900) or The Nest Group (catalog number SMM S010), or an equivalent.

[6] A suitable cartridge to remove free 2-AB is Glyko GlycoClean S Cartridge from Prozyme (catalog number GKI-4726) or an equivalent.

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APTS-labeled standard solution: Add 2 µL of the *APTS labeling reagent* and 2 µL of *1 M sodium cyanoborohydride* to a vial of USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS. Incubate at 55° for 90 min. Add 46 µL of water to quench the reaction and mix. Transfer 5 µL of the APTS-labeled USP RS to 1.995 mL of *Sample buffer* before separation. [NOTE—The volume of *Sample buffer* may need to be adjusted so that the fluorescence signals of the oligosaccharides are similar to those from the *APTS-labeled sample solution*.]

APTS-labeled sample solution: Add 2 µL of *APTS labeling reagent* and 2 µL of *1 M sodium cyanoborohydride* to the dried glycan sample after enzymatic release and separation steps. Incubate at 55° for 90 min. Add 46 µL of water to quench the reaction and mix. Transfer 5 µL of the APTS-labeled glycan solution to 95 µL of *Sample buffer* before separation.

[NOTE—Follow the guidelines listed in *Table 1* to select the appropriate USP RS and label.]

SEPARATION AND IDENTIFICATION OF OLIGOSACCHARIDES

• **NORMAL PHASE CHROMATOGRAPHY/HILIC**

Procedure 1

1.4 M formic acid solution: Mix 273 mL of water with 15 mL of 98%–100% formic acid.

1.4 M ammonia solution: Mix 155 mL of water with 40 mL of 26% ammonia solution.

Ammonium formate buffer: Add *1.4 M ammonia solution* to *1.4 M formic acid solution* until a pH of 4.4 is obtained.

Solution A: Acetonitrile, *Ammonium formate buffer*, and water (75: 4.3: 20.7)

Solution B: Acetonitrile, *Ammonium formate buffer*, and water (54: 8.3: 37.7)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	79	21
80.0	47	53
81.0	0	100
92.0	0	100
93.0	79	21
113.0	79	21

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 µL of water. [NOTE—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [NOTE—Use a ratio of 30 µL of water per 100 µg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Chromatographic system

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

Mode: LC

Detector: Fluorescence (250-nm excitation wavelength and 428-nm emission wavelength)

Column: 2.0-mm × 15-cm; 3-µm packing L68

Temperatures

Column: 45°

Autosampler: 2°–8°

Flow rate: 0.2 mL/min

Injection volume: 2 µL

System suitability

Samples: *Standard solution* and *Blank solution*

Suitability requirements

Blank: No peak in the chromatogram of the *Blank solution* within the retention time window at 5–113 min

Chromatogram similarity: The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS.

Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G0, G0F, G1Fa, G1Fb, G2F, A1F,* and *A2F* (see *Table 3*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to oligomannose (MAN) *MAN-5, MAN-6, MAN-7, MAN-8,* and *MAN-9* (see *Table 4*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 3* or *Table 4*

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Table 3. Standard Solution Using USP Oligosaccharide System Suitability Mixture A RS

N-Glycan	Approximate Relative Retention Time
G0 (NGA2)	0.47
G0F (NGA2F)	0.57
G1Fa (NA1F, FA2G1, or NA2G1F)	0.75
G1Fb (NA1F, FA2G1, or NA2G1F)	0.78
G2F (NA2F)	1.00
A1F (G2fS1)	1.39
A2F (G2fS2)	1.76

Table 4. Standard Solution Using USP Oligosaccharide System Suitability Mixture B RS

N-Glycan	Approximate Relative Retention Time
MAN-5	0.74
MAN-6	1.00
MAN-7	1.29
MAN-8	1.60
MAN-9	1.85

Analysis: Equilibrate the *Chromatographic system* for at least 1 h with starting conditions described in *Table 2*. Inject the *Blank solution* until the baseline is stable (1–3 injections).

Sample: *Sample solution*

Integrate peaks in the resulting chromatogram, and report the relative peak areas of glycan structures relevant to the product. A baseline is drawn from the first to the last peak. For general information on chromatographic peak integration, see ⟨621⟩.

Procedure 2

Solution A: 250 mM ammonium formate, pH 4.0, prepared as follows. Mix 500 mL of water with 9.8 mL of 96% formic acid. Adjust with 30% ammonium hydroxide to a pH of 4.0 ± 0.1. Dilute with water to a final volume of 1000 mL. Pass the solution through a 0.22-µm pore size filter composed of cellulose acetate membrane.

Solution B: Acetonitrile

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0.0	20	80
2.0	30	70
67.0	52	48
67.1	80	20
73.0	80	20
73.1	20	80
85.0	20	80

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NLT 500 µL of water. [NOTE—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [NOTE—Use a ratio of 50 µL of water per 100 µg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Chromatographic system

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

Mode: LC

Detector: Fluorescence; set the excitation and emission wavelengths as listed in *Table 6*.

Table 6

Time (min)	Excitation (nm)	Emission (nm)
0.00	330	420

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Table 6 *(continued)*

Time (min)	Excitation (nm)	Emission (nm)
1.00	330	420
1.01	400	500
10.00	400	500
10.01	330	420
85.00	330	420

Column: 4.6-mm × 25-cm; 5-µm packing L68
Temperatures
Column: 35°
Autosampler: 2°–8°
Flow rate: 0.4 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Chromatogram similarity: The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS.
Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G0*, *G0F*, *G1Fa*, *G1Fb*, *G2*, *G2F*, *A1F*, and *A2F* (see *Table 7*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to *MAN-5*, *MAN-6*, *MAN-7*, *MAN-8*, and *MAN-9* (see *Table 8*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.
Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 7* or *Table 8*

Table 7. Standard Solution Using USP Oligosaccharide System Suitability Mixture A RS

N-Glycan	Approximate Relative Retention Time
G0 (NGA2)	0.78
G0F (NGA2F)	0.83
G1Fa (NA1F, FA2G1, or NA2G1F)	0.91
G1Fb (NA1F, FA2G1, or NA2G1F)	0.92
G2 (NA2)	0.96
G2F (NA2F)	1.00
A1F (G2fS1)	1.11
A2F (G2fS2)	1.20

Table 8. Standard Solution Using USP Oligosaccharide System Suitability Mixture B RS

N-Glycan	Approximate Relative Retention Time
MAN-5	0.77
MAN-6	0.86
MAN-7	0.93
MAN-8	1.00
MAN-9	1.05

Analysis
Sample: *Sample solution*
Integrate peaks in the resulting chromatogram and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see ⟨621⟩.

• **HIGH-PERFORMANCE ANION EXCHANGE LIQUID CHROMATOGRAPHY WITH FLUOROMETRIC DETECTION**
Procedure 1
Solution A: Water; degas before use.

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Solution B: 0.5 M sodium acetate, prepared as follows. Dissolve 20.5 g of anhydrous sodium acetate in 450 mL of water and mix well. Dilute with water to a final volume of 500 mL. Filter the solution through a membrane of NMT 0.45-µm pore size, and degas before use.
Solution C: 0.5 M sodium acetate, prepared as follows. To 972 mL of water, add 26 mL of 50% (w/w) sodium hydroxide solution. Pass the solution through an alkaline-resistant filter membrane of NMT 0.45-µm pore size, and degas before use.
Mobile phase: See *Table 9*.

Table 9

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Elution
0.0	70	0	30	Initial condition
15.0	70	0	30	0–75 mM sodium acetate, isocratic 0.15 N sodium hydroxide
65.0	55	15	30	
66.0	0	50	50	250 mM sodium acetate, 0.25 N sodium hydroxide wash
74.0	0	50	50	
75.0	70	0	30	Re-equilibrium
90.0	70	0	30	

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 µL of water. [NOTE—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]
Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [NOTE—Use a ratio of 20 µL of water per 100 µg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]
Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: Fluorescence (330-nm excitation wavelength and 420-nm emission wavelength)
Columns
Guard: 4.0-mm × 5-cm; 10-µm packing L46
Analytical: 4.0-mm × 25-cm; 10-µm packing L46
Temperatures
Column: 25°
Autosampler: 4°
Flow rate: 0.8 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Chromatogram similarity: The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS.
Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G0F, G1F, G0, G2F, A1F,* and *A2F* (see *Table 10*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to *MAN-5, MAN-6, MAN-7, MAN-8,* and *MAN-9* (see *Table 11*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.
Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 10* or *Table 11*

Table 10. Standard Solution Using USP Oligosaccharide System Suitability Mixture A RS

N-Glycan	Approximate Relative Retention Time
G0F (NGA2F)	0.67
G1F (NA1F, FA2G1, or NA2G1F)	0.86
G0 (NGA2)	0.92
G2F (NA2F)	1.00
A1F (G2fS1)	1.75
A2F (G2fS2)	2.22

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Table 11. Standard Solution Using USP Oligosaccharide System Suitability Mixture B RS

N-Glycan	Approximate Relative Retention Time
MAN-5	0.86
MAN-6	1.00
MAN-7	1.09
MAN-8	1.15
MAN-9	1.24

Analysis: Equilibrate the *Chromatographic system* with the initial *Mobile phase* conditions described in *Table 9* for a minimum of 15 min. Inject 50 µL of water for the first injection.

Sample: *Sample solution*

Integrate peaks in the resulting chromatogram and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see ⟨621⟩.

Procedure 2

Solution A: Water; degas before use

Solution B: 0.5 M sodium acetate, prepared as follows. Dissolve 41.0 g of sodium acetate anhydrous in 900 mL of water. Transfer the solution to a 1-L volumetric flask, and bring with water to volume. Filter the solution through a nylon membrane with NMT 0.45-µm pore size, and degas before use.

Solution C: 0.5 M sodium hydroxide, prepared as follows. To 900 mL of water, add 26 mL of 50% (w/w) sodium hydroxide solution, and mix well. Bring the solution with water to the final volume of 1000 mL. Filter the solution through an alkaline-resistant nylon membrane with NMT 0.45-µm pore size, and degas before use.

Mobile phase: See *Table 12*.

Table 12

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Elution
0.0	80	10	10	Initial condition
15.0	80	10	10	50–150 mM sodium acetate, isocratic 0.05 N sodium hydroxide
70.0	60	30	10	150–450 mM sodium acetate, isocratic 0.05 N sodium hydroxide
94.0	0	90	10	Sodium acetate wash, no gradient
99.0	0	90	10	
105	0	10	90	Sodium hydroxide wash, no gradient
110	00	10	90	
111	80	10	10	Re-equilibrium
130	80	10	10	

Standard solution: Reconstitute the *Dried 2-AB labeled standard* with NMT 500 µL of water. [NOTE—The volume of water may need to be adjusted so that the fluorescence signals from the *Standard solution* are similar to those from the *Sample solution*.]

Sample solution: Reconstitute the *Dried 2-AB labeled sample* with an appropriate volume of water. [NOTE—Use a ratio of 20 µL of water per 100 µg of glycoprotein used in the *Enzymatic Release of N-Glycans* test as a starting point.]

Blank solution: Buffer matrix of sample carried through the *Sample Preparation* procedure.

Chromatographic system

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

Mode: LC

Detector: Fluorescence (330-nm excitation wavelength and 420-nm emission wavelength)

Columns

Guard: 4.0-mm × 5-cm; 10-µm packing L46

Analytical: 4.0-mm × 25-cm; 10-µm packing L46

Temperatures

Column: 25°

Autosampler: 4°

Flow rate: 0.5 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution* and *Blank solution*

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Suitability requirements

Blank: The chromatogram with *Blank solution* must not have any interfering components in the regions of *N*-glycan elution.

Chromatogram similarity: The chromatogram obtained from the *Standard solution* corresponds to that of the reference chromatogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture C RS or USP Oligosaccharide System Suitability Mixture D RS.

Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture C RS is used, identify peaks corresponding to *A1* (peak 1 and peak 2), *A2*, and *A3* (see *Table 13*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the Certificate. If USP Oligosaccharide System Suitability Mixture D RS is used, identify peaks corresponding to *A1* (peak 1), *A2*, *A3*, and *A3G3S4* (see *Table 14*; see also *Appendix*) in the chromatogram obtained from the *Standard solution* by comparing the reference chromatogram provided with the Certificate.

Relative retention times: Corresponding to the relative retention times of *N*-glycans listed in *Table 13* or *Table 14*

Table 13. Standard Solution Using USP Oligosaccharide System Suitability Mixture C RS

N-Glycan	Approximate Relative Retention Time
A1 (G1S1), peak 1	0.31
A1 (G1S1), peak 2	0.32
A2 (G2S2)	0.73
A3 (G3S3)	1.00

Table 14. Standard Solution Using USP Oligosaccharide System Suitability Mixture D RS

N-Glycan	Approximate Relative Retention Time
A1 (G1S1), peak 1	0.23
A2 (G2S2)	0.57
A3 (G3S3)	0.78
A3G3S4 (S4NA3, or A3 + Sa)	1.00

Analysis: [NOTE—Equilibrate the column with initial mobile phase conditions for a minimum of 15 min.]

Sample: *Sample solution*

Inject 25 µL of water, and run the gradient program at least once to equilibrate the column and system. Integrate peaks in the resulting chromatogram, and report the relative peak areas of glycan structures relevant to the product. For general information on chromatographic peak integration, see ⟨621⟩.

• **CAPILLARY ELECTROPHORESIS**

Run buffer: Dissolve 1.492 g of TEA and 10 g of glycerol in 80 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.5 and dilute with water to a final volume of 100 mL.

Standard solution: *APTS-labeled standard solution* prepared as directed in the test for *APTS Labeling for CE Separation*

Sample solution: *APTS-labeled sample solution* prepared as directed in the test for *APTS Labeling for CE Separation*

Blank solution: Buffer matrix of glycoprotein sample carried through the *Sample Preparation* procedure

Instrumental conditions

Mode: CE

Detector: Laser-induced fluorescence (488-nm excitation wavelength and 520-nm emission wavelength)

Capillary: 50-µm inner diameter bare fused-silica with 50 cm total length and 40 cm separation length

Preconditioning of the capillary: Between each run, rinse for 5 min at 40 psi with 0.5 N sodium hydroxide, followed by water for 1 min at 40 psi. [NOTE—Precondition a new capillary or as needed: Rinse for 5 min at 20 psi with methanol, followed by water for 1 min at 50 psi, 1 N hydrochloric acid for 5 min at 20 psi, water for 1 min at 50 psi, 0.5 N sodium hydroxide for 25 min at 20 psi, and water for 5 min at 50 psi.]

Prefilling of the capillary: Rinse for 5 min at 40 psi with *Run buffer.*

Sample injection: 10 s hydrodynamic (pressure) injection

Voltage: 22 kV for 60 min

Temperatures

Capillary cartridge: 18°

Sample storage: 20°

System suitability

Sample: *Standard solution*

Suitability requirements

Electropherogram similarity: The electropherogram obtained from the *Standard solution* corresponds to that of the reference electropherogram provided with the Certificate for either USP Oligosaccharide System Suitability Mixture A RS or USP Oligosaccharide System Suitability Mixture B RS.

Presence of *N*-glycan species: If USP Oligosaccharide System Suitability Mixture A RS is used, identify peaks corresponding to *G2F*, *G2*, *G1Fa*, *G1Fb*, *G0F*, *MAN-5*, *G0*, and *A1F* (see *Table 15*; see also *Appendix*) in the electropherogram obtained from the *Standard solution* by comparing the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture A RS. If USP Oligosaccharide System Suitability Mixture B RS is used, identify peaks corresponding to *MAN-5*, *MAN-6*, *MAN-7*, *MAN-8*, and *MAN-9* (see

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Table 16; see also *Appendix*) in the electropherogram obtained from the *Standard solution* by comparing the reference electropherogram provided with the USP Certificate for USP Oligosaccharide System Suitability Mixture B RS.

Relative migration times: Corresponding to relative migration times listed in *Table 15* or *Table 16*

Table 15. Standard Solution Using USP Oligosaccharide System Suitability Mixture A RS

N-Glycan	Approximate Relative Migration Time
G2F (NA2F)	0.79
G2 (NA2)	0.84
G1Fb (NA1F, FA2G1, or NA2G1F)	0.87
G1Fa (NA1F, FA2G1, or NA2G1F)	0.90
G0F (NGA2F)	1.00
MAN-5	1.03
G0 (NGA2)	1.12
A1F (G2fS1)	1.18

Table 16. Standard Solution Using USP Oligosaccharide System Suitability Mixture B RS

N-Glycan	Approximate Relative Migration Time
MAN-9	0.82
MAN-8	0.85
MAN-7	0.91, 0.92, 0.94
MAN-6	1.00
MAN-5	1.11

Analysis

Sample: *Sample solution*

Integrate peaks in the resultant electropherogram and report the relative peak areas of glycan structures relevant to the product.

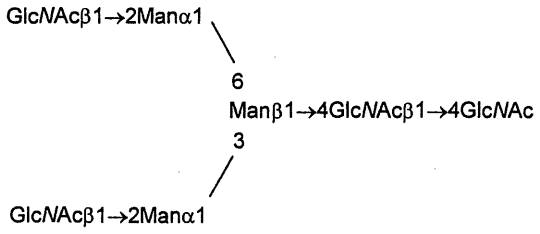
ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Oligosaccharide System Suitability Mixture A RS
USP Oligosaccharide System Suitability Mixture B RS
USP Oligosaccharide System Suitability Mixture C RS
USP Oligosaccharide System Suitability Mixture D RS

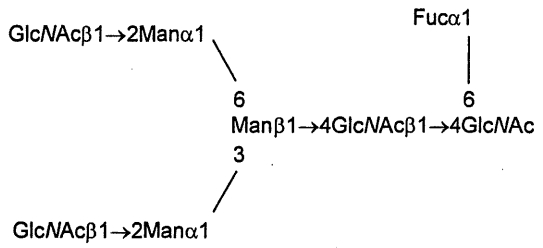
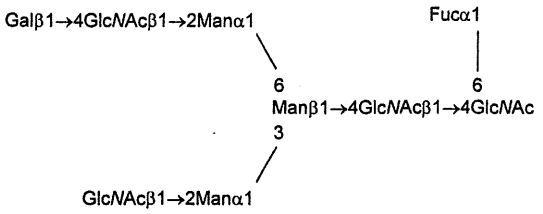
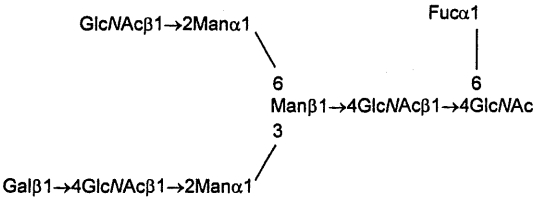
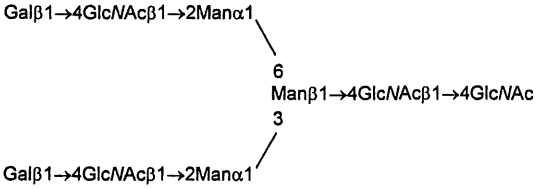
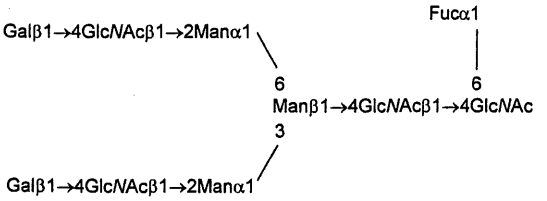
APPENDIX

Table 17. Glycan Descriptions

Glycan	Description
G0 (NGA2)	Asialo-, agalacto- biantennary oligosaccharide 

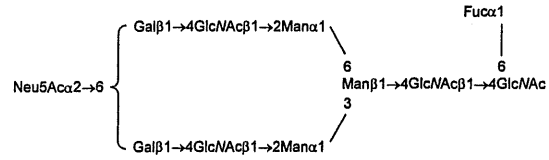
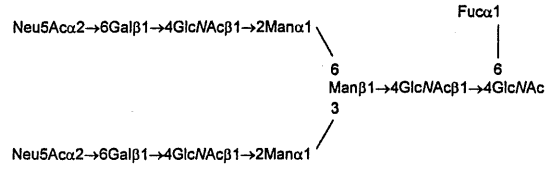
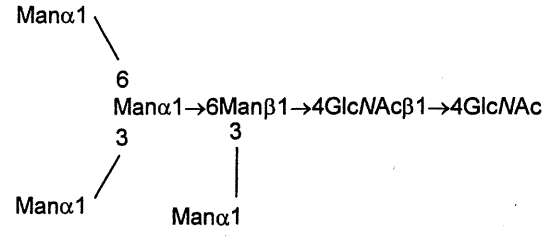
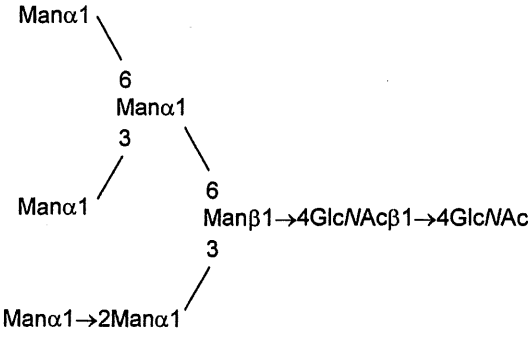
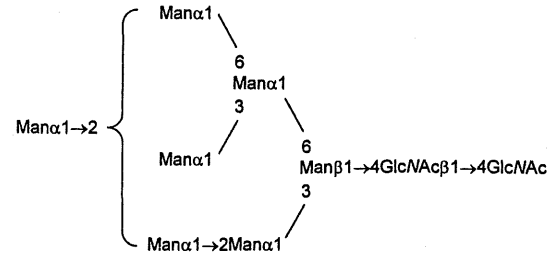
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Table 17. Glycan Descriptions *(continued)*

Glycan	Description
G0F (NGA2F)	Asialo-agalacto-fucosylated biantennary oligosaccharide 
G1Fa (NA1F, FA2G1, or NA2G1F) with the α(1,6) linkage	Asialo-, monogalactosylated, fucosylated biantennary oligosaccharide with galactosylation at the α(1,6) linkage 
G1Fb (NA1F, FA2G1, or NA2G1F) with the α(1,3) linkage	Asialo-, monogalactosylated, fucosylated biantennary oligosaccharide with galactosylation at the α(1,3) linkage 
G2 (NA2)	Asialo, galactosylated, biantennary oligosaccharide 
G2F (NA2F)	Asialo-fucosylated biantennary oligosaccharide 

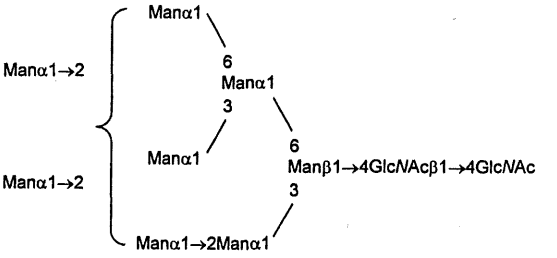
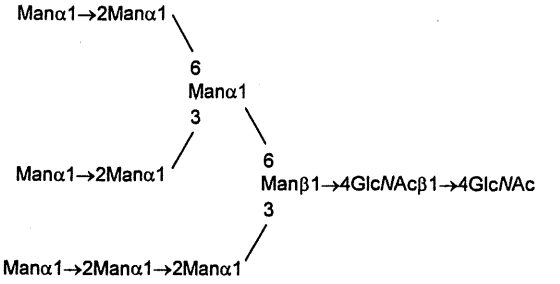
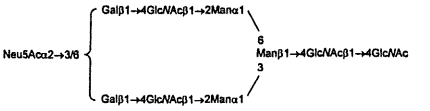
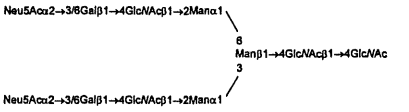
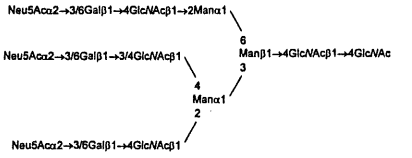
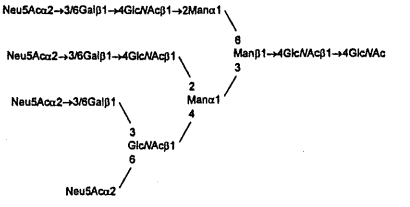
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Table 17. Glycan Descriptions *(continued)*

Glycan	Description
A1F (G2fS1)	Monosialo-fucosylated biantennary oligosaccharide 
A2F (G2fS2)	Disialo-fucosylated biantennary oligosaccharide 
MAN-5	Oligomannose 5 Oligomannose *N*-linked oligosaccharide with 5 mannosyl residues 
MAN-6	Oligomannose 6 Oligomannose *N*-linked oligosaccharide with 6 mannosyl residues 
MAN-7	Oligomannose 7 Oligomannose *N*-linked oligosaccharide with 7 mannosyl residues 

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Table 17. Glycan Descriptions *(continued)*

Glycan	Description
MAN-8	Oligomannose 8 Oligomannose *N*-linked oligosaccharide with 8 mannosyl residues 
MAN-9	Oligomannose 9 Oligomannose *N*-linked oligosaccharide with 9 mannosyl residues 
A1 (G1S1)	Mono-sialylated, galactosylated, biantennary oligosaccharide 
A2 (G2S2)	Di-sialylated, galactosylated, biantennary oligosaccharide 
A3 (G3S3)	Tri-sialylated, galactosylated, triantennary oligosaccharide 
A3G3S4 (S4NA3, or A3 + Sa)	Tetra-sialylated, galactosylated, triantennary oligosaccharide 

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⟨221⟩ CHLORIDE AND SULFATE

Change to read:

The following limit tests are provided as general procedures for use where limits for chloride and sulfate are specified in the individual monographs.

Perform the tests and the controls in glass cylinders of the same diameter and matched as closely as practicable in other respects (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). Use the same quantities of the same reagents for both the solution under test and the control solution containing the specified volume of chloride or sulfate. If, after acidification, the solution is not perfectly clear, pass it through a filter paper that gives negative tests for chloride and sulfate. Add the precipitant, silver nitrate TS or barium chloride TS as required, to both the test solution and the control solution in immediate sequence.

Where the individual monograph calls for applying the test to a specific volume of a solution of the substance, and the limit for chloride or sulfate corresponds to 0.20 mL or less of 0.020 N hydrochloric acid or sulfuric acid, respectively, apply the test to the solution without further dilution. In such cases maintain the same volume relationships for the control solution as specified for the solution under test. In applying the test to the salts of heavy metals, which normally show an acid reaction, omit the acidification and do not neutralize the solution. Dissolve bismuth salts in a few mL of water and 2 mL of nitric acid before treating with the precipitant.

CHLORIDE—Dissolve the specified quantity of the substance under test in 30 to 40 mL of water, or, where the substance is already in solution, add water to make a total volume of 30 to 40 mL, and, if necessary, neutralize the solution with nitric acid to litmus. Add 1 mL each of nitric acid and of silver nitrate TS and sufficient water to make 50 mL. Mix, and allow to stand for 5 minutes protected from direct sunlight. Unless otherwise specified in the monograph, compare the turbidity, if any, with that produced in a solution containing the volume of 0.020 N hydrochloric acid specified in the monograph.

SULFATE—Dissolve the specified quantity of the substance under test in 30 to 40 mL of water, or, where the substance is already in solution, add water to make a total volume of 30 to 40 mL, and, if necessary, neutralize the solution with hydrochloric acid to litmus. Add 1 mL of 3 N hydrochloric acid, 3 mL of barium chloride TS, and sufficient water to make 50 mL. Mix, and allow to stand for 10 minutes. Unless otherwise specified in the monograph, compare the turbidity, if any, with that produced in a solution containing the volume of 0.020 N sulfuric acid specified in the monograph.

⟨223⟩ DIMETHYLANILINE

The following limit test is provided as a general procedure, when specified in the individual monographs for the gas chromatographic determination in compendial articles of traces of dimethylaniline, a hydrochloric acid scavenger that may have been carried over during processing.

PROCEDURE

Internal standard solution: Unless otherwise specified in the individual monograph, prepare a solution of naphthalene in cyclohexane containing about 50 µg per mL.

Standard preparation: Unless otherwise specified in the individual monograph, transfer 50.0 mg of *N,N*-dimethylaniline to a 50-mL volumetric flask, add 25 mL of 1 N hydrochloric acid, swirl to dissolve, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 250-mL volumetric flask, dilute with water to volume, and mix. To a suitable centrifuge tube add 1.0 mL of this solution, 5.0 mL of 1 N sodium hydroxide, and 1.0 mL of *Internal standard solution*, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as the *Standard preparation.*

Test preparation: Unless otherwise specified in the individual monograph, transfer 1.0 g of the substance to be tested to a suitable centrifuge tube, add 5 mL of 1 N sodium hydroxide, swirl to dissolve the specimen, add 1.0 mL of *Internal standard solution*, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as the *Test preparation.*

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The gas chromatograph is equipped with a flame-ionization detector, maintained at about 250°, and a 0.53-mm × 30-m fused silica capillary column bonded with a 1.0-µm film of phase G42. The carrier gas is helium, with a linear velocity of about 30 cm per second and a split ratio of 10:1. The column temperature is maintained at 110° for the first 4 minutes after an injection is made, then increased from 110° to 200° at 8° per minute, and then held at 200° for 5 minutes. The injection port temperature is maintained at 250°. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure* identify the dimethylaniline and naphthalene peaks by their relative retention times, which are 1.0 and 1.3, respectively. The signal-to-noise ratio for the dimethylaniline peak is not less than 10.

Procedure: Inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The ratio of the response of any dimethylaniline peak to the response of the naphthalene peak obtained from the *Test preparation* is not greater than that obtained from the *Standard preparation* (0.002%).

⟨226⟩ 4-EPIANHYDROTETRACYCLINE

This chromatographic procedure is provided to demonstrate that the content of 4-epianhydrotetracycline, a degradation product of tetracycline, does not exceed the limit given in the individual monograph.

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PROCEDURE

EDTA buffer: Dissolve 37.2 g of edetate disodium in 800 mL of water, adjust with ammonium hydroxide to a pH of 7.8, dilute with water to 1000 mL, and mix.

Support phase: Add 5 mL of *EDTA buffer* to 10 g of acid-washed chromatographic siliceous earth for column chromatography, and mix until the siliceous earth is uniformly moistened.

Test solution: Prepare as directed in the individual monograph.

Procedure: Prepare a 15-mm × 170-mm chromatographic tube with a 4-mm × 50-mm outlet by packing it, in increments, with *Support phase*, firmly tamping down each increment, until the tube is filled to a height of about 10 cm. In a beaker, prepare a mixture of 1 g of acid-washed chromatographic siliceous earth for column chromatography and 1 mL of *Test solution*. Transfer the mixture to the top of the column. Dry-wash the beaker with *Support phase*, and transfer to the column to provide an additional 1-cm layer on top of the mixture containing the *Test solution*. Within 30 minutes, pass chloroform through the column, and collect successive fractions of 5.0 mL, 5.0 mL, 10.0 mL, 10.0 mL, and 5.0 mL. Observe the column during elution, and note the appearance of two separate yellow bands. The fraction or fractions containing the first yellow band contain the anhydrotetracyclines. Discard these fractions. The fractions after the first yellow band contain the 4-epianhydrotetracycline. Determine the absorbance of each 4-epianhydrotetracycline fraction at the wavelength of maximum absorbance at about 438 nm, with a suitable spectrophotometer, diluting each fraction, if necessary, with chloroform, and using chloroform as the blank. Calculate the quantity, in mg, of 4-epianhydrotetracycline in each fraction by the formula:

$$\text{Result} = A_U VD/A_S$$

A_U = absorbance of the fraction taken
V = volume of the fraction taken (mL)
D = dilution factor, if the fraction was diluted
A_S = absorptivity of 4-epianhydrotetracycline at 438 nm, 20.08

From the sum of the quantities of 4-epianhydrotetracycline found in the fractions, calculate the percentage of 4-epianhydrotetracycline in relation to the tetracycline hydrochloride equivalent contained in the *Test solution*.

⟨227⟩ 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS

INTRODUCTION

This chapter provides a procedure and acceptance criterion (limit) to control the principal degradation product of acetaminophen, 4-aminophenol, an impurity that can form by hydrolysis of acetaminophen.

SOLUTION PREPARATIONS

All solution preparations that contain acetaminophen or 4-aminophenol should be protected from light and should be stored only for as long as can be supported by solution stability data acquired during verification under actual conditions of use.

Buffer: 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

Diluent: Acetonitrile and *Buffer* (10:90)

Solution A: 10 mM phosphate buffer prepared as follows. Add 0.60 g of monobasic potassium phosphate and 0.82 g of anhydrous dibasic sodium phosphate to a 1-L volumetric flask. Dissolve and dilute with water to volume to a pH of 7.0.

Solution B: Water

Solution C: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	90	5	5
5	90	5	5
7	10	10	80
7.1	90	5	5
10	90	5	5

Standard stock solution: 25 µg/mL of USP 4-Aminophenol RS in *Diluent*. Prepare fresh in conjunction with the other solution preparations described below. Discard after 4 h or as supported by solution stability data. [NOTE—See *Chromatographic Adjustments*, solution stability, below.]

System suitability solution: 2.5 µg/mL of USP 4-Aminophenol RS in *Diluent*, from the *Standard stock solution*

Sample stock solution: Nominally 10 mg/mL of acetaminophen from a suitable quantity of drug product in *Diluent*. [NOTE—Either component of the *Diluent* may be introduced to the drug product first, followed by addition of the other

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component to maintain the proportions of acetonitrile and *Buffer* and to achieve the appropriate final volume defined for the *Diluent*.]

[NOTE—It is recommended that the *Sample solution* and *Standard solution* be prepared concurrently within a narrow window of time (e.g., 30 min) for each drug product sample.]

Standard solution: Add 25.0 mL of the *Sample stock solution* and 15.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

Sample solution: Add 25.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

CHROMATOGRAPHIC METHOD

Chromatographic system

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

Mode: LC

Detector: UV 300 nm

Column: 4.6-mm × 15-cm; 5-µm packing L85

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The typical retention time for 4-aminophenol is about 4.2–5.3 min.]

Suitability requirements

Resolution: NLT 1.0 between 4-aminophenol and the nearest peak, *Standard solution*

Tailing factor: NMT 1.5 for the 4-aminophenol peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 20 for the 4-aminophenol peak, *System suitability solution*

ANALYSIS

Samples: *Standard solution* and *Sample solution*

Inject the *Sample solution* and *Standard solution* for each drug product sample sequentially, i.e., back-to-back.

Calculate the percentage of 4-aminophenol (C_6H_7NO) relative to acetaminophen in the portion of drug product taken:

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

r_U = peak response of 4-aminophenol from the *Sample solution*

r_S = peak response of 4-aminophenol from the *Standard solution*

W_S = amount of USP 4-Aminophenol RS added to the *Standard solution* (mg)

W_U = amount of acetaminophen in the *Sample solution* (mg)

Acceptance criteria (unless otherwise stated in the monograph): NMT 0.15% of 4-aminophenol relative to acetaminophen

CHROMATOGRAPHIC ADJUSTMENTS

The retention time of 4-aminophenol can be tuned to achieve specificity for a given product matrix. This allowance supersedes provisions in ⟨621⟩ for adjusting chromatographic conditions and is intended to provide a measure of flexibility when needed. Suggestions for changing 4-aminophenol retention are given in *Table 2*. The use of a ternary mobile phase system affords ready changes to the ionic strength (water from *Solution B*) and organic strength (acetonitrile from *Solution C*), but this can be simplified to a binary mobile phase system.

Table 2

Condition Change	Change in 4-Aminophenol Retention
Increase in organic strength (*Solution C*)	Decreases 4-aminophenol retention
Decrease in pH (*Solution A*)	Increases 4-aminophenol retention
Increase in ionic strength (*Solution B*)	Decreases 4-aminophenol retention
Increase in column temperature	Increases 4-aminophenol retention

Adjustments to the chromatographic procedure may require verification or validation. See *Validation of Compendial Procedures* ⟨1225⟩ and *Verification of Compendial Procedures* ⟨1226⟩ for guidance. Adjusted chromatographic conditions must meet all system suitability requirements.

Solution stability must be verified under actual conditions of use to ensure 4-aminophenol is stable in the *Sample solution* and the *Standard solution* as evidenced by NMT ±10% change of the 4-aminophenol peak areas.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 4-Aminophenol RS

4-Amino-1-hydroxybenzene.

C_6H_7NO

109.13

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⟨228⟩ ETHYLENE OXIDE AND DIOXANE

The following procedure is used to determine the contents of residual ethylene oxide and dioxane in the products prepared from ethylene oxide. Unless otherwise directed in the individual monograph, use *Method I*.

Method I

[CAUTION—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate between 4° and 8°.]

[NOTE—Before using the polyethylene glycol 200 in this test, remove any volatile components from it by placing 500 mL of polyethylene glycol 200 in a 1000-mL round-bottom flask and attaching the flask to a rotary evaporator maintained at 60° and under a vacuum of 10–20 mm Hg for 6 h.]

Acetaldehyde solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]

Ethylene oxide stock solution: 2.5 mg/g of ethylene oxide.

Prepare as follows: Tare a glass-stoppered conical flask, add 50 mL of polyethylene glycol 200, and reweigh the flask. Transfer 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in a mixture of sodium chloride and ice (1:3). Transfer 300 µL (corresponding to 250 mg) of liquid ethylene oxide to the polyethylene glycol 200, and swirl gently to mix. Replace the stopper, reweigh the flask, and determine the amount of ethylene oxide absorbed by weight difference. Adjust the weight of the mixture with polyethylene glycol 200 to 100.0 g, replace the stopper, and swirl gently to mix. [NOTE—Fill a chilled pressure bottle with liquid ethylene oxide, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Use an adequately chilled apparatus where appropriate. Prepare this stock solution immediately prior to use, and store in a refrigerator after preparation.]

Ethylene oxide solution: Tare a glass-stoppered conical flask, and chill it in a refrigerator. Add 35 mL of polyethylene glycol 200, and reweigh the flask. Transfer 1 g of chilled *Ethylene oxide stock solution* to the tared conical flask. Adjust the weight of the solution with polyethylene glycol 200 to 50.0 g, replace the stopper, and swirl gently to mix. Transfer 10 g of this solution to a 50-mL volumetric flask. Add 30 mL of water, and mix. Dilute with water to volume, and mix to obtain a solution containing 10 µg/mL of ethylene oxide. [NOTE—Use an adequately chilled apparatus where appropriate. Prepare immediately before use.]

Dioxane solution: 500 µg/mL of dioxane

Standard solution A: Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial. [NOTE—Other sizes such as a 22-mL pressure headspace vial may be used, depending on operating conditions; however, the same size must be used for *Standard solution A*, *Standard solution B*, and the *Sample solution*.] Add 0.1 mL of *Acetaldehyde solution* and 0.1 mL of *Dioxane solution*, seal the vial, and mix.

Standard solution B: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, and add 0.1 mL of *Ethylene oxide solution*, 0.1 mL of *Dioxane solution*, and 1.0 mL of *N,N*-dimethylacetamide. Seal the vial, and mix.

Sample solution: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, and add 1.0 mL of *N,N*-dimethylacetamide and 0.2 mL of water. Seal the vial, and mix.

Chromatographic system

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

Mode: Headspace GC

Detector: Flame ionization

Column: 0.32-mm × 30-m glass or quartz capillary; 1.0-µm layer of phase G1

Temperature

Injector port: 150°

Detector: 250°

Column: See the column temperature table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	5
50	5	180	—
180	30	230	5

Carrier gas: Helium

Linear velocity: 20 cm/s

Injection volume: 1 mL (gaseous headspace)

Injection type: Split ratio 20:1

Headspace sampler

Temperature equilibration time: 45 min

Equilibration temperature

70° for *Standard solution A*

90° for *Standard solution B*

90° for *Sample solution*

Transfer line temperature: 150°

Pressurization time: 1 min

Injection time: 12 s

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System suitability
Sample: *Standard solution A*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.94 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between acetaldehyde and ethylene oxide
Signal-to-noise ratio: NLT 5, determined from the dioxane peak
Relative standard deviation: NMT 15%
Analysis
Samples: *Standard solution B* and *Sample solution*
[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 2.5, respectively.]
Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_E \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_E = quantity of ethylene oxide added to *Standard solution B* (µg)
r_U = ethylene oxide peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_D \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_D = quantity of dioxane added to *Standard solution B* (µg)
r_U = dioxane peak responses from the *Sample solution*
r_S = dioxane peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Method II
Ethylene oxide standard solution: Dilute 0.5 mL of ethylene oxide in methylene chloride (50 mg/mL)[1] with water to 50.0 mL. [NOTE—The solution is stable for 3 months if stored in vials with polytetrafluoroethylene (polytef)-coated silicon membrane crimped caps at –20°.] Allow to reach room temperature. Dilute 1.0 mL with water to 250.0 mL to obtain a solution having a concentration of 2 µg/mL of ethylene oxide. [NOTE—Use this solution immediately after preparation.]
Dioxane standard solution: 0.05 µL/mL of dioxane
Acetaldehyde standard solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]
Resolution solution: Add 2.0 mL of *Acetaldehyde standard solution* and 2.0 mL of *Ethylene oxide standard solution* to a 10-mL headspace vial. Seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.
Standard solution A: 0.48 µg/mL of ethylene oxide, from *Ethylene oxide standard solution*, and 0.005 µL/mL of dioxane, from *Dioxane standard solution*, in water
Standard solution B: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of *Standard solution A*, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.
Sample solution: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of water, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: Headspace GC
Detector: Flame ionization
Column: 0.53-mm × 50-m fused-silica capillary column; 5.0-µm layer of phase G27
Temperature
Injector port: 85°
Detector: 250°
Column: See the column temperature table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	10	250	5

Carrier gas: Helium
Flow rate: 4 mL/min
Injection volume: 1 mL (gaseous headspace)
Injection type: Split ratio 3.5 : 1
Headspace sampler
Temperature equilibration time: 30 min
Equilibration temperature: 80°

[1] This is a commercially available solution.

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System suitability
Sample: *Resolution solution*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between acetaldehyde and ethylene oxide
Analysis
Samples: *Standard solution B* and *Sample solution*
[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 1.9, respectively.]
Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_E \times V \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_E = concentration of ethylene oxide in the *Standard solution A* (µg/mL)
V = volume of *Standard solution A* added to *Standard solution B* (2.0 mL)
r_U = ethylene oxide peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_D \times V \times \rho \times F \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_D = concentration of dioxane in *Standard solution A* (µL/mL)
V = volume of *Standard solution A* added to *Standard solution B* (2.0 mL)
ρ = density of dioxane (1.03 g/mL = 1.03 mg/µL)
F = conversion factor (1000 µg/mg)
r_U = dioxane peak responses from the *Sample solution*
r_S = dioxane peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

⟨232⟩ ELEMENTAL IMPURITIES—LIMITS

INTRODUCTION

This chapter specifies limits for the amounts of elemental impurities in drug products. Regardless of the approach used, compliance with the limits specified is required for all drug products unless otherwise specified in an individual monograph or specifically excluded in this *Introduction*.

Elemental impurities include catalysts and environmental contaminants that may be present in drug substances, excipients, or drug products. These impurities may occur naturally, be added intentionally, or be introduced inadvertently (e.g., by interactions with processing equipment and the container–closure system). When elemental impurities are known to be present, have been added, or have the potential for introduction, assurance of compliance to the specified levels is required. A risk-based control strategy may be appropriate when analysts determine how to assure compliance with this standard. Due to the ubiquitous nature of arsenic, cadmium, lead, and mercury, they (at the minimum) must be considered in the risk assessment.

This chapter does not apply to:

- Radiopharmaceuticals
- Articles intended only for veterinary use
- Vaccines
- Cell metabolites
- DNA products
- Allergenic extracts
- Cells, whole blood, cellular blood components, or blood derivatives, including plasma and plasma derivatives
- Products based on genes (gene therapy)
- Cells (cell therapy)
- Tissue (tissue engineering)
- Dialysate solutions not intended for systemic circulation
- Total parenteral nutritions (TPNs)
- Elements that are intentionally included in the drug product for therapeutic benefit
- Dietary supplements and their ingredients, which are addressed in *Elemental Contaminants in Dietary Supplements* ⟨2232⟩

The limits presented in this chapter do not apply to excipients and drug substances, except where specified in an individual monograph. However, manufacturers of pharmaceutical products need certain information about the content of elemental impurities in drug substances or excipients in order to meet the criteria of this chapter. Drug product manufacturers can use

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elemental impurity test data on components from tests performed by drug substance or excipient manufacturers, who may provide test data, or if applicable, risk assessments. Elemental impurity data generated by a qualified supplier of drug product components are acceptable for use by a drug product manufacturer to demonstrate compliance with this chapter in the final drug product. Drug substance or excipient manufacturers who choose to perform a risk assessment must conduct that risk assessment using *Table 2* in this chapter. Elements that are inherent in the nature of the material, as in the case of some naturally-sourced materials, must be considered in the risk assessment.

SPECIATION

The determination of the oxidation state, organic complex, or combination is termed "speciation". Each of the elemental impurities has the potential to be present in differing oxidation or complexation states. However, arsenic and mercury are of particular concern because of the differing toxicities of their inorganic and complexed organic forms.

The arsenic limits are based on the inorganic (most toxic) form. Arsenic can be measured using a total-arsenic procedure under the assumption that all arsenic contained in the material under test is in the inorganic form. Where the limit is exceeded using a total-arsenic procedure, it may be possible to show, via a procedure that quantifies the different forms, that the inorganic form meets the specification.

The mercury limits are based upon the inorganic (2^+) oxidation state. The methyl mercury form (most toxic) is rarely an issue for pharmaceuticals. Thus, the limit was established assuming the most common (mercuric) inorganic form. Limits for articles that have the potential to contain methyl mercury (e.g., materials derived from fish) are to be provided in the monograph.

ROUTES OF EXPOSURE

The elements included in the tables below have been placed into three classes, based on their toxicity and likelihood of occurrence in the drug product. The classification scheme is intended to focus the risk assessment on those elements that are the most toxic but also have a reasonable probability of inclusion in the drug product (see *Table 2*).

The toxicity of an elemental impurity is related to its extent of exposure (bioavailability). The extent of exposure has been determined for each of the elemental impurities of interest for three routes of administration: oral, parenteral, and inhalational. These limits are based on chronic exposure. Consider the oral permissible daily exposures (PDEs) in *Table 1* as a starting point in developing specific PDEs for other routes of administration, except where otherwise stated in the individual monograph.

[NOTE—The routes of administration of drug products are defined in *Pharmaceutical Dosage Forms* ⟨1151⟩.]

DRUG PRODUCTS

The limits described in the third through fifth columns of *Table 1* are the base daily dose PDEs of the elemental impurities of interest for a drug product taken by a patient according to indicated routes of administration.

Parenteral Products

Parenteral drug products with maximum daily volumes up to 2 L may use the maximum daily volume to calculate permissible concentrations from PDEs. For products whose daily volumes, as specified by labeling and/or established by clinical practice, may exceed 2 L (e.g., saline, dextrose, and solutions for irrigation), a 2-L volume may be used to calculate permissible concentrations from PDEs.

Table 1. Permitted Daily Exposures for Elemental Impurities

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
Cadmium	1	5	2	2
Lead	1	5	5	5
Arsenic	1	15	15	2
Mercury	1	30	3	1
Cobalt	2A	50	5	3
Vanadium	2A	100	10	1
Nickel	2A	200	20	5
Thallium	2B	8	8	8
Gold	2B	100	100	1
Palladium	2B	100	10	1
Iridium	2B	100	10	1
Osmium	2B	100	10	1
Rhodium	2B	100	10	1

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Table 1. Permitted Daily Exposures for Elemental Impurities *(continued)*

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
Ruthenium	2B	100	10	1
Selenium	2B	150	80	130
Silver	2B	150	10	7
Platinum	2B	100	10	1
Lithium	3	550	250	25
Antimony	3	1200	90	20
Barium	3	1400	700	300
Molybdenum	3	3000	1500	10
Copper	3	3000	300	30
Tin	3	6000	600	60
Chromium	3	11000	1100	3

Recommendations for Elements to be Considered in the Risk Assessment

Table 2 identifies elemental impurities for inclusion in the risk assessment. This table can be applied to all sources of elemental impurities in the drug product.

Table 2. Elements to be Considered in the Risk Assessment

Element	Class	If Intentionally Added (All Routes)	If Not Intentionally Added		
			Oral	Parenteral	Inhalation
Cadmium	1	Yes	Yes	Yes	Yes
Lead	1	Yes	Yes	Yes	Yes
Arsenic	1	Yes	Yes	Yes	Yes
Mercury	1	Yes	Yes	Yes	Yes
Cobalt	2A	Yes	Yes	Yes	Yes
Vanadium	2A	Yes	Yes	Yes	Yes
Nickel	2A	Yes	Yes	Yes	Yes
Thallium	2B	Yes	No	No	No
Gold	2B	Yes	No	No	No
Palladium	2B	Yes	No	No	No
Iridium	2B	Yes	No	No	No
Osmium	2B	Yes	No	No	No
Rhodium	2B	Yes	No	No	No
Ruthenium	2B	Yes	No	No	No
Selenium	2B	Yes	No	No	No
Silver	2B	Yes	No	No	No
Platinum	2B	Yes	No	No	No
Lithium	3	Yes	No	Yes	Yes
Antimony	3	Yes	No	Yes	Yes
Barium	3	Yes	No	No	Yes
Molybdenum	3	Yes	No	No	Yes
Copper	3	Yes	No	Yes	Yes
Tin	3	Yes	No	No	Yes
Chromium	3	Yes	No	No	Yes

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Options for Demonstrating Compliance

DRUG PRODUCT ANALYSIS OPTION

The results obtained from the analysis of a typical dosage unit, scaled to a maximum daily dose, are compared to the *Daily Dose PDE*.

$$\textit{Daily Dose PDE} \geq \text{measured value } (\mu g/g) \times \text{maximum daily dose } (g/day)$$

The measured amount of each impurity is NMT the *Daily Dose PDE*, unless otherwise stated in the individual monograph.

SUMMATION OPTION

Separately, add the amounts of each elemental impurity (in µg/g) present in each of the components of the drug product:

$$\textit{Daily Dose PDE} \geq [\Sigma^{M}_{1}(C_M \times W_M)] \times D_D$$

M = each ingredient used to manufacture a dosage unit
C_M = element concentration in component (drug substance or excipient) (µg/g)
W_M = weight of component in a dosage unit (g/dosage unit)
D_D = number of units in the maximum daily dose (unit/day)

The result of the summation of each impurity is NMT the *Daily Dose PDE*, unless otherwise stated in the individual monograph. Before products can be evaluated using this option, the manufacturer must ensure that additional elemental impurities cannot be inadvertently added through the manufacturing process or via the container–closure system over the shelf life of the product.

INDIVIDUAL COMPONENT OPTION

For drug products with a daily dose of NMT 10 g, if all drug substances and excipients in a formulation meet the concentration limits shown in *Table 3*, then these components may be used in any proportion. No further calculation is necessary. While elemental impurities derived from the manufacturing process or the container–closure system are not specifically provided for in the *Individual Component Option*, it is expected that the drug product manufacturer will ensure that these sources do not contribute significantly to the total content of elemental impurities.

DRUG SUBSTANCE AND EXCIPIENTS

The acceptable levels of elemental impurities depend on the material's ultimate use. Therefore, manufacturers of pharmaceutical products need certain information about the content of elemental impurities in drug substances or excipients in order to meet the criteria of this chapter. Drug product manufacturers can use elemental impurity test data on components from tests performed by drug substance manufacturers or excipient manufacturers, who may provide test data, or, if applicable, risk assessments. Elemental impurity data generated by a qualified supplier of drug product components are acceptable for use by a drug product manufacturer to demonstrate compliance with this chapter in the final drug product. Drug substance or excipient manufacturers who choose to perform a risk assessment must conduct that risk assessment using *Table 2* in this chapter. Elements that are inherent in the nature of the material, as in the case of some naturally-sourced materials, must be considered in the risk assessment.

The values provided in *Table 3* are example concentration limits for components (drug substances and excipients) of drug products dosed at a maximum daily dose of 10 g/day. These values serve as default concentration limits to aid discussions between drug product manufacturers and the suppliers of the components of their drug products.

[NOTE—Individual components may need to be limited at levels different from those in the table depending on monograph-specific mitigating factors.]

Table 3. Permitted Concentrations of Elemental Impurities for Individual Component Option

Element	Class	Oral Concentration (µg/g)	Parenteral Concentration (µg/g)	Inhalation Concentration (µg/g)
Cadmium	1	0.5	0.2	0.2
Lead	1	0.5	0.5	0.5
Arsenic	1	1.5	1.5	0.2
Mercury	1	3	0.3	0.1
Cobalt	2A	5	0.5	0.3
Vanadium	2A	10	1	0.1
Nickel	2A	20	2	0.5
Thallium	2B	0.8	0.8	0.8
Gold	2B	10	10	0.1

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Table 3. Permitted Concentrations of Elemental Impurities for Individual Component Option *(continued)*

Element	Class	Oral Concentration (µg/g)	Parenteral Concentration (µg/g)	Inhalation Concentration (µg/g)
Palladium	2B	10	1	0.1
Iridium	2B	10	1	0.1
Osmium	2B	10	1	0.1
Rhodium	2B	10	1	0.1
Ruthenium	2B	10	1	0.1
Selenium	2B	15	8	13
Silver	2B	15	1	0.7
Platinum	2B	10	1	0.1
Lithium	3	55	25	2.5
Antimony	3	120	9	2
Barium	3	140	70	30
Molybdenum	3	300	150	1
Copper	3	300	30	3
Tin	3	600	60	6
Chromium	3	1100	110	0.3

ANALYTICAL TESTING

If, by process monitoring and supply-chain control, manufacturers can demonstrate compliance, then further testing may not be needed. When testing is done to demonstrate compliance, proceed as directed in *Elemental Impurities—Procedures* ⟨233⟩.

⟨233⟩ ELEMENTAL IMPURITIES—PROCEDURES

INTRODUCTION

This chapter describes two analytical procedures (*Procedures 1* and *2*) for the evaluation of the levels of the elemental impurities. The chapter also describes criteria for acceptable alternative procedures. By means of validation studies, analysts will confirm that the analytical procedures described herein are suitable for use on specified material.

Use of Alternative Procedures

The chapter also describes criteria for acceptable alternative procedures. Alternative procedures that meet the validation requirements herein may be used in accordance with *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*. Information on the *Requirements for Alternate Procedure Validation* is provided later in this chapter.

Speciation

The determination of the oxidation state, organic complex, or combination is termed *speciation*. Analytical procedures for speciation are not included in this chapter, but examples may be found elsewhere in *USP–NF* and in the literature.

PROCEDURES

- COMPENDIAL PROCEDURES 1 AND 2

System standardization and suitability evaluation using applicable reference materials should be performed on the day of analysis.

Procedure and detection technique: *Procedure 1* can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP–AES or ICP–OES). *Procedure 2* can be used for elemental impurities generally amenable to detection by ICP–MS. Before initial use, the analyst should verify that the procedure is appropriate for the instrument and sample used (procedural verification) by meeting the alternative procedure validation requirements below.

Sample preparation: Forms of sample preparation include *Neat, Direct aqueous solution, Direct organic solution,* and *Indirect solution*. The selection of the appropriate sample preparation depends on the material under test and is the responsibility of the analyst. When a sample preparation is not indicated in the monograph, an analyst may use any of the following appropriately validated preparation procedures. In cases where spiking of a material under test is necessary to provide an acceptable signal intensity, the blank should be spiked with the same *Target elements*, and where possible, using the same spiking solution. Standard solutions may contain multiple *Target elements*. [NOTE—All liquid samples should be weighed.]

Neat: Used for liquids or alternative procedures that allow the examination of unsolvated samples.

Direct aqueous solution: Used when the sample is soluble in an aqueous solvent.

Direct organic solution: Used where the sample is soluble in an organic solvent.

Indirect solution: Used when a material is not directly soluble in aqueous or organic solvents. Total metal extraction is the preferred sample preparation approach to obtain an *Indirect solution*. Digest the sample using the *Closed vessel*

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digestion procedure provided below or one similar to it. The sample preparation scheme should yield sufficient sample to allow quantification of each element at the limit specified in the corresponding monograph or chapter.

Closed vessel digestion: This sample preparation procedure is designed for samples that must be digested in a *Concentrated acid* using a closed vessel digestion apparatus. *Closed vessel digestion* minimizes the loss of volatile impurities. The choice of a *Concentrated acid* depends on the sample matrix. The use of any of the *Concentrated acids* may be appropriate, but each introduces inherent safety risks. Therefore, appropriate safety precautions should be used at all times. [NOTE—Weights and volumes provided may be adjusted to meet the requirements of the digestion apparatus used.]

An example procedure that has been shown to have broad applicability is the following. Dehydrate and predigest 0.5 g of primary sample in 5 mL of freshly prepared *Concentrated acid*. Allow to sit loosely covered for 30 min in a fume hood. Add an additional 10 mL of *Concentrated acid*, and digest, using a closed vessel technique, until digestion or extraction is complete. Repeat, if necessary, by adding an additional 5 mL of *Concentrated acid*. [NOTE—Where closed vessel digestion is necessary, follow the manufacturer's recommended procedures to ensure safe use.]

Alternatively, leachate extraction may be appropriate with justification following scientifically validated metal disposition studies, which may include animal studies, speciation, or other means of studying disposition of the specific metal in the drug product.

Reagents: All reagents used for the preparation of sample and standard solutions should be free of elemental impurities, in accordance with *Plasma Spectrochemistry* ⟨730⟩.

• **PROCEDURE 1: ICP–OES**

Standardization solution 1: 1.5*J* of the *Target element(s)* in a *Matched matrix*

Standardization solution 2: 0.5*J* of the *Target element(s)* in a *Matched matrix*

Sample stock solution: Proceed as directed in *Sample preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample solution: Dilute the *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target elements* at NMT 1.5*J*.

Blank: *Matched matrix*

Elemental spectrometric system

(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: ICP

Detector: Optical detection system

Rinse: Diluent used

Standardization: *Standardization solution 1*, *Standardization solution 2*, and *Blank*

System suitability

Sample: *Standardization solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization solution 1* before and after the analysis of the *Sample solution*.

Suitability criteria: NMT 20% for each *Target element*. [NOTE—If samples are high in mineral content, rinse system well before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences (e.g., wavelength overlaps).]

• **PROCEDURE 2: ICP–MS**

Standardization solution 1: 1.5*J* of the *Target element(s)* in a *Matched matrix*

Standardization solution 2: 0.5*J* of the *Target element(s)* in a *Matched matrix*

Sample stock solution: Proceed as directed for *Sample preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer.

Sample solution: Dilute the *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target elements* at NMT 1.5*J*.

Blank: *Matched matrix*

Elemental spectrometric system

(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: ICP. [NOTE—An instrument with a cooled spray chamber is recommended. (A collision cell or reaction cell may also be beneficial.)]

Detector: Mass spectrometer

Rinse: Diluent used

Standardization: *Standardization solution 1*, *Standardization solution 2*, and *Blank*

System suitability

Sample: *Standardization solution 1*

Suitability requirements

Drift: Compare results obtained from *Standardization solution 1* before and after the analysis of the *Sample solution*.

Suitability criteria: *Drift* NMT 20% for each *Target element*. [NOTE—If samples are high in mineral content, rinse system well before introducing the *Sample* in order to minimize carryover.]

Analysis: Analyze according to the manufacturer's suggestions for program and *m/z*. Calculate and report results based on the original sample size. [NOTE—Appropriate measures must be taken to correct for matrix-induced interferences (e.g., argon chloride interference with arsenic determinations).]

REQUIREMENTS FOR ALTERNATE PROCEDURE VALIDATION

If the specified compendial procedures do not meet the needs of a specific application, an alternative procedure may be developed (see *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*). Alternative procedures must be validated and shown to be acceptable, in accordance with the validation requirements for alternative procedures as

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described below. The level of validation necessary to ensure that an alternative procedure is acceptable depends on whether a limit test or a quantitative determination is specified in the monograph. The requirements for the validation of an elemental impurities procedure for each type of determination are described below. Any alternative procedure that has been validated and meets the acceptance criteria that follow is considered to be suitable for use.

LIMIT PROCEDURES

The following section defines the validation parameters for the acceptability of alternative limit procedures. Meeting these requirements must be demonstrated experimentally using an appropriate system suitability procedure and reference material.

The suitability of the method must be determined by conducting studies with the material or mixture under test supplemented with known concentrations of each *Target element* of interest at the appropriate acceptance limit concentration. The material or mixture under test must be spiked before any sample preparation steps are performed.

- **DETECTABILITY**

Standard solution: A preparation of reference materials for the *Target element(s)* at the *Target concentration*

Spiked sample solution 1: Prepare a solution of sample under test, spiked with appropriate reference materials for the *Target elements* at the *Target concentration*, solubilized or digested as described in *Sample preparation*.

Spiked sample solution 2: Prepare a solution of the sample under test, spiked with appropriate reference materials at 80% of the *Target concentration* for the *Target elements*, solubilized or digested as described in *Sample preparation*.

Unspiked sample solution: A sample of material under test, solubilized or digested in the same manner as the *Sample solutions*

Acceptance criteria

Non-instrumental procedures: *Spiked sample solution 1* provides a signal or intensity equivalent to or greater than that of the *Standard solution*. *Spiked sample solution 2* must provide a signal or intensity less than that of *Spiked sample solution 1*. [NOTE—The signal from each *Spiked sample solution* is NLT the *Unspiked sample solution* determination.]

Instrumental procedures: The average value of the three replicate measurements of *Spiked sample solution 1* is within ±15% of the average value obtained for the replicate measurements of the *Standard solution*. The average value of the replicate measurements of *Spiked sample solution 2* must provide a signal intensity or value less than that of the *Standard solution*. [NOTE—Correct the values obtained for each of the spiked solutions using the *Unspiked sample solution*.]

- **PRECISION FOR INSTRUMENTAL METHODS (REPEATABILITY)**

[NOTE—Non-instrumental precision is demonstrated by meeting the *Detectability* requirement above.]

Sample solutions: Six independent samples of the material under test, spiked with appropriate reference materials for the *Target elements* at the *Target concentration*

Acceptance criteria

Relative standard deviation: NMT 20% for each *Target element*

- **SPECIFICITY**

The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) each *Target element* in the presence of components that may be expected to be present, including other *Target elements*, and matrix components.

QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of alternative quantitative procedures. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference materials. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure for the purpose of quantifying the *Target elements*.

- **ACCURACY**

Standard solutions: Prepare solutions containing the *Target elements* at concentrations ranging from 50% to 150% of *J*, using appropriate reference materials.

Test samples: Prepare samples of the material under test spiked with appropriate reference materials before any sample preparation steps (digestion or solubilization) at concentrations ranging from 50% to 150% of *J* for each *Target element*.

Acceptance criteria

Spike recovery: 70%–150% for the mean of three replicate preparations at each concentration

- **PRECISION**

Repeatability

Test samples: Six independent samples of material under test (taken from the same lot) spiked with appropriate reference materials for the *Target element(s)* at the indicated level

Acceptance criteria

Relative standard deviation: NMT 20% ($N = 6$) for each *Target element*

Intermediate precision (ruggedness)

Perform the *Repeatability* analysis again either on a different day, with a different instrumentation, with a different analyst, or a combination thereof. Combine the results of this analysis with the *Repeatability* analysis so the total number of analyses is 12.

Acceptance criteria

Relative standard deviation: NMT 25% ($N = 12$) for each *Target element*

- **SPECIFICITY**

The procedure must be able to unequivocally assess (see ⟨1225⟩) each *Target element* in the presence of components that may be expected to be present, including other *Target elements*, and matrix components.

- **LIMIT OF QUANTITATION, RANGE, AND LINEARITY**

Demonstrated by meeting the *Accuracy* requirement.

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GLOSSARY

Concentrated acid: Concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or *Aqua regia*

Aqua regia: *Aqua regia* is a mixture of concentrated hydrochloric and nitric acids, typically at ratios of 3:1 or 4:1, respectively.

Matched matrix: Solutions having the same solvent composition as the *Sample solution*. In the case of an aqueous solution, *Matched matrix* would indicate that the same acids, acid concentrations, and mercury stabilizer are used in both preparations.

Target elements: Elements with the potential of being present in the material under test. Include arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in the target element evaluation when testing is done to demonstrate compliance. *Target elements* should also include any elements that may be added through material processing or storage.

Target limit or Target concentration: The acceptance value for the elemental impurity being evaluated. Exceeding the *Target limit* indicates that a material under test exceeds the acceptable value. The determination of compliance is addressed in other chapters. [NOTE—When applying this chapter to *Elemental Impurities—Limits* ⟨232⟩ and *Elemental Contaminants in Dietary Supplements* ⟨2232⟩, *Target limits* can be approximated by dividing the *Daily Dose PDEs* by the maximum daily dose for the *Drug Product Analysis Option* in ⟨232⟩ or the *Daily Serving PDE* divided by the maximum daily serving size in ⟨2232⟩.]

J: The concentration (w/w) of the element(s) of interest at the *Target limit*, appropriately diluted to the working range of the instrument. For example, if the target elements are lead and arsenic for an analysis of an oral solid drug product with a daily dose of 10 g/day using inductively coupled plasma–mass spectrometry (ICP–MS), the target limit for these elements would be 0.5 µg/g and 1.5 µg/g (see *Table 2* in ⟨232⟩). However, in this case, the linear dynamic range of the ICP–MS is known to extend from 0.01 ng/mL to 0.1 µg/mL for these elements. Therefore, a dilution factor of at least 1:100 is required to ensure that the analysis occurs in the linear dynamic range of the instrument. *J* would thus equal 5 ng and 15 ng/mL for lead and arsenic, respectively, when the dilution factor is added.

Appropriate reference materials: Where *Appropriate reference materials* are specified in the chapter, certified reference materials (CRM) from a national metrology institute (NMI), or reference materials that are traceable to the CRM of an NMI should be used. An example of an NMI in the United States is the National Institute of Standards and Technology.

⟨241⟩ IRON

This limit test is provided to demonstrate that the content of iron, in either the ferric or the ferrous form, does not exceed the limit for iron specified in the individual monograph. The determination is made by concomitant visual comparison with a control prepared from a standard iron solution.

SPECIAL REAGENTS

Standard Iron Solution

Dissolve 863.4 mg of ferric ammonium sulfate [$FeNH_4(SO_4)_2 \cdot 12H_2O$] in water, add 10 mL of 2 N sulfuric acid, and dilute with water to 100.0 mL. Pipet 10 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, dilute with water to volume, and mix. This solution contains the equivalent of 0.01 mg (10 µg) of iron per mL.

Ammonium Thiocyanate Solution

Dissolve 30 g of ammonium thiocyanate in water to make 100 mL.

STANDARD PREPARATION

Into a 50-mL color-comparison tube pipet 1 mL of *Standard Iron Solution* (10 µg of Fe), dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

TEST PREPARATION

Into a 50-mL color comparison tube place the solution prepared for the test as directed in the individual monograph and if necessary dilute with water to 45 mL; or, dissolve in water, and dilute with water to 45 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$1.0/(1000L)$$

in which L is the *Iron* limit in percentage. Add 2 mL of hydrochloric acid, and mix.

PROCEDURE

To each of the tubes containing the *Standard Preparation* and the *Test Preparation* add 50 mg of ammonium peroxydisulfate crystals and 3 mL of *Ammonium Thiocyanate Solution*, and mix: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

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⟨251⟩ LEAD

The imposition of stringent limits on the amounts of lead that may be present in pharmaceutical products has resulted in the use of two methods, of which the one set forth in this chapter depends upon extraction of lead by solutions of dithizone.

Select all reagents for this test to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Thoroughly rinse all glassware with warm dilute nitric acid (1 in 2), followed by water.

SPECIAL REAGENTS

Ammonia-cyanide solution: Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

Ammonium citrate solution: Dissolve 40 g of citric acid in 90 mL of water. Add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a reddish color. Remove any lead that may be present by extracting the solution with 20-mL portions of *Dithizone extraction solution* (see below), until the dithizone solution retains its orange-green color.

Diluted standard lead solution: Dilute an accurately measured volume of standard lead solution TS (containing 10 µg of lead per mL), with 9 volumes of dilute nitric acid (1 in 100) to obtain a solution that contains 1 µg of lead per mL.

Dithizone extraction solution: Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of alcohol. Store the solution in a refrigerator.

Before use, shake a suitable volume of the dithizone extraction solution with about half its volume of dilute nitric acid (1 in 100), discarding the nitric acid.

Hydroxylamine hydrochloride solution: Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make approximately 65 mL. Transfer to a separator, add 5 drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 min. Extract this solution with successive 10- to 15-mL portions of chloroform until a 5-mL portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 3 N hydrochloric acid until the solution is pink (if necessary, add 1 or 2 drops more of thymol blue TS), then dilute with water to 100 mL.

Potassium cyanide solution: Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from this solution by extraction with successive portions of *Dithizone extraction solution*, as described in *Ammonium citrate solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

Standard dithizone solution: Dissolve 10 mg of dithizone in 1000 mL of chloroform. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light, and store in a refrigerator.

PROCEDURE

Test preparation or Sample solution

[NOTE—If, in the following preparation, the substance under test reacts too rapidly and begins charring with 5 mL of sulfuric acid before heating, instead use 10 mL of cooled dilute sulfuric acid (1 in 2), and add a few drops of hydrogen peroxide before heating.]

Where the monograph does not specify preparation of a solution, prepare a *Test preparation* or *Sample solution* as follows. [**CAUTION**—*Exercise safety precautions in this procedure, because some substances may react with explosive violence when digested with hydrogen peroxide.*]

Transfer 1.0 g of the substance under test to a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest on a hot plate in a hood until charring begins. Other suitable means of heating may be substituted. (Add additional sulfuric acid, if necessary, to wet the substance completely, but do not add more than a total of 10 mL.) Add, dropwise and with caution, 30% hydrogen peroxide, allowing the reaction to subside and heat between drops. Add the first few drops very slowly, mix carefully to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls of the flask.

[NOTE—Add peroxide whenever the mixture turns brown or darkens.]

Continue the digestion until the substance is completely destroyed, copious fumes of sulfur trioxide are evolved, and the solution is colorless. Cool, and cautiously add 10 mL of water. Evaporate until sulfur trioxide again is evolved, and cool. Repeat this procedure with another 10 mL of water to remove any traces of hydrogen peroxide. Cautiously dilute with 10 mL of water, and cool.

Analysis: Transfer the *Test preparation* or *Sample solution*, rinsing with 10 mL of water, or the volume of the prepared sample specified in the monograph to a separator, and, unless otherwise directed in the monograph, add 6 mL of *Ammonium citrate solution* and 2 mL of *Hydroxylamine hydrochloride solution*. (For the determination of lead in iron salts, use 10 mL of *Ammonium citrate solution*.) Add 2 drops of phenol red TS, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution if necessary, and add 2 mL of *Potassium cyanide solution*. Immediately extract the solution with 5-mL portions of *Dithizone extraction solution*, draining off each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 mL of dilute nitric acid (1 in 100), and discard the chloroform layer. Add to the acid solution 5.0 mL of *Standard dithizone solution* and 4 mL of *Ammonia-cyanide solution*, and shake for 30 s.

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Acceptance criteria: The color of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *Diluted standard lead solution* equivalent to the amount of lead permitted in the sample under examination and made with the same quantities of the same reagents and in the same manner as in the test with the sample.

⟨261⟩ MERCURY

METHOD I

[NOTE—Mercuric dithizonate is light-sensitive. Perform this test in subdued light.]

Reagents

DITHIZONE STOCK SOLUTION

Dissolve 40 mg of dithizone in 1000 mL of chloroform.

DITHIZONE TITRANT

Dilute 30.0 mL of *Dithizone Stock Solution* with chloroform to 100.0 mL. This solution contains approximately 12 mg of dithizone per L.

MERCURY STOCK SOLUTION

Transfer 135.4 mg of mercuric chloride to a 100-mL volumetric flask, and dilute with 1 N sulfuric acid to volume. This solution contains the equivalent of 100 mg of Hg in 100 mL.

MERCURY SOLUTION FOR STANDARDIZING DITHIZONE TITRANT

Transfer 2.0 mL of *Mercury Stock Solution* to a 100-mL volumetric flask, and dilute with 1 N sulfuric acid to volume. Each mL of this solution contains the equivalent of 20 µg of Hg.

The following solutions are called for in the limit test for mercury that is specified in the monographs on Ferrous Fumarate, Ferrous Sulfate, and Dried Ferrous Sulfate.

HYDROXYLAMINE HYDROCHLORIDE SOLUTION

Prepare as directed in the test for *Lead* ⟨251⟩.

STANDARD MERCURY SOLUTION

On the day of use, quantitatively dilute 1.0 mL of *Mercury Stock Solution* with 1 N sulfuric acid to 1000 mL. Each mL of the resulting solution contains the equivalent of 1 µg of mercury.

DITHIZONE EXTRACTION SOLUTION Prepare as directed in the test for *Lead* ⟨251⟩.

DILUTED DITHIZONE EXTRACTION SOLUTION

Just prior to use, dilute 5 mL of *Dithizone Extraction Solution* with 25 mL of chloroform.

Standardization of Dithizone Titrant

Transfer 1.0 mL of *Mercury Solution for Standardizing Dithizone Titrant* to a 250-mL separator, and add 100 mL of 1 N sulfuric acid, 90 mL of water, 1 mL of glacial acetic acid, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Titrate the solution with *Dithizone Titrant* from a 10-mL microburet, shaking the mixture 20 times after each addition and allowing the chloroform layer to separate, then discarding the chloroform layer. Continue until a final addition of *Dithizone Titrant* is green in color after shaking. Calculate the quantity, in µg, of Hg equivalent to each mL of *Dithizone Titrant* by the formula:

$$20/V$$

in which V is the volume, in mL, of *Dithizone Titrant* added.

Test Preparation

Transfer about 2 g of the substance under test, accurately weighed, to a glass-stoppered, 250-mL conical flask, add 20 mL of a mixture of equal volumes of nitric acid and sulfuric acid, attach a suitable condenser, reflux the mixture for 1 hour, cool, cautiously dilute with water, and boil until fumes of nitrous acid no longer are noticeable. Cool the solution, cautiously dilute with water, transfer to a 200-mL volumetric flask, dilute with water to volume, mix, and filter.

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Procedure

Transfer 50.0 mL of *Test Preparation* to a 250-mL separator, and extract with successive small portions of chloroform until the last chloroform extract remains colorless. Discard the chloroform extract, and add to the extracted *Test Preparation* 50 mL of 1 N sulfuric acid, 90 mL of water, 1 mL of glacial acetic acid, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Proceed as directed under *Standardization of Dithizone Titrant*, beginning with "Titrate the solution." Calculate the amount of mercury.

METHOD IIA AND METHOD IIB

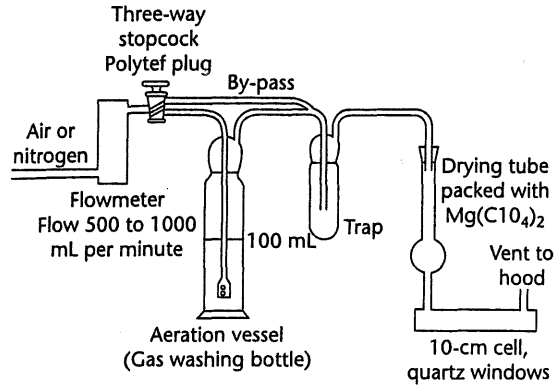
Mercury Detection Instrument

Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder and capable of measuring the radiation absorbed by mercury vapors at the mercury resonance line of 253.6 nm.

[NOTE—Wash all glassware associated with the test with nitric acid, and rinse thoroughly with water before use.]

Aeration Apparatus

The apparatus (see *accompanying diagram*) consists of a flowmeter capable of measuring flow rates from 500 to 1000 mL per minute, connected via a three-way stopcock fitted with a polytef plug to an aeration vessel (250-mL gas washing bottle), followed by a trap, a drying tube packed with magnesium perchlorate, a 10-cm × 25-mm flow-through cell with quartz windows, and terminating with a vent to a fume hood.



Connections are glass or polyvinyl chloride

Mercury Aeration Apparatus

Reagents

POTASSIUM PERMANGANATE SOLUTION

Dissolve 5 g of potassium permanganate in 100 mL of water.

HYDROXYLAMINE HYDROCHLORIDE SOLUTION

Dissolve 10 g of hydroxylamine hydrochloride in 100 mL of water.

STANNOUS CHLORIDE SOLUTION

Dissolve 10 g of $SnCl_2 \cdot 2H_2O$ in 20 mL of warm hydrochloric acid, and add 80 mL of water. Prepare fresh each week.

STANDARD MERCURY SOLUTION

Prepare from *Mercury Stock Solution* as directed under *Method I*. Each mL of the *Standard Mercury Solution* contains the equivalent of 1 µg of mercury.

Test Preparation

Unless otherwise directed in the individual monograph, use the quantity, in g, of the test substance calculated by the formula:

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$$2.0/L$$

in which L is the mercury limit, in ppm.

METHOD IIA

Standard Preparation

Pipet 2.0 mL of *Standard Mercury Solution* into a 100-mL beaker, and add 35 mL of water, 3 mL of sulfuric acid, and 1 mL of potassium permanganate solution. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Test Preparation

Transfer the calculated amount of the test substance to a 100-mL beaker, and add 35 mL of water. Stir, and warm to assist solution, if necessary. Add 2 drops of phenolphthalein TS, and, as necessary, slowly neutralize with constant stirring, using 1 N sodium hydroxide or 1 N sulfuric acid. Add 3 mL of sulfuric acid and 1 mL of *Potassium Permanganate Solution*. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Procedure

Assemble the *Aeration Apparatus* as shown in the accompanying diagram, with the aeration vessel and the trap empty, and the stopcock in the bypass position. Connect the apparatus to the absorption cell, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a smooth baseline reading at 253.6 nm, following the manufacturer's instructions for operating the instrument.

Treat the *Standard Preparation* and the *Test Preparation* similarly, as follows. Destroy the excess permanganate by adding *Hydroxylamine Hydrochloride Solution*, dropwise, until the solution is colorless. Immediately wash the solution into the aeration vessel with water, and dilute with water to 100 mL. Add 2 mL of *Stannous Chloride Solution*, and immediately reconnect the aeration vessel to the aeration apparatus. Turn the stopcock from the bypass position to the aerating position, and continue the aeration until the absorption peak has been passed and the recorder pen returns to the baseline. Disconnect the aeration vessel from the apparatus, and wash with water after each use. After correcting for any reagent blank, any absorbance produced by the *Test Preparation* does not exceed that produced by the *Standard Preparation*.

METHOD IIB

[**CAUTION**—Some substances may react with explosive violence when digested with hydrogen peroxide. Exercise safety precautions at all times.]

Standard Preparation

Pipet 2.0 mL of *Standard Mercury Solution* into a 125-mL conical flask, add 3 mL each of nitric acid and sulfuric acid, mix, and add an amount of 30 percent hydrogen peroxide equal to the total amount used in preparing the *Test Preparation*. Attach a suitable water-cooled condenser with a standard-taper joint to fit the flask, and reflux the mixture in a fume hood for 1 hour. Turn off the water circulating through the condenser, and heat until white fumes appear in the flask. Cool, and cautiously add 10 mL of water through the condenser, while swirling the flask. Again heat until white fumes appear, cool, and add an additional 15 mL of water. Remove the condenser, and rinse the sides of the flask to obtain a volume of 35 mL. Add 1 mL of *Potassium Permanganate Solution*, boil for a few seconds, and cool.

Test Preparation

Transfer the calculated amount of the test substance to a 125-mL conical flask. Add 5 mL each of nitric acid and sulfuric acid and a few glass beads. Attach a suitable water-cooled condenser with a standard-taper joint to fit the flask, and digest in a fume hood, preferably on a hot plate, and at a temperature not exceeding 120°, until charring begins. (If additional sulfuric acid is necessary to wet the specimen completely, add it carefully through the condenser, but do not allow the total volume added to exceed 10 mL.) After the test substance has been decomposed by the acid, cautiously add, dropwise through the condenser, 30 percent hydrogen peroxide, allowing the reaction to subside and again heating between drops (add the first few drops very slowly with sufficient mixing, in order to prevent a rapid reaction; discontinue heating if foaming becomes excessive). When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the specimen from caking on glass exposed to the heating unit. Maintain oxidizing conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens. Continue the digestion until the organic matter is destroyed, and then reflux the mixture for 1 hour. Turn off the water circulating through the condenser, and heat until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, and cautiously add 10 mL of water through the condenser, while swirling the flask. Again heat until white fumes appear. Cool, and cautiously add 15 mL of water. Remove the condenser, and rinse the sides of the flask with a few mL of water to obtain a volume of 35 mL. Add 1 mL of *Potassium Permanganate Solution*, boil for a few seconds, and cool.

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Procedure

Proceed as directed for *Procedure* under *Method IIa.*

⟨267⟩ POROSIMETRY BY MERCURY INTRUSION

In general, different types of pores may be pictured as apertures, channels, or cavities within a solid body or as space (i.e., interstices or voids) between solid particles in a bed, compact, or aggregate. *Porosity* is a term that is often used to indicate the porous nature of solid material and is more precisely defined as the ratio of the volume of accessible pores and voids to the total volume occupied by a given amount of the solid. In addition to the accessible pores, a solid may contain closed pores, which are isolated from the external surface and into which fluids are not able to penetrate. The characterization of closed pores, i.e., cavities with no access to an external surface, is not covered in this general chapter.

Porous materials may take the form of fine or coarse powders, compacts, extrudates, sheets, or monoliths. Their characterization usually involves the determination of the total pore volume or porosity as well as the pore size distribution.

It is well established that the performance of a porous solid (e.g., its strength, reactivity, permeability, or adsorbent power) is dependent on its pore structure. Many different methods have been developed for the characterization of pore structure. In view of the complexity of most porous solids, it is not surprising to find that the results obtained are not always in agreement and that no single technique can be relied upon to provide a complete picture of the pore structure. The choice of the most appropriate method depends on the application of the porous solid, its chemical and physical nature, and the range of pore size.

This chapter provides guidance for measurement of porosity and pore size distribution by mercury porosimetry. It is a comparative test, usually destructive, in which the volume of mercury penetrating a pore or void is determined as a function of an applied hydrostatic pressure that can be related to a pore diameter. Other information such as pore shape and interconnectivity, the internal and external surface area, powder granulometry, and bulk and tapped density can also be inferred from volume–pressure curves; however, these aspects of the technique do not fall under the scope of this chapter.

Practical considerations presently limit the maximum applied absolute pressure reached by some equipment to about 400 MPa, corresponding to a minimum equivalent pore diameter of approximately 0.003 µm. The maximum diameter will be limited for samples having a significant depth because of the difference in the hydrostatic head of mercury from the top to the bottom of the sample. For most purposes this limit may be regarded as 400 µm.

Interparticle and intraparticle porosity can be determined, but the method does not distinguish between these porosities where they coexist.

The method is suitable for the study of most porous materials. Samples that amalgamate with mercury, such as certain metals, may be unsuitable for this technique or may require a preliminary passivation. Other materials may deform or compact under the applied pressure. In some cases, it may be possible to apply sample compressibility corrections, and useful comparative data may still be obtained.

The mercury porosimetry technique should be considered to be comparative, because for most porous media, a theory is not available to allow an absolute calculation of results of pore size distribution. Therefore, this technique is mainly recommended for development studies.

Mercury is toxic. Appropriate precautions must be observed to safeguard the health of the operator and others working in the area. Waste material must also be disposed of in a suitable manner, according to local regulations.

PRINCIPLE

The technique is based on the measurement of the mercury volume intruded into a porous solid as a function of the applied pressure. The measurement includes only those pores into which mercury can penetrate at the pressure applied.

A nonwetting liquid penetrates into a porous system only under pressure. The pressure applied is in inverse proportion to the inner width of the pore aperture. In the case of cylindrical pores, the correlation between pore diameter and pressure is given by the Washburn equation:

$$d_p = -\frac{4 \cdot \sigma}{p} \cos\theta \qquad (1)$$

p = applied pressure, in pascals
d_p = pore diameter, in meters
σ = surface tension of mercury, in newtons per meter
θ = contact angle of mercury on the sample, in degrees

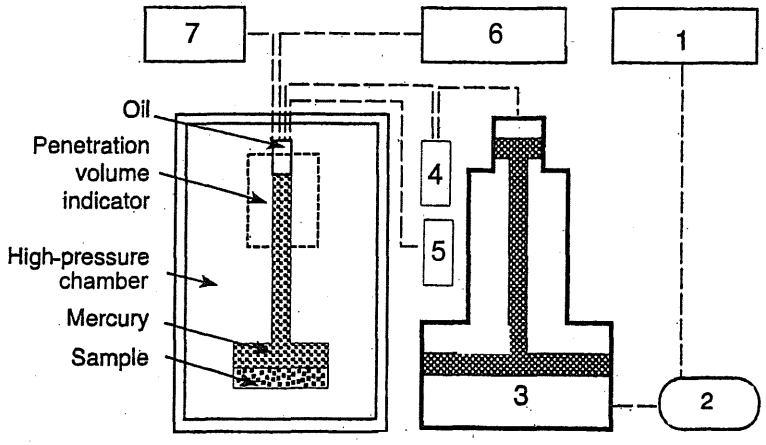
APPARATUS

The sample holder, referred to as a penetrometer or dilatometer, has a calibrated capillary tube, through which the sample can be evacuated and through which mercury can enter. The capillary tube is attached to a wider tube in which the test sample is placed. The change in the volume of mercury intruded is usually measured by the change in capacitance between the mercury column in the capillary tube and a metal sleeve around the outside of the capillary tube. If precise measurements are required, the internal volume of the capillary tube should be between 20% and 90% of the expected pore and void volume of the sample.

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Because different materials exhibit a wide range of open porosities, a number of penetrometers with different diameter capillary tubes and sample volumes may be required. A typical setup for a mercury porosimeter instrument is given in *Figure 1*. The porosimeter may have separate ports for high- and low-pressure operation, or the low-pressure measurement may be carried out on a separate unit.

The pressure range is typically 4 kPa to 300 kPa for low-pressure operation and above 300 kPa for high-pressure operation, depending on the design of the particular apparatus and on the intended use.



1. Low-pressure hydraulic fluid reservoir
2. Hydraulic pump
3. Pressure multiplier
4. Pressure transducer
5. High-pressure hydraulic fluid reservoir
6. Vacuum pump with gauge
7. Mercury reservoir

Figure 1. Example of the setup of a mercury porosimeter instrument.

METHOD

Sample Preparation

The sample is pretreated to remove adsorbed material that can obscure its accessible porosity by either heating and/or evacuation or by flowing inert gas. It may be possible to passivate the surface of wettable or amalgam-forming solids by producing a thin layer of oxide, or by coating with stearate. The sample of the pretreated solid is weighed and transferred to the penetrometer. The pore system of the sample is then degassed in a vacuum to a maximum residual pressure of 7 Pa.

Filling the Penetrometer with Mercury

Use mercury of analytical quality. The sample is overlaid with mercury under vacuum. The vacuum is required to ensure the transfer of mercury from the reservoir to the penetrometer. In a filled penetrometer, the filling pressure is comprised of the applied pressure plus the pressure contribution created by the head of mercury contacting the sample. A typical filling pressure would be about 4 kPa. The hydrostatic pressure of the mercury over the sample can be minimized by filling the penetrometer in the horizontal positions.

Low-Pressure Measurement

Admit air or nitrogen in a controlled manner to increase the pressure either in stages corresponding to the particular pore sizes of interest, or continuously at a slow rate. The concomitant change in the length of the mercury column in the capillary tube is recorded. When the maximum required pressure has been reached, reduce the pressure to ambient.

High-Pressure Measurement

After measurement at low-pressure conditions, the penetrometer filled with mercury is transferred to the high-pressure port or unit of the instrument and overlaid with hydraulic fluid. Mercury is intruded into the pore system via the hydraulic fluid. Increase the pressure in the system to the maximum pressure reached in the low-pressure measurement, and record the intrusion volume at this pressure, because subsequent intrusion volumes are calculated from this initial volume. Increase the pressure either in stages corresponding to the particular pore sizes of interest, or continuously at a slow rate. The fall in the mercury column is measured up to the maximum required pressure. If required, the pressure may be decreased either in stages or continuously at a slow rate to determine the mercury extrusion curve. Make corrections to take account of changes in the

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volume of the mercury, the penetrometer, and other components of the volume detector system under elevated pressure. The extent of the corrections may be determined by means of blank measurements under the same conditions. An experimentally determined volume–pressure curve is shown in *Figure 2*.

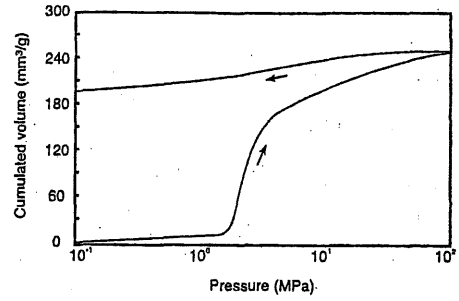


Figure 2. Volume–pressure curve as semilogarithmic plot.

REPORTING OF RESULTS

The pressure readings are converted to pore diameter by means of the Washburn equation or by another model.

The surface tension of mercury, σ, depends not only on the temperature and the material, but also—in the case of markedly curved surface areas—on the radius of curvature. In general, values between 0.41 $N \cdot m^{-1}$ and 0.52 $N \cdot m^{-1}$ are measured at room temperature. If the value is not known, $\sigma = 0.48\ N \cdot m^{-1}$ can be used.

The contact angle of mercury θ in most cases is more than 90°. It may be determined using a contact angle instrument. If the θ value is not known, 130° can be used. Report the values of contact angle, surface tension, and the model used in the calculation. Visualization of the data can be done with several types of graphs. Frequently, in a graphical representation, the pore diameter is plotted on the abscissa and the dependent intruded specific volume on the ordinate to give the pore size distribution. It is appropriate here to choose a logarithmic scale for the abscissa (see *Figure 3*). The spaces between the particles of the solid sample are included as pores in the calculation. If the pores differ in size from the voids, the latter can be separated by choosing the relevant pore size range.

Extrusion curves may not be used for calculating the pore size distribution (for hysteresis, see *Figure 2*), because an intruded part of the mercury always remains in the pore system. The retention ratio may be useful for the qualitative characterization of pores that are only accessible via narrow openings ("ink-bottle pores").

The most common characteristic values, such as the total intruded specific volume, the mean, and the median pore diameter are calculated from the pore size distribution. Moreover, sufficient information should be documented about the sample, the sample preparation, the evacuation conditions, and the instrument used.

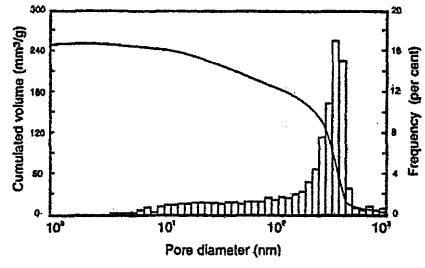


Figure 3. Pore volume distribution as semilogarithmic plot.

CONTROL OF THE INSTRUMENT'S PERFORMANCE

As the mercury porosimetry technique is considered as a comparative test, no details are given in this chapter. However, it is recommended that a stable comparison material should be tested on a regular basis to monitor instrument calibration and performance.

⟨268⟩ POROSITY BY NITROGEN ADSORPTION–DESORPTION

INTRODUCTION

Porosity is a term typically used to indicate the porous nature of solid material and is more precisely defined as the ratio of the volume of accessible pores and voids to the total volume occupied by a given amount of the solid. Closed or inaccessible

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pores that are isolated from the external surface are excluded from this definition of pore volume. Pores (or voids) may consist of apertures, channels, or cavities within a solid body or spaces between solid particles in a compact or aggregate. Pores exist in a variety of solid materials beyond compacts and aggregates, such as powders and tablets, and their characterization usually involves the determination of the total pore volume or porosity, as well as the pore size distribution. Pores generally are classified by size into the following groups:

- Micropores—less than 2 nm
- Mesopores—2 to 50 nm
- Macropores—greater than 50 nm

The method in *Porosity by Nitrogen Adsorption–Desorption* ⟨268⟩ is complementary to that in general chapter *Porosimetry by Mercury Intrusion* ⟨267⟩. Mercury porosimetry may, in principle (in theory), be used with pore diameters from 3 nm to 400 µm but is most applicable in the range from 100 nm to 200 µm. Nitrogen adsorption–desorption may be used to characterize pores smaller than about 300 nm but is most appropriate for the analysis of mesopores and into the low macropore range from 2 to 100 nm.

APPARATUS

Measurements are most commonly performed using the static volumetric procedure, although dynamic flow methods also may be employed. Users of commercially available equipment should refer to the manufacturer's literature and manual for a description of their particular apparatus. For example, a static volumetric apparatus must provide: evacuation of the system to a pressure of less than 10 Pa, delivery of known volumes of high-purity nitrogen and helium, accurate measurement of pressure and temperature, and a means of cooling the sample to liquid nitrogen temperature.

MEASUREMENT PRINCIPLE

The adsorption of an inert gas onto solid surfaces at low temperatures is a well-known phenomenon and is the basis for measurement of the surface area of solids (see general chapter *Specific Surface Area* ⟨846⟩). As gas adsorbs to a surface, it can condense into accessible pores. The total pore volume and pore size distribution can be derived from the gas adsorption isotherm, which is the measure of amount adsorbed as a function of adsorbate partial pressure. Adsorption isotherms fall into six general categories, depending on the relative energetics of adsorption and the presence of pores. *Figure 1* shows six general categories of adsorption isotherms.

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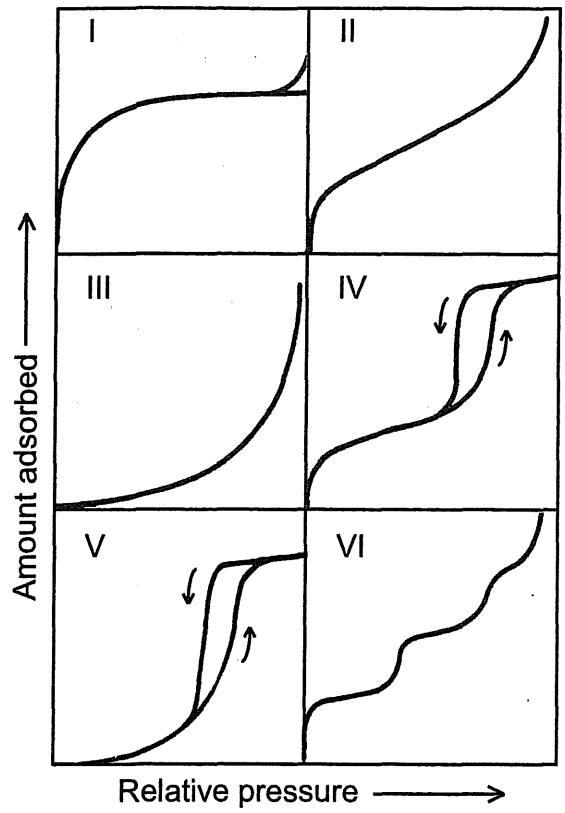


Figure 1. Isotherm Types. [Reproduced with permission and modification from: Sing KSW, Everett DH, Haul RAW, et al. Reporting physisorption for gas/solid systems with special reference to the determination of surface area and porosity (recommendations 1984). *Pure Appl Chem.* 1985;57(4):603–619, *Figure 2.*]

Micropores (pore sizes less than 2 nm) frequently give rise to type I isotherms. Mesopores and macropores normally yield type IV isotherms, but for pores greater than about 100 nm hysteresis may be difficult to observe, thus yielding a type II isotherm. Although some information, such as the total porosity, can be derived for microporous materials, the determination of pore size distributions in that size range is outside the scope of this chapter.

The preferred adsorbate is nitrogen, and the isotherm is determined at liquid nitrogen temperature (77.4 K). Other adsorbates may be used for special purposes but are not discussed in this chapter.

PROCEDURE

Sample Preparation

Before analysis, analysts should outgas the sample to remove gases and vapors that may have become physically adsorbed onto the surface. The outgassing conditions must be demonstrated to yield reproducible adsorption–desorption, a constant sample weight, and no detectable physical or chemical changes in the sample. Outgassing of many substances often is achieved by applying a vacuum, by purging the sample in a flowing stream of a nonreactive, dry gas, or by applying an adsorption–desorption cycling procedure. If appropriate, analysts can apply elevated temperatures to increase the rate at which the contaminants leave the surface. Analysts should be cautious when outgassing samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample. If heating is employed, the recommended temperature and time of outgassing should be the minimum needed to achieve reproducible measurement of the adsorption–desorption isotherm.

Analysts should determine the sample mass after outgassing or, alternatively, should determine the mass after the adsorption–desorption measurement. The total surface area of the sample should be greater than 1 m^2 and preferably greater than 5 m^2.

Isotherm Measurement

Specific details of the measurement process depend on the procedure used. Analysts should follow the manufacturer's instructions for the particular instrument used. The following description is applicable generally:

- Analysts should determine the saturated vapor pressure of the adsorbate, p_0. It is preferable to determine p_0 experimentally at the time of measurement, but analysts can use a calculated value.

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- Analysts should determine the nitrogen sorption isotherm and should measure the volume adsorbed, V_a, at the lowest desired relative pressure (p/p_0, the ratio of the measured adsorbate pressure to its saturated vapor pressure).
- Analysts repeat the measurement of V_a at successively higher relative pressure values to the maximum desired relative pressure (generally 0.99). Then they successively decrease the relative pressure to determine sorbed amounts on the desorption portion of the isotherm. Analysts should measure at least 20 points on both the adsorption and desorption segments, covering a relative pressure (p/p_0) range of approximately 0.05–0.99. The p/p_0 values can be distributed to achieve the best resolution of the pore size distribution. If only the desorption segment is being used to calculate the pore size distribution, fewer points can be used on the adsorption segment.

DATA ANALYSIS

Examination of the Isotherm

The isotherm is depicted as a plot of quantity of nitrogen adsorbed (as volume, V_a, or moles, n_a) vs. p/p_0. The isotherm data also can be presented in tabular form. From the graph, determine the isotherm and hysteresis types by comparison to the examples in *Figures 1* and *2*. A type I isotherm is common for microporous materials. A type IV isotherm usually is exhibited by materials that contain mesopores or small macropores.

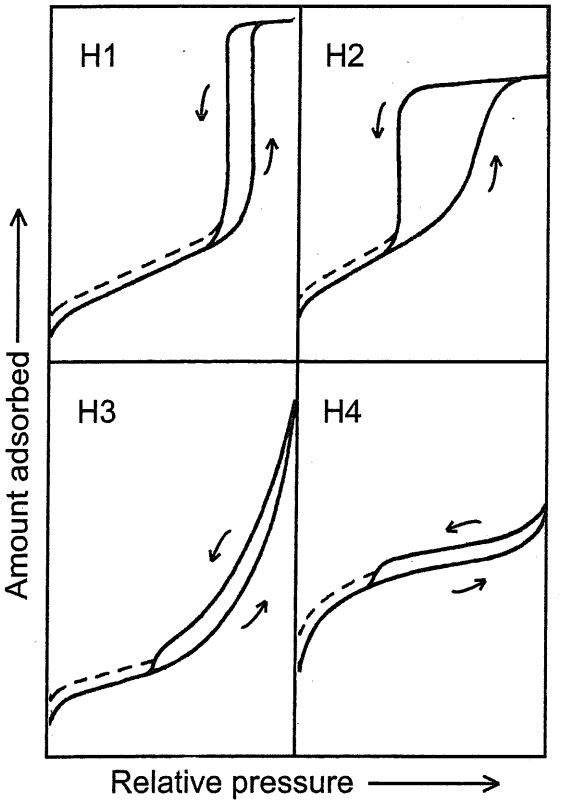


Figure 2. Hysteresis Loop Types. [Reproduced with permission and modification from: Sing KSW, Everett DH, Haul RAW, et al. Reporting physisorption for gas/solid systems with special reference to the determination of surface area and porosity (recommendations 1984). *Pure Appl Chem.* 1985;57(4):603–619, *Figure 3*.]

Constructing a *t*-plot or α_s-plot to compare the isotherm of the test sample to that of a reference isotherm also helps to illustrate the presence of micro- and mesoporosity. The reference isotherm may be calculated using a mathematical expression, but it is recommended that an experimentally determined reference isotherm be used when the adsorbent has chemical properties that are similar to those of the test sample.

The *t*-plot method is based on the *t*-curve, which is a plot of the amount of nitrogen adsorbed on the nonporous solid as a function of *t*, the statistical thickness of the adsorbed layer. The *t* value is calculated:

$$t = \frac{n_a \times \delta_a}{n_m}$$

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n_m = monolayer amount

δ_a = thickness of a single molecular layer, usually taken as 0.354 nm for nitrogen

In the α_s-plot method, the amount of nitrogen adsorbed by the reference nonporous solid is normalized by using the amount adsorbed at some fixed relative pressure ($n'_{a,x}$), often taken as 0.4. The normalized adsorption α_s (equal to $n_a/n'_{a,x}$) then is plotted against p/p_0 to obtain an α_s-curve.

The *t*-plot or α_s-plot is constructed by plotting the amount of nitrogen adsorbed by the test sample against *t* or α_s for the reference material, rather than p/p_0. The conversion of p/p_0 to *t* or α_s is carried out by reference to the *t*-curve or α_s-curve. The shape of the plot depends on the nature of the porosity present in the test sample, as follows:

1. if the *t*- or α_s-plot is linear and passes through the origin, the test sample is nonporous or macroporous
2. if the test sample contains mesopores, the plot shows an upward deviation at the relative pressure corresponding to the onset of capillary condensation in the smallest mesopores
3. if the test sample contains micropores, the plot exhibits a downward deviation because multilayers cannot fully develop within the constricted space inside the micropores.

Some materials contain combinations of pores, which may result in a complex plot that is difficult to interpret. In such cases analysts should be cautious when they analyze the isotherm.

Calculation of Pore Size Distribution

This analysis is valid only for calculations of the size distributions of mesopores.

Calculation of the pore size distribution is based on the Kelvin equation:

$$r_k = \frac{2 \times \sigma_l \times v_l \times \cos(\theta) \times 10^3}{R \times T \times \ln(p/p_0)}$$

r_K = core radius of the pore (or Kelvin radius) (nm)

σ_l = adsorbate (nitrogen) liquid surface tension (N/m)

v_l = molar volume of the condensed adsorbate (nitrogen) (cm^3/mol)

R = universal gas constant, 8.3144 ($J \cdot K^{-1} \cdot mol^{-1}$)

T = temperature (K)

θ = adsorbate contact angle (0 for a wetted surface)

For nitrogen, equation 2 reduces to:

$$r_k = \frac{-0.953}{\ln(p/p_0)}$$

The actual pore radius, r_p, is calculated from the Kelvin radius by correcting for the thickness, *t*, of the adsorbate on the pore walls. For cylindrical pores, $r_p = r_k + t$, and the pore diameter, d_p, is given by $d_p = 2(r_k + t)$. Because of the different geometry of parallel-sided slit-shaped pores, the slit width is given by $r_k + 2t$.

Analysts can calculate the volume pore size distribution using the method of Barrett, Joyner, and Halenda. This model assumes that the pores are rigid and of regular shape (e.g., cylindrical or slit-shaped), micropores are absent, and the pore size distribution does not extend continuously above the largest pores measurable by this procedure, which implies that all of the pores evaluated are filled at the highest relative pressure.

Porosity and pore size distribution calculations that employ the Kelvin equation must be performed using the desorption isotherm. The Kelvin equation was derived for macroscopic systems and is not strictly valid at the molecular scale. Thus, the Kelvin equation relies on an intact meniscus in order to accurately describe experimental phenomena. For the systems discussed in this chapter, this is achieved only for the desorption isotherm. However, for desorption the application of the Kelvin equation at lower pore sizes is limited by the surface tension of the adsorbate. The limit is illustrated by the point of closure of the hysteresis loop in the isotherm. For nitrogen, this point occurs at a relative pressure of about 0.45, corresponding to a limiting cylindrical pore radius of about 2 nm. Thus the Kelvin equation is not applicable for micropores.

Calculation of Micropore Volume

If the *t*- or α_s-plot indicates the presence of micropores, the micropore volume can be obtained from the intercept of the extrapolated linear portion of the curve.

Reported Results

Typically reported results may include total pore volume or porosity, micropore volume, median or mean pore diameter, pore size distribution, and pore surface area.

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CALIBRATION AND VERIFICATION OF SYSTEM PERFORMANCE

Analysts should carry out calibration of individual components in accordance with the manufacturer's recommendations. Calibration of pressure transducers and temperature sensors is accomplished with reference to standard pressure- and temperature-measuring devices that have calibrations traceable to national standards. Manifold volume calibration is achieved by appropriate pressure and temperature measurements using constant-temperature volumetric spaces or solids of known, traceable volume.

A certified reference material or locally defined reference material that is traceable to a certified reference material should be tested on a regular basis in order to monitor instrument calibration and performance.

⟨271⟩ READILY CARBONIZABLE SUBSTANCES TEST

In tests for readily carbonizable substances, unless otherwise directed, add the specified quantity of the substance, finely powdered if in solid form, in small portions to the comparison container, which is made of colorless glass resistant to the action of sulfuric acid and contains the specified volume of sulfuric acid (see under *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*).

Stir the mixture with a glass rod until solution is complete, allow the solution to stand for 15 minutes, unless otherwise directed, and compare the color of the solution with that of the specified Matching Fluid (see *Color and Achromicity* ⟨631⟩) in a comparison container, which also is of colorless glass and has the same internal and cross-section dimensions, viewing the fluids transversely against a background of white porcelain or white glass.

When heat is directed in order to effect solution of the substance in the sulfuric acid, mix the sample and the acid in a test tube, heat as directed, and transfer the solution to the comparison container for matching with the designated Matching Fluid (see *Color and Achromicity* ⟨631⟩).

⟨281⟩ RESIDUE ON IGNITION

Portions of this general chapter have been harmonized with the corresponding texts of the *European Pharmacopoeia* and the *Japanese Pharmacopoeia*. The portions that are not harmonized are marked with symbols (•,). The harmonized texts of these pharmacopeias are therefore interchangeable, and the methods of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* may be used for demonstration of compliance instead of the present *United States Pharmacopeia* general chapter. These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.

The *Residue on Ignition/Sulfated Ash* test uses a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

PROCEDURE

Ignite a suitable crucible (for example, silica, platinum, quartz, or porcelain) at 600 ± 50° for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant), and weigh it accurately. Weigh accurately •1 to 2 g of the substance, or,• the amount specified in the individual monograph, in the crucible.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. Cool; then,•unless otherwise directed in the individual monograph,• moisten the residue with a small amount (usually 1 mL) of sulfuric acid; heat gently until white fumes are no longer evolved; and ignite at 600 ± 50°, •unless another temperature is specified in the individual monograph,• until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately, and calculate the percentage of residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and igniting as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

•Conduct the ignition in a well-ventilated hood, but protected from air currents, and at as low a temperature as is possible to effect the complete combustion of the carbon. A muffle furnace may be used, if desired, and its use is recommended for the final ignition at 600 ± 50°.

Calibration of the muffle furnace may be carried out using an appropriate digital temperature meter and a working thermocouple probe calibrated against a standard thermocouple traceable to the National Institute of Standards and Technology.

Verify the accuracy of the measuring and controlling circuitry of the muffle furnace by checking the positions in the furnace at the control set point temperature of intended use. Select positions that reflect the eventual method of use with respect to location of the specimen under test. The tolerance is ±25° at each position measured.•

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⟨291⟩ SELENIUM

STOCK SOLUTION

Dissolve 40.0 mg of metallic selenium in 100 mL of dilute nitric acid (1 in 2) in a 1000-mL volumetric flask, warming gently on a steam bath if necessary to effect solution, add water to volume, and mix. Pipet 5 mL of this solution into a 200-mL volumetric flask, add water to volume, and mix. Each mL of the resulting solution contains the equivalent of 1 µg of selenium (Se).

DIAMINONAPHTHALENE SOLUTION

Dissolve 100 mg of 2,3-diaminonaphthalene and 500 mg of hydroxylamine hydrochloride in 0.1 N hydrochloric acid to make 100 mL. Prepare this solution fresh on the day of use.

STANDARD SOLUTION

Pipet 6 mL of *Stock Solution* into a 150-mL beaker, and add 25 mL of dilute nitric acid (1 in 30) and 25 mL of water.

TEST SOLUTION

Clean combustion of the test material is an important factor in conducting the test. For compounds that burn poorly and produce soot, the addition of magnesium oxide usually results in more thorough combustion and reduces soot formation. Where the need to add magnesium oxide has been identified, it is specified in the individual monograph. Using a 1000-mL combustion flask and using 25 mL of dilute nitric acid (1 in 30) as the absorbing liquid, proceed as directed under *Oxygen Flask Combustion* ⟨471⟩, using a test specimen weighing 100 to 200 mg, unless directed otherwise in the individual monograph. Upon completion of the combustion, place a few mL of water in the cup, loosen the stopper, and rinse the stopper, the specimen holder, and the sides of the flask with about 10 mL of water. Transfer the solution with the aid of about 20 mL of water to a 150-mL beaker, and heat gently to the boiling temperature. Boil for 10 minutes, and allow the solution to cool to room temperature.

PROCEDURE

Treat the *Standard Solution*, the *Test Solution*, and the reagent blank consisting of 25 mL of dilute nitric acid (1 in 30) and 25 mL of water, concomitantly and in parallel, as follows. Add ammonium hydroxide solution (1 in 2) to adjust to a pH of 2.0 ± 0.2. Dilute with water to 60 mL, and transfer to a low-actinic separator with the aid of 10 mL of water, adding the 10 mL of rinsings to the separator. Add 200 mg of hydroxylamine hydrochloride, swirl to dissolve, immediately add 5.0 mL of *Diaminonaphthalene Solution*, insert the stopper, and swirl to mix. Allow the solution to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, and allow the layers to separate. Discard the aqueous layer, and centrifuge the cyclohexane extract to remove any dispersed water. Determine the absorbances of the cyclohexane extracts of the *Test Solution* and the *Standard Solution* in a 1-cm cell at the wavelength of maximum absorbance at about 380 nm, with a suitable spectrophotometer, using the cyclohexane extract of the reagent blank as the blank, and compare the absorbances: the absorbance of the *Test Solution* is not greater than that of the *Standard Solution* where a 200-mg test specimen has been taken, or is not greater than one-half that of the *Standard Solution* where a 100-mg test specimen has been taken.

OTHER TESTS AND ASSAYS

⟨301⟩ ACID-NEUTRALIZING CAPACITY

[NOTE—All tests shall be conducted at a temperature of 37 ± 3°.]

Standardization of pH meter: Standardize a pH meter using the 0.05 m potassium biphthalate and 0.05 m potassium tetraoxalate standardizing buffers as described under *pH* ⟨791⟩.

Magnetic stirrer: Transfer 100 mL of water to a 250-mL beaker containing a 40- × 10-mm (or other suitable size) magnetic stirring bar that is coated with solid perfluorocarbon and has a spin ring at its center. Adjust the power setting of the magnetic stirrer to produce a stirring rate of 300 ± 30 rpm when the stirring bar is centered in the beaker, as determined by a suitable optical tachometer.

Test preparation

Powders: Transfer the accurately weighed portion of the substance specified in the individual monograph to a 250-mL beaker, add 70 mL of water, and mix on the *Magnetic stirrer* for 1 minute.

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Effervescent solids: Transfer an accurately weighed quantity, equivalent to the minimum labeled dosage, to a 250-mL beaker, add 10 mL of water, and swirl the beaker gently while allowing the reaction to subside. Add another 10 mL of water, and swirl gently. Wash the walls of the beaker with 50 mL of water, and mix on the *Magnetic stirrer* for 1 minute.

Suspensions and other liquids: Shake the container until the contents are uniform, and determine the density. Transfer an accurately weighed quantity of the uniform mixture, equivalent to the minimum labeled dosage, to a 250-mL beaker, add water to make a total volume of about 70 mL, and mix on the *Magnetic stirrer* for 1 minute.

Lozenges: Accurately weigh not fewer than 20 lozenges, and determine the average weight. Select and weigh 2 lozenges, and transfer them to a 250-mL beaker containing 70 mL of water.

Nonchewable tablets: Weigh not fewer than 20 tablets, and determine the average tablet weight. Grind the tablets to a fine powder, mix to obtain a uniform mixture, and transfer an accurately weighed quantity of it, equivalent to the minimum labeled dosage, to a 250-mL beaker. If wetting is desired, add not more than 5 mL of alcohol (neutralized to an apparent pH of 3.5), and mix to wet the specimen thoroughly. Add 70 mL of water, and mix on the *Magnetic stirrer* for 1 minute.

Chewable tablets: Prepare as directed for *Nonchewable tablets*.

Tablets that are required to be chewed: Transfer 1 Tablet to a 250-mL beaker, add 50 mL of water, and mix on the *Magnetic stirrer* for 1 minute.

Capsules: Weigh accurately not fewer than 20 capsules. Remove the capsule contents completely, with the aid of a cotton swab if necessary. Accurately weigh the empty capsules, and determine the average weight of the contents per capsule. Mix the combined capsule contents to obtain a uniform mixture, and proceed as directed for *Nonchewable tablets*, beginning with "transfer an accurately weighed quantity of it."

PROCEDURES

• **PROCEDURE FOR POWDERS, EFFERVESCENT SOLIDS, SUSPENSIONS AND OTHER LIQUIDS, LOZENGES, NONCHEWABLE TABLETS, CHEWABLE TABLETS, AND CAPSULES**

Pipet 30.0 mL of 1.0 N hydrochloric acid VS into the *Test preparation* while continuing to stir with the *Magnetic stirrer*. [NOTE—Where the acid-neutralizing capacity of the specimen under test is greater than 25 mEq, use 60.0 mL of 1.0 N hydrochloric acid VS, and make the appropriate modifications in the calculation.] Stir for 15 minutes, accurately timed, after the addition of the acid, begin to titrate immediately, and in a period not to exceed an additional 5 minutes, titrate the excess hydrochloric acid with 0.5 N sodium hydroxide VS to attain a stable (for 10 to 15 seconds) pH of 3.5. Calculate the number of mEq of acid consumed by the formula:

$$\text{Total mEq} = (30 \times N_{HCl}) - (V_{NaOH} \times N_{NaOH})$$

N_{HCl} = normality of hydrochloric acid VS
V_{NaOH} = volume of sodium hydroxide VS used for titration
N_{NaOH} = normality of sodium hydroxide VS

Express the result in terms of mEq of acid consumed per g of the substance tested.

• **PROCEDURE FOR TABLETS THAT ARE REQUIRED TO BE CHEWED**

Pipet 30.0 mL of 1.0 N hydrochloric acid VS into the *Test preparation* while continuing to stir with the *Magnetic stirrer* for 10 minutes, accurately timed, after the addition of the acid. Discontinue stirring briefly, and without delay remove any gum base from the beaker using a long needle. Promptly rinse the needle with 20 mL of water, collecting the washing in the beaker, and resume stirring for 5 minutes, accurately timed, then begin to titrate immediately, and in a period not to exceed an additional 5 minutes, titrate the excess hydrochloric acid with 0.5 N sodium hydroxide VS to attain a stable (for 10 to 15 seconds) pH of 3.5. Calculate the number of mEq of acid consumed by the Tablet tested by the formula:

$$\text{Total mEq} = (30 \times N_{HCl}) - (V_{NaOH} \times N_{NaOH})$$

⟨311⟩ ALGINATES ASSAY

APPARATUS

The required apparatus (see *Figure 1*) contains a capillary metering valve, A, followed by a flowmeter, B, to control and monitor the flow of nitrogen through the system. Halogenated vinyl plastic tubing* and a rubber fitting, C, are used to connect the flowmeter to a sidearm of a reaction flask, D. Flask D is a 250-mL round-bottom, boiling flask, resting in a suitable heating mantle, E. Flask D is provided with a 225-mm Hopkins coil reflux condenser, F. The condenser terminates in a U-shaped trap, G, which contains two 25-g bands of 20-mesh zinc, the bands being bounded and separated by three 3-inch plugs of glass wool. The trap terminates in an adapter, H, that by means of a halogenated vinyl plastic tubing and a twistcock connector, I, connects with a 250-mL gas washing bottle, J. The inlet (bubbling) tube extends almost to the bottom of the gas washing bottle, and it terminates in a fritted disk having a coarse porosity. The size of all glass joints is $^{24}/_{40}$, except for the $^{45}/_{50}$ joint of the gas washing bottle.

* This type of tubing is commonly referred to as Tygon tubing. This note is added for clarity and it does not constitute USP's endorsement of this product.

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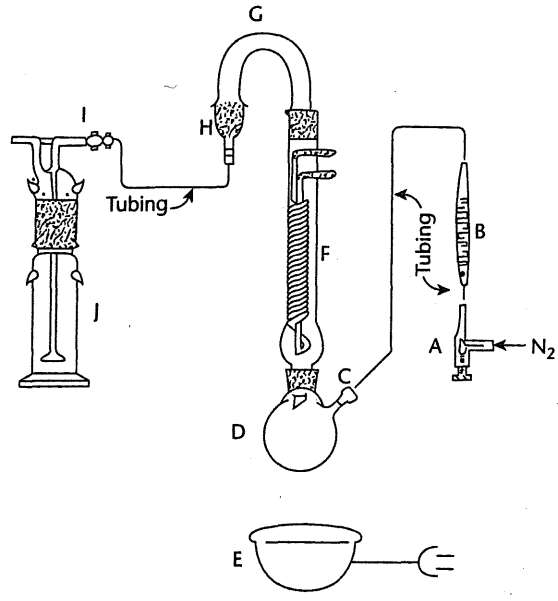


Figure 1. Apparatus for Alginates Assay

SYSTEM SUITABILITY

Using D-glucuronolactone as the standard, proceed as directed for *Procedure,* but do not perform the preboiling steps. The system is suitable if the following criteria are met: (1) a blank determination results in a net titration value, C, between 0.02 and 0.06 mEq, calculated as follows:

$$A_b - B_b$$

in which A_b is the number of mEq of 0.25 N sodium hydroxide in the 25 mL used, and B_b is the number of mEq of 0.1 N hydrochloric acid used in the blank titration; and (2) the percentage of carbon dioxide, CO_2, obtained from the standard is between 24.2% and 25.7%.

PROCEDURE

Unless otherwise directed in the individual monograph, transfer a specimen of about 250 mg, accurately weighed, into the reaction flask, D, add 50 mL of 0.1 N hydrochloric acid, insert several boiling chips, and connect the flask to the reflux condenser, F, using phosphoric acid as a lubricant. [NOTE—Stopcock grease may be used for the other connections.] Connect the nitrogen line to the sidearm of the flask, and adjust the flow of cooling water to about 2 L per minute.

[NOTE—The following preboiling steps, outlined in this paragraph, are optional and need only be performed when the presence of inorganic carbonates is suspected.] Maintain the flow of nitrogen through the apparatus at 90 to 100 mL per minute. Raise the heating mantle, E, to the flask, heat the specimen to boiling, and boil gently for 2 minutes. Turn the heat off, lower the mantle, E, and allow to cool for about 10 minutes.

Connect the empty gas washing bottle assembly, J, and sweep the system with nitrogen at a rate of 90 to 100 mL per minute for 5 minutes. Reduce the nitrogen flow to 60 to 65 mL per minute, add 10 drops of butyl alcohol, 25.0 mL of 0.25 N sodium hydroxide VS, and 50 mL of distilled water into the bottle, rinsing down the inside of the gas washing bottle, and replace the cap. Detach the rubber fitting, C, from sidearm, and add 46 mL of hydrochloric acid through the sidearm of the boiling flask. Reattach the nitrogen line, raise the heating mantle, and heat the reaction mixture to boiling. After 2 hours of boiling, increase the nitrogen flow to 90 to 100 mL per minute, discontinue the heating, and lower the mantle. Allow to cool for 10 minutes. Disconnect, and disassemble the gas washing bottle. Using a directed stream of distilled water, thoroughly rinse all parts of the bubbling tube and cap, collecting the washings in the gas washing bottle. Use nitrogen to gently force all water out of the bubbling tube. To the bottle immediately add 10 mL of 10% barium chloride solution and a stirring bar. Insert a tight stopper, and stir gently for 1 minute. Allow to stand for at least 5 minutes. Add three drops of phenolphthalein TS, and titrate with 0.1 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Calculate the percentage of carbon dioxide, CO_2, by the formula:

$$2200[(A - B) - C]/(1000W)(1 - D)$$

in which A is the number of mEq of 0.25 N sodium hydroxide in the 25 mL used; B is the number of mEq of 0.1 N hydrochloric acid used for the titration of the sample or the standard; C is the net titration value calculated in the blank determination; W is the weight, in g, of the sample or the standard taken; and D is the percentage expressed as a decimal (1 decimal place), obtained in the test for *Loss on drying* for the sample or for the standard.

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⟨341⟩ ANTIMICROBIAL AGENTS—CONTENT

An essential component of Injections preserved in multiple-dose containers is the antimicrobial agent or antimicrobial agents present to reduce the hazard of having introduced, in the course of removing some of the contents, accidental microbial contamination of the contents remaining. It is a Pharmacopeial requirement that the presence and amount added of such antimicrobial agent(s) be declared on the label of the container. This general chapter provides methods for the most commonly used antimicrobial agents. These methods or other suitably validated methods are to be used to demonstrate that the declared antimicrobial agent is present but does not exceed the labeled amount by more than 20%.

The concentration of an antimicrobial preservative added to a multiple-dose or single-dose parenteral, otic, nasal, and ophthalmic preparation may diminish during the shelf life of the product. Therefore, the manufacturer shall determine the lowest level at which the preservative is effective, and the product should be so formulated as to assure that this level is exceeded throughout the product's shelf life. At the time of its manufacture, the product should contain the declared amount of antimicrobial preservative (within ±20% to allow for manufacturing and analytical variations). The quantitative label statement of the preservative content is not intended to mean that the labeled quantity is retained during the shelf life of the product; rather, it is a statement of the amount added, within process limits, and which is not exceeded by more than 20%. An example of such a label statement is "____(unit) added as preservative". [NOTE—"____(unit)" would be a number followed by the unit of measurement, e.g., 0.015 mg/mL or 0.1%.]

The most commonly used antimicrobial agents include benzyl alcohol; chlorobutanol; phenol; the four homologous esters of *p*-hydroxybenzoic acid (methyl, ethyl, propyl, and butyl parabens); and the two mercurials, phenylmercuric nitrate and thimerosal. The method used for phenylmercuric nitrate is polarographic, whereas quantitative liquid chromatography was used for thimerosal and the four homologous esters of *p*-hydroxybenzoic acid. Gas chromatography is used in the determination of phenol, benzyl alcohol, and chlorobutanol.

GENERAL GAS AND LIQUID CHROMATOGRAPHIC METHODS

The general gas chromatography procedures set forth in the following paragraphs are applicable to the quantitative determination of benzyl alcohol, chlorobutanol, and phenol. Prepare the *Internal standard solution* and the *Standard solution* for each antimicrobial agent as directed below. Unless otherwise directed by the individual monograph, prepare the *Sample solution* from accurately measured portions of the sample under test and the *Internal standard solution* such that the concentration of the antimicrobial agent and the composition of the solvent correspond closely to the concentration and composition of the *Standard solution*. Suggested operating parameters of the gas chromatograph are provided in this section.

The general high-pressure liquid chromatography (HPLC) procedures set forth in the following paragraphs are applicable to the quantitative determination of parabens and *Thimerosal*. Prepare the *Internal standard solution* and the *Standard solution* for each antimicrobial agent as directed below. Unless otherwise directed, prepare the *Sample solution* from accurately measured portions of the sample under test and the *Internal standard solution*, if applicable, such that the concentration of the antimicrobial agent and the composition of the solvent is about the same as the concentration and composition of the *Standard solution*. Suggested operating parameters of the liquid chromatograph are provided in this section.

• **BENZYL ALCOHOL**

Diluent: Methanol and water (20:80)

Internal standard solution: 3.8 mg/mL of phenol prepared as follows. Dissolve a suitable amount of phenol in 10% of the flask volume of methanol, and dilute with water to volume.

Standard solution: 1.8 mg/mL of USP Benzyl Alcohol RS and 1.5 mg/mL of phenol prepared as follows. Dissolve 180 mg of USP Benzyl Alcohol RS in 20 mL of methanol contained in a 100-mL volumetric flask. Add 40.0 mL of *Internal standard solution*, and dilute with water to volume.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.32-mm fused-silica; bonded with a 0.5-µm film of phase G16

Temperatures

Injection port: 200°

Detector: 310°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	5
150	10	230	7

Carrier gas: Helium

Flow rate (constant): 2 mL/min

Injection volume: 1 µL

Split ratio: 10:1

Run time: 20 min

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzyl alcohol and phenol are about 1.0 and 1.25, respectively.]

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Suitability requirements
Resolution: NLT 2.0 between the benzyl alcohol and phenol peaks
Tailing factor: NMT 2.0 for the benzyl alcohol peak
Relative standard deviation: NMT 2.0% for the peak response ratio of benzyl alcohol to phenol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzyl alcohol (C_7H_8O) in the portion of the sample taken:

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

R_U = peak response ratio of benzyl alcohol to phenol from the *Sample solution*
R_S = peak response ratio of benzyl alcohol to phenol from the *Standard solution*
C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution*
C_U = nominal concentration of benzyl alcohol in the *Sample solution*

• **CHLOROBUTANOL**
Diluent: Methanol and water (50:50)
Internal standard solution: 10 mg/mL of 2,2,2-trichloroethanol in *Diluent*
Standard stock solution: 5 mg/mL of USP Chlorobutanol RS in methanol
Standard solution: 1.25 mg/mL of USP Chlorobutanol RS and 2 mg/mL of 2,2,2-trichloroethanol prepared as follows. Transfer 2.5 mL of *Standard stock solution*, 2.0 mL of *Internal standard solution*, and 0.5 mL of methanol to a 10-mL volumetric flask. Dilute with water to volume.
Sample stock solution: Quantitatively dilute, if necessary, an accurately measured volume corresponding to 2.5 mg/mL of chlorobutanol in water.
Sample solution: Combine 5.0 mL of *Sample stock solution* with 2.0 mL of *Internal standard solution* in a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 30-m × 0.32-mm fused-silica; bonded with a 0.25-µm film of phase G16
Temperatures
Injection port: 260°
Detector: 280°
Column: 135°
Carrier gas: Helium
Flow rate: 1 mL/min
Injection volume: 0.5 µL
Split ratio: 10:1
Run time: 12 min
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for chlorobutanol and 2,2,2-trichloroethanol are about 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 2.0 between chlorobutanol and 2,2,2-trichloroethanol
Relative standard deviation: NMT 1.0% for the peak response ratio of chlorobutanol to 2,2,2-trichloroethanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlorobutanol ($C_4H_7Cl_3O$), on the anhydrous basis, in the portion of the sample taken:

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

R_U = peak response ratio of chlorobutanol to 2,2,2-trichloroethanol from the *Sample solution*
R_S = peak response ratio of chlorobutanol to 2,2,2-trichloroethanol from the *Standard solution*
C_S = concentration of USP Chlorobutanol RS in the *Standard solution*
C_U = nominal concentration of chlorobutanol in the *Sample solution*

• **PHENOL**
Internal standard solution: 2 mg/mL of USP Benzyl Alcohol RS in methanol
Standard stock solution: 4 mg/mL of USP Phenol RS in water
Standard solution: 0.4 mg/mL each of USP Phenol RS and USP Benzyl Alcohol RS prepared as follows. Combine 5.0 mL of *Standard stock solution* with 10.0 mL of *Internal standard solution* in a 50-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 30-m × 0.32-mm fused-silica; bonded with a 0.5-µm film of phase G16

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Temperatures
Injection port: 200°
Detector: 310°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	5
150	10	230	7

Carrier gas: Helium
Flow rate (constant flow): 2 mL/min
Injection volume: 1 µL
Split ratio: 10:1
Run time: 20 min

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and phenol are about 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between benzyl alcohol and phenol
Tailing factor: NMT 2.0 for the phenol peak
Relative standard deviation: NMT 1.0% for the peak response ratio of phenol to benzyl alcohol

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of phenol (C_6H_6O) in the portion of the sample taken:

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

R_U = peak response ratio of phenol to benzyl alcohol from the *Sample solution*
R_S = peak response ratio of phenol to benzyl alcohol from the *Standard solution*
C_S = concentration of USP Phenol RS in the *Standard solution*
C_U = nominal concentration of phenol in the *Sample solution*

• **METHYLPARABEN AND PROPYLPARABEN**
Buffer: 7 g/L of monobasic potassium phosphate in water
Mobile phase: Methanol and *Buffer* (65:35)
Internal standard solution: 0.013 mg/mL of USP Ethylparaben RS in *Mobile phase*
System suitability solution: 0.01 mg/mL each of USP Butylparaben RS, USP Propylparaben RS, USP Ethylparaben RS, USP Methylparaben RS, and *p*-hydroxybenzoic acid in *Mobile phase*
Standard stock solution: 0.2 mg/mL of USP Methylparaben RS and 0.03 mg/mL of USP Propylparaben RS in *Mobile phase*
Standard solution: Combine 5 mL of *Standard stock solution* with 5 mL of *Internal standard solution*, and extract three times with 10-mL aliquots of diethyl ether. Filter the combined ether layers through anhydrous sodium sulfate. Evaporate the ether extract to dryness, and dissolve the residue in 50 mL of *Mobile phase*.
Sample solution: Combine 5 mL of the specimen under test with 5 mL of *Internal standard solution*, and extract three times with 10-mL aliquots of diethyl ether. Filter the combined ether layers through anhydrous sodium sulfate. Evaporate the ether extract to dryness, and dissolve the residue in 50 mL of *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Columns
Guard: 4.0-mm × 3-mm; packing L1
Analytical: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 10 µL
Run time: 10 min

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for *p*-hydroxybenzoic acid, methylparaben, ethylparaben, and propylparaben are about 0.58, 1.0, 1.4, and 2.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between *p*-hydroxybenzoic acid and methylparaben, NLT 2.0 between methylparaben and ethylparaben; *System suitability solution*
Tailing factor: NMT 2.0 for the methylparaben and propylparaben peaks, *Standard solution*
Relative standard deviation: NMT 2.0% for the peak response ratio of methylparaben to ethylparaben, NMT 2.0% for the peak response ratio of propylparaben to ethylparaben; *System suitability solution*

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylparaben ($C_8H_8O_3$) in the portion of the sample taken:

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

R_U = peak response ratio of methylparaben to ethylparaben from the *Sample solution*
R_S = peak response ratio of methylparaben to ethylparaben from the *Standard solution*
C_S = concentration of USP Methylparaben RS in the *Standard solution*
C_U = nominal concentration of methylparaben in the *Sample solution*

Calculate the percentage of the labeled amount of propylparaben ($C_{10}H_{12}O_3$) in the portion of the sample taken:

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

R_U = peak response ratio of propylparaben to ethylparaben from the *Sample solution*
R_S = peak response ratio of propylparaben to ethylparaben from the *Standard solution*
C_S = concentration of USP Propylparaben RS in the *Standard solution*
C_U = nominal concentration of propylparaben in the *Sample solution*

Ethylparaben and butylparaben may be determined in a similar manner using appropriate internal standard solutions. However, because the extraction recovery is matrix dependent, the user should verify the suitability of the procedure for their drug product and for different product formulations.

• **THIMEROSAL**

Solution A: Trifluoroacetic acid and water (0.5: 1000)

Mobile phase: Methanol and *Solution A* (60:40)

Standard solution: 25 µg/mL of USP Thimerosal RS in water

Chromatographic system

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

Mode: LC

Detector: UV 222 nm

Column: 2.1-mm × 10-cm; 2-µm packing L1

Autosampler temperature: 4°

Flow rate: 0.35 mL/min

Injection volume: 2.5 µL

System suitability

Sample: *Standard solution*

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of thimerosal ($C_9H_9HgNaO_2S$) in the portion of the sample taken:

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

r_U = peak response of thimerosal from the *Sample solution*
r_S = peak response of thimerosal from the *Standard solution*
C_S = concentration of USP Thimerosal RS in the *Standard solution*
C_U = nominal concentration of thimerosal in the *Sample solution*

POLAROGRAPHIC METHOD

• **PHENYLMERCURIC NITRATE**

Standard stock solution: 0.1 mg/mL of phenylmercuric nitrate in sodium hydroxide solution (1 in 250). Warm, if necessary, to dissolve.

Standard solution: Pipet 10 mL of *Standard stock solution* into a 25-mL volumetric flask, and proceed as directed under *Sample solution* beginning with "add 2 mL of potassium nitrate solution (1 in 100)".

Sample solution: Pipet 10 mL of the specimen under test into a 25-mL volumetric flask, add 2 mL of potassium nitrate solution (1 in 100) and 10 mL of pH 9.2 alkaline borate buffer (see in *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and adjust to a pH of 9.2, if necessary, by the addition of 2 N nitric acid. Add 1.5 mL of freshly prepared gelatin solution (1 in 1000), then add the pH 9.2 alkaline borate buffer to volume.

Analysis: Pipet a portion of the *Sample solution* into the polarographic cell, and deaerate by bubbling nitrogen through the solution for 15 min. Insert the dropping mercury electrode of a suitable polarograph (see *Polarography* ⟨801⟩), and record the polarogram from −0.6 to −1.5 volts versus the saturated calomel electrode.

Calculate the quantity, in µg/mL, of phenylmercuric nitrate ($C_6H_5HgNO_3$) in the portion of the sample taken:

$$\text{Result} = 2.5C[(i_d)_U/(i_d)_S]$$

C = concentration of phenylmercuric nitrate in the *Standard solution* (µg/mL)

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$(i_d)_U$ = diffusion current of the *Sample solution*, as the difference between the residual current and the limiting current
$(i_d)_S$ = diffusion current of the *Standard solution*, as the difference between the residual current and the limiting current

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzyl Alcohol RS
USP Butylparaben RS
USP Chlorobutanol RS
USP Ethylparaben RS
USP Methylparaben RS
USP Phenol RS
USP Propylparaben RS
USP Thimerosal RS

⟨345⟩ ASSAY FOR CITRIC ACID/CITRATE AND PHOSPHATE

INTRODUCTION

The following ion chromatographic general procedure is provided for the determination of citric acid/citrate and phosphate in compendial articles, when specified in the individual monographs. See *Ion Chromatography* ⟨1065⟩ for discussion of the theory and principles of measurements using ion chromatography.

ASSAY

• **PROCEDURE**

Mobile phase: 20 mM sodium hydroxide or potassium hydroxide from an appropriate volume of carbonate-free sodium hydroxide or potassium hydroxide solution of known concentration and water (resistivity NLT 18 megohm-cm). Alternatively, *Mobile phase* can be generated electrolytically using an automatic eluent generator. Protect the *Mobile phase* from atmospheric carbon dioxide.

Standard solution 1 (for the assay of citric acid/citrate only): 20 µg/mL of citrate ($C_6H_5O_7$) in freshly prepared 1 mM sodium hydroxide from USP Citric Acid RS

Standard solution 2 (for the concomitant assay of citrate and phosphate): 20 µg/mL of citrate ($C_6H_5O_7$) and 12 µg/mL of phosphate (PO_4) in freshly prepared 1 mM sodium hydroxide from USP Citric Acid RS and monobasic sodium phosphate

Sample solution (for the assay of citric acid/citrate): Nominally 20 µg/mL of citrate in freshly prepared 1 mM sodium hydroxide, unless otherwise stated in the monograph

Sample solution (for the assay of phosphate): Nominally 12 µg/mL of phosphate in freshly prepared 1 mM sodium hydroxide, unless otherwise stated in the monograph

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity with suppression
Columns
Analytical: 4-mm × 25-cm; 13-µm packing L61
Guard: 4-mm × 5-cm; 13-µm packing L61
Temperatures
Column: 30°
Detector: 35°
Suppressor: 4-mm membrane anionic autosuppressor or a suitable chemical suppression system
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Samples: *Standard solution 1* and/or *Standard solution 2*, as appropriate
[NOTE—The relative retention times for phosphate and citrate are 0.57 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for the citrate and/or phosphate peaks, as appropriate
Relative standard deviation: NMT 1.5% for six replicate injections for the citrate and/or phosphate peaks, as appropriate

Analysis
Samples: *Standard solution 1* and/or *Standard solution 2*, and *Sample solution*
Unless otherwise stated in the monograph, calculate the concentration of citrate or phosphate in the portion of the *Sample solution* taken:

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

r_U = peak response of the citrate or phosphate peak from the *Sample solution*
r_S = peak response of the citrate or phosphate peak from *Standard solution 1* or *Standard solution 2*
C_S = concentration of citrate or phosphate from *Standard solution 1* or *Standard solution 2* (µg/mL)

USP Reference Standards ⟨11⟩
USP Citric Acid RS

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⟨351⟩ ASSAY FOR STEROIDS

The following procedure is applicable for determination of those Pharmacopeial steroids that possess reducing functional groups such as α-ketols.

STANDARD PREPARATION

Dissolve in alcohol a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with alcohol to obtain a solution having a concentration of about 10 µg per mL. Pipet 20 mL of this solution into a glass-stoppered, 50-mL conical flask.

ASSAY PREPARATION

Prepare as directed in the individual monograph.

PROCEDURE

To each of the two flasks containing the *Assay Preparation* and the *Standard Preparation,* respectively, and to a similar flask containing 20.0 mL of alcohol to serve as the blank, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Then to each flask add 2.0 mL of a mixture of alcohol and tetramethylammonium hydroxide TS (9:1), mix, and allow to stand in the dark for 90 minutes. Without delay, concomitantly determine the absorbances of the solutions from the *Assay Preparation* and the *Standard Preparation* at about 525 nm, with a suitable spectrophotometer, against the blank. Calculate the result by the formula given in the individual monograph, in which C is the concentration, in µg per mL, of the Reference Standard in the *Standard Preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay Preparation* and the *Standard Preparation,* respectively.

⟨381⟩ ELASTOMERIC CLOSURES FOR INJECTIONS

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in the general test chapter *Injections and Implanted Drug Products* ⟨1⟩ are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

This chapter applies to closures used for long-term storage of preparations defined in the general test chapter *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging.* Such closures are typically used as part of a vial, bottle, or pre-fill syringe package system.

This chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer; however, it does apply to closures treated with silicone (e.g., Dimethicone, *NF*). When performing the tests in this chapter, it is not required that closures be treated with silicone, although there is no restriction prohibiting the use of siliconized closures.

This chapter also applies to closures coated with other lubricious materials (e.g., materials chemically or mechanically bonded to the closure) that are not intended to, and in fact do not provide, a barrier to the base elastomer. When performing the tests, closures with lubricious nonbarrier coatings are to be tested in their coated state.

The following comments relate solely to closures laminated or coated with materials intended to provide, or in fact function as, a barrier to the base elastomer (e.g., PTFE or lacquer coatings). It is not permissible to use a barrier material in an attempt to change a closure that does not meet compendial requirements to one that does conform. Therefore, all *Physicochemical Tests* apply to the base formula of such closures, as well as to the coated or laminated closure. To obtain *Physicochemical Tests* results, the tests are to be performed on uncoated or nonlaminated closures of the same elastomeric compound, as well as to the laminated or coated closure. The *Functionality Tests* apply to and are to be performed using the laminated or coated elastomeric closure. *Biological Tests* apply to the lamination or coating material, as well as to the base formula. *Biological Tests* may be performed on the laminated or coated closure, or they may be performed on the laminate/coating material and the uncoated or nonlaminated closures of the same elastomeric compound. In the latter case, the results are to be reported separately. The base formula used for physicochemical or biological tests intended to support the compendial compliance of a barrier-coated closure should be similar to the corresponding coated closure in configuration and size.

For all *Nephelometry, Turbidimetry, and Visual Comparison* ⟨855⟩ tests performed on any closure type, it is important to document the closure being tested, including a full description of the elastomer, and any lubrication, coating, laminations, or treatments applied.

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are typically used for aqueous preparations. Type II closures are typically intended for nonaqueous preparations and are those which, having properties

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optimized for special uses, may not meet all requirements listed for Type I closures because of physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements, but still meets the Type II requirements for the test(s), the closure is assigned a final classification of Type II. All elastomeric closures suitable for use with injectable preparations must comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.

It is appropriate to use this chapter when identifying elastomeric closures that might be acceptable for use with injectable preparations on the basis of their biological reactivity, their aqueous extract physicochemical properties, and their functionality.

The following closure evaluation requirements are beyond the scope of this chapter:

- The establishment of closure identification tests and specifications
- The verification of closure–product physicochemical compatibility
- The identification and safety determination of closure leachables found in the packaged product
- The verification of packaged product closure functionality under actual storage and use conditions

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, compatibility testing must be repeated, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and, for aseptic processes, sterilized prior to use in packaging injectable products.

CHARACTERISTICS

Elastomeric closures are translucent or opaque and have no characteristic color, the latter depending on the additives used. They are homogeneous and practically free from flash and adventitious materials (e.g., fibers, foreign particles, and waste rubber.)

IDENTIFICATION

Closures are made of a wide variety of elastomeric materials and optional polymeric coatings. For this reason, it is beyond the scope of this chapter to specify identification tests that encompass all possible closure presentations. However, it is the responsibility of the closure supplier and the injectable product manufacturer (the end user) to verify the closure elastomeric formulation and any coating or laminate materials used according to suitable identification tests. Examples of some of the analytical test methodologies that may be used include specific gravity, percentage of ash analysis, sulfur content determination, FTIR-ATR test, thin-layer chromatography of an extract, UV absorption spectrophotometry of an extract, or IR absorption spectrophotometry of a pyrolysate.

TEST PROCEDURES

Elastomeric closures shall conform to biological, physicochemical, and functionality requirements both as they are shipped by the closure supplier to the injectable product manufacturer (the end user), and in their final ready-to-use state by the end user.

For those elastomeric closures processed by the supplier prior to distribution to the end user, the supplier shall demonstrate compendial conformance of closures exposed to such processing and/or sterilization steps. Similarly, if elastomeric closures received by the end user are subsequently processed or sterilized, the end user is responsible for demonstrating the continued conformance of closures to compendial requirements subsequent to such processing and/or sterilization conditions (i.e., in their ready-to-use state). This is especially important if closures shall be exposed to processes or conditions that may significantly impact the biological, physicochemical, or functionality characteristics of the closure (e.g., gamma irradiation).

For closures that are normally lubricated with silicone prior to use, it is permissible to perform physicochemical testing on nonlubricated closures, in order to avoid potential method interference and/or difficulties in interpreting test results. For closures supplied with other lubricious nonbarrier coatings, all tests are to be performed using the coated closure.

For closures coated or laminated with coatings intended to provide a barrier function (e.g., PTFE or lacquer coatings), physicochemical compendial tests apply to the uncoated base elastomer, as well as to the coated closure. In this case, suppliers are responsible for demonstrating physicochemical compendial compliance of the coated closure, as well as of the uncoated closure, processed or treated in a manner simulating conditions typically followed by the supplier for such coated closures prior to shipment to the end user. The uncoated closure subject to physicochemical tests should be similar to the corresponding coated closure in size and configuration. End users of coated closures are also responsible for demonstrating the continued physicochemical compendial conformance of the coated closure, processed or treated in a manner simulating conditions typically employed by the end user prior to use.

In all cases, it is appropriate to document all conditions of closure processing, pretreatment, sterilization, or lubrication when reporting test results.

Table 1 summarizes the testing requirements of closures, and the responsibilities of the supplier and the end user.

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Table 1

Closure Types (As Supplied or Used)	Test Requirements		
	Physicochemical Tests	**Functionality Tests**	**Biological Tests**
Closure with or without Silicone Coating	• Tests are to be performed.	• Tests are to be performed.	• Tests are to be performed.
	• Silicone use is optional.	• Silicone use is optional.	• Silicone use is optional.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with Lubricious Coating (Nonbarrier Material; Not Silicone)	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with Barrier Coating	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user		OR:
	AND:		• Tests are to be performed on uncoated closures (base formula) and the laminate/coating material (report results separately).
	• Tests are to be performed on uncoated closures (base formula).	• Responsibility: supplier and end user	
	• Responsibility: supplier		• Responsibility: supplier and end user

BIOLOGICAL TESTS

Two stages of testing are indicated. The first stage is the performance of an in vitro test procedure as described in general test chapter *Biological Reactivity Tests, In Vitro* ⟨87⟩. Materials that do not meet the requirements of the in vitro test are subjected to the second stage of testing, which is the performance of the in vivo tests, according to the procedures set forth in the general test chapter *Biological Reactivity Tests, In Vivo* ⟨88⟩, *Systemic Injection Test* and *Intracutaneous Test*. Materials that meet the requirements of the in vitro test are not required to undergo in vivo testing.

Type I and Type II closures must both conform to the requirements of either the in vitro or the in vivo biological reactivity tests. [NOTE—Also see the general information chapter *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* ⟨1031⟩.]

Change to read:

PHYSICOCHEMICAL TESTS

Preparation of Solution S

Place whole, uncut closures corresponding to a surface area of 100 ± 10 cm^2 into a suitable glass container. Cover the closures with 200 mL of *Purified Water* or *Water for Injection*. If it is not possible to achieve the prescribed closure surface area (100 ± 10 cm^2) using uncut closures, select the number of closures that will most closely approximate 100 cm^2, and adjust the volume of water used to the equivalent of 2 mL per each 1 cm^2 of actual closure surface area used. Boil for 5 minutes, and rinse five times with cold *Purified Water* or *Water for Injection*.

Place the washed closures into a Type I glass wide-necked flask (see *Containers—Glass* ⟨660⟩), add the same quantity of *Purified Water* or *Water for Injection* initially added to the closures, and weigh. Cover the mouth of the flask with a Type I glass beaker. Heat in an autoclave so that a temperature of 121 ± 2° is reached within 20 to 30 minutes, and maintain this temperature for 30 minutes. Cool to room temperature over a period of about 30 minutes. Add *Purified Water* or *Water for Injection* to bring it up to the original mass. Shake, and immediately decant and collect the solution.

[NOTE—This solution must be shaken before being used in each of the tests.]

Preparation of Blank

Prepare a blank solution similarly, using 200 mL of *Purified Water* or *Water for Injection* omitting the closures.

APPEARANCE OF SOLUTION (TURBIDITY/OPALESCENCE AND COLOR)

Determination of turbidity (opalescence): [NOTE—The determination of turbidity may be performed by visual comparison (*Procedure A*), or instrumentally using a suitable ratio turbidimeter (*Procedure B*). For a discussion of turbidimetry, see *Nephelometry, Turbidimetry, and Visual Comparison* ⟨855⟩. Instrumental assessment of clarity provides a more discriminatory test that does not depend on the visual acuity of the analyst.]

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water and dilute with water to 100.0 mL. Allow to stand for 4 to 6 hours.

Hexamethylenetetramine solution: Dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water in a 100-mL glass-stoppered flask.

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Opalescence stock suspension: Add 25.0 mL of *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the flask. Mix, and allow to stand for 24 hours. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard suspension: Prepare a suspension by diluting 15.0 mL of the *Opalescence stock suspension* with water to 1000.0 mL. *Opalescence standard suspension* is stable for about 24 hours after preparation.

Reference suspensions: Prepare according to *Table 2*. Mix and shake before use. [NOTE—Stabilized formazin suspensions that can be used to prepare stable, diluted turbidity standards are available commercially and may be used after comparison with the standards prepared as described.]

Table 2

	Reference Suspension A	Reference Suspension B	Reference Suspension C	Reference Suspension D
Standard of Opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water	95.0 mL	90.0 mL	70.0 mL	50.0 mL
Nephelometric Turbidity Units	3 NTU	6 NTU	18 NTU	30 NTU

Procedure A: visual comparison: Use identical test tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm. Fill one tube to a depth of 40 mm with *Solution S,* one tube to the same depth with water, and four others to the same depth with *Reference suspensions A, B, C,* and *D*. Compare the solutions in diffuse daylight 5 minutes after preparation of the *Reference suspensions,* viewing vertically against a black background. The light conditions shall be such that *Reference suspension A* can be readily distinguished from water and that *Reference suspension B* can be readily distinguished from *Reference suspension A*.

REQUIREMENT: *Solution S* is not more opalescent than *Reference suspension B* for Type I closures, and not more opalescent than *Reference suspension C* for Type II closures. *Solution S* is considered clear if its clarity is the same as that of water when examined as described above, or if its opalescence is not more pronounced than that of *Reference suspension A* (refer to *Table 3*).

Procedure B: instrumental comparison: Measure the turbidity of the *Reference suspensions* in a suitable calibrated turbidimeter (see ⟨855⟩). The blank should be run and the results corrected for the blank. *Reference suspensions A, B, C,* and *D* represent 3, 6, 18, and 30 Nephelometric Turbidity Units (NTU), respectively. Measure the turbidity of *Solution S* using the calibrated turbidimeter.

REQUIREMENT: The turbidity of *Solution S* is not greater than that for *Reference suspension B* (6 NTU FTU) for Type I closures, and is not greater than that for *Reference suspension C* (18 NTU FTU) for Type II closures (refer to *Table 3*).

Table 3

Comparison Method		
Opalescence Requirements	Procedure A (Visual)	Procedure B (Instrumental)
Type I closures	No more opalescent than Suspension B	No more than 6 NTU
Type II closures	No more opalescent than Suspension C	No more than 18 NTU

Determination of color

Color standard: Prepare a solution by diluting 3.0 mL of *Color and Achromicity* ⟨631⟩, *Color Determination and Standards, Matching Fluids, Matching Fluid O* with 97.0 mL of diluted hydrochloric acid.

procedure: Use identical tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm. Fill one tube to a depth of 40 mm with *Solution S,* and the second with the *Color standard.* Compare the liquids in diffuse daylight, viewing vertically against a white background.

requirement: Solution S is not more intensely colored than the *Color standard.*

Acidity or Alkalinity

BROMOTHYMOL BLUE SOLUTION

Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of alcohol. Dilute with water to 100 mL.

PROCEDURE

To 20 mL of *Solution S* add 0.1 mL of *Bromothymol blue solution.* If the solution is yellow, titrate with 0.01 N sodium hydroxide until a blue endpoint is reached. If the solution is blue, titrate with 0.01 N hydrochloric acid until a yellow endpoint is reached. If the solution is green, it is neutral and no titration is required.

BLANK CORRECTION

Test 20 mL of *Blank* similarly. Correct the results obtained for *Solution S* by subtracting or adding the volume of titrant required for the *Blank,* as appropriate. (*Titrimetry* ⟨541⟩.)

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REQUIREMENT

Not more than 0.3 mL of 0.01 N sodium hydroxide produces a blue color, or not more than 0.8 mL of 0.01 N hydrochloric acid produces a yellow color, or no titration is required.

Absorbance

PROCEDURE

[NOTE—Perform this test within 5 hours of preparing *Solution S*.] Pass *Solution S* through a 0.45-µm pore size filter, discarding the first few mL of filtrate. Measure the absorbance of the filtrate at wavelengths between 220 and 360 nm in a 1-cm cell using the blank in a matched cell in the reference beam. If dilution of the filtrate is required before measurement of the absorbance, correct the test results for the dilution.

REQUIREMENT

The absorbances at these wavelengths do not exceed 0.2 for Type I closures or 4.0 for Type II closures.

Reducing Substances

PROCEDURE

[NOTE—Perform this test within 4 hours of preparing *Solution S*.] To 20.0 mL of *Solution S* add 1 mL of diluted sulfuric acid and 20.0 mL of 0.002 M potassium permanganate. Boil for 3 minutes. Cool, add 1 g of potassium iodide, and titrate immediately with 0.01 M sodium thiosulfate, using 0.25 mL of starch solution TS as the indicator. Perform a titration using 20.0 mL of blank and note the difference in volume of 0.01 M sodium thiosulfate required.

REQUIREMENT

The difference between the titration volumes is not greater than 3.0 mL for Type I closures and not greater than 7.0 mL for Type II closures.

Heavy Metals

▲LEAD NITRATE STOCK SOLUTION

Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

STANDARD LEAD SOLUTION

On the day of use, dilute 10.0 mL of *Lead nitrate stock solution* with water to 100.0 mL.

pH 3.5 ACETATE BUFFER

Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

STANDARD PREPARATION

Into a 50-mL color-comparison tube pipet 2 mL of *Standard lead solution* (20 µg of Pb) and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH of between 3.0 and 4.0, dilute with water to 40 mL, and mix.

TEST PREPARATION

Into a 50-mL color-comparison tube ▲pipet 10.0 mL of *Solution S* and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH of between 3.0 and 4.0, dilute with water to 40 mL, and mix.▲ (ERR 1-Sep-2018)

PROCEDURE

To each of the two tubes containing the *Standard preparation* and the *Test preparation*, add 2 mL of *pH 3.5 acetate buffer*, then add 1.2 mL of thioacetamide–glycerin base TS. [NOTE—In countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.] Dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation*.▲ (RB 1-Jan-2018)

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Requirement

Solution S contains not more than 2 ppm of heavy metals as lead.

Extractable Zinc

Test Solution

Prepare a *Test solution* by diluting 10.0 mL of *Solution S* to 100 mL with 0.1 N hydrochloric acid. Prepare a test blank similarly, using the *Blank* for *Solution S*.

Zinc Standard Solution

Prepare a solution (10 ppm Zn) by dissolving zinc sulfate in 0.1 N hydrochloric acid.

Reference Solutions

Prepare not fewer than three *Reference solutions* by diluting the *Zinc standard solution* with 0.1 N hydrochloric acid. The concentrations of zinc in these *Reference solutions* are to span the expected limit of the *Test solution*.

Procedure

Use a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a zinc hollow-cathode lamp and an air–acetylene flame. An alternative procedure such as an appropriately validated inductively coupled plasma analysis (ICP) may be used.

Test each of the *Reference solutions* at the zinc emission line of 213.9 nm at least three times. Record the steady readings. Rinse the apparatus with the test blank solution each time, to ensure that the reading returns to initial blank value. Prepare a calibration curve from the mean of the readings obtained for each *Reference solution*. Record the absorbance of the *Test solution*. Determine the ppm zinc concentration of the *Test solution* using the calibration curve.

Requirement

Solution S contains not more than 5 ppm of extractable zinc.

Ammonium

Alkaline Potassium Tetraiodomercurate Solution

Prepare a 100-mL solution containing 11 g of potassium iodide and 15 g of mercuric iodide in water. Immediately before use, mix 1 volume of this solution with an equal volume of a 250 g per L solution of sodium hydroxide.

Test Solution

Dilute 5 mL of *Solution S* to 14 mL with water. Make alkaline if necessary by adding 1 N sodium hydroxide, and dilute with water to 15 mL. Add 0.3 mL of *Alkaline potassium tetraiodomercurate solution*, and close the container.

Ammonium Standard Solution

Prepare a solution of ammonium chloride in water (1 ppm NH_4). Mix 10 mL of the 1 ppm ammonium chloride solution with 5 mL water and 0.3 mL of *Alkaline potassium tetraiodomercurate solution*. Close the container.

Requirement

After 5 minutes, any yellow color in the *Test solution* is no darker than the *Ammonium standard solution* (no more than 2 ppm of NH_4 in *Solution S*).

Volatile Sulfides

Procedure

Place closures, cut if necessary, with a total surface area of 20 ± 2 cm^2 in a 100-mL flask, and add 50 mL of a 20 g per L citric acid solution. In the same manner and at the same time, prepare a control solution in a separate 100-mL flask by dissolving 0.154 mg of sodium sulfide in 50 mL of a 20 g per L citric acid solution. Place a piece of lead acetate paper over the mouth of each flask, and hold the paper in position by placing over it an inverted weighing bottle. Heat the flasks in an autoclave at 121 ± 2° for 30 minutes.

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REQUIREMENT

Any black stain on the paper produced by the test solution is not more intense than that produced by the control substance.

FUNCTIONALITY TESTS

[NOTE—Samples treated as described for preparation of *Solution S* and air-dried should be used for *Functionality Tests* of *Penetrability*, *Fragmentation*, and *Self-Sealing Capacity*. *Functionality Tests* are performed on closures intended to be pierced by a hypodermic needle. The *Self-Sealing Capacity* test is required only for closures intended for multiple-dose containers. The needle specified for each test is a lubricated long bevel (bevel angle 12 ± 2°) hypodermic needle[1] .]

Penetrability

PROCEDURE

Fill 10 suitable vials to the nominal volume with water, fit the closures to be examined, and secure with a cap. Using a new hypodermic needle as described above for each closure, pierce the closure with the needle perpendicular to the surface.

REQUIREMENT

The force for piercing is no greater than 10 N (1 kgf) for each closure, determined with an accuracy of ±0.25 N (25 gf).

Fragmentation

CLOSURES FOR LIQUID PREPARATIONS

Fill 12 clean vials with water to 4 mL less than the nominal capacity. Fit the closures to be examined, secure with a cap, and allow to stand for 16 hours.

CLOSURES FOR DRY PREPARATIONS

Fit closures to be examined into 12 clean vials, and secure each with a cap.

PROCEDURE

Using a hypodermic needle as described above fitted to a clean syringe, inject into each vial 1 mL of water while removing 1 mL of air. Repeat this procedure four times for each closure, piercing each time at a different site. Use a new needle for each closure, checking that it is not blunted during the test. Filter the total volume of liquid in all the vials through a single filter with a nominal pore size no greater than 0.5 µm. Count the rubber fragments on the surface of the filter visible to the naked eye.

REQUIREMENT

There are no more than five fragments visible. This limit is based on the assumption that fragments with a diameter >50 µm are visible to the naked eye. In case of doubt or dispute, the particles are examined microscopically to verify their nature and size.

Self-Sealing Capacity

PROCEDURE

Fill 10 suitable vials with water to the nominal volume. Fit the closures that are to be examined, and cap. Using a new hypodermic needle as described above for each closure, pierce each closure 10 times, piercing each time at a different site. Immerse the 10 vials in a solution of 0.1% (1 g per L) methylene blue, and reduce the external pressure by 27 kPa for 10 minutes. Restore to atmospheric pressure, and leave the vials immersed for 30 minutes. Rinse the outside of the vials.

REQUIREMENT

None of the vials contain any trace of blue solution.

[1] Refer to ISO 7864, Sterile hypodermic needles for single use with an external diameter of 0.8 mm (21 gauge).

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⟨391⟩ EPINEPHRINE ASSAY

ASSAY

Ferro-citrate solution: On the day needed, dissolve 1.5 g of ferrous sulfate in 200 mL of water to which have been added 1.0 mL of dilute hydrochloric acid (1 in 12) and 1.0 g of sodium bisulfite. Dissolve 500 mg of sodium citrate in 10 mL of this solution, and mix.

Buffer solution: In a 50-mL volumetric flask mix 4.2 g of sodium bicarbonate, 5.0 g of potassium bicarbonate, and 18 mL of water (not all of the solids will dissolve at this stage). To another 18 mL of water add 3.75 g of aminoacetic acid and 1.7 mL of 6 N ammonium hydroxide, mix to dissolve, and transfer this solution to the 50-mL volumetric flask containing the other mixture. Dilute with water to volume, and mix until solution is complete.

Standard preparation: Transfer about 18 mg of USP Epinephrine Bitartrate RS, accurately weighed, to a 100-mL volumetric flask with the aid of 20 mL of sodium bisulfite solution (1 in 50), dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with sodium bisulfite solution (1 in 500) to volume, and mix. [NOTE—Make the final dilution when the assay is carried out.] The concentration of USP Epinephrine Bitartrate RS in the *Standard preparation* is about 18 µg per mL.

Assay preparation: Transfer to a 50-mL volumetric flask an accurately measured volume of the Injection under assay, equivalent to about 500 µg of epinephrine, dilute with sodium bisulfite solution (1 in 500) to volume, if necessary, and mix. [NOTE—The final concentration of sodium bisulfite is in the range of 1 to 3 mg per mL, any bisulfite present in the Injection under assay being taken into consideration.]

Procedure: Into three 50-mL glass-stoppered conical flasks transfer, separately, 20.0-mL aliquots of the *Standard preparation*, the *Assay preparation*, and sodium bisulfite solution (1 in 500) to provide the blank. To each flask add 200 µL of *Ferro-citrate solution* and 2.0 mL of *Buffer solution*, mix, and allow the solutions to stand for 30 minutes. Determine the absorbances of the solutions in 5-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using the blank to set the instrument.

Calculate the quantity, in mg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula, in which 183.21 and 333.30 are the molecular weights of epinephrine and epinephrine bitartrate, respectively:

$$\text{Result} = (183.21/333.30)(0.05C/V)(A_U/A_S)$$

C = concentration of USP Epinephrine Bitartrate RS in the *Standard preparation* (µg/mL)
V = volume of Injection taken (mL)
A_U = absorbance of the *Assay preparation*
A_S = absorbance of the *Standard preparation*

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Epinephrine Bitartrate RS

⟨401⟩ FATS AND FIXED OILS

The following definitions and general procedures apply to fats, fixed oils, waxes, resins, balsalms, and similar substances.

Preparation of Specimen

If a specimen of oil shows turbidity owing to separated stearin, warm the container in a water bath at 50° until the oil is clear, or if the oil does not become clear on warming, pass it through dry filter paper in a funnel contained in a hot-water jacket. Mix thoroughly, and weigh at one time as many portions as are needed for the various determinations, preferably using a bottle that has a pipet dropper or a weighing buret. If the specimen is solid at room temperature, keep it melted until the desired portions of specimen are withdrawn.

PROCEDURES

- **SPECIFIC GRAVITY:** Determine the specific gravity of a fat or oil as directed in *Specific Gravity* ⟨841⟩.
- **MELTING TEMPERATURE:** Determine the melting temperature as directed for *Class II* substances (see *Melting Range or Temperature* ⟨741⟩).
- **ACID VALUE (FREE FATTY ACIDS)**

The acidity of fats and fixed oils in this pharmacopeia may be expressed as the number of mL of 0.1 N alkali required to neutralize the free acids in 10.0 g of substance. Acidity frequently is expressed as the Acid Value, which is the number of mg of potassium hydroxide required to neutralize the free acids in 1.0 g of the substance. Unless directed otherwise in the individual monograph, use *Method I*.

Method I

Procedure: Unless directed otherwise, dissolve about 10.0 g of the substance, accurately weighed, in 50 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide, unless otherwise specified) contained in a flask. If the test specimen does not dissolve in the cold solvent, connect the flask with a suitable condenser and warm slowly, with frequent shaking, until the specimen dissolves. Add 1 mL of phenolphthalein TS, and titrate with 0.1 N potassium hydroxide VS or 0.1 N sodium hydroxide VS until the solution remains faintly pink after shaking for 30 s. Calculate either the Acid Value or the volume of 0.1 N alkali required to neutralize 10.0 g of specimen (free fatty acids), whichever is appropriate. Calculate the Acid Value:

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$$\text{Result} = (M_r \times V) \times (N/W)$$

M_r = molecular weight of potassium hydroxide, 56.11
V = volume (mL)
N = normality of the potassium hydroxide solution or the sodium hydroxide solution
W = weight of the sample taken (g)

If the volume of 0.1 N potassium hydroxide VS or 0.1 N sodium hydroxide VS required for the titration is less than 2 mL, a more dilute titrant may be used, or the sample size may be adjusted accordingly. The results may be expressed in terms of the volume of titrant used or in terms of the equivalent volume of 0.1 N potassium hydroxide or 0.1 N sodium hydroxide.

If the oil has been saturated with carbon dioxide for the purpose of preservation, gently reflux the alcohol–ether solution for 10 min before titration. The oil may be freed from carbon dioxide also by exposing it in a shallow dish in a vacuum desiccator for 24 h before weighing the test specimens.

Method II

Procedure: Prepare 125 mL of a solvent mixture consisting of equal volumes of isopropyl alcohol and toluene. Before use, add 2 mL of a 1% solution of phenolphthalein in isopropyl alcohol to the 125-mL mixture, and neutralize with alkali to a faint but permanent pink color. Accurately weigh the appropriate amount of well-mixed liquid sample indicated in *Table 1*, and dissolve it in the neutralized solvent mixture. If the test specimen does not dissolve in the cold solvent, connect the flask with a suitable condenser and warm slowly, with frequent shaking, until the specimen dissolves. Shake vigorously while titrating with 0.1 N potassium hydroxide VS or 0.1 N sodium hydroxide VS to the first permanent pink of the same intensity as that of the neutralized solvent before mixing with the sample. Calculate the Acid Value as indicated in *Method I*.

Table 1

Acid Value	Sample Weight (g)
0–1	20
1–4	10
4–15	2.5
15–74.9	0.5
≥75.0	0.1

• **ESTER VALUE**

The Ester Value is the number of mg of potassium hydroxide required to saponify the esters in 1.0 g of the substance. If the Saponification Value and the Acid Value have been determined, the difference between these two represents the Ester Value, i.e., Ester Value = Saponification Value – Acid Value.

Procedure: Place 1.5–2 g of the substance, accurately weighed, in a tared, 250-mL flask, add 20–30 mL of neutralized alcohol, and shake. Add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS until the free acid is neutralized. Add 25.0 mL of 0.5 N alcoholic potassium hydroxide VS, and proceed as directed in *Saponification Value*, beginning with "Heat the flask" and omitting the further addition of phenolphthalein TS. Calculate the Ester Value:

$$\text{Result} = [M_r \times (V_B - V_T) \times N]/W$$

M_r = molecular weight of potassium hydroxide, 56.11
V_B = volume of 0.5 N hydrochloric acid consumed in the blank test (mL)
V_T = volume of 0.5 N hydrochloric acid consumed in the actual test (mL)
N = exact normality of the hydrochloric acid
W = weight of the substance taken for the test (g)

• **HYDROXYL VALUE**

The Hydroxyl Value is the number of mg of potassium hydroxide equivalent to the hydroxyl content of 1.0 g of the substance.

Pyridine–acetic anhydride reagent: Just before use, mix 3 volumes of freshly opened or freshly distilled pyridine with 1 volume of freshly opened or freshly distilled acetic anhydride.

Procedure: Transfer a quantity of the substance, determined by reference to *Table 2* and accurately weighed, to a glass-stoppered, 250-mL conical flask, and add 5.0 mL of *Pyridine–acetic anhydride reagent*. Transfer 5.0 mL of *Pyridine–acetic anhydride reagent* to a second glass-stoppered, 250-mL conical flask to provide the reagent blank. Fit both flasks with suitable glass-jointed reflux condensers, heat on a steam bath for 1 h, add 10 mL of water through each condenser, and heat on the steam bath for 10 min more. Cool, and to each add 25 mL of butyl alcohol, previously neutralized to phenolphthalein TS with 0.5 N alcoholic potassium hydroxide, by pouring 15 mL through each condenser and, after removing the condensers, washing the sides of both flasks with the remaining 10-mL portions. To each flask add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS, recording the volume, in mL, consumed by the residual acid in the test solution as *T* and that consumed by the blank as *B*. In a 125-mL conical flask, mix about 10 g of the substance, accurately weighed, with 10 mL of freshly distilled pyridine, previously neutralized to phenolphthalein TS, add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS, recording the volume, in mL, consumed by the free acid in the test specimen as *A*. Calculate the Hydroxyl Value:

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$$\text{Result} = [(M_r \times N)/W] \times \{B + [(W \times A)/C] - T\}$$

M_r = molecular weight of potassium hydroxide, 56.11
N = exact normality of the alcoholic potassium hydroxide
W = weight of the substances taken for the acetylation (g)
C = weight of the substances taken for the free acid determination (g)

If the Acid Value for the test substance is known, calculate the Hydroxyl Value:

$$\text{Result} = [(M_r \times N)/W] \times (B - T) + \text{Acid Value}$$

M_r = molecular weight of potassium hydroxide, 56.11
N = exact normality of the alcoholic potassium hydroxide
W = weight of the substance taken for the acetylation (g)

Table 2

Hydroxyl Value Range	Weight of Test Specimen (g)
0–20	10
20–50	5
50–100	3
100–150	2
150–200	1.5
200–250	1.25
250–300	1.0
300–350	0.75

• **IODINE VALUE**

The Iodine Value represents the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the substance. Unless otherwise specified in the individual monograph, determine the Iodine Value by *Method I*.

Method I (Hanus method)

Procedure: Transfer an accurately weighed quantity of sample, as determined from *Table 3*, into a 250-mL iodine flask, dissolve it in 10 mL of chloroform, add 25.0 mL of iodobromide TS, insert the stopper in the vessel securely, and allow it to stand for 30 min protected from light, with occasional shaking. Then add, in the order named, 30 mL of potassium iodide TS and 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, shaking thoroughly after each addition of thiosulfate. When the iodine color becomes quite pale, add 3 mL of starch TS, and continue the titration with 0.1 N sodium thiosulfate VS until the blue color is discharged. Perform a blank test at the same time with the same quantities of the same reagents and in the same manner (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Calculate the Iodine Value:

$$\text{Result} = [A_r \times (V_B - V_S) \times N]/(10 \times W)$$

A_r = atomic weight of iodine, 126.90
V_B = volume of 0.1 N sodium thiosulfate VS consumed by the blank test (mL)
V_S = volume of 0.1 N sodium thiosulfate VS consumed by the actual test (mL)
N = exact normality of the sodium thiosulfate VS
W = weight of the substance taken for the test (g)

[NOTE—If more than half of the iodobromide TS is absorbed by the portion of the substance taken, repeat the determination, using a smaller portion of the substance under examination.]

Table 3. Sample Weights

Iodine Value Expected	Weight (g) ±0.1
<5	3.0
5–20	1.0
21–50	0.4
51–100	0.2
101–150	0.13
151–200	0.1

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Method II

Potassium iodide solution: Dissolve 10.0 g of potassium iodide in water to make 100 mL. Store in light-resistant containers.

Starch indicator solution: Mix 1 g of soluble starch with sufficient cold water to make a thin paste. Add, while stirring, to 100 mL of boiling water. Mix, and cool. Use only the clear solution.

Procedure: Melt the sample, if it is not already liquid. [NOTE—The temperature during melting should exceed the melting point of the sample by NMT 10°.] Pass through two pieces of filter paper to remove any solid impurities and the last traces of moisture. The filtration may be performed in an air oven at 100° but should be completed within 5 min ± 30 s. The sample must be absolutely dry. All glassware must be absolutely clean and completely dry. After filtration, allow the filtered sample to achieve a temperature of 68°–71 ± 1° before weighing the sample. Once the sample has achieved a temperature of 68°–71 ± 1°, immediately weigh the sample into a 500-mL iodine flask, using the weights and weighing accuracy noted in the accompanying table. [NOTE—The weight of the substance must be such that there will be an excess of iodochloride TS of 50%–60% of the amount added, that is, 100%–150% of the amount absorbed.] Add 15 mL of a fresh mixture of cyclohexane and glacial acetic acid (1:1), and swirl to dissolve the sample. Add 25.0 mL of iodochloride TS, insert the stopper securely in the flask, and swirl to mix. Allow it to stand at 25 ± 5°, protected from light, with occasional shaking, for 1.0 or 2.0 h, depending on the Iodine Value (IV) of the sample: IV < 150, 1.0 h; IV ≥ 150, 2.0 h. Then, within 3 min after the indicated reaction time, add, in the order named, 20 mL of *Potassium iodide solution* and 150 mL of recently boiled and cooled water, and mix. Within 30 min, titrate the liberated iodine with 0.1 N sodium thiosulfate VS, while stirring by mechanical means after each addition of thiosulfate. When the yellow iodine color has almost disappeared, add 1–2 mL of *Starch indicator solution*, and continue the titration with 0.1 N sodium thiosulfate VS until the blue color is discharged. Perform a blank test at the same time with the same quantities of the same reagents and in the same manner (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Calculate the Iodine Value as indicated in *Method I*.

• **PEROXIDE VALUE**

The Peroxide Value is the number that expresses, in milliequivalents of active oxygen, the quantity of peroxide contained in 1000 g of the substance. [NOTE—This test must be performed promptly after sampling to avoid oxidation of the test specimen.]

Procedure: Unless otherwise directed, place about 5 g of the substance, accurately weighed, in a 250-mL conical flask fitted with a ground-glass stopper. Add 30 mL of a mixture of glacial acetic acid and chloroform (3:2), shake to dissolve, and add 0.5 mL of saturated potassium iodide solution. Shake for exactly 1 min, and add 30 mL of water. Titrate with 0.01 N sodium thiosulfate VS, adding the titrant slowly with continuous shaking, until the yellow color is almost discharged. Add 5 mL of starch TS, and continue the titration, shaking vigorously, until the blue color is discharged. Perform a blank determination under the same conditions. [NOTE—The volume of titrant used in the blank determination must not exceed 0.1 mL.]

Calculate the Peroxide Value:

$$\text{Result} = [1000\ (V_T - V_B) \times N]/W$$

V_T = volume of 0.01 N sodium thiosulfate consumed in the actual test (mL)
V_B = volume of 0.01 N sodium thiosulfate consumed in the blank test (mL)
N = exact normality of the sodium thiosulfate solution
W = weight of the substance taken for the test (g)

• **SAPONIFICATION VALUE**

The Saponification Value is the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters contained in 1.0 g of the substance.

Procedure: Place 1.5–2 g of the substance in a tared, 250-mL flask, weigh accurately, and add to it 25.0 mL of 0.5 N alcoholic potassium hydroxide. Heat the flask on a steam bath, under a suitable condenser to maintain reflux for 30 min, frequently rotating the contents. [NOTE—Reflux time can be up to 90 min to ensure complete saponification, depending on the type of ester to be tested.] Then add 1 mL of phenolphthalein TS, and titrate the excess potassium hydroxide with 0.5 N hydrochloric acid VS. Perform a blank determination under the same conditions (see *Titrimetry* ⟨541⟩, *Residual Titrations*). The titration also can be carried out potentiometrically. Calculate the Saponification Value:

$$\text{Result} = [M_r \times (V_B - V_T) \times N]/W$$

M_r = molecular weight of potassium hydroxide, 56.11
V_B = volume of 0.5 N hydrochloric acid consumed in the blank test (mL)
V_T = volume of 0.5 N hydrochloric acid consumed in the actual test (mL)
N = exact normality of the hydrochloric acid
W = weight of the substance taken for the test (g)

If the oil has been saturated with carbon dioxide for the purpose of preservation, expose it in a shallow dish in a vacuum desiccator for 24 h before weighing the test specimens.

• **UNSAPONIFIABLE MATTER**

The term "unsaponifiable matter" in oils or fats refers to those substances that are not saponifiable by alkali hydroxides but are soluble in the ordinary fat solvents, and to products of saponification that are soluble in such solvents.

Procedure: Transfer about 5.0 g of the oil or fat, accurately weighed, to a 250-mL conical flask, add 50 mL of an alcoholic potassium hydroxide solution prepared by dissolving 12 g of potassium hydroxide in 10 mL of water and diluting this solution with alcohol to 100 mL, and heat the flask on a steam bath under a suitable condenser to maintain reflux for 1 h,

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swirling frequently. Cool to a temperature below 25°, and transfer the contents of the flask to a separator with a polytetrafluoroethylene stopcock, rinsing the flask with two 50-mL portions of water that are added to the separator (do not use grease on stopcock). Extract with three 100-mL portions of ether, combining the ether extracts in another separator containing 40 mL of water. Gently rotate or shake the separator for a few minutes. [NOTE—Violent agitation may result in the formation of a difficult-to-separate emulsion.] Allow the mixture to separate, and discard the lower aqueous phase. Wash the ether extract with two additional 40-mL portions of water, and discard the lower aqueous phase. Wash the ether extract successively with a 40-mL portion of potassium hydroxide solution (3 in 100) and a 40-mL portion of water. Repeat this potassium hydroxide solution–water wash sequence three times. Wash the ether extract with 40-mL portions of water until the last washing is not reddened by the addition of 2 drops of phenolphthalein TS. Transfer the ether extract to a tared flask, and rinse the separator with 10 mL of ether, adding the rinsings to the flask. Evaporate the ether on a steam bath, and add 6 mL of acetone to the residue. Remove the acetone in a current of air, and dry the residue at 105° until successive weighings differ by NMT 1 mg. Calculate the percentage of unsaponifiable matter in the portion of oil or fat taken:

$$\text{Result} = 100 \times (W_R/W_S)$$

W_R = weight of the residue (g)
W_S = weight of the oil or fat taken for the test (g)

Dissolve the residue in 20 mL of alcohol, previously neutralized to the phenolphthalein endpoint, add phenolphthalein TS, and titrate with 0.1 N alcoholic sodium hydroxide VS to the first appearance of a faint pink color that persists for NLT 30 s. If the volume of 0.1 N alcoholic sodium hydroxide required is greater than 0.2 mL, the separation of the layers was incomplete; the residue weighed cannot be considered "unsaponifiable matter," and the test must be repeated.

• **SOLIDIFICATION TEMPERATURE OF FATTY ACIDS**

Preparation of the fatty acids: Heat 75 mL of glycerin–potassium hydroxide solution (made by dissolving 25 g of potassium hydroxide in 100 mL of glycerin) in an 800-mL beaker to 150°, and add 50 mL of the clarified fat, melted if necessary. Heat the mixture for 15 min with frequent stirring, but do not allow the temperature to rise above 150°. Saponification is complete when the mixture is homogeneous and no particles cling to the beaker at the meniscus. Pour the contents of the beaker into 500 mL of nearly boiling water in an 800-mL beaker or casserole, add slowly 50 mL of dilute sulfuric acid [made by adding water and sulfuric acid (3:1)], and heat the solution, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the acids with boiling water until free from sulfuric acid, collect them in a small beaker, place on a steam bath until the water has settled and the fatty acids are clear, filter into a dry beaker while hot, and dry at 105° for 20 min. Place the warm fatty acids in a suitable container, and cool in an ice bath until they congeal.

Test for complete saponification: Place 3 mL of the dry acids in a test tube, and add 15 mL of alcohol. Heat the solution to boiling, and add an equal volume of 6 N ammonium hydroxide. A clear solution results.

Procedure: Using an apparatus similar to the *Congealing Temperature Apparatus* specified therein, proceed as directed for *Procedure* in *Congealing Temperature* ⟨651⟩, reading "solidification temperature" for "congealing point" (the terms are synonymous). The average of NLT four consecutive readings of the highest point to which the temperature rises is the solidification temperature of the fatty acids.

• **FATTY ACID COMPOSITION**

Standard solution: Prepare an ester mixture of known composition containing the esters required in the individual monograph. This *Standard solution* may contain other components. [NOTE—Ester mixtures are available commercially from Nu-Chek-Prep, Inc., PO Box 295, Elysian, MN 56028. Typical Nu-Chek-Prep ester mixtures useful in this test include Nu-Chek 17A and Nu-Chek 19A.] Nu-Chek mixture 17A has the following composition:

Table 4

Percentage	Fatty Acid Ester	Carbon-Chain Length	Number of Double Bonds
1.0	Methyl myristate	14	0
4.0	Methyl palmitate	16	0
3.0	Methyl stearate	18	0
3.0	Methyl arachidate	20	0
3.0	Methyl behenate	22	0
3.0	Methyl lignocerate	24	0
45.0	Methyl oleate	18	1
15.0	Methyl linoleate	18	2
3.0	Methyl linolenate	18	3
20.0	Methyl erucate	22	1

Nu-Chek mixture 19A has the following composition:

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Table 5

Percentage	Fatty Acid Ester	Carbon-Chain Length	Number of Double Bonds
7.0	Methyl caprylate	8	0
5.0	Methyl caprate	10	0
48.0	Methyl laurate	12	0
15.0	Methyl myristate	14	0
7.0	Methyl palmitate	16	0
3.0	Methyl stearate	18	0
12.0	Methyl oleate	18	1
3.0	Methyl linoleate	18	2

0.5 N Methanolic sodium hydroxide solution: Dissolve 2 g of sodium hydroxide in 100 mL of methanol.

Test solution: [NOTE—If fatty acids containing more than 2 double bonds are present in the test specimen, remove air from the flask by purging it with nitrogen for a few minutes.] Transfer about 100 mg of the test specimen to a 50-mL conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar. Add 4 mL of *0.5 N Methanolic sodium hydroxide solution,* and reflux until fat globules disappear (usually 5–10 min). Add 5 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL, swirl to mix, and reflux for 2 min. Add 4 mL of chromatographic *n*-heptane through the condenser, and reflux for 1 min. Cool, remove the condenser, add about 15 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Pass the *n*-heptane layer through 0.1 g of anhydrous sodium sulfate (previously washed with chromatographic *n*-heptane) into a suitable flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with chromatographic *n*-heptane to volume, and mix.

System suitability solution: Transfer about 20 mg each of stearic acid, palmitic acid, and oleic acid to a 25-mL conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar, and proceed as directed for *Test solution* beginning with "Add 5.0 mL of a solution prepared by dissolving".

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The gas chromatograph is equipped with a flame-ionization detector, maintained at a temperature of about 260°, a splitless injection system, and a 0.53-mm × 30-m fused-silica capillary column bonded with a 1.0-µm layer of phase G16. The chromatograph is programmed to maintain the column temperature at 70° for about 2 min after injection, then to increase the temperature at the rate of 5°/min to 240°, and finally to maintain this temperature for 5 min. The injection port temperature is maintained at about 220°. The carrier gas is helium with a linear velocity of about 50 cm/s.

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.87 for methyl palmitate, 0.99 for methyl stearate, and 1.0 for methyl oleate; the resolution, *R,* between methyl stearate and methyl oleate is NLT 1.5; and the relative standard deviation of the peak area responses for the palmitate and stearate peaks for replicate injections is NMT 6.0%. The relative standard deviation of the peak area response ratio of the palmitate to stearate peaks from these replicate injections is NMT 1.0%.

Procedure: Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the fatty acid ester peaks in the chromatogram of the *Test solution* by comparing the retention times of these peaks with those in the chromatogram of the *Standard solution,* and measure the peak areas for all of the fatty acid ester peaks in the chromatogram from the *Test solution.* Calculate the percentage of each fatty acid component in the test specimen:

$$\text{Result} = 100 \times (A/B)$$

A = area of the peak response obtained for each individual fatty acid ester component

B = sum of the peak areas of all of the peaks, excluding the solvent peak, in the chromatogram from the *Test solution*

• **OMEGA-3 FATTY ACIDS DETERMINATION AND PROFILE**

The following procedure may be used for the determination of eicosapentaenoic acid (EPA) (C20:5 n-3), docosahexaenoic acid (DHA) (C22:6 n-3), and total omega-3 acids obtained from fish, plant, or microbial sources in bulk oils and encapsulated oil, either as triglycerides or as ethyl esters. The term "triglyceride" is applicable to algal oils, fish oils, fish liver oils, and products containing omega-3-acids in triglycerides form. The results are expressed as free fatty acids or ethyl esters. Perform the tests as rapidly as possible. Protect the solutions from actinic light, oxidizing agents, oxidation catalysts, and air.

Content of EPA and DHA

Antioxidant solution: Dissolve an accurately weighed quantity of butylated hydroxytoluene in 2,2,4-trimethylpentane to obtain a solution with a concentration of 0.05 mg per mL.

Internal standard solution: Transfer an accurately weighed quantity of USP Methyl Tricosanoate RS to a volumetric flask. Dissolve in *Antioxidant solution,* and dilute with the same solvent to obtain a solution with a concentration of about 7.0 mg/mL. [NOTE—Guard the solution against evaporation during use.]

Test solution 1 (for triglycerides): In a 10-mL volumetric flask, dissolve the mass of sample to be examined, according to *Table 6,* in *Antioxidant solution,* and dilute with the same solution to volume. Transfer 2.0 mL of this solution to a glass

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tube, and evaporate the solvent with a gentle stream of nitrogen. Add 1.5 mL of a 2% (w/v) solution of sodium hydroxide in methanol, cap tightly with a polytetrafluoroethylene-lined cap, mix, and heat in a boiling water bath for 7 min. Cool, add 2 mL of boron trichloride–methanol solution (120 g in 1000 mL of methanol), cover with nitrogen, cap tightly, mix, and heat in a boiling water bath for 30 min. Cool to 40°–50°, add 1.0 mL of 2,2,4-trimethylpentane, cap, and mix on a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated sodium chloride solution containing 1 volume of sodium chloride and 2 volumes of water. [NOTE—Shake from time to time. Before use, decant the solution from any undissolved substance, and filter if necessary.] Cover with nitrogen, cap, and mix on a vortex mixer or shake thoroughly for at least 15 s. Allow the upper layer to become clear, and transfer to a separate tube. Shake the methanol layer once more with 1.0 mL of 2,2,4-trimethylpentane, and combine the 2,2,4-trimethylpentane extracts. Wash the combined extracts with two quantities, 1 mL each, of water, and dry over anhydrous sodium sulfate.

Table 6

Approx. Sum EPA + DHA	Amount of Sample to Be Weighed (g)
30%–50%	0.4–0.5
50%–70%	0.3
>70%	0.25

Test solution 2 (for triglycerides): Transfer the equivalent amount of sample used to prepare *Test solution 1* to a 10-mL volumetric flask, and dissolve in and dilute with *Internal standard solution* to volume. Gentle heating (up to 60°) may be applied to obtain a clear solution. Then proceed as directed in *Test solution 1* starting with "Transfer 2.0 mL".

Test solution 3 (for ethyl esters): In a 10-mL volumetric flask, dissolve the mass of sample to be examined, according to *Table 6*, in the *Internal standard solution*, and dilute with the same solution to volume. Gentle heating (up to 60°) may be applied to obtain a clear solution.

Test solution 4 (for ethyl esters): Transfer the equivalent amount of sample used to prepare *Test solution 3* to a 10-mL volumetric flask, and dissolve in and dilute with *Antioxidant solution* to volume.

Standard solution 1a: Transfer 60 mg of USP Docosahexaenoic Acid Ethyl Ester RS, accurately weighed, to a 10-mL volumetric flask, and dissolve in and dilute with *Internal standard solution* to volume. Gentle heating (up to 60°) may be applied to obtain a clear solution.

Standard solution 1b: Transfer 90 mg of USP Eicosapentaenoic Acid Ethyl Ester RS, accurately weighed, to a 10-mL volumetric flask, and dissolve in and dilute with *Internal standard solution* to volume. Gentle heating (up to 60°) may be applied to obtain a clear solution.

Standard solution 1a and *Standard solution 1b* are ready for ethyl esters analysis. For triglycerides analysis, continue with *Standard solution 2a* and *Standard solution 2b*.

Standard solution 2a: Transfer 2.0 mL of *Standard solution 1 a* to a glass tube, and evaporate the solvent with a gentle stream of nitrogen. Then proceed as directed for *Test solution 1* starting with, "Add 1.5 mL".

Standard solution 2b: Transfer 2.0 mL of *Standard solution 1b* to a glass tube, and evaporate the solvent with a gentle stream of nitrogen. Then proceed as directed for *Test solution 1* starting with, "Add 1.5 mL".

System suitability solution 1: Transfer 0.300 g of methyl palmitate, 0.300 g of methyl stearate, 0.300 g of methyl arachidate, and 0.300 g of methyl behenate, accurately weighed, to a 10-mL volumetric flask, and dissolve in and dilute with *Antioxidant solution* to volume.

System suitability solution 2: [NOTE—This solution must be prepared only for triglycerides and only if tetracos-15-enoic acid methyl ester is not clearly observed in the chromatogram obtained with *Test solution 2*.] Transfer 55.0 mg of docosahexaenoic acid methyl ester and about 5.0 mg of tetracos-15-enoic acid (nervonic acid) methyl ester, accurately weighed, to a 10-mL volumetric flask, and dissolve in and dilute with *Antioxidant solution* to volume.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 25-m fused-silica capillary column bonded with a 0.20-µm film of phase G16

Temperatures

Injection port

Split injection: 250°

Splitless injection: 90°–250°

Detector: 270°

Column: See *Table 7a* or *Table 7b*.

Table 7a (Split Injection)

Initial Temperature (°)	Hold Time at 170° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	2	3	240	2.5

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Table 7b (Splitless Injection)

Initial Temperature (°)	Hold Time at 90° (min)	Temperature Ramp Number 1 (°/min)	To Temperature (°)	Temperature Ramp Number 2 (°/min)	To Final Temperature (°)	Hold Time at Final Temperature (min)
90	2	30	170	3	240	2

Carrier gas: Helium
Flow rate: 1 mL/min
Split flow ratio: 200:1. [NOTE—If necessary, adjust the split ratio and/or sample dilution to obtain a tailing factor of 0.8–1.5 for the fatty acid methyl ester peaks in *System suitability solution 1*, the methyl or ethyl ester peaks of eicosapentaenoic acid and docosahexaenoic acid in *Test solution 1* and *Test solution 4*, at the same time observing that for *Test solution 1* or *Test solution 4* any peaks due to the corresponding esters of C18:3 n-3, C18:4 n-3, C20:4 n-3, C21:5 n-3, and C22:5 n-3 are clearly detectable. If splitless injection mode is used, solutions should be further diluted 1 in 200 with *Antioxidant solution*.]
Injection size: 1 µL

System suitability (for triglycerides)
Samples: *System suitability solution 1* and *Test solution 2* or *System suitability solution 2* (if applicable)

System suitability (for ethyl esters)
Sample: *System suitability solution 1*

System suitability requirements
Theoretical area percentages (System suitability solution 1): The area percentages, after adjusting for the actual weights, of the peaks of methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate, each should be within ±1.0% (absolute) of theoretical values provided in *Table 8*.

Table 8

Fatty Acid Methyl Ester	Theoretical Area (%) in a Solution of Equal Weights of Methyl Palmitate, Methyl Stearate, Methyl Arachidate, and Methyl Behenate
Methyl palmitate	24.37
Methyl stearate	24.84
Methyl arachidate	25.23
Methyl behenate	25.56

[NOTE—The theoretical area (%) in a solution of equal weights (*Table 8*) is derived from the calculated theoretical response factors, which are 1.049, 1.029, 1.013, and 1.000 for methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate, respectively.]
Resolution: For triglycerides (*Test solution 2* or *System suitability solution 2*): NLT 1.2 between the docosahexaenoic acid methyl ester and tetracos-15-enoic acid methyl ester peaks

Analysis (for triglycerides)
Samples: *Standard solution 2a, Standard solution 2b, Test solution 1*, and *Test solution 2*
Inject each sample in duplicate and measure the peak responses. Compare the chromatographic profile of *Test solution 2* with that of *Test solution 1* and identify the internal standard peak present in *Test solution 2*. Calculate the percentage of EPA or DHA in the triglycerides sample taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times F \times 100$$

R_U = ratio of peak response of either EPA or DHA to the corrected peak response of the internal standard in the chromatogram of *Test solution 2*, calculated:

[NOTE—If $r_{U1} = 0$ due to no peak observed at the locus of the internal standard in the chromatogram of *Test solution 1*, then $R_U = r_{T2}/r_{U2}$.]

$$\text{Result} = 1/[(r_{U2}/r_{T2}) - (r_{U1}/r_{T1})]$$

r_{U2} = peak response of the internal standard in the chromatogram of *Test solution 2*
r_{T2} = peak response of EPA or DHA in the chromatogram of *Test solution 2*
r_{U1} = peak response of any peak at the locus of the internal standard in the chromatogram of *Test solution 1*
r_{T1} = peak response of EPA or DHA in the chromatogram of *Test solution 1*
R_S = ratio of peak response of either DHA or EPA to the peak response of the internal standard in the chromatogram of *Standard solution 2a* or *Standard solution 2b*
W_S = weight of DHA taken for preparing *Standard solution 1a* or weight of EPA taken for preparing *Standard solution 1b* (mg)
W_U = weight of sample taken for preparing *Test solution 2* (mg)

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F = factor to express content of DHA as free fatty acids, 0.921; and factor to express content of EPA as free fatty acids, 0.915

Analysis (for ethyl esters)

Samples: *Standard solution 1a, Standard solution 1b, Test solution 3,* and *Test solution 4*

Inject each sample in duplicate and measure the peak responses. Compare the chromatographic profile of *Test solution 3* with that of *Test solution 4* and identify the internal standard peak present in *Test solution 3*. Calculate the percentage of EPA or DHA in the ethyl esters sample taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = ratio of peak response of either EPA or DHA to the corrected peak response of the internal standard in the chromatogram of *Test solution 3*, calculated as follows:

$$\text{Result} = 1/[(r_{U3}/r_{T3}) - (r_{U4}/r_{T4})]$$

[NOTE—If $r_{U4} = 0$ due to no peak observed at the locus of the internal standard in the chromatogram of *Test solution 4*, then $R_U = r_{T3}/r_{U3}$.]

r_{U3} = peak response of the internal standard in the chromatogram of *Test solution 3*
r_{T3} = peak response of EPA or DHA in the chromatogram of *Test solution 3*
r_{U4} = peak response of any peak at the locus of the internal standard in the chromatogram of *Test solution 4*
r_{T4} = peak response of EPA or DHA in the chromatogram of *Test solution 4*
R_S = ratio of peak response of either DHA or EPA to the peak response of the internal standard in the chromatogram of *Standard solution 1a* or *Standard solution 1b*
W_S = weight of DHA taken for preparing *Standard solution 1a* or weight of EPA taken for preparing *Standard solution 1b* (mg)
W_U = weight of sample taken for preparing *Test solution 3* (mg)

Content of total omega-3 acids (for triglycerides)

Calculate the percentage of the total omega-3 acids in the portion of triglycerides sample taken:

$$\text{Result} = \text{EPA} + \text{DHA} + [(A_{n-3} \times (\text{EPA} + \text{DHA})/(A_{EPA} + A_{DHA})]$$

EPA = content of EPA, from the test for *Content of EPA and DHA* (%)
DHA = content of DHA, from the test for *Content of EPA and DHA* (%)
A_{n-3} = sum of the areas of the peaks corresponding to C18:3 n-3, C18:4 n-3, C20:4 n-3, C21:5 n-3, and C22:5 n-3 methyl esters in the chromatogram obtained with *Test solution 1*
A_{EPA} = area of the peak corresponding to the EPA methyl ester in the chromatogram obtained with *Test solution 1*
A_{DHA} = area of the peak corresponding to the DHA methyl ester in the chromatogram obtained with *Test solution 1*

Content of total omega-3 acids (for ethyl esters)

Calculate the percentage of the total omega-3 acids in the portion of ethyl esters sample taken:

$$\text{Result} = \text{EPA} + \text{DHA} + [(A_{n-3} \times (\text{EPA} + \text{DHA})/(A_{EPA} + A_{DHA})]$$

EPA = content of EPA, from the test for *Content of EPA and DHA* (%)
DHA = content of DHA, from the test for *Content of EPA and DHA* (%)
A_{n-3} = sum of the areas of the peaks corresponding to C18:3 n-3, C18:4 n-3, C20:4 n-3, C21:5 n-3, and C22:5 n-3 ethyl esters in the chromatogram obtained with *Test solution 4*
A_{EPA} = area of the peak corresponding to the EPA ethyl ester in the chromatogram obtained with *Test solution 4*
A_{DHA} = area of the peak corresponding to the DHA ethyl ester in the chromatogram obtained with *Test solution 4*

• **WATER AND SEDIMENT IN FIXED OILS**

Apparatus: The preferred centrifuge has a diameter of swing (d = distance from tip to tip of whirling tubes) of 38–43 cm and is operated at a speed of about 1500 rpm. If a centrifuge of different dimensions is used, calculate the desired rate of revolution:

$$\text{rpm} = 1500\sqrt{40.6/d}$$

The centrifuge tubes are pear-shaped and are shaped to accept closures. The total capacity of each tube is about 125 mL. The graduations are clear and distinct, reading upward from the bottom of the tube according to the scale shown in *Table 9*.

Table 9

Volume (mL)	Scale Division (mL)
0–3	0.1
3–5	0.5

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Table 9 *(continued)*

Volume (mL)	Scale Division (mL)
5–10	1.0
10–25	5.0
25–50	25.0
50–100	50.0

Procedure: Place 50.0 mL of benzene in each of two centrifuge tubes, and to each tube add 50.0 mL of the oil, warmed if necessary to re-incorporate separated stearin, and mixed thoroughly at 25°. Insert the stopper tightly into the tubes, shake them vigorously until the contents are mixed thoroughly, and then immerse the tubes in a water bath at 50° for 10 min. Centrifuge for 10 min. Read the combined volume of water and sediment at the bottom of each tube. Centrifuge repeatedly for 10-min periods until the combined volume of water and sediment remains constant for three consecutive readings. The sum of the volumes of combined water and sediment in the two tubes represents the percentage, by volume, of water and sediment in the oil.

• **ANISIDINE VALUE**

The Anisidine Value is defined as 100 times the optical density measured in a 1-cm cell of a solution containing 1 g of the substance to be examined in 100 mL of a mixture of solvents and reagents according to the following method described. [NOTE—Carry out the operations as rapidly as possible, avoiding exposure to actinic light.]

Test solution A: Dissolve 0.500 g of the substance to be examined in isooctane, and dilute with the same solvent to 25.0 mL.

Test solution B: To 5.0 mL of *Test solution A* add 1.0 mL of a 2.5-g/L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Standard solution: To 5.0 mL of isooctane add 1.0 mL of a 2.5-g/L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Procedure: Measure the absorbance of *Test solution A* at 350 nm using isooctane as the blank. Measure the absorbance of *Test solution B* at 350 nm exactly 10 min after its preparation, using the *Standard solution* as the compensation liquid. Calculate the Anisidine Value from the expression:

$$\text{Result} = [25 \times (1.2A_S - A_B)]/m$$

A_S = absorbance of *Test solution B* at 350 nm
A_B = absorbance of *Test solution A* at 350 nm
m = weight of the substance to be examined in *Test solution A* (g)

• **TOTAL OXIDATION VALUE (TOTOX)**

Total Oxidation Value is defined:

$$\text{Result} = 2PV + AV$$

PV = Peroxide Value
AV = Anisidine Value

• **TRACE METALS**

Apparatus

The apparatus typically consists of the following:

Digestion flasks: Use a polytetrafluoroethylene flask with a volume of about 120 mL, fitted with an airtight closure, a valve to adjust the pressure inside the container, and a polytetrafluoroethylene tube to allow the release of gas.

System: Make the flask airtight, using the same torsional force for each of them.

Microwave oven: It has a magnetron frequency of 2450 MHz with a selectable output from 0 to 630 ± 70 W in 1% increments, a programmable digital computer, a polytetrafluoroethylene-coated microwave cavity with a variable speed exhaust fan, a rotating turntable drive system, and exhaust tubing to vent fumes.

Atomic absorption spectrometer: It is equipped with a hollow-cathode lamp as the source of radiation and a deuterium lamp as a background corrector. The system is fitted with the following:

1. A graphite furnace as the atomization device for cadmium, copper, iron, lead, nickel, and zinc.
2. An automated continuous-flow hydride vapor generation system for arsenic and mercury.

General procedure

[**CAUTION**—When using closed high-pressure digestion vessels and microwave laboratory equipment, the safety precautions and operating instructions given by the manufacturer must be followed.]

[NOTE—If an alternative apparatus is used, adjustment of the instrument parameters may be necessary.]

Cleaning: Clean all the glassware and laboratory equipment with a 10-mg/mL solution of nitric acid before use.

Trace metal–free nitric acid: Nitric acid meets the requirements with the maximum values for arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), lead (Pb), nickel (Ni), and zinc (Zn) equal to 0.005, 0.005, 0.001, 0.02, 0.002, 0.001, 0.005, and 0.01 ppm, respectively.

Trace metal–free hydrochloric acid: Hydrochloric acid meets the requirements with the maximum values for As, Cd, Cu, Fe, Hg, Pb, Ni, and Zn equal to 0.005, 0.003, 0.003, 0.05, 0.005, 0.001, 0.004, and 0.005 ppm, respectively.

Trace metal–free sulfuric acid: Sulfuric acid meets the requirements with the maximum values for As, Cd, Cu, Fe, Hg, Pb, Ni, and Zn equal to 0.005, 0.002, 0.001, 0.05, 0.005, 0.001, 0.002, and 0.005 ppm, respectively.

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Test stock solution: In a digestion flask place about 0.5 g of fatty oil, accurately weighed, as indicated in each individual monograph. Add 6 mL of *Trace metal–free nitric acid* and 4 mL of *Trace metal–free hydrochloric acid.* Close the flask.

Blank stock solution: Mix 6 mL of *Trace metal–free nitric acid* and 4 mL of *Trace metal–free hydrochloric acid* in a digestion flask.

Test solution 1: Place the digestion flask containing the *Test stock solution* in the microwave oven. Carry out the digestion in three steps according to the following program: 80% power for 15 min, 100% power for 5 min, and 80% power for 20 min.

At the end of the cycle allow the flask to cool. Add 4 mL of *Trace metal–free sulfuric acid* to the flask. Repeat the digestion program. After completing the digestion, allow the flask to cool to room temperature. Open the digestion flask, and transfer the clear, colorless solution obtained into a 50-mL volumetric flask. Rinse the digestion flask with 2 quantities, 15 mL each, of water, and collect the rinsings in the volumetric flask. Add 1.0 mL of a 10-mg/mL solution of magnesium nitrate and 1.0 mL of a 100-mg/mL solution of ammonium dihydrogen phosphate to the volumetric flask. Dilute with water to volume, and mix. This solution is *Test solution 1.*

Blank solution 1: Place the digestion flask containing *Blank stock solution* in the microwave oven. Proceed as directed in *Test solution 1* beginning with "Carry out the digestion in three steps according to the following program".

Direct calibration: [NOTE—Concentrations of the standard solutions will depend on the metal contents of the test substance.] For routine measurements, 3 standard solutions, *Blank solution 1,* and *Test solution 1* are prepared and examined. Use *Test solution 1* and *Blank solution 1* as prepared previously or as indicated in the monograph. Prepare NLT 3 standard solutions containing all the metal elements to be tested. The expected absorbance value in *Test solution 1* for each metal element should be within its corresponding calibrated absorbance range, preferably in the middle of the calibrated absorbance range. Any reagents used in the preparation of *Test solution 1* are added at the same concentration to the standard solutions.

Introduce each of the solutions into the instrument using the same number of replicates for each of the solutions to obtain a steady reading.

Prepare a calibration curve from the mean of the readings obtained with the standard solutions by plotting the means as a function of concentration. Determine the concentration of the element in *Test solution 1* from the curve obtained.

Standard additions: Add to at least four identical volumetric flasks equal volumes of *Test solution 1* as prepared previously or as indicated in the monograph. Add to all but one of the flasks progressively larger volumes of a standard solution containing a known concentration of the test element to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve. Dilute the contents of each flask with the solvent specified in the monograph to volume, and mix. The flask without an addition of standard solution is labeled as the test solution.

Introduce each of the solutions into the instrument, using the same number of replicates for each of the solutions, to obtain a steady reading.

Plot the absorbances of the standard solutions and the test solution versus the added quantity of test element. [NOTE—The test solution should be plotted as if it had a content of added test element equivalent to 0 mg or µg.] Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of test element in the test solution.

Specific Tests

Cadmium (cd), copper (cu), iron (fe), lead (pb), nickel (ni), and zinc (zn)

Standard stock solution: Prepare a solution containing known concentrations of 5 µg/mL for each test element.

Standard solutions: In three identical 10-mL volumetric flasks introduce 10, 20, and 40 µL of *Standard stock solution,* respectively. To each flask add 5.0 mL of *Test solution 1,* dilute with water to volume, and mix.

Test solution 2: In a 10-mL volumetric flask add 5.0 mL of *Test solution 1,* dilute with water to volume, and mix.

Blank solution 2: In a 10-mL volumetric flask add 5.0 mL of *Blank solution 1,* dilute with water to volume, and mix.

Procedure: Measure the content of Cd, Cu, Fe, Pb, Ni, and Zn using a suitable graphite furnace atomic absorption spectrophotometer. Concomitantly determine the absorbances of *Blank solution 2,* the *Standard solutions,* and *Test solution 2* at least three times each. The absorbance value of *Blank solution 2* is subtracted from the value obtained using the *Standard solutions* and *Test solution 2.* Proceed as directed in the *Standard additions* method in *General Procedure.* See *Table 10* for instrumental parameters that may be used.

Table 10

	Cd	Cu	Fe	Pb	Ni	Zn
Wavelength (nm)	228.8	324.8	248.3	283.5	232	213.9
Slit (nm)	0.5	0.5	0.2	0.5	0.2	0.5
Lamp current (mA)	6	7	5	5	10	7
Ignition temperature (°)	800	800	800	800	800	800
Atomization temperature (°)	1800	2300	2300	2200	2500	2000
Background corrector	On	Off	Off	Off	Off	Off
Nitrogen flow (L/min)	3	3	3	3	3	3

Arsenic and mercury

Measure the content of arsenic and mercury against their standard solutions at a known concentration using the *Direct calibration* method in *General Procedure,* with an automated continuous-flow hydride vapor generation system.

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For the 1 ppm arsenic specification limit and 1 ppm mercury specification limit, prepare three working calibration solutions with known concentrations of 5, 10, and 20 ng/mL for each test element, respectively.

The absorbance value of the blank solution is automatically subtracted from the value obtained using the test solution.

Arsenic

Blank solution 3: Add 1.0 mL of a 200-mg/mL solution of potassium iodide to 19.0 mL of *Blank solution 1* prepared previously. Allow this solution to stand at room temperature for about 50 min or at 70° for about 4 min.

Test solution 3: Add 1.0 mL of a 200-mg/mL solution of potassium iodide to 19.0 mL of *Test solution 1* prepared previously. Allow this solution to stand at room temperature for about 50 min or at 70° for about 4 min.

Acid reagent 1: *Trace metal-free hydrochloric acid*

Reducing reagent 1: A 6-mg/mL solution of sodium tetrahydroborate in a 5-mg/mL solution of sodium hydroxide

The instrumental parameters in *Table 11* may be used.

Mercury

Blank solution 4: Proceed as directed in *Blank solution 3*.

Test solution 4: Proceed as directed in *Test solution 3*.

Acid reagent 2: A 515-mg/mL solution of *Trace metal–free hydrochloric acid*

Reducing reagent 2: A 10-mg/mL solution of stannous chloride in a 200-mg/mL solution of *Trace metal–free hydrochloric acid*

The instrumental parameters in *Table 11* may be used.

Table 11

	As	Hg
Wavelength (nm)	193.7	253.7
Slit width (nm)	0.2	0.5
Lamp current (mA)	10	4
Acid reagent flow rate (mL/min)	1.0	1.0
Reducing reagent flow rate (mL/min)	1.0	1.0
Flow rate for the blank, standard, test solutions (mL/min)	7.0	7.0
Absorption cell	Quartz (heated)	Quartz (unheated)
Background corrector	Off	Off
Nitrogen flow rate (L/min)	0.1	0.1

• **STEROL COMPOSITION**

Separation of the sterol fraction

Reference solution A: Dissolve an accurately weighed quantity of cholesterol in chloroform to obtain a solution of 5% (w/v).

Developing solvent system: A mixture of toluene and acetone (95:5) or a mixture of hexane and ether (65:35)

Test solution A: Accurately weigh 5 g of the test substance into a 250-mL flask. Add 50 mL of alcoholic potassium hydroxide TS 2 (2 N alcoholic potassium hydroxide), and heat to gentle boiling with continuous vigorous stirring until saponification takes place (the solution becomes clear). Continue heating for a further 20 min, and add 50 mL of water from the top of the condenser. Cool the flask to approximately 30°. Transfer the contents of the flask to a 500-mL separating funnel with several rinses of water, amounting in all to about 50 mL. Add approximately 80 mL of ether, shake vigorously for approximately 30 s, and allow to settle. [NOTE—Any emulsion can be destroyed by adding small quantities of ethyl or methyl alcohol by means of a spray.] Separate the lower aqueous phase, and collect it into a second separating funnel. Perform two further extractions on the water–alcohol phase in the same way, using 60–70 mL of ether on each occasion. Pool the ether extracts into a single separating funnel, and wash with water, 50 mL at a time, until the wash water is no longer alkaline to phenolphthalein. Dry the ether phase with anhydrous sodium sulfate, and filter on anhydrous sodium sulfate into a previously weighed 250-mL flask, washing the funnel and filter with small quantities of ether. Distill the ether down to a few mL, and bring to dryness under a slight vacuum or in a stream of nitrogen. Complete the drying at 100° for approximately 15 min, and then weigh after cooling in a desiccator. Dissolve the unsaponifiables so obtained in chloroform to obtain a solution with a concentration of approximately 5%.

Test solution B: Treat 5 g of canola oil in the same way as prescribed for the test substance in *Test solution A*, beginning with "Add 50 mL of alcoholic potassium hydroxide TS 2 (2 N alcoholic potassium hydroxide)".

Test solution C: Treat 5 g of sunflower oil in the same way as prescribed for the test substance in *Test solution A*, beginning with "Add 50 mL of alcoholic potassium hydroxide TS 2 (2 N alcoholic potassium hydroxide)".

Procedure: Immerse the thin-layer chromatographic plate (see ⟨621⟩), 20-cm × 20-cm silica gel on polyester with a layer thickness of 200 µm and particle size of 5–17 µm,[1] completely in the 0.2 N alcoholic potassium hydroxide for 10 s, allow to dry in a fume cupboard for 2 h, and finally place at 100° for 1 h. [NOTE—Remove from the validated heating device, and keep the plate in a desiccator until required for use. The plates must be used within 15 days. Thin-layer chromatographic plates that do not require the preconditioning also are commercially available.] Use a separate plate for each test solution.

Place a mixture of toluene and acetone (95:5) or a mixture of hexane and ether (65:35) in the chamber to a depth of approximately 1 cm. Close the chamber with the appropriate cover, and leave for at least 30 min. Strips of filter

[1] A commercial TLC plate may be obtained from Sigma-Aldrich, catalog #z122785.

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paper dipping into the eluant may be placed on the internal surfaces of the chamber. [NOTE—The developing mixture should be replaced for every test to ensure reproducible elution conditions.] Apply 0.3 mL of *Test solution A* approximately 2 cm from the lower edge in a streak that is as thin and as uniform as possible. In line with the streak, place 2–3 µL of *Reference solution A* at one end of the plate. Develop the chromatograms in an equilibrated chamber with the *Developing solvent system* until the solvent front reaches approximately 1 cm from the upper edge of the plate. Remove the plate from the developing chamber, and evaporate the solvent under a current of hot air [NOTE—avoid excessive heat] or by leaving the plate for a short while under a hood. Spray the plate with a 0.2% alcoholic solution of 2,7-dichlorofluorescein, and examine in UV light at 254 nm. [NOTE—The plates pretreated with UV indicator also are commercially available and are used equivalently.] In each of the plates, mark the limits of the sterol band identified by being aligned with the stain obtained from *Reference solution A* along the edges of the fluorescence, and additionally include the area of the zones 2–3 mm above and below the visible zones corresponding to *Reference solution A*. Remove the silica gel in the marked area into a filter funnel with a G3 porous septum.[2] Add 10 mL of hot chloroform, mix carefully with the metal spatula, filter under vacuum, and collect the filtrate in the conical flask attached to the filter funnel. Wash the residue in the funnel three times with ether, about 10 mL each time, and collect the filtrate in the same flask attached to the funnel. Evaporate the filtrate to a volume of 4–5 mL, transfer the residual solution to a previously weighed 10-mL test tube with a tapering bottom and a sealing stopper, and evaporate to dryness by mild heating in a gentle stream of nitrogen. Dissolve the residue in a few drops of acetone, and evaporate again to dryness. Place at 105° for approximately 10 min, allow to cool in a desiccator, and weigh.

Treat *Test solution B* and *Test solution C* the same way as directed for *Test solution A*.

Determination of the Sterols

Test solution D: To the test tube containing the sterol fraction separated from the test substance by thin-layer chromatography add a freshly prepared mixture of anhydrous pyridine, hexamethyldisilazane, and chlorotrimethylsilane (9:3:1) [NOTE—This reagent also is commercially available and is used equivalently.] in the ratio of 50 µL for every mg of sterols, avoiding any uptake of moisture. Insert the stopper into the test tube, and shake carefully until the sterols are completely dissolved. Allow it to stand for at least 15 min at ambient temperature, and centrifuge for a few minutes if necessary. Use the supernatant. [NOTE—The slight opalescence that may form is normal and does not cause an anomaly. However, the formation of a white floc or the appearance of a pink color is indicative of the presence of moisture or deterioration of the reagent. If these occur, the test must be repeated.]

Reference solution E: To 9 parts of the sterols separated from canola oil by thin-layer chromatography, add 1 part of cholesterol. Treat the mixture in the same way as directed in *Test solution D*.

Reference solution F: Treat the sterols separated from sunflower oil by thin-layer chromatography in the same way as directed in *Test solution D*.

Chromatographic system

(See *Chromatography* ⟨621⟩.)

The gas chromatograph is equipped with a flame-ionization detector and a glass or fused-silica capillary column 20–30 m in length, internal diameter 0.25–0.32 mm, entirely coated with a 0.10- to 0.30-µm layer of stationary phase G27 or G36. The injection port temperature is maintained at 280°, the detector temperature is maintained at 290°, and the column temperature is maintained at 260 ± 5°. The carrier gas is either helium with a linear velocity of 20–35 cm/s or hydrogen with a linear velocity of 30–50 cm/s. A split ratio of 1:50 to 1:100 is used. Chromatograph *Reference solution E* and *Reference solution F*, and record the peak responses as directed in *Procedure:* the retention time should be 20 ± 5 min for β-sitosterol, and all the sterols present must be separated. The chromatogram obtained with *Reference solution E* shows four principal peaks corresponding to cholesterol, brassicasterol, campesterol, and β-sitosterol; and the chromatogram obtained with *Reference solution F* shows four principal peaks corresponding to campesterol, stigmasterol, β-sitosterol, and Δ7-stigmastenol. The retention times of the sterols with reference to β-sitosterol are given in *Table 12*.

Table 12. Relative Retention Times of Sterols for Two Different Columns

Identification	G36 Column	G27 Column
Cholesterol	0.67	0.63
Brassicasterol	0.73	0.71
24-Methylene-cholesterol	0.82	0.80
Campesterol	0.83	0.81
Campestanol	0.85	0.82
Stigmasterol	0.88	0.87
Δ7-Campesterol	0.93	0.92
Δ5,23-Stigmastadienol	0.95	0.95
Clerosterol	0.96	0.96
β-Sitosterol	1.00	1.00
Sitostanol	1.02	1.02
Δ5-Avenasterol	1.03	1.03

[2] A commercial product may be obtained from Kimble/Kontes as a filter, buchner with fritted disc, Kimax 28400-152.

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Table 12. Relative Retention Times of Sterols for Two Different Columns *(continued)*

Identification	G36 Column	G27 Column
Δ5,24-Stigmastadienol	1.08	1.08
Δ7-Stigmastenol	1.12	1.12
Δ7-Avenasterol	1.16	1.16

Procedure: Separately inject equal volumes (about 1 µL) of *Test solution D*, *Reference solution E*, and *Reference solution F* into the chromatograph, record the chromatograms, and measure the peak areas for the sterols. Calculate the percentage of each individual sterol in the sterol fraction of the test substance taken:

$$\text{Result} = 100 \times (A/S)$$

A = area of the peak due to the sterol component to be determined
S = sum of the areas of the peaks due to the components indicated in *Table 12*

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** ⟨11⟩
  USP Docosahexaenoic Acid Ethyl Ester RS
  USP Eicosapentaenoic Acid Ethyl Ester RS
  USP Methyl Tricosanoate RS

⟨411⟩ FOLIC ACID ASSAY

INTRODUCTION

The following liquid chromatographic procedures are provided for the determination of folic acid as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component of dietary supplements or pharmaceutical dosage forms.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by using low-actinic glassware.

ASSAY

- **Procedure 1**

This procedure can be used to determine folic acid in the following:

- Oil- and Water-Soluble Vitamins with Minerals Tablets
- Oil- and Water-Soluble Vitamins with Minerals Capsules
- Oil- and Water-Soluble Vitamins Tablets
- Oil- and Water-Soluble Vitamins Capsules
- Water-Soluble Vitamins with Minerals Tablets
- Water-Soluble Vitamins with Minerals Capsules
- Water-Soluble Vitamins Tablets
- Water-Soluble Vitamins Capsules

This procedure involves the extraction of analytes from the formulation by using an *Internal standard solution* that contains methylparaben, tetrabutylammonium hydroxide, and pentetic acid in alcoholic phosphate buffer and by mechanically shaking to release the analytes from the matrices.

Unless specified in the individual monographs, the reagent solutions, *Internal standard solution*, *Standard solution*, and *Sample solutions* are prepared as follows.

Reagent A: 25% Solution of tetrabutylammonium hydroxide in methanol

Reagent B: Transfer 5.0 g of pentetic acid to a 50-mL volumetric flask. Using sonication if necessary, dissolve in and dilute with 1 N sodium hydroxide to volume.

Mobile phase: 2 g of monobasic potassium phosphate in 650 mL of water. Add 12.0 mL of *Reagent A*, 7.0 mL of 3 N phosphoric acid, and 240 mL of methanol. Cool to room temperature, adjust with phosphoric acid or ammonia TS to a pH of 7.0, dilute with water to 1000 mL, and filter. Recheck the pH before use by adding water or methanol to the prepared *Mobile phase* to obtain baseline separation of folic acid and the internal standard. The pH may be increased up to 7.15 to obtain better separation. [Note—The methanol and water content may be varied (1%–3%).]

Internal standard solution: Transfer 40 mg of methylparaben to a 1000-mL volumetric flask, and add 220 mL of methanol to dissolve. Dissolve 2.0 g of monobasic potassium phosphate in 300 mL of water in a separate beaker, quantitatively transfer this solution to the flask containing the methylparaben solution, and add an additional 300 mL of water. Add 19 mL of *Reagent A*, 7 mL of 3 N phosphoric acid, and 30 mL of *Reagent B*. Adjust with ammonia TS to a pH of 9.8, bubble nitrogen through the solution for 30 min, dilute with water to volume, and mix.

Standard solution: 0.016 mg/mL of USP Folic Acid RS in the *Internal standard solution*

Sample solution for Tablets: Crush NLT 30 Tablets until finely powdered. Transfer a portion of powder, equivalent to 0.4 mg of folic acid, to a 50-mL amber-colored centrifuge tube. Add 25.0 mL of the *Internal standard solution*, shake by mechanical means for 10 min, and centrifuge. Filter a portion of the clear supernatant, and use the filtrate.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a

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current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer an amount of the Capsule contents to a suitable centrifuge tube, and add a volume of *Internal standard solution* to obtain a concentration of 0.016 mg/mL of folic acid. Shake by mechanical means for 10 min, and centrifuge. Filter a portion of the clear supernatant, and use the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for folic acid and methylparaben are about 0.8 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of the *Sample* taken:

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

R_U = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the appropriate *Sample solution*

R_S = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Standard solution*

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

C_U = nominal concentration of folic acid in the appropriate *Sample solution* (µg/mL)

• **PROCEDURE 2**

This procedure can be used to determine folic acid in the following:

- Oil- and Water-Soluble Vitamins with Minerals Tablets
- Oil- and Water-Soluble Vitamins with Minerals Capsules
- Oil- and Water-Soluble Vitamins Tablets
- Oil- and Water-Soluble Vitamins Capsules
- Water-Soluble Vitamins with Minerals Tablets
- Water-Soluble Vitamins with Minerals Capsules
- Water-Soluble Vitamins Tablets
- Water-Soluble Vitamins Capsules

This procedure involves the extraction of analytes from the formulation by using an extracting solution that contains either a mixture of edetate disodium and ammonium hydroxide or a mixture of methanol, glacial acetic acid, and ethylene glycol, and by mechanically shaking to release the analytes from the matrices.

Unless specified in the individual monographs, the reagent solution, *Diluent, Standard stock solution, Standard solutions*, and *Sample solutions* are prepared as follows.

Reagent: Dissolve 7.5 g of edetate disodium, with stirring, in 500 mL of water containing 10 mL of ammonium hydroxide.

Diluent: 60 µg/mL of ammonium hydroxide

Mobile phase: Transfer 0.4 mL of triethylamine, 15 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask, and dilute with 0.008 M sodium 1-hexanesulfonate to volume.

Standard stock solution: 60 µg/mL of USP Folic Acid RS in *Diluent*. Prepare this solution fresh daily.

Standard solution for Tablets: Mix 5.0 mL of the *Standard stock solution* with 10.0 mL of methanol and 35.0 mL of *Reagent*. Shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Standard solution for Capsules: Mix 5.0 mL of the *Standard stock solution* with 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Sample solution for Tablets: Transfer a portion of finely powdered Tablets, equivalent to 0.3 mg of folic acid, to a 125-mL stoppered flask. Add 10.0 mL of methanol and 35.0 mL of *Reagent*. Shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer an amount of the Capsule contents, equivalent to 0.3 mg of folic acid, to a 125-mL stoppered flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

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Column: 4.6-mm × 25-cm; packing L7
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: Appropriate *Standard solution* and appropriate *Sample solution*
Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of the *Sample* taken:

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

r_U = peak response of folic acid from the appropriate *Sample solution*
r_S = peak response of folic acid from the appropriate *Standard solution*
C_S = concentration of USP Folic Acid RS in the appropriate *Standard solution* (µg/mL)
C_U = nominal concentration of folic acid in the appropriate *Sample solution* (µg/mL)

• **PROCEDURE 3**
This procedure can be used to determine folic acid in the following:
- Active pharmaceutical ingredient
- Dietary ingredient

This procedure involves the dissolution of analytes and internal standard in *Mobile phase*.
Unless specified in the individual monographs, the reagent solutions, *Internal standard solution, Standard stock solution, Standard solution, Sample stock solution,* and *Sample solution* are prepared as follows.
3 N phosphoric acid: 98 g/L of phosphoric acid in water
6 N ammonium hydroxide: Dilute 40 mL of ammonium hydroxide with water to 100 mL.
Mobile phase: Transfer 2.0 g of monobasic potassium phosphate into a 1000-mL volumetric flask, and dissolve in 650 mL of water. Add 15.0 mL of a solution of 0.5 M tetrabutylammonium hydroxide in methanol, 7.0 mL of *3 N phosphoric acid,* and 270 mL of methanol. Cool to room temperature, adjust with *3 N phosphoric acid* or *6 N ammonium hydroxide* to a pH of 5.0, and dilute with water to volume. Recheck the pH before use.
Internal standard solution: 2 mg/mL of methylparaben in *Mobile phase*. Dissolve the methylparaben first with methanol (about 4% of the final volume), and dilute with *Mobile phase* to volume.
Standard stock solution: 1 mg/mL of USP Folic Acid RS in *Mobile phase*. Dissolve the folic acid with the aid of 10% ammonium hydroxide (about 1% of the final volume), and dilute with *Mobile phase* to volume.
Standard solution: Transfer 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample stock solution: Transfer 100 mg of folic acid to a 100-mL volumetric flask, and dissolve in 40 mL of *Mobile phase* and 1 mL of 10% ammonium hydroxide. Dilute with *Mobile phase* to volume.
Sample solution: Transfer 4.0 mL of *Sample stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.6 between methylparaben and folic acid
Relative standard deviation: NMT 2.0% for the ratios of the folic acid peak response to the internal standard peak response
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of the *Sample* taken:

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

R_U = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Standard solution*
C_S = concentration of USP Folic Acid RS in the *Standard stock solution* (mg/mL)
C_U = concentration of folic acid in the *Sample stock solution* (mg/mL)

• **PROCEDURE 4**
This procedure can be used to determine folic acid in the following:

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- Folic Acid Tablets
- Folic Acid Injection

This procedure involves the dissolution of analytes in a *Diluent* that contains 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL of water.

Unless specified in the individual monographs, the *Diluent, System suitability solution, Standard solution,* and *Sample solutions* are prepared as follows.

Mobile phase: Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1-L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.

Diluent: Aqueous solution containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL

System suitability solution: 0.2 mg/mL each of USP Folic Acid RS and USP Folic Acid Related Compound A RS in *Diluent.* [NOTE—Before use, pass through a filter of 1-µm or finer pore size.]

Standard solution: 0.20 mg/mL of USP Folic Acid RS in *Diluent*

Sample solution for Tablets: Equivalent to 0.2 mg/mL of folic acid from NLT 20 powdered Tablets in *Diluent.* Shake gently to aid dissolution, and filter, discarding the first few mL of the filtrate.

Sample solution for Injection: Dilute with *Diluent* an accurately measured volume of Injection, quantitatively and stepwise, to obtain a solution having a concentration close to that of the *Standard solution* and between 0.20 and 0.80 mg/mL.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

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

Suitability requirements

Resolution: NLT 3.6 between folic acid related compound A and folic acid, *System suitability solution*

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

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

Calculate the percentage of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of Tablets taken:

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

r_U = peak response of folic acid from the *Sample solution for Tablets*
r_S = peak response of folic acid from the *Standard solution*
C_S = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of folic acid in the *Sample solution for Tablets* (mg/mL)

Calculate the quantity, in mg, of folic acid ($C_{19}H_{19}N_7O_6$) in each mL of Injection taken:

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

r_U = peak response of folic acid from the *Sample solution for Injection*
r_S = peak response of folic acid from the *Standard solution*
C_S = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)
V_U = volume of Injection taken for preparation of the *Sample solution for Injection* (mL)
V = total volume used to dilute the Injection (mL)

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Folic Acid RS
USP Folic Acid Related Compound A RS

Calcium formyltetrahydrofolate.

⟨413⟩ IMPURITIES TESTING IN MEDICAL GASES

INTRODUCTION

This general test chapter defines the safe and proper means to sample high-pressure gas containers of different medical gas compositions using the detector tube manufacturer's suggested total gas volume for the purpose of conducting detector tube analysis to satisfy the *USP* monographs. See *Reagents* in the section *Reagents, Indicators, and Solutions* for information on each referenced detector tube.

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There are two types of detector tubes currently manufactured: those to be used with a manual hand pump of fixed volume (e.g., 100 mL/pump stroke) and those used in a continuous flow system that can be set to pass a volume of gas through the detector tube at approximately 1 atmosphere. It is important to match the appropriate detector tube type to the mode of gas volume exchange.

To ensure that the required gas volume has passed through the detector tube, measure the gas volume at the time of the analysis either by using a hand pump or using a flowmeter that is calibrated to the subject gas or corrected via a calibration chart. Flowmeter manufacturers generally provide a chart of the gas volume flow of common gases for each of the flow tubes identified in this general chapter.

[NOTE—See general chapter *Medical Gases Assay* ⟨415⟩ for sampling.]

CONTINUOUS FLOW SYSTEM

Identify the gas contained in the container, and select the appropriate gas regulator. Secure the regulator to the gas container. Do not apply lubricant or Teflon tape to the container-to-regulator connections. Purge the regulator with the gas under test. Select float setting to obtain the proper flow rate to achieve total gas volume as recommended by the manufacturer. Attach the detector tube, then adjust the flow rate to the required level as indicated by the charts that accompany the flowmeter and the detector tube. Time the gas flow to achieve the desired total gas volume ±10 s. At the allotted time close the regulator valve, then the main container valve. Observe the tube while it is still attached to determine the degree of color change, and record the result. Remove the tube, and disconnect the apparatus, vent the regulator gas pressure to atmosphere, and remove the regulator from the container. Dispose of the tube after use.

HAND PUMP—FIXED VOLUME

The alternative approach is to apply the gas detector hand pump. The system draws a consistent volume with each pump stroke. To ensure the accuracy of the total gas volume, the user must follow the pump manufacturer's suggested recommendation about how to pump.

⟨415⟩ MEDICAL GASES ASSAY

INTRODUCTION

The evaluation of the purity of a gas used for medical treatment or as a component of a pharmaceutical process is the purpose of a *USP* medical gas monograph. The purity generally is evaluated by an assay for the content of the article and by analyses for trace impurities. The application of gas chromatography, paramagnetic analysis, and detector tubes to medical gases is somewhat different from traditional procedures used for analytes in the liquid phase and therefore warrants a separate description. This general test chapter focuses on the assay for content tests. Sampling for impurities is addressed in general chapter *Impurities Testing in Medical Gases* ⟨413⟩.

This chapter includes sampling and qualification aspects of gas chromatographic and paramagnetic analyses of medical gases. In addition, it includes a description of the initial set-up, validation, and calibration of these instruments. The specific assay procedures are defined in the specific monograph for that gas.

The basic definitions of instrumental qualification and validation are included in general information chapters *Analytical Instrument Qualification* ⟨1058⟩ and *Validation of Compendial Procedures* ⟨1225⟩, respectively, and will not be repeated. However, when variations of the materials presented in these chapters are necessary due to the character of the analyte, this chapter will define those variations.

METHODS

Gas Chromatography (GC)

See *Chromatography* ⟨621⟩.

Detectors for Medical Gases Assay

The two most common detectors used in the analyses of medical gases are the thermal conductivity detector (TCD) and flame ionization detector (FID).

The TCD will detect any gas or vapor that has a thermal conductivity (TC) that differs significantly from the high TC of the reference gas, usually helium, therefore it is virtually universal. However, the generally accepted lower detection limit for the TCD is 50 ppm v/v. This represents a limitation for the evaluation of trace impurities in medical gases.

The FID is also used for the evaluation of trace impurities in medical gases, because it is more sensitive to organic compounds but does not produce a signal for most common medical gases.

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QUALIFICATION

Installation Qualification (IQ)

The IQ requirements ensure the gas chromatograph hardware and software (or readout device) is installed safely and in accordance with the GC manufacturer's instructions.

Consideration should be given to the following as applicable:

- Suitability of the sample system (including connections);
- Leakage (should be leak free);
- Representative sampling;
- Sample flow rates;
- Response time;
- Correct output signals;
- Power supply (including voltage regulation); and
- Appropriate environmental conditions of the instrument and of the sample itself (e.g., temperature and pressure).

Operational Qualification (OQ)

OQ verifies that the GC performs as intended within its anticipated operating range. For medical gas final product testing, the GC is tested to ensure repeatability (verification that relative standard deviation is consistent with claims) for each analyte of interest. Due to the specific nature of medical gas testing and the limited number of analytes, routine calibration and periodic calibration verification of a GC instrument and the testing procedure may be used in place of initial or periodic OQ. When an instrument is used for a broader range of analytes, the replacement of OQ with calibration and periodic calibration verification is inappropriate.

Performance Qualification (PQ)

For medical gas final product testing, the GC is periodically checked at appropriate intervals during analytical runs with a calibration gas (i.e., verifying that the results are consistent with a named concentration within acceptable accuracy and precision ranges after a specific number of sample injections).

Paramagnetic Oxygen Measurement

THEORY

The paramagnetic analyzer measures the displacement of a diamagnetic gas (nitrogen) by a paramagnetic gas (oxygen), in a strong magnetic field. A measuring cell typically employs a glass dumbbell with nitrogen-filled spheres that is suspended on a torsion strip between magnets that concentrate the flux around the dumbbell. When oxygen molecules enter the measuring cell, the dumbbell is deflected by the force exerted by the oxygen molecules that are attracted to the strongest part of the magnetic field. By using optical sensors, a feedback coil, and suitable electronics, analysts measure an output that is directly proportional to the partial pressure of oxygen.

Oxygen is the only paramagnetic gas present above trace levels in the atmosphere. However, paramagnetic analyzers can be affected by the magnetic susceptibility of the background gas. Therefore changes to background gases in *USP* monographs should be avoided.

Design Considerations

The design considerations for the purchase of new instruments may include the following parameters.

DRIFT

A change of the output of the instrument for a given concentration over a stated period of time under constant conditions and without any adjustments being made to the instrument by external means. Drift is the summation of two components, zero drift and span drift. Drift determines the frequency of instrument calibration.

ZERO DRIFT

A change in the output when zero gas is being measured.

SPAN DRIFT

A change in the output at the level of oxygen concentration that is being measured.

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OPERATING TEMPERATURE

The ambient temperature range for which the stated performance specification of the instrument will remain valid. A larger temperature coefficient will indicate that a smaller change in ambient temperature is permitted before re-calibration is required.

OPERATING PRESSURE

The instrument should operate at the inlet pressures of the samples to be tested.

Qualification Aspects

INSTALLATION QUALIFICATION (IQ)

The IQ requirements ensure the oxygen analyzer hardware and software (or readout device) is installed safely and in accordance with the oxygen analyzer manufacturer's instructions.

Consideration should be given to the following as applicable:

- Suitability of the sample system (including connections);
- Leakage (should be leak free);
- Representative sampling;
- Sample flow rates;
- Response time;
- Correct output signals;
- Power supply (including voltage regulation); and
- Appropriate environmental conditions of the instrument and of the sample itself (e.g., temperature and pressure).

OPERATIONAL QUALIFICATION (OQ)

The OQ requirements verify that the paramagnetic analyzer performs as intended within its anticipated operating range and is suitable for the actual conditions of use. Instruments and apparatus should be calibrated and used in accordance with the oxygen analyzer manufacturer's instructions. Because of the specific nature of the instrument, routine calibration may be used in place of initial or periodic OQ testing.

PERFORMANCE QUALIFICATION (PQ)

The PQ requirements verify that the paramagnetic analyzer performs as intended in its normal operating environment. For medical gas final product testing, the paramagnetic analyzer is initially calibrated (zeroed and spanned using a certified standard) in accordance with the oxygen analyzer manufacturer's instructions and is periodically recalibrated to ensure continued acceptable performance.

ZEROING THE INSTRUMENT (ESTABLISHING THE LOWER LIMIT)

Using the certified standard defined in the monograph, establish a zero setting on the analyzer by passing the zero gas into the analyzer at the oxygen analyzer manufacturer's suggested flow rate. Maintain the flow until a stable reading is observed on the instrument. As necessary, adjust the zero setting to a value of 0.0% according to the oxygen analyzer manufacturer's instructions. Confirm the reading is stable.

[NOTE—Depending on the intended use of the instrument, zeroing to a setting other than 0.0% is an acceptable alternative to this procedure if it provides greater measurement precision.]

SPANNING THE RANGE OF USE

Establish the upper limit (span) with a span gas defined in the monograph and appropriate for the range of use. Pass the span gas through the instrument at the manufacturer's suggested flow rate. Confirm the reading is stable. Adjust the span setting to the certified value of the reference standard according to the oxygen analyzer manufacturer's instructions. Confirm the reading is stable.

VALIDATION

Validation of this instrument is generally completed during the (IQ/OQ) process. Routine verification is performed as described in the OQ/PQ sections of this chapter; and, therefore, specific information on instrument validation is unnecessary.

PROCEDURE

For Off-line Instrument

Before analysis, the instrument is calibrated by zeroing and spanning as described in the PQ section.

[NOTE—The calibration need not be run concomitantly with the test samples.]

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Connect the sample gas to the instrument, and establish a constant flow into the analyzer at the analyzer manufacturer's suggested flow rate. Maintain the flow until a constant reading is observed on the instrument. The definition of a constant reading is included in the analyzer manufacturer's instructions or in the user's instrument qualification documentation.

For On-line Instrument

The calibration intervals are defined by the analyzer manufacturer, by past history, or by statistical means. Establish a constant flow into the analyzer at the manufacturer's suggested flow rate.

SAMPLING

Sampling from Liquid Phase

Cylinders containing a dip tube allow a liquid sample to be obtained from the valve outlet with the cylinder in the upright position. If a dip tube is not present, the cylinder should be placed in an inverted position with the cylinder and main valve safely supported (so the liquid phase is in contact with the valve).

Sampling of medical gases should always be conducted using the required regulator. Regulators should be purged with the gas that will be sampled. When necessary, the flow to the analyzer should be measured using a calibrated flow-measuring device.

Sampling from Gaseous Phase

Cylinders that do not contain a dip tube allow a gaseous sample to be obtained from the valve outlet with the cylinder in the upright position. If a dip tube is present, the cylinder should be in an inverted position with the cylinder and main valve safely supported (so the gaseous phase is in contact with the end of the dip tube). Sampling of medical gases should always be conducted using the required regulator.

CERTIFIED STANDARDS FOR MEDICAL GAS ANALYSIS

USP monographs for medical gases require tests that use certified standards for instrument calibration and analytical determinations. Such compendial testing may be conducted using reference materials that are traceable to the U.S. National Institute of Standards and Technology or other National standards-setting organizations, e.g., Institute for National Measurement Standards (Canada). The individual monographs and the reagents, indicators, and solutions section refer to the nominal percent of various certified standards required to perform medical gas analysis. The requirements for the actual certified concentrations in terms of variance from the nominal value are indicated in the *Reagents, Indicators, and Solutions* section for each respective certified standard.

⟨425⟩ IODOMETRIC ASSAY—ANTIBIOTICS

The following method is provided for the assay of most of the Pharmacopeial penicillin antibiotic drugs and their dosage forms, for which iodometric titration is particularly suitable.

ASSAY

Standard preparation: Dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about that specified in the table. Pipet 2.0 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Solvents and Final Concentrations

Antibiotic	Solvent*	Final Concentration
Amoxicillin	Water	1.0 mg/mL
Ampicillin	Water	1.25 mg/mL
Ampicillin Sodium	*Buffer B.1*	1.25 mg/mL
Cloxacillin Sodium	Water	1.25 mg/mL
Cyclacillin	Water	1.0 mg/mL
Dicloxacillin Sodium	*Buffer B.1*	1.25 mg/mL
Methicillin Sodium	*Buffer B.1*	1.25 mg/mL
Nafcillin Sodium	*Buffer B.1*	1.25 mg/mL

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Solvents and Final Concentrations *(continued)*

Antibiotic	Solvent*	Final Concentration
Oxacillin Sodium	*Buffer B.1*	1.25 mg/mL
Penicillin G Potassium	*Buffer B.1*	2,000 units/mL
Penicillin G Sodium	*Buffer B.1*	2,000 units/mL
Penicillin V Potassium	*Buffer B.1*	2,000 units/mL
Phenethicillin Potassium	*Buffer B.1*	2,000 units/mL

* Unless otherwise noted, the *Buffers* are the potassium phosphate buffers defined in the section *Media and Diluents* under *Antibiotics—Microbial Assays* ⟨81⟩, except that sterilization is not required before use.

Assay preparation: Unless otherwise specified in the individual monograph, dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity, accurately weighed, of the specimen under test, and dilute quantitatively with the same solvent to obtain a solution having a known final concentration of about that specified in the table. Pipet 2 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Procedure

Inactivation and titration: To 2.0 mL of the *Standard preparation* and of the *Assay preparation*, in respective flasks, add 2.0 mL of 1.0 N sodium hydroxide, mix by swirling, and allow to stand for 15 minutes. To each flask add 2.0 mL of 1.2 N hydrochloric acid, add 10.0 mL of 0.01 N iodine VS, immediately insert the stopper, and allow to stand for 15 minutes. Titrate with 0.01 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, and continue the titration to the discharge of the blue color.

Blank determination: To a flask containing 2.0 mL of the *Standard preparation* add 10.0 mL of 0.01 N iodine VS. If the *Standard Preparation* contains amoxicillin or ampicillin, immediately add 0.1 mL of 1.2 N hydrochloric acid. Immediately titrate with 0.01 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, and continue the titration to the discharge of the blue color. Similarly treat a flask containing 2.0 mL of the *Assay preparation.*

Calculations

Calculate the microgram (or unit) equivalent (F) of each mL of 0.01 N sodium thiosulfate consumed by the *Standard preparation* by the formula:

$$\text{Result} = (2CP)/(B - I)$$

C = concentration of Reference Standard in the *Standard preparation* (mg/mL)
P = potency of the Reference Standard (µg/mg or units/mg)
B = volume of 0.01 N sodium thiosulfate consumed in the *Blank determination* (mL)
I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and titration* (mL)

Calculate the potency of the specimen under test by the formula given in the individual monograph.

⟨429⟩ LIGHT DIFFRACTION MEASUREMENT OF PARTICLE SIZE

INTRODUCTION

The method is based on the ISO standards 13320-1(1999) and 9276-1(1998).

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia.*

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider angular range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by single particles and scattering by clusters of primary particles, i.e., by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary particles before measurement.

For nonspherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g., sedimentation, sieving).

This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems (e.g., powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids), through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products.

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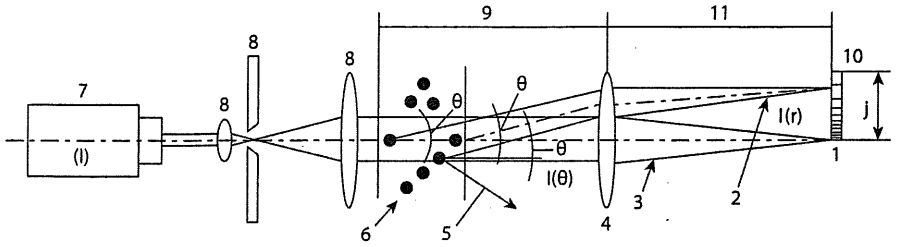
PRINCIPLE

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for subsequent analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

INSTRUMENT

The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity, or direct bright light.

An example of a setup of a laser light diffraction instrument is given in *Figure 1*. Other equipment may be used.



1. Obscuration detector
2. Scattered beam
3. Direct beam
4. Fourier lens
5. Scattered light not collected by lens (4)
6. Particle ensemble
7. Light source laser
8. Beam processing unit
9. Working distance lens (4)
10. Multi-element detector
11. Focal distance of lens (4)

Figure 1. Example of a set-up of a laser light diffraction instrument.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in two positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus in a converging beam. The advantage of the conventional setup is that a reasonable path length for the sample is allowed within the working distance of the lens. The second setup allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

DEVELOPMENT OF THE METHOD

The measurement of particle size by laser diffraction can give reproducible data, even in the submicron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g., dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 µm to 3 mm. Because of recent advances in lens and equipment design, newer instruments are routinely capable of exceeding this range. With the validation report, the user demonstrates the applicability of the method for its intended use.

Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle-size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

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Evaluation of the Dispersion Procedure

The sample to be analyzed is inspected, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method, it is highly advisable to check that comminution of the particles does not occur and, conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g., crystallization, milling) of the material has changed, the applicability of the method must be verified (e.g., by microscopic comparison).

Sprays, aerosols, and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes, and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g., agitation, sonication) are often applied for deagglomeration or deaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing is most commonly used. Nonrecirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers that apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol that is blown through the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free-flowing, coarser particles or granules, the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving, and the mass and percentage of removed material are reported. However, after presieving, note that the sample is no longer representative, unless proven otherwise.

Optimization of the Liquid Dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must

- be transparent at the laser wavelength and practically free from air bubbles or particles;
- have a refractive index that differs from that of the test material;
- be a nonsolvent of the test material (pure liquid or prefiltered, saturated solution);
- not alter the size of the test materials (e.g., by solubility, solubility enhancement, or recrystallization effects);
- favor easy formation and stability of the dispersion;
- be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.); and
- possess a suitable viscosity to facilitate recirculation, stirring, and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH, respectively, can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore, a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

Optimization of the Gas Dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water, and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

Determination of the Concentration Range

In order to produce an acceptable signal-to-noise ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. [NOTE—In different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g., obscuration, optical concentration, proportional number of total mass.]

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Determination of the Measuring Time

The time of measurement, the reading time of the detector, and the acquisition frequency is determined experimentally in accordance with the required precision. Generally, the time for measurement permits a large number of detector scans or sweeps at short time intervals.

Selection of an Appropriate Optical Model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly larger amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, because small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about 0.01–0.1 i) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g., shape, surface roughness, and porosity) bear upon the final result.

Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision, and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH is not applicable as it is not possible to discriminate different components into a sample, nor is it possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor signal-to-noise ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly on the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for repeatability such as % RSD $\leq$ 10% [n = 6] for any central value of the distribution (e.g., for x_{50}). Values at the sides of the distribution (e.g., x_{10} and x_{90}) are oriented towards less stringent acceptance criteria such as % RSD $\leq$ 15% [n = 6]. Below 10 µm, these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

MEASUREMENT

Precautions

The instructions given in the instrument manual are followed:

- never look into the direct path of the laser beam or its reflections;
- earth all instrument components to prevent ignition of solvents or dust explosions;
- check the instrument set-up (e.g., warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight); and
- in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent airflow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

Measurement of the Light Scattering of Dispersed Sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to subtract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated, sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity, and the quantum efficiency. The coordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The

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ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and hence the particle concentration.

Conversion of Scattering Pattern Into Particle-Size Distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of deviations between measured and calculated scattering patterns (e.g., least squares), some constraints (e.g., non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each make and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

REPORTING RESULTS

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol x is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. Q3(x) denotes the volume fraction undersize at the particle size x. In a graphical representation, x is plotted on the abscissa and the dependent variable Q3 on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as x_{10}, x_{50}, and x_{90}, respectively) are frequently used. x_{50} is also known as the median particle size. It is recognized that the symbol d is also widely used to designate the particle size, thus the symbol x may be replaced by d.

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. Because the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

CONTROL OF THE INSTRUMENT PERFORMANCE

Use the instrument according to the manufacturer's instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument meets the requirements if the mean value of x_{50} from at least three independent measurements does not deviate by more than 3% from the certified range of values of the certified reference material. The mean values for x_{10} and x_{90} must not deviate by more than 5% from the certified range of values. Below 10 µm, these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, nonspherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same nonspherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

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Qualification of the System

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software, and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is considered to meet the requirements if the x_{50} value does not deviate by more than 10% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g., x_{10} and x_{90}), then these values must not deviate by more than 15% from the certified range of values. Below 10 µm, these values must be doubled.

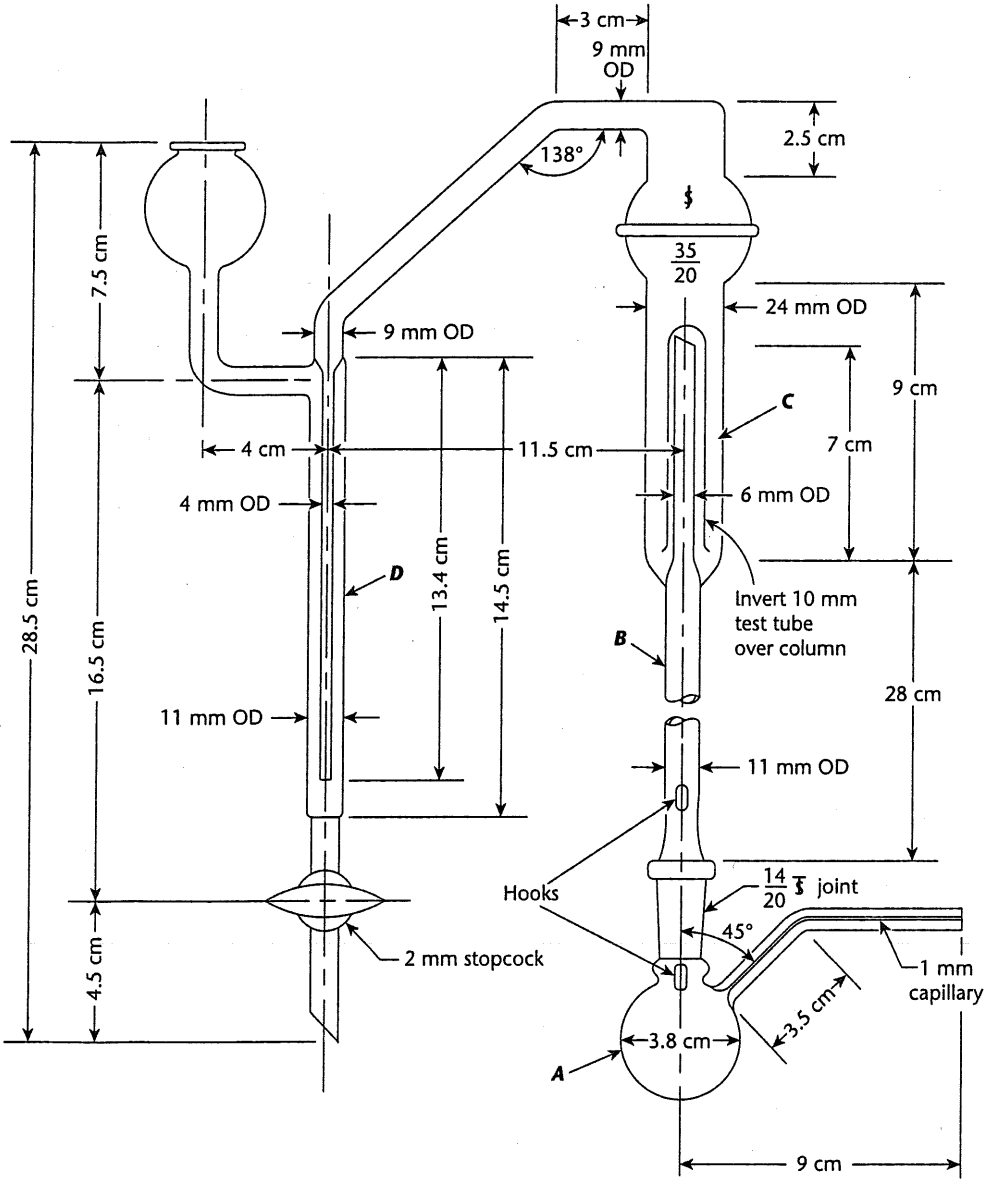
[NOTE—For calibration of the instrument, stricter requirements are laid down in the paragraph on *Calibration*.]

⟨431⟩ METHOXY DETERMINATION

APPARATUS

The apparatus for methoxy determination is shown diagrammatically in the *accompanying figure.*

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Apparatus for Methoxy Determination

The boiling flask, A, is fitted with a capillary side-arm for the introduction of carbon dioxide or nitrogen and is connected to a column, B, which serves to separate aqueous hydriodic acid from the more volatile methyl iodide. The methyl iodide passes through water in a scrubber trap, C, and is finally absorbed in the bromine–acetic acid solution in absorption tube D. The carbon dioxide or nitrogen is introduced through a pressure-regulating device and connected to the apparatus by a small capillary containing a small cotton pledget.

[NOTE—Avoid the use of organic solvents in cleaning this apparatus, since traces remaining may interfere with the determination. This test is used also for ethoxy determination with an 80-minute reaction time and a titrant equivalent of 0.751 mg of (OC_2H_5).]

For greater convenience in use and cleaning, a ground-glass ball joint connects the two upright columns of the apparatus. The top of the scrubber C consists of a 35/20 ball joint, the upper half of which is connected to the side-arm leading into tube D. This permits taking the apparatus apart and facilitates adding the water to the trap. Also, it allows access to the loose inverted (10-mm) test tube that serves as the trap over the inner tube of the scrubber C.

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REAGENTS

Bromine-Acetic Acid Solution

Dissolve 100 g of potassium acetate in 1000 mL of a solution consisting of 900 mL of glacial acetic acid and 100 mL of acetic anhydride. On the day of use, to 145 mL of this solution add 5 mL of bromine.

Hydriodic Acid

A colorless, or nearly colorless, constant-boiling reagent solution, prepared for this purpose, is available commercially. If not obtained commercially, it may be prepared by distilling hydriodic acid over red phosphorus, passing carbon dioxide or nitrogen through the apparatus during the distillation. Use the constant-boiling mixture (between 55% and 58% of HI) distilling between 126° and 127°, which is colorless or nearly colorless. [**CAUTION**—Exercise safety precautions when distilling Hydriodic Acid.] Place the acid in small, amber, glass-stoppered bottles previously flushed with carbon dioxide, or nitrogen, seal with paraffin, and store in a cool, dark place.

PROCEDURE

Prepare the apparatus by disconnecting the ball joint and pouring water into trap C until it is half-full. Connect the two parts, using a minimal amount of a suitable silicone grease to seal the ball joint. Add 7 mL of *Bromine-Acetic Acid Solution* to absorption tube D. Weigh the sample in a tared gelatin capsule, and add it to the boiling flask along with a few boiling chips or pieces of porous plate. Finally add 6 mL of *Hydriodic Acid* and attach the flask to the column, using a minimal amount of a suitable silicone grease to seal the junction. Bubble the carbon dioxide or nitrogen through the apparatus at the rate of 2 bubbles per second, place the boiling flask in an oil bath or heating mantle heated to 150°, and continue the reaction for 40 minutes for methoxy determination, or 80 minutes for ethoxy determination. Drain the contents of the absorption tube into a 500-mL conical flask containing 10 mL of sodium acetate solution (1 in 4). Rinse the tube with water, adding the rinsings to the flask, and finally dilute with water to about 125 mL. Add formic acid, dropwise, with swirling, until the reddish brown color of the bromine is discharged, then add 3 additional drops. A total of 12 to 15 drops usually is required. Allow to stand for 3 minutes, and add 15 mL of diluted sulfuric acid and 3 g of potassium iodide, and titrate immediately with 0.1 N sodium thiosulfate VS, using 3 mL of starch TS as the indicator. Perform a blank determination, including also a gelatin capsule, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 0.517 mg of (OCH_3).

⟨441⟩ NIACIN OR NIACINAMIDE ASSAY

ASSAY

Chemical Method

• **PROCEDURE**

The following photometric procedure involves the colorimetric reaction of niacin or niacinamide with cyanogen bromide in the presence of sulfanilic acid. It can be used for the determination of niacin or niacinamide in the monographs such as *Niacin Injection*, *Niacinamide Injection*, and *Niacinamide Tablets*.

[NOTE—Determine from the labeling if the vitamin in the assay specimen is niacin or niacinamide, and use the corresponding standard preparation (either *Standard niacin preparation* or *Standard niacinamide preparation*) as directed in the *Procedure*.]

Cyanogen bromide solution: Dissolve 5 g of cyanogen bromide in water to make 50 mL. [**CAUTION**—Prepare this solution under a hood, as cyanogen bromide volatilizes at room temperature, and the vapor is highly irritating and poisonous.]

Sulfanilic acid solution: To 2.5 g of sulfanilic acid add 15 mL of water and 3 mL of 6 N ammonium hydroxide. Mix, and add while stirring, more 6 N ammonium hydroxide, if necessary, until the acid dissolves. Adjust the solution with 3 N hydrochloric acid to a pH of about 4.5 using bromocresol green TS as an external indicator, and dilute with water to 25 mL.

Standard niacin stock solution: Transfer 25.0 mg of USP Niacin RS to a 500-mL volumetric flask, dissolve in alcohol solution (1 in 4), dilute with alcohol solution (1 in 4) to volume, and mix. Store in a refrigerator. Each mL of this solution contains 50 µg of USP Niacin RS.

Standard niacin preparation: Transfer 10.0 mL of *Standard niacin stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 5 µg of USP Niacin RS.

Standard niacinamide stock solution: Transfer 50.0 mg of USP Niacinamide RS to a 500-mL volumetric flask, dissolve in alcohol solution (1 in 4), dilute with alcohol solution (1 in 4) to volume, and mix. Store in a refrigerator. Each mL of this solution contains 100 µg of USP Niacinamide RS.

Standard niacinamide preparation: Transfer 10.0 mL of *Standard niacinamide stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 10 µg of USP Niacinamide RS.

Assay preparation: Prepare as directed in the individual monograph.

Procedure: Pipet into four marked tubes the quantities of the appropriate *Standard preparation*, *Assay preparation*, *Ammonia dilution*, and *Water* indicated in *Table 1*. Then add the other constituents, respectively, as listed in the table, according to the directions given herein.

Chemical Tests

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Table 1. Reaction Mixtures for Niacin or Niacinamide Assay—Chemical Method

Constituent	Tube 1 (mL)	Tube 2 (mL)	Tube 3 (mL)	Tube 4 (mL)
Standard preparation	1.0	1.0	—	—
Assay preparation	—	—	1.0	1.0
Ammonia dilution (ammonium hydroxide, diluted to 1 in 50)	0.5	0.5	0.5	0.5
Water	6.5	1.5	6.5	1.5
Cyanogen bromide solution	—	5.0	—	5.0
Sulfanilic acid solution	2.0	2.0	2.0	2.0
Hydrochloric acid	1 drop	—	1 drop	—

To *Tube 1* add the *Sulfanilic acid solution*, shake well, and add the *Hydrochloric acid*. Mix, place in a suitable spectrophotometer, and adjust to zero absorbance at 450 nm. To *Tube 2* add the *Cyanogen bromide solution*, mix, and 30 s accurately timed after completion of the addition of the cyanogen bromide, add the *Sulfanilic acid solution*, with swirling. Close the tube, place it in the spectrophotometer, and after 2 min measure its absorbance at 450 nm against *Tube 1* as a blank, designating the absorbance as A_S. Repeat the procedure with *Tube 3* (as blank) and *Tube 4*, designating the absorbance of *Tube 4* as A_U. Calculate the quantity of niacin or niacinamide in the sample as directed in the individual monograph.

Chromatographic Methods

The following liquid chromatographic procedures are provided for the determination of niacin or niacinamide as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in the dietary supplements or pharmaceutical dosage forms.

Use the appropriate USP Reference Standard for the niacin or niacinamide present in the formulation.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware.

- **PROCEDURE 1**
  - This procedure can be used to determine niacin or niacinamide in:
    - Oil- and water-soluble vitamins with minerals tablets
  - This procedure involves the extraction of analytes from the formulation by hot water or mechanical shaking
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Diluent: Acetonitrile, glacial acetic acid, and water (5:1:94)

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL

Standard solution: Transfer 80 mg of either USP Niacin RS or USP Niacinamide RS to a 200-mL volumetric flask, and add 180 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution: For tablets, finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 10 mg of niacinamide and 2.5 mg each of pyridoxine hydrochloride, riboflavin, and thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas for niacin or niacinamide.

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*

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r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

• **PROCEDURE 2**

- This procedure can be used to determine niacin or niacinamide in:
  - Oil- and water-soluble vitamins with minerals tablets
- This procedure involves the extraction of analytes from the formulation by the *Extraction solvent*, heat, and mechanical shaking
- Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: Transfer 1 mL of glacial acetic acid and 2.5 g of edetate disodium to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.
Extraction solvent: *Solution A* and methanol (3:1)
Mobile phase: 0.1 M sodium acetate solution (13.6 mg/mL of sodium acetate in water). Adjust with acetic acid to a pH of 5.4. [NOTE—A small amount of methanol (up to 1%) may be added to the *Mobile phase* to improve resolution.]
Standard stock solution: 1 mg/mL of USP Niacin RS or USP Niacinamide RS in *Extraction solvent*
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask, and dilute with *Extraction solvent* to volume.
Sample solution: [NOTE—This preparation is suitable for the determination of niacin or niacinamide, pyridoxine, and riboflavin, when present in the formulation.] For tablets, finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 2 mg of riboflavin, to a 200-mL volumetric flask. If riboflavin is not present in the formulation, transfer a portion of the powder, equivalent to 2 mg of pyridoxine. If pyridoxine is not present in the formulation, transfer a portion of the powder, equivalent to 20 mg of niacin or niacinamide. Add 100.0 mL of *Extraction solvent*, and mix for 20 min, using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, cool to room temperature, and filter. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% [NOTE—If necessary, flush the column with methanol between injections.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*
r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

• **PROCEDURE 3**

- This procedure can be used to determine niacin or niacinamide in:
  - Oil- and water-soluble vitamins with minerals tablets
- This procedure involves the extraction of analytes from the formulation by mixtures of organic solvents, heat, and mechanical shaking
- Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Reagent: 25 mg/mL of edetate disodium in water
Mobile phase: Transfer 0.4 mL of triethylamine, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask. Dilute with 0.008 M sodium 1-hexanesulfonate to volume.
Standard stock solution: 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS in the *Reagent*, with heating if necessary
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.
Sample solution: For tablets, weigh and finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 7.5 mg of niacin or niacinamide, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; packing L7
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*
r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

• **PROCEDURE 4**

- This procedure can be used to describe niacin as:
  - An active pharmaceutical ingredient
  - A dietary supplement ingredient
  - An ingredient in the niacin extended-release tablets
- This procedure involves either dissolving the analyte in the *Diluent*, a mixture of methanol and water (82:18), or extracting the analyte from the formulation with *Diluent* and mechanical shaking
- Unless specified in the individual monographs, the *System suitability solution*, *Standard solution A*, *Standard solution B*, *Sample solution A*, *Sample solution B*, and reagent solutions are prepared as follows.

Diluent: Methanol and water (82:18)
Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05
System suitability solution: 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.10 mg/mL of pyridine in *Diluent*
Standard solution A: For active pharmaceutical ingredient or dietary supplement ingredient, use 0.25 mg/mL of USP Niacin RS in *Diluent*.
Standard solution B: For extended-release tablets, use 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.0978 mg/mL of pyridine in *Diluent*.
Sample solution A: For active pharmaceutical ingredient or dietary supplement ingredient, use 0.25 mg/mL of Niacin in *Diluent*.
Sample solution B: For extended-release tablets, transfer a quantity of powder, equivalent to 50 mg of niacin from NLT 20 finely powdered tablets, to a suitable flask. Add *Diluent*, and stir for 2 h. Dilute with *Diluent* to a final concentration of 0.25 mg/mL of niacin.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution A* or *Standard solution B*
[NOTE—The relative retention times for pyridine, 6-hydroxynicotinic acid, and niacin are 0.14, 0.64, and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid, and NLT 1.5 between 6-hydroxynicotinic acid and niacin, *System suitability solution*
Relative standard deviation: NMT 2.0% for the niacin peak from replicate injections, *Standard solution A* or *Standard solution B*
Analysis
Samples: *Standard solution A* and *Sample solution A* or *Standard solution B* and *Sample solution B*
Calculate the percentage of niacin ($C_6H_5NO_2$) in the portion of the *Sample* taken:

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

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r_U = peak response of niacin from *Sample solution A* or *Sample solution B*
r_S = peak response of niacin from *Standard solution A* or *Standard solution B*
C_S = concentration of USP Niacin RS in *Standard solution A* or *Standard solution B* (mg/mL)
C_U = concentration of Niacin in *Sample solution A* or nominal concentration of niacin in *Sample solution B* (mg/mL)

- **PROCEDURE 5**
  - This procedure can be used to determine niacinamide as:
    - An active pharmaceutical ingredient
    - A dietary supplement ingredient
  - This procedure involves dissolving the niacinamide as the active pharmaceutical ingredient or a dietary supplement ingredient in the *Mobile phase*
  - Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Niacin solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: Methanol and 0.005 M sodium 1-heptanesulfonate (30:70)
Standard solution: Transfer 50 mg of USP Niacinamide RS to a 100-mL volumetric flask. Add 3 mL of water to dissolve, and dilute with *Mobile phase* to volume. Dilute with *Mobile phase* to 0.04 mg/mL.
Niacin solution: Prepare as directed in the *Standard solution*, using USP Niacin RS instead of USP Niacinamide RS.
System suitability solution: Mix equal volumes of the *Standard solution* and *Niacin solution*.
Sample solution: Prepare as directed in the *Standard solution*, using Niacinamide instead of USP Niacinamide RS.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between niacin and niacinamide, *System suitability solution*
Relative standard deviation: NMT 2.0% for the niacinamide peak from replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

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

- **PROCEDURE 6**
  - This procedure can be used to determine niacin as:
    - An active ingredient in niacin tablets
  - This procedure involves the extraction of the niacin from the formulation by water, heat, and mechanical shaking
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: 5-mM solution of sodium 1-hexanesulfonate in water
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Solution A* (14:7:1:78)
Standard solution: 0.050 mg/mL of USP Niacin RS in water. Dissolve with the aid of heat in a steam bath.
Sample solution: For tablets, transfer an equivalent to 500 mg of niacin from NLT 20 finely powdered tablets to a suitable flask. Add 50 mL of water, and heat on a steam bath for 30 min. Sonicate for 2 min, shake by mechanical means for 15 min, and cool to room temperature. Dilute with water to 0.050 mg/mL, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 262 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.3 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates for the niacin peak
Tailing factor: NMT 2.0 for the niacin peak
Relative standard deviation: NMT 2.0% for the niacin peak from replicate injections, *Standard solution*

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of the *Sample* taken:

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

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

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP 6-Hydroxynicotinic Acid RS
(see *Procedure 4*)
USP Niacin RS
USP Niacinamide RS

[NOTE—The previously dried Reference Standards may be stored in a desiccator over silica gel, protected from light.]

⟨451⟩ NITRITE TITRATION

The following general method is provided for the determination of most of the Pharmacopeial sulfonamide drugs and their dosage forms, as well as of other Pharmacopeial drugs for which nitrite titration is particularly suitable.

PROCEDURE

Accurately weigh about 500 mg in the case of a sulfonamide, or otherwise the quantity specified in the individual monograph, and transfer to a suitable open vessel. Add 20 mL of hydrochloric acid and 50 mL of water, stir until dissolved, cool to about 15°, and slowly titrate with 0.1 M sodium nitrite VS that previously has been standardized against USP Sulfanilamide RS. Determine the endpoint electrometrically, using suitable electrodes (platinum-calomel or platinum-platinum). Place the buret tip below the surface of the solution to eliminate air oxidation of the sodium nitrite, and stir the solution gently, using a magnetic stirrer, without pulling a vortex of air under the surface, maintaining the temperature at about 15°. The titration may be carried out manually, or by means of an automatic titrator. In performing it manually, add the titrant until the titration is within 1 mL of the endpoint, and then add it in 0.1-mL portions, allowing not less than 1 minute between additions. (The instrument needle deflects and then returns to approximately its original position until the endpoint is reached.)

The weight, in mg, of the substance to which each mL of 0.1 M sodium nitrite VS is equivalent is as stated in the individual monograph.

For the assay of Tablets of the sulfonamides or other drugs, reduce not less than 20 tablets to a fine powder, weigh accurately a portion of the powder, equivalent to about 500 mg if a sulfonamide, or the quantity of drug specified in the individual monograph, and proceed as directed in the foregoing, beginning with "transfer to a suitable open vessel."

For the assay of Injections and other liquid forms where the nitrite titration is specified, pipet a portion, equivalent to about 500 mg if a sulfonamide, or the quantity of drug specified in the individual monograph, into a suitable open vessel, and proceed as directed in the foregoing, beginning with "Add 20 mL of hydrochloric acid."

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sulfanilamide RS

⟨461⟩ NITROGEN DETERMINATION

Some alkaloids and other nitrogen-containing organic compounds fail to yield all of their nitrogen upon digestion with sulfuric acid; therefore these methods cannot be used for the determination of nitrogen in all organic compounds.

METHOD I

Nitrates and Nitrites Absent

Place about 1 g of the substance, accurately weighed, in a 500-mL Kjeldahl flask of hard borosilicate glass. The material to be tested, if solid or semisolid, may be wrapped in a sheet of nitrogen-free filter paper for convenience in transferring it to the flask. Add 10 g of powdered potassium sulfate or anhydrous sodium sulfate, 500 mg of powdered cupric sulfate, and 20 mL of sulfuric acid. Incline the flask at an angle of about 45°, and gently heat the mixture, keeping the temperature below the boiling point until frothing has ceased. Increase the heat until the acid boils briskly, and continue the heating until the solution has been clear green in color or almost colorless for 30 minutes. Allow to cool, add 150 mL of water, mix the contents of the flask, and again cool. Add cautiously 100 mL of sodium hydroxide solution (2 in 5), in such manner as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution. Immediately add a few pieces of granulated zinc, and without delay connect the flask to a Kjeldahl connecting bulb (trap), previously attached to a condenser, the delivery tube from

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which dips beneath the surface of 100 mL of boric acid solution (1 in 25) contained in a conical flask or a wide-mouth bottle of about 500-mL capacity. Mix the contents of the Kjeldahl flask by gentle rotation, and distill until about four-fifths of the contents of the flask has distilled over. Titrate with 0.5 N sulfuric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sulfuric acid VS is equivalent to 7.003 mg of nitrogen.

When the nitrogen content of the substance is known to be low, the 0.5 N sulfuric acid VS may be replaced by 0.1 N sulfuric acid VS. Each mL of 0.1 N sulfuric acid VS is equivalent to 1.401 mg of nitrogen.

Nitrates and Nitrites Present

Place a quantity of the substance, accurately weighed, corresponding to about 150 mg of nitrogen, in a 500-mL Kjeldahl flask of hard borosilicate glass, and add 25 mL of sulfuric acid in which 1 g of salicylic acid previously has been dissolved. Mix the contents of the flask, and allow the mixture to stand for 30 minutes with frequent shaking. To the mixture add 5 g of powdered sodium thiosulfate, again mix, then add 500 mg of powdered cupric sulfate, and proceed as directed under *Nitrates and Nitrites Absent*, beginning with "Incline the flask at an angle of about 45°."

When the nitrogen content of the substance is known to exceed 10%, 500 mg to 1 g of benzoic acid may be added, prior to digestion, to facilitate the decomposition of the substance.

METHOD II

Apparatus

Select an appropriate 300-mL Kjeldahl flask, from which the nitrogen is first liberated by acid digestion and then transferred quantitatively to the titration vessel by steam distillation.

Procedure

Place an accurately weighed or measured quantity of the material, equivalent to 2 to 3 mg of nitrogen, in the digestion flask of the apparatus. Add 1 g of a powdered mixture of potassium sulfate and cupric sulfate (10:1), and wash down any adhering material from the neck of the flask with a fine jet of water. Add 7 mL of sulfuric acid, allowing it to rinse down the wall of the flask, then, while swirling the flask, add 1 mL of 30 percent hydrogen peroxide cautiously down the side of the flask. (Do not add hydrogen peroxide during the digestion.)

Heat the flask over a free flame or an electric heater until the solution has a clear blue color and the sides of the flask are free from carbonaceous material. Cautiously add to the digestion mixture 70 mL of water, cool the solution, and arrange for steam distillation. Add through a funnel 30 mL of sodium hydroxide solution (2 in 5) in such manner as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution, rinse the funnel with 10 mL of water, tightly close the apparatus, and begin the distillation with steam immediately. Receive the distillate in 15 mL of boric acid solution (1 in 25), to which has been added 3 drops of methyl red-methylene blue TS and sufficient water to cover the end of the condensing tube. Continue the distillation until the distillate measures 80 to 100 mL. Remove the absorption flask, rinse the end of the condensing tube with a small quantity of water, and titrate the distillate with 0.01 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N sulfuric acid VS is equivalent to 140.1 µg of nitrogen.

When a quantity of material containing more than 2 to 3 mg of nitrogen is taken, 0.02 N or 0.1 N sulfuric acid may be employed, provided that at least 15 mL is required for the titration. If the total dry weight of material taken is greater than 100 mg, increase proportionately the quantities of sulfuric acid and sodium hydroxide.

⟨466⟩ ORDINARY IMPURITIES

This test, where called for in the individual monograph, is provided to evaluate the presence of ordinary impurities in official articles. Ordinary impurities are defined as those species in drug substances and/or drug products that have no significant, undesirable biological activity in the amounts present. These impurities may arise out of the synthesis, preparation, or degradation of compendial articles. In certain instances, impurities that pose a potential health risk may be detected. Because these impurities would not be individually identified by the strict use of this General Chapter, a separate evaluation may be necessary to ensure that the detected impurities fit the requirements set forth in the definition of Ordinary Impurities. Selections of tests and assays allow for anticipated amounts of impurities that are unobjectionable for the customary use of the article.

REPORTING AND SPECIFICATIONS

The value of 2.0%, unless otherwise specified in the individual monograph, was selected as the general limit for the total amount of ordinary impurities in monographs where documentation did not support adoption of other values.

Where a monograph sets limits on concomitant components and/or specified impurities/degradation products, these species are not to be included in the estimation of ordinary impurities unless so stated in the individual monograph. Concomitant components are defined as species characteristic of many drug substances that are not considered to be impurities in the Pharmacopeial sense. Examples of concomitant components are geometric and optical isomers (or racemates) and antibiotics that are mixtures. Any component that can be considered a toxic impurity because of significant undesirable biological effect is not considered to be a concomitant component.

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METHODOLOGY

Unless otherwise specified in an individual monograph, estimation of the amount and number of ordinary impurities is made by relative methods rather than by strict comparison to individual Reference Standards. Nonspecific detection of ordinary impurities is also consistent with this classification.

Typical evaluation methods used for ordinary impurities are thin-layer chromatographic (TLC) techniques. See *Chromatography* ⟨621⟩ for a general discussion of the thin-layer chromatographic technique. Tests for related substances or chromatographic purity may also be used to evaluate the presence of ordinary impurities. Other methods (e.g., HPLC, HPTLC, etc.) may also be used with adequate justification as an alternate method. Unless otherwise specified in the individual monograph, use the following method.

Test Solution

Prepare, in the solvent specified in the monograph, a solution of the substance under test having an accurately known final concentration of about 10 mg per mL. [NOTE—Heat or sonication may be used to dissolve the drug substance where use of such does not adversely affect the compound.]

Standard Solutions

Prepare, in the solvent specified in the monograph, solutions of the USP Reference Standard or designated substance having accurately known concentrations of 0.01 mg per mL, 0.05 mg per mL, 0.1 mg per mL, and 0.2 mg per mL.

[NOTE—Heat or sonication may be used to dissolve the drug substance where use of such does not adversely affect the compound.]

Procedure

Use a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, and the *Eluant* specified in the monograph. Apply equal volumes (20 µL) of the *Test Solution* and *Standard Solutions* to the plate, using a stream of nitrogen to dry the spots.

Allow the chromatogram to develop in a pre-equilibrated chamber until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and air-dry. View the plate using the visualization technique(s) specified. Locate any spots other than the principal spot in the chromatogram of the *Test Solution,* and determine their relative intensities by comparison with the chromatograms of the appropriate *Standard Solutions.* See discussion above with regard to reporting and specifying total ordinary impurities.

Change to read:

KEY FOR VISUALIZATION TECHNIQUES

1. Use UV light at 254 nm and at about 366 nm.
2. Use Iodoplatinate TS.

▲3. *Solution A*—Mix 850 mg of bismuth subnitrate with 40 mL of water and 10 mL of glacial acetic acid. *Solution B*—Dissolve 8 g of potassium iodide in 20 mL of water. Mix A and B together to obtain a Stock Solution which can be stored for several months in a dark bottle. Mix 10 mL of the Stock Solution with 20 mL of glacial acetic acid, and dilute with water to make 100 mL, to prepare the spray reagent.

4.▲ (ERR 1-Oct-2018) *Ninhydrin Spray*—Dissolve 200 mg of ninhydrin in 100 mL of alcohol. Heat the plate after spraying.

▲5.▲ (ERR 1-Oct-2018) *Acid Spray*—In an ice bath, add slowly and cautiously, with stirring, 10 mL of sulfuric acid to 90 mL of alcohol. Spray the plate, and heat until charred.

▲6.▲ (ERR 1-Oct-2018) *Acid–Dichromate Spray*—Add sufficient potassium dichromate to 100 mL of sulfuric acid to make a saturated solution. Spray the plate, and heat until charred.

▲7.▲ (ERR 1-Oct-2018) *Vanillin*—Dissolve 1 g of vanillin in 100 mL of sulfuric acid.

▲8.▲ (ERR 1-Oct-2018) *Chloramine T–Trichloroacetic Acid*—Mix 10 mL of a 3% aqueous solution of chloramine T with 40 mL of a 25% alcoholic solution of trichloroacetic acid. Prepare immediately before use.

▲9.▲ (ERR 1-Oct-2018) *Folin-C*—Add 10 g of sodium tungstate and 2.5 g of sodium molybdate to 70 mL of water, add 5 mL of 85% phosphoric acid and 10 mL of 36% hydrochloric acid, and reflux this solution for 10 hours.

▲10.▲ (ERR 1-Oct-2018) *$KMnO_4$*—Dissolve 100 mg of Potassium Permanganate in 100 mL of water.

▲11.▲ (ERR 1-Oct-2018) *DAB*—Mix 1 g of *p*-dimethylaminobenzaldehyde in 100 mL of 0.6 N hydrochloric acid.

▲12.▲ (ERR 1-Oct-2018) *DAC*—Mix 100 mg of *p*-dimethylaminocinnamaldehyde in 100 mL of 1 N hydrochloric acid.

▲13.▲ (ERR 1-Oct-2018) *Ferricyanide*—Mix equal volumes of a 1% ferric chloride solution and a 1% potassium ferricyanide solution. Use immediately.

▲14.▲ (ERR 1-Oct-2018) *Fast Blue B*—Reagent A—Dissolve 500 mg of Fast Blue B Salt in 100 mL of water.Reagent B—0.1 N sodium hydroxide.
Spray first with A, then with B.

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▲15.▲ (ERR 1-Oct-2018) *Alkaline Ferric Cyanide*—Dilute 1.5 mL of a 1% potassium ferricyanide solution with water to 20 mL, and add 10 mL of 15% sodium hydroxide solution.

▲16.▲ (ERR 1-Oct-2018) *Iodine Spray*—Prepare a 0.5% solution of iodine in chloroform.

▲17.▲ (ERR 1-Oct-2018) Expose the plate for 10 minutes to iodine vapors in a pre-equilibrated closed chamber, on the bottom of which there are iodine crystals.

▲18.▲ (ERR 1-Oct-2018) *Solution A*—Dissolve 0.5 g of potassium iodide in 50 mL of water. *Solution B*—Prepare a solution of 0.5 g of soluble starch in 50 mL of hot water.
Just prior to use, mix equal volumes of *Solution A* and *Solution B*.

▲19.▲ (ERR 1-Oct-2018) *PTSS*—Dissolve 20 g of *p*-toluenesulfonic acid in 100 mL of alcohol, spray the plate, dry for 15 minutes at 110°, and view under UV light at 366 nm.

▲20.▲ (ERR 1-Oct-2018) *o-Tolidine Spray*—Dissolve 160 mg of *o*-tolidine in 30 mL of glacial acetic acid, dilute with water to make 500 mL, add 1 g of potassium iodide, and mix until the potassium iodide has dissolved.

▲21.▲ (ERR 1-Oct-2018) Mix 3 mL of chloroplatinic acid solution (1 in 10) with 97 mL of water, followed by the addition of 100 mL of potassium iodide solution (6 in 100) to prepare the spray reagent.

▲22.▲ (ERR 1-Oct-2018) *Iodine–Methanol Spray*—Prepare a mixture of iodine TS and methanol (1:1).

⟨467⟩ RESIDUAL SOLVENTS

1. INTRODUCTION
2. CLASSIFICATION OF RESIDUAL SOLVENTS BY RISK ASSESSMENT
3. CONTROL STRATEGY
4. LIMITS OF RESIDUAL SOLVENTS
   4.1 Class 1: Solvents to Be Avoided
   4.2 Class 2: Solvents to Be Limited
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GLOSSARY
APPENDICES
   1. *Appendix 1: Residual Solvents Included in this General Chapter*
   2. *Appendix 2: Additional Background*
   3. *Appendix 3: Procedures for Establishing Exposure Limits*
USP Reference Standards ⟨11⟩

Change to read:

▲1. INTRODUCTION

For pharmacopeial purposes, residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacturing of drug substances, excipients, or dietary ingredients, or in the preparation of drug products or dietary supplement products. Appropriate selection of the solvent for the synthesis of a drug substance or an excipient may enhance the yield or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical element in the synthetic process and may not be completely removed by the manufacturing process. Because residual solvents do not provide therapeutic benefit, they should be removed, to the extent possible, to meet safety-based limits, ingredient and product specifications, good manufacturing practices, or other quality-based requirements.

The objective of this general chapter is to define acceptable amounts of residual solvents in pharmaceutical drug products and dietary supplement products for the safety of the patient. Tests for residual solvents are not generally mentioned in specific

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monographs because the solvents used may vary from one manufacturer to another; however, the limits to be applied must comply with those specified in this general chapter.

This chapter provides procedures for the analysis of residual solvents, although alternative validated methodologies may also be used to demonstrate compliance with the defined limits. For guidance on verification of *USP* procedures or validation of alternative methods for residual solvents, see *Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures* ⟨1467⟩. This chapter does not address solvents deliberately used as excipients, nor does it address solvates. The limits specified in this chapter do not apply directly to excipients, drug substances, or dietary ingredients except where specified in the individual monographs. However, residual solvent levels present in drug substances, excipients, and dietary ingredients may be used to demonstrate compliance as an integral part of the control strategy, thereby reducing or eliminating the need for analysis in the product (see *3. Control Strategy*).

Information on residual solvents in coating materials, colorants, flavors, capsules, and imprinting inks is generally not needed unless Class 1 solvents are used in the manufacture of these components.

Throughout this chapter, the term "likely to be present" (LTBP) refers to 1) solvents used or produced in the final manufacturing step; 2) solvents used or produced in earlier manufacturing steps that are not consistently removed by a validated process; and 3) solvents properly declared by a validated supplier of a drug substance, excipient, or dietary ingredient.

2. CLASSIFICATION OF RESIDUAL SOLVENTS BY RISK ASSESSMENT

Residual solvents assessed in this general chapter are listed in *Appendix 1* by common names and structures. USP is aligned with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline for Residual Solvents Q3C (R5) approach for the classification of residual solvents (see *Appendix 2* for additional details). These solvents were evaluated for their possible risk to human health and were placed into one of three classes based on their toxicity data and their environmental impact as shown in *Table 1*.

Table 1. Classification of Residual Solvents and Their Assessments

Residual Solvent Classes	Assessment
Class 1 (solvents to be avoided)	Known human carcinogens
	Strongly suspected human carcinogens
	Solvents particularly known to have ozone-depleting properties
Class 2 (solvents to be limited)	Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity
	Solvents suspected of other significant but reversible toxicities
Class 3 (solvents with low toxic potential)	Solvents with low toxic potential to humans; no health-based exposure limit is needed

The limits for Class 2 solvents are based on the toxicological permitted daily exposure (PDE), calculated as defined in *Appendix 3*, whereas Class 3 solvents are considered less toxic, and control to 50 mg/day or less for each of these solvents is acceptable without justification. The lists in this chapter are not exhaustive and are subject to revision. For solvents not listed, *Appendix 3* may be used to define PDE limits if sufficient toxicological data are available. A flow diagram for potential sources of residual solvents in pharmaceutical drug products and dietary supplements is shown in *Figure 1*.

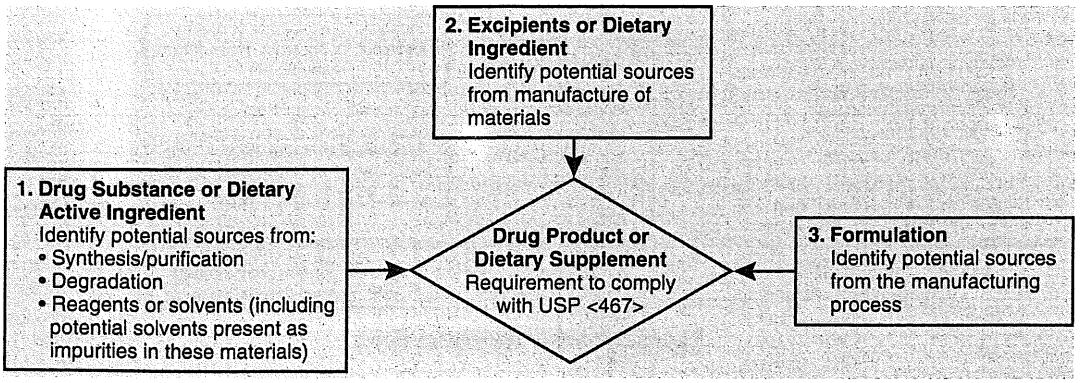


Figure 1. Potential sources of residual solvents in pharmaceutical drug products and dietary supplements.

There are three potential sources of residual solvents in the pharmaceutical drug products and dietary supplement products that should be considered (see *Figure 1*):

1. Drug substance or dietary active ingredient

   Potential solvent sources include their use or formation during the synthesis or purification of the drug substance; their presence in raw materials or reagents used in their synthesis; or degradation of the drug substance.
2. Excipients and/or dietary ingredient

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Potential solvent sources include their use or formation during the manufacture or purification of excipients.

3. Formulation

Potential solvent sources are associated with their use or formation during the official product manufacturing process.

The potential presence of other solvents as impurities in the solvents used must be taken into consideration in the assessment of solvents LTBP. Because of the high toxicity of benzene and other Class 1 solvents, all likely sources of these solvents must be considered. For example, potential sources of benzene may include its presence as an impurity in a solvent used in the manufacturing process; its use in the manufacture of starting material; or its production as a reaction by-product. In the event that the user has insufficient information to complete a thorough assessment of the potential sources of residual solvents (or as an alternative to performing this assessment), solvent screening may be used (see *8. Analytical Procedures for Class 1 and Class 2 Residual Solvents*).

Pharmaceutical drug products and dietary supplement products should contain no higher levels of residual solvents than can be supported by safety data. All solvents included in this general chapter are listed in *Appendix 1*. Those solvents that show toxicity of special concern or carcinogenicity, and/or atmospheric ozone-depletion effects (Class 1, see *Table 2*), should be avoided in the production of drug substances, dietary supplement ingredients, excipients, pharmaceutical drug products, and dietary supplement products unless their use can be strongly justified in a risk-benefit assessment. Those solvents associated with less severe but still significant toxicity (Class 2, see *Table 3*) should be limited to protect patients from potential adverse effects. Whenever it is practicable, less toxic solvents (Class 3, see *Table 4*) should be used. For the purposes of this pharmacopeia, when a manufacturer has received approval from a regulatory authority for the use of a solvent not currently listed in this chapter, or for a level of a listed solvent higher than the limit currently given in this chapter, it is the responsibility of that manufacturer to notify USP of the identity and level of the solvent, and the appropriate test procedure. USP will then address the information in the individual monograph. A new solvent or revised limit that has been approved through the ICH process will be added to the appropriate list in this general chapter. See *Appendix 2* for additional background information related to residual solvents.

3. CONTROL STRATEGY

The user may choose to demonstrate compliance with this chapter by analysis for residual solvents in the official product or by analysis of the official substances. These control strategy options are presented in *Figure 2*.

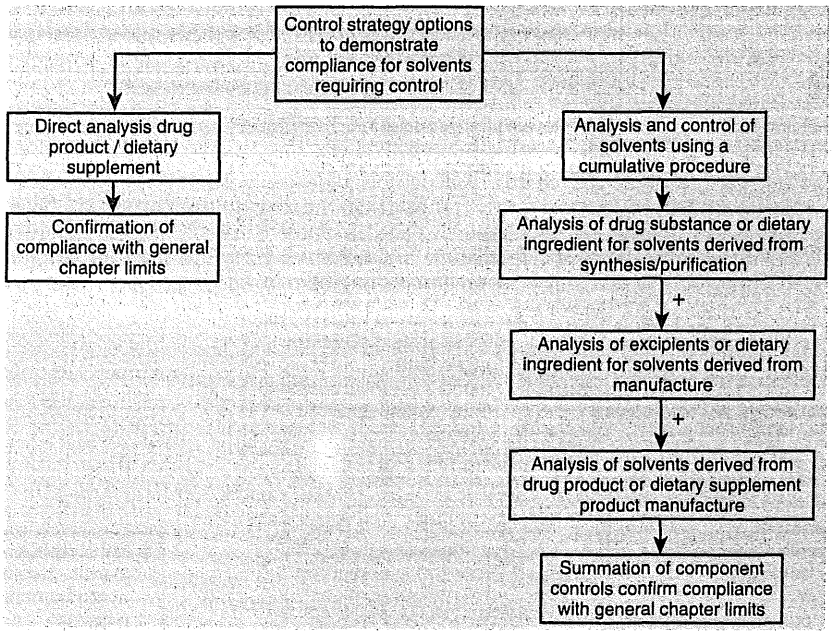


Figure 2. Control strategy options.

Compliance with the chapter requires that all solvents LTBP comply with the control limits.
The following options are to be considered when designing the control strategy.

1. **Testing the official product:** This approach is acceptable in all cases.
2. **Analysis of the official substances and using a cumulative approach to determine the solvent content in the official product:**
   A. **If no solvent(s) are used during the manufacture of the official product,** use the cumulative approach to determine the solvent content in the active pharmaceutical ingredient (API) and excipients or dietary ingredients to calculate solvent levels in the official product.

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B. **If solvent(s) are used during the manufacture of the official product,** use the cumulative approach as described in (a) above. Then, for each solvent used in the manufacture, determine the content in the finished product or after the manufacturing step where the solvent(s) were used.

In all cases, the solvent levels in the final official product must not exceed the limits defined in the chapter.

4. LIMITS OF RESIDUAL SOLVENTS

4.1 Class 1: Solvents to Be Avoided

Class 1 residual solvents (*Table 2*) should not be used in the manufacture of drug substances, excipients, dietary ingredients, or official products because of their unacceptable toxicities or deleterious environmental effects. However, if their use in order to produce an official product with a significant therapeutic advance is unavoidable, their levels should be restricted as shown in *Table 2*, unless otherwise stated in the individual monograph. The solvent 1,1,1-trichloroethane is included in *Table 2* because it is a severe environmental hazard. The stated limit of 1500 ppm is based on a review of safety data.

The procedures described in *7. Identification, Control, and Quantification of Residual Solvents* in this general chapter are to be applied wherever possible. Otherwise an appropriate validated procedure is to be used.

Table 2. Control Limits for Class 1 Residual Solvents in Official Products: Solvents to Be Avoided

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

4.2 Class 2: Solvents to Be Limited

Class 2 residual solvents (*Table 3*) should be limited in drug substances, excipients, dietary ingredients, and official products because of the inherent toxicities of these residual solvents. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The method used to establish PDEs for residual solvents is presented in *Appendix 3*.

Table 3. Class 2 Residual Solvents in Official Products

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600

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Table 3. Class 2 Residual Solvents in Official Products *(continued)*

Solvent	PDE (mg/day)	Concentration Limit (ppm)
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
Trichloroethylene	0.8	80
Xylene[a]	21.7	2170

[a] Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

4.3 Class 3: Solvents with Low Toxic Potential

Class 3 solvents (*Table 4*) are regarded as less toxic and of lower risk to human health than Class 1 and Class 2 residual solvents. Class 3 includes no solvent known to be a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the residual solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies.

It is considered that amounts of these residual solvents of 50 mg/day or less of each solvent (corresponding to 5000 ppm or 0.5% w/w in *Option 1*, which is described below) would be acceptable for each solvent without justification. Higher amounts may also be acceptable, provided that they are realistic in relation to manufacturing capability and good manufacturing practice. If a Class 3 solvent limit in an individual monograph is greater than 0.5%, that residual solvent should be identified and quantified. The procedures described in *7. Identification, Control, and Quantification of Residual Solvents*, with appropriate modifications to the standard solutions, are to be applied wherever possible. Otherwise, an appropriate validated procedure is to be used.

Table 4. Class 3 Residual Solvents in Official Products

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	Methylisobutylketone
Dimethyl sulfoxide	2-Methyl-1-propanol
Ethanol	Pentane
Ethyl acetate	1-Pentanol
Ethyl ether	1-Propanol
Ethyl formate	2-Propanol
Formic acid	Propyl acetate

5. OPTIONS FOR DESCRIBING LIMITS OF CLASS 2 AND CLASS 3 RESIDUAL SOLVENTS

This chapter provides two options for establishing compliance. Both *Option 1* and *Option 2* are based on the PDE.

5.1 Option 1—Concentration Limit

The concentration limits in ppm stated in *Table 3* for Class 2 solvents and the general requirement for Class 3 (5000 ppm, equivalent to 0.5% w/w) are used. The values for Class 2 solvents were calculated using the equation below by assuming a product weight of 10 g administered daily.

$$\text{Concentration (ppm)} = (1000\ \mu g/mg \times \text{PDE})/\text{dose}$$

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Here, PDE is given in terms of milligrams per day (mg/day), and dose is given in grams per day (g/day). These limits are considered acceptable for all drug substances, excipients, dietary ingredients, and official products. Therefore, *Option 1* may be applied if the daily amount is not known or does not exceed 10 g. If all official substances (drug substances, excipients, and/or dietary ingredients) in a formulation or dietary supplement meet the limits given in *Option 1*, these components may be used in any proportions. No further calculation is necessary, provided that the daily amount does not exceed 10 g. Products that are administered in doses (daily intake for dietary supplements) greater than 10 g/day are to be considered under *Option 2*.

5.2 Option 2—Summation of Components Content

Option 2 uses the PDE and the actual maximum daily amount of the product to calculate solvent exposure and assess compliance.

Option 2 must be used to demonstrate compliance with this chapter where the maximum daily dose of the official product exceeds 10 g/day or where at least one component in the formulation exceeds the *Option 1* limits.

These limits are applied by summing the amounts of a residual solvent present in each of the components of the official product. The contribution of each solvent per day should result in a total amount that does not exceed the PDE.

Consider the example of the application of *Option 1* and *Option 2* limits to acetonitrile concentration in an official product. The PDE for acetonitrile is 4.1 mg/day, thus the *Option 1* limit is 410 ppm. The maximum administered daily mass of an official product is 5.0 g, and the official product contains two excipients. Assuming that there is no other source of acetonitrile in the manufacturing process, the calculated official product content of acetonitrile and the daily exposure are given in *Table 5*.

Table 5. Example of Meeting the Requirement for Acetonitrile as per Option 2

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	400	0.36
Excipient 2	3.8	800	3.04
Official product	5.0	728	3.64

Excipient 1 meets the *Option 1* limit, but the official substance, *Excipient 2*, and *Official product* do not meet the *Option 1* limit. Nevertheless, the product meets the *Option 2* limit of 4.1 mg/day and thus conforms to the recommendations in this chapter.

Consider another example using acetonitrile as a residual solvent. The maximum administered daily mass of an official product is 5.0 g, and the official product contains two excipients. Assuming no other source of acetonitrile in the manufacturing process, the *Official product's* content of acetonitrile and the maximum content of residual acetonitrile are given in *Table 6*.

Table 6. Example of Failing the Requirement for Acetonitrile as per Option 2

Component	Amount in Formulation (g)	Acetonitrile Content (ppm)	Daily Exposure (mg)
Drug substance	0.3	800	0.24
Excipient 1	0.9	2000	1.8
Excipient 2	3.8	800	3.04
Official product	5.0	1016	5.08

In this example, the product meets neither the *Option 1* nor the *Option 2* limit according to the summation. Compliance with the chapter may still be demonstrated via analysis of the *Official product* as it is possible that the formulation process may have reduced the level of acetonitrile below the calculated content. If the level of acetonitrile was not reduced during formulation to the allowed limit, then the manufacturer of the *Official product* should take other steps to reduce the amount of acetonitrile in the official product. If the manufacturer receives approval from the regulatory authority for such a higher level of residual solvent, it is the responsibility of that manufacturer to notify USP regarding the identity of this solvent and the approved residual solvent limit in the article. USP will then address this topic in the individual monograph.

6. REPORTING LEVELS OF RESIDUAL SOLVENTS

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in drug substances and excipients to meet the criteria of this chapter. The following statements are given as acceptable examples of the information that could be provided from a supplier of drug substances or excipients to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

- If Class 1 solvents are present, they should be identified and quantified.

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- Only Class 2 solvents X, Y,... are LTBP. All are below the *Option 1* limit. (Here the supplier would name the Class 2 solvents represented by X, Y,...)
- Only Class 2 solvents X, Y,..., and Class 3 solvents are LTBP. Residual Class 2 solvents are below the *Option 1* limit and residual Class 3 solvents are below 0.5% each.
- Only Class 3 solvents are LTBP. Loss on drying (LOD) is not greater than 0.5%.
- If only Class 3 solvents are LTBP and LOD is more than 0.5%, they should be identified and quantified.
- If solvents of Class 2 or Class 3 are present at greater than their *Option 1* limits or 0.5%, respectively, they should be identified and quantified to allow compliance with *Option 2*.

The term LTBP as used in the above examples refers to the solvent used or produced in the final manufacturing step and to solvents used or produced in earlier manufacturing steps and not removed consistently by a validated process.

7. IDENTIFICATION, CONTROL, AND QUANTIFICATION OF RESIDUAL SOLVENTS

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. The control strategy may involve analysis of official products, active ingredients, excipients, and dietary ingredients. In some cases the sample does not dissolve completely. In such cases it may be acceptable to pulverize the sample into a fine powder carefully, avoiding the generation of frictional heat and in a manner that minimizes the loss of volatile solvents. This technique ensures that any residual solvent that may be present can be released. It should be performed as fast as possible immediately prior to dispersion in the sample solvent, to minimize the loss of volatile solvents during the procedure.

[NOTE—The organic-free water specified in the following procedures produces no significantly interfering peaks when chromatographed.]

7.1 Class 1 and Class 2 Residual Solvents

This chapter describes analytical procedures (*Procedures A, B,* and *C*) for evaluating the levels of all Class 1 and the majority of Class 2 residual solvents. For each test matrix, verification is needed to demonstrate reliability of the compendial procedure, as described in ⟨1467⟩.

When the solvents that are present or potentially present in the sample are known, they can be determined using a limit test such as *Procedure A* or *Procedure B*, or by a quantitative test, such as *Procedure C*.

When the solvents LTBP are not known, use the screening tests of *Procedure A* and *Procedure B* as required. *Procedure A* and *Procedure B* can separate most of the solvents listed in this chapter. Additionally, *Figure 3* can be used as a decision tree to demonstrate compliance.

In a screening test, if the article does not meet the acceptance criteria of *Procedure A*, then *Procedure B* is to be used to demonstrate compliance. If the article does not meet the criteria using *Procedure A* and *Procedure B*, then *Procedure C* must be used to quantify the residual solvent(s) present in the article.

When the information about solvents LTBP in the material is known, the system suitability requirements only need to be demonstrated for the solvents expected to be present. Several residual solvents listed in this chapter are not detected at the limit concentration using the analytical procedure described below. Those solvents include formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone, sulfolane, *N,N*-dimethylacetamide, and *N,N*-dimethylformamide. Those solvents should be determined using an alternative method that has been appropriately validated to demonstrate that the method is suitable for its intended purpose, i.e., quantitative determination of residual solvent or limit tests for them (see *Category II* in *Validation of Compendial Procedures* ⟨1225⟩, *Table 2*, which provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic). Chapter ⟨1467⟩ provides additional guidance on the specific validation criteria for alternative methods used for identification and quantification of residual solvents.

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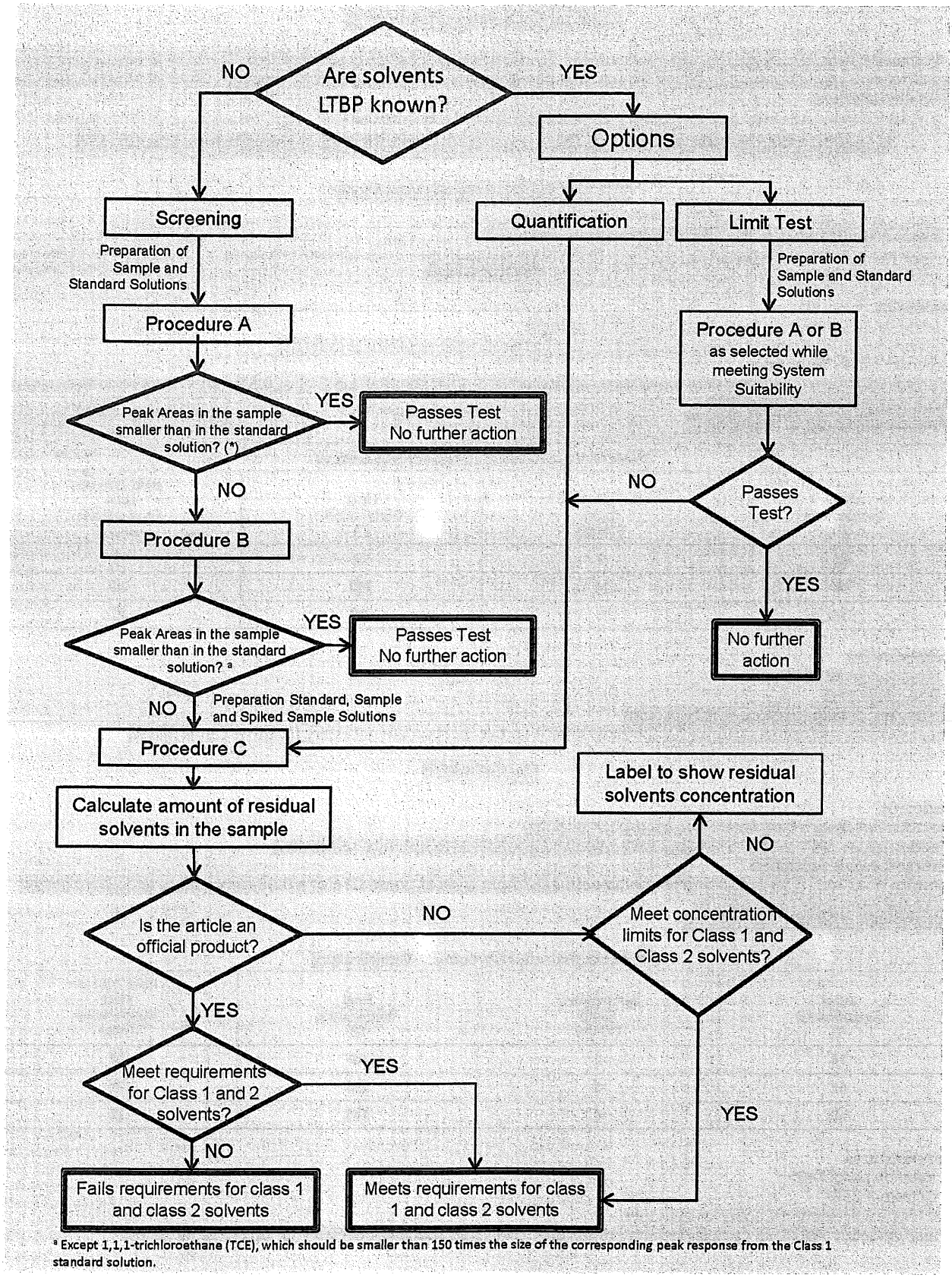


Figure 3. Determination of residual solvents and decision tree using *Procedures A, B,* and *C*.

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7.2 Class 3 Residual Solvents

Procedures A and *B* can separate most solvents in Classes 1, 2, and 3. However, the procedures have not been validated for Class 3 solvents, and appropriate validation will be required (see the entry for alternative methods under *7.1 Class 1 and Class 2 Residual Solvents*).

8. ANALYTICAL PROCEDURES FOR CLASS 1 AND CLASS 2 RESIDUAL SOLVENTS

8.1 Chromatographic Systems

(See *Chromatography* ⟨621⟩, *System Suitability* and *Figure 4*.)

PROCEDURE A

Mode: GC
Injector: Headspace (see *Table 9* for headspace settings)
Injection type: Split ratio, 1:5. [NOTE—Split ratio can be modified to optimize sensitivity.]
Detector: Flame ionization
Column: 0.32-mm × 30-m capillary fused silica coated with 1.8-µm layer of phase G43 or 0.53-mm × 30-m wide-bore coated with a 3.0-µm layer of phase G43
Column temperature: See *Table 7*.

Table 7. Procedure A Column Temperature

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	10	240	—
240	0	240	20

Temperatures
Injection port: 140°
Detector: 250°
Carrier gas: Helium, nitrogen, or hydrogen
Linear velocity: About 35 cm/s for helium (adjustment and verification are necessary for other carrier gases)

PROCEDURE B

Mode: GC
Injector: Headspace (see *Table 9* for headspace settings)
Injection type: Split ratio, 1:5. [NOTE—Split ratio can be modified to optimize sensitivity.]
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica coated with a 0.25-µm layer of phase G16 or a 0.53-mm × 30-m wide-bore coated with a 0.25-µm layer of phase G16
Column temperature: See *Table 8*.

Table 8. Procedure B Column Temperature

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	20
50	6	165	—
165	0	165	20

Temperatures
Injection port: 140°
Detector: 250°
Carrier gas: Helium, nitrogen, or hydrogen
Linear velocity: About 35 cm/s for helium (adjustment and verification are necessary for other carrier gases)

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PROCEDURE C

Use the chromatographic conditions, from either *Procedure A* or *Procedure B*, that provide the best performance for the solvent(s) being tested.

HEADSPACE PARAMETER SETTINGS

There are three general sets of headspace operating parameters that are used for *Procedures A* and *B* (*Table 9*).

Table 9. Headspace Operating Parameters

Procedure Parameters	Headspace Instrumental Setting		
	Set 1	Set 2	Set 3
Equilibration temperature (°)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°) [if appropriate][a]	85	110	105
Syringe temperature (°) [if appropriate]	80–90	105–115	80–90
Carrier gas: helium, nitrogen,[b] or hydrogen[b] at an appropriate pressure[b]	—	—	—
Pressurization time(s) (sec) [if appropriate]	≥60	≥60	≥60
Injection volume (mL)[c]	1	1	1

[a] It is recommended to increase the temperature of the transfer line between runs to eliminate any potential condensation of solvents.
[b] The use of nitrogen or hydrogen as the carrier gas requires adjustment and verification.
[c] Injection volume may be expressed in terms of the injection time.

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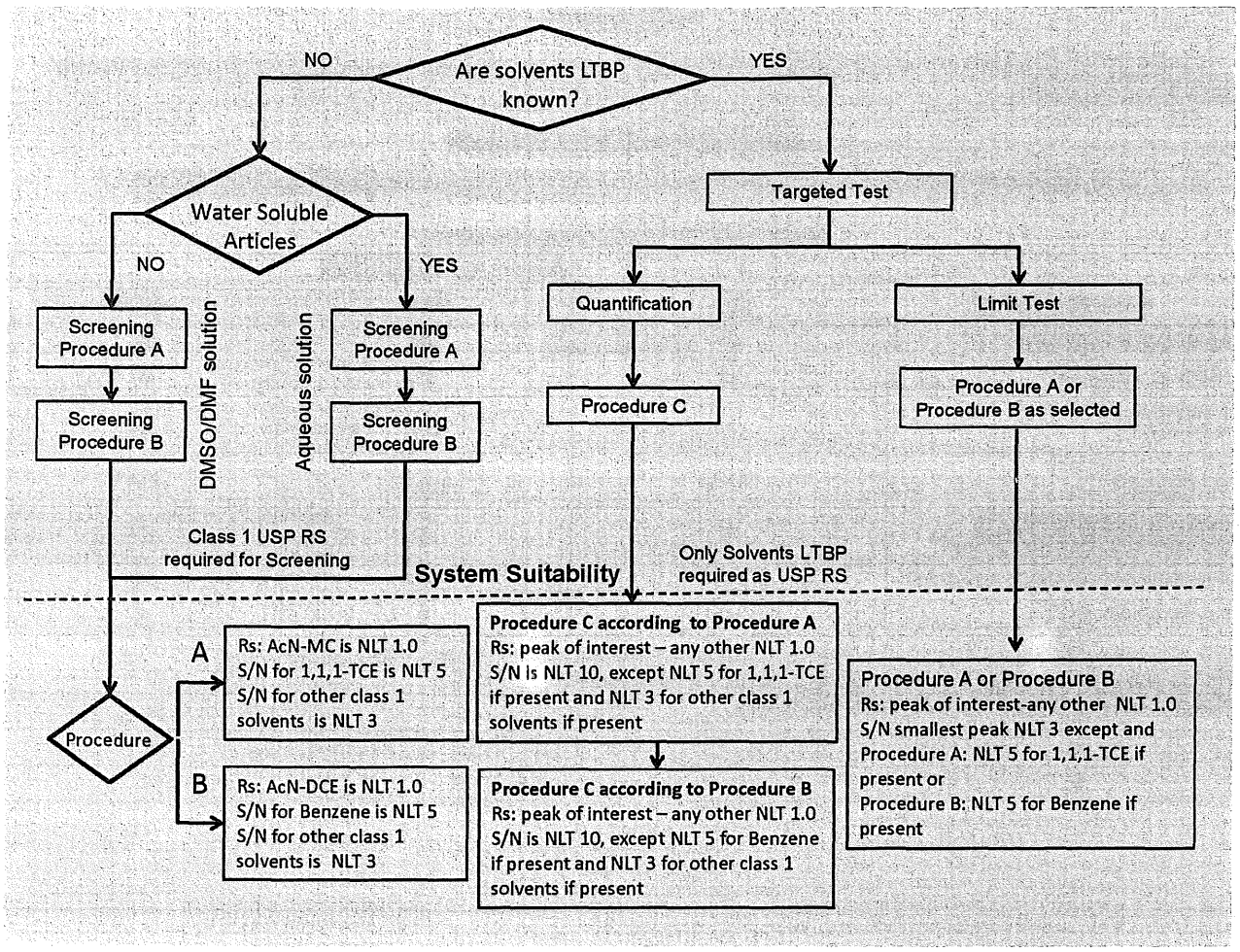


Figure 4. System suitability requirements based on the method of choice and previous knowledge about solvents LTBP. The acceptance criteria are based on the solvents LTBP (AcN: acetonitrile; 1,1,1-TCE: 1,1,1-trichloroethane; DCE: *cis*-dichloroethene; MC: methylene chloride).

8.2 Screening of Water-Soluble Articles

STOCK SOLUTIONS

Class 1 standard stock solution: [NOTE—When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.] Transfer 1.0 mL of USP Residual Solvents Mixture—Class 1 RS to a 100-mL volumetric flask containing about 9 mL of dimethylsulfoxide, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing about 50 mL of water, dilute with water to volume, and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask containing about 50 mL of water, dilute with water to volume, and mix.
Class 2 mixture A standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture A RS to a 100-mL volumetric flask, dilute with water to volume, and mix.
Class 2 mixture B standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture B RS to a 100-mL volumetric flask, dilute with water to volume, and mix.
Sample stock solution: Transfer about 250 mg of the article under test, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

SYSTEM SUITABILITY SOLUTION

Class 1 system suitability solution: Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial, add 5.0 mL of *Sample stock solution*, apply the stopper, cap, and mix.

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STANDARD SOLUTIONS

Class 1 standard solution: [NOTE—When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.] Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial containing 5.0 mL of water. Apply the stopper, cap, and mix.
Class 2 mixture A standard solution: Transfer 1.0 mL of *Class 2 mixture A standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.
Class 2 mixture B standard solution: Transfer 5.0 mL of *Class 2 mixture B standard stock solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

SAMPLE SOLUTION

Sample solution: Transfer 5.0 mL of *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of water, apply the stopper, cap, and mix.

PROCEDURE A

Analysis: Using *Procedure A Chromatographic System*, separately inject (using one of the headspace operating parameter sets described in *Table 9*) equal volumes of headspace of the *Class 1 standard solution*, *Class 1 system suitability solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements (see *Figure 4*)**:** The signal-to-noise (S/N) ratio for 1,1,1-trichloroethane in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3, and the resolution between acetonitrile and methylene chloride in the *Class 2 mixture A standard solution* is NLT 1.0.
Acceptance criteria: If a response of any peak, other than the peak for 1,1,1-TCE, in the *Sample solution* is greater than or equal to a corresponding peak in either the *Class 1 standard solution*, or either *Class 2 mixture A standard solution* or *Class 2 mixture B standard solution*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 standard solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

PROCEDURE B

Analysis: Using *Procedure B Chromatographic System*, separately inject (using one of the headspace operating parameter sets described in *Table 9*) equal volumes of headspace of the *Class 1 standard solution*, *Class 1 system suitability solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements (see *Figure 4*)**:** The S/N ratio for benzene in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3, and the resolution between acetonitrile and *cis*-dichloroethene in the *Class 2 mixture A standard solution* is NLT 1.0.
Acceptance criteria: If the response(s) in the *Sample solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to corresponding peak(s) in either the *Class 1 standard solution*, or either *Class 2 mixture A standard solution* or *Class 2 mixture B standard solution*, proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

8.3 Screening of Water-Insoluble Articles

STOCK SOLUTIONS

[NOTE—Dimethyl sulfoxide may be substituted as an alternative solvent to dimethylformamide. When transferring solutions, place the tip of the pipet just below the surface of the liquid and mix.]
Class 1 standard stock solution: Transfer 1.0 mL of USP Residual Solvents Mixture—Class 1 RS to a 100-mL volumetric flask previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, previously filled with about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix (reserve a portion of this solution for the *Class 1 system suitability solution*). Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.
Class 2 mixture A standard stock solution: Transfer 1.0 mL of USP Residual Solvents Class 2—Mixture A RS to a 100-mL volumetric flask containing about 80 mL of dimethylformamide, dilute with dimethylformamide to volume, and mix.
Class 2 mixture B standard stock solution: Transfer 0.5 mL of USP Residual Solvents Class 2—Mixture B RS to a 10-mL volumetric flask, dilute with dimethylformamide to volume, and mix.
Sample stock solution: Transfer about 500 mg of the article under test, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix.

SYSTEM SUITABILITY SOLUTION

Class 1 system suitability solution: Mix 5 mL of *Sample stock solution* with 0.5 mL of the intermediate dilution reserved from *Class 1 standard stock solution*. Transfer 1 mL of this solution to an appropriate headspace vial containing 5 mL of water. Apply the stopper, cap, and mix.

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STANDARD SOLUTIONS

Class 1 standard solution: Transfer 1.0 mL of *Class 1 standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.
Class 2 mixture A standard solution: Transfer 1.0 mL of *Class 2 mixture A standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.
Class 2 mixture B standard solution: Transfer 1.0 mL of *Class 2 mixture B standard stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

SAMPLE SOLUTION

Sample solution: Transfer 1.0 mL of *Sample stock solution* to an appropriate headspace vial containing 5.0 mL of water, apply the stopper, cap, and mix.

PROCEDURE A

Analysis: Proceed as directed for *Procedure A* in *8.2 Screening of Water-Soluble Articles* with a split ratio of 1:3. [NOTE—The split ratio can be modified in order to optimize sensitivity.] Separately inject (using one of the headspace operating parameter sets described in *Table 9* with a recommended vial pressure of 10 psi) equal volumes of headspace of the *Class 1 standard solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.
System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3; and the resolution between acetonitrile and methylene chloride in the *Class 2 mixture A standard solution* is NLT 1.0.
Acceptance criteria: If a response of any peak, other than the peak for 1,1,1-TCE in the *Sample solution* is greater than or equal to a corresponding peak in either the *Class 1 standard solution*, or either *Class 2 mixture A standard solution* or *Class 2 mixture B standard solution*, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the *Class 1 standard solution*, proceed to *Procedure B* to verify the identity of the peak; otherwise the article meets the requirements of this test.

PROCEDURE B

Analysis: Proceed as directed for *Procedure B* in *8.2 Screening of Water-Soluble Articles* with a split ratio of 1:3. Separately inject (using one of the headspace operating parameter sets described in *Table 9* with a recommended vial pressure of 10 psi) equal volumes of headspace of the *Class 1 standard solution*, *Class 2 mixture A standard solution*, *Class 2 mixture B standard solution*, *Sample solution*, and *Class 1 system suitability solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements (see *Figure 4*): The S/N ratio for benzene in the *Class 1 standard solution* is NLT 5. The S/N ratio of each peak in the *Class 1 system suitability solution* is NLT 3; and the resolution between acetonitrile and *cis*-dichloroethene in the *Class 2 mixture A standard solution* is NLT 1.0.
Acceptance criteria: If the peak response(s) in the *Sample solution* of the peak(s) identified in *Procedure A* is/are greater than or equal to corresponding peak(s) in either the *Class 1 standard solution*, or either *Class 2 mixture A standard solution* or *Class 2 mixture B standard solution*, proceed to *Procedure C* to quantify the peak(s); otherwise the article meets the requirements of this test.

8.4 Limit Tests When Solvents LTBP Are Known

SOLUTIONS

Prepare the standard solution and the *Sample solution* as directed in *8.2 Screening of Water-Soluble Articles* or *8.3 Screening of Water-Insoluble Articles* according to the procedure chosen based on the sample solubility. Use USP Reference Standards for those solvents LTBP to prepare the standard solution.

When solvents LTBP are known, only the standards of those solvents LTBP are needed, and determination may be done using a limit test or a quantitative test. Only quantitative tests can be used for *Option 2*.
System suitability solution: Prepare the *System suitability solution* according to the solubility of the sample for screening for water-soluble or water-insoluble articles at the concentration of the standard solutions there. Use only those solvents LTBP.

PROCEDURE A

Analysis (limit test): Separately inject (using one of the headspace operating parameter sets described in *Table 9*) equal volumes of headspace of the *System suitability solution*, *Standard solution*, and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements (see *Figure 4*): The S/N ratio for 1,1,1-trichloroethane, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.
Acceptance criteria: If the peak response of every peak of interest in the *Sample solution* is smaller than the corresponding peak in the *Standard solution*, the article meets the requirements of this test.

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PROCEDURE B

Analysis (limit test): Separately inject (following one of the headspace operating parameter sets described in *Table 9*) equal volumes of headspace of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements (see *Figure 4*): The S/N ratio for benzene, if present, is NLT 5. The S/N ratio is NLT 3 for any other solvent. If there is more than one solvent LTBP, the resolution between a peak of interest and any other peak is NLT 1.0.
Acceptance criteria: If the peak response of every peak of interest in the *Sample solution* is smaller than the corresponding peak in the *Standard solution*, the article meets the requirements of this test.

8.5 Quantitative Tests—Procedure C

QUANTIFICATION FOR WATER-SOLUBLE ARTICLES

(Use the requirement for *General* or *Targeted test*, as applicable.)
Sample stock solution: Prepare a *Sample stock solution* as directed in *8.2 Screening of Water-Soluble Articles, Stock Solutions*. Only the Reference Standards for those solvents identified by *Procedure A* or *Procedure B* or LTBP need to be used.
Standard stock solution: [NOTE—Prepare a separate *Standard stock solution* for each solvent LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*. For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 standard stock solution*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak LTBP or identified and verified by *Procedure A* and *Procedure B* to a suitable container, and dilute quantitatively and stepwise, if necessary, with water to obtain a solution with a final concentration of 1/20 of the value stated in *Table 2* or *Table 3* under *Concentration Limit*.
Standard solution: Transfer 1.0 mL of *Standard stock solution* to an appropriate headspace vial, add 5.0 mL of water, apply the stopper, cap, and mix.
Sample solution: Prepare as directed in *8.2 Screening of Water-Soluble Articles, Sample Solution*.
Spiked sample solution: [NOTE—Prepare a separate *Spiked sample solution* for each peak LTBP or potentially LTBP or identified and verified by *Procedure A* and *Procedure B*.] Transfer 5.0 mL of each *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of the *Standard stock solution*, apply the stopper, cap, and mix. The use of a vial containing the sample spiked with multiple standards is permitted, provided that the procedure is validated accordingly.
Analysis: Proceed as directed for *Procedure A*.
[NOTE—If the performance of *Procedure B* is better than that shown by *Procedure A*, then *Procedure B* may be used for quantification.]
Separately inject (using one of the headspace operating parameter sets described in *Table 9* with a recommended vial pressure of 10 psi) equal volumes of headspace of the *Standard solution*, *Sample solution*, and *Spiked sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest.
System suitability requirements for Procedure A (see *Figure 4*)
General: Same as in *8.2 Screening of Water-Soluble Articles, Procedure A*.
Targeted test: The S/N ratio is NLT 10 for any solvent except NLT 5 for 1,1,1-trichloroethane and NLT 3 for any other Class 1 solvent, if present. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.
System suitability requirements for Procedure B (see *Figure 4*)
General: Same as in *8.2 Screening of Water-Soluble Articles, Procedure B*.
Targeted test: The S/N ratio is NLT 10 for any solvent except NLT 5 for benzene and NLT 3 for any other Class 1 solvent, if present. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.
Calculate the amount, in ppm, of each residual solvent found in the article under test:

$$\text{Result} = 5(C/W)[r_U/(r_{ST} - r_U)]$$

C = concentration of the appropriate USP Reference Standard in the *Standard stock solution* (µg/mL)
W = weight of the article under test taken to prepare the *Sample stock solution* (g)
r_U = peak responses of each residual solvent obtained from the *Sample solution*
r_{ST} = peak responses of each residual solvent obtained from the *Spiked sample solution*

QUANTIFICATION FOR WATER-INSOLUBLE ARTICLES

(Use the requirement for *General* or *Targeted test* as applicable.)
Sample stock solution: Prepare a *Sample stock solution* as directed in *8.3 Screening of Water-Insoluble Articles, Stock Solutions*. Only the Reference Standard for those solvents identified by *Procedure A* or *Procedure B* or LTBP need to be used.
Standard stock solution: [NOTE—Prepare a separate *Standard stock solution* for each solvent LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*. For the Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under *Class 1 standard stock solution*.] Transfer an accurately measured volume of each individual USP Reference Standard corresponding to each residual solvent peak LTBP or identified and verified by *Procedure A* and *Procedure B* to a suitable container, and dilute quantitatively and stepwise, if necessary, with water to obtain a solution with a final concentration of 1/20 of the value stated in *Table 2* or *Table 3* under *Concentration Limit*.
Standard solution: Transfer 1.0 mL of *Standard stock solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.

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Sample solution: Transfer 1.0 mL of *Sample stock solution* to an appropriate headspace vial, containing 5.0 mL of water, apply the stopper, cap, and mix.
Spiked sample solution: [NOTE—Prepare a separate *Spiked sample solution* for each peak LTBP or potentially LTBP, or identified and verified by *Procedure A* and *Procedure B*.] Transfer 1.0 mL of each *Sample stock solution* to an appropriate headspace vial, add 1.0 mL of *Standard stock solution* and 4.0 mL of water, and apply the stopper, cap, and mix. The use of a vial containing the sample spiked with multiple standards is permitted, provided that the procedure is validated accordingly.
Analysis: Proceed as directed for *Procedure A*.
[NOTE—If the performance of *Procedure B* is better than that shown by *Procedure A*, then *Procedure B* may be used for quantification.]
Separately inject (using one of the headspace operating parameter sets described in *Table 9* with a recommended vial pressure of 10 psi) equal volumes of headspace of the *Standard solution*, *Sample solution*, and *Spiked sample solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks of interest. If Class 1 solvents are LTBP, also inject the *Class 1 system suitability solution*.
System suitability requirements for Procedure A (see *Figure 4*):
General: Same as in *8.3 Screening of Water-Insoluble Articles, Procedure A*.
Targeted test: The S/N ratio is NLT 10 for any solvent except NLT 5 for 1,1,1-trichloroethane and NLT 3 for any other Class 1 solvent, if present. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.
System suitability requirements for Procedure B (see *Figure 4*):
General: Same as in *8.3 Screening of Water-Insoluble Articles, Procedure B*.
Targeted test: The S/N ratio is NLT 10 for any solvent except NLT 5 for benzene and NLT 3 for any other Class 1 solvent, if present. If there is more than one solvent LTBP, the resolution between a peak of interest and any adjacent peak is NLT 1.0.
Calculate the amount, in ppm, of each residual solvent found in the article under test:

$$\text{Result} = 10(C/W)[r_U/(r_{ST} - r_U)]$$

C = concentration of the appropriate USP Reference Standard in the *Standard stock solution* (µg/mL)
W = weight of the article under test taken to prepare the *Sample stock solution* (g)
r_U = peak responses of each residual solvent obtained from the *Sample solution*
r_{ST} = peak responses of each residual solvent obtained from the *Spiked sample solution*

9. ANALYTICAL PROCEDURES FOR CLASS 3 RESIDUAL SOLVENTS

If only Class 3 solvents are present, and the monograph for the article under test contains an LOD procedure specifying an upper limit of NMT 0.5% (per *Option 1* in this chapter), then the level of residual solvent can be determined as directed in *Loss on Drying* ⟨731⟩. If Class 3 solvents are present with other solvents, e.g., Class 1 or Class 2 solvents, or if there is no LOD procedure in the monograph for the article under test, or if the limit for Class 3 solvent(s) in an individual monograph is greater than 50 mg/day (corresponding to 5000 ppm or 0.5% under *Option 1*), then the Class 3 residual solvent or solvents present in the article under test should be identified and quantified. In that case, the procedures described in this chapter, with appropriate adjustments, are to be applied wherever possible and USP Reference Standards, where available, should be used in these procedures. Such procedures should be properly validated as described in ⟨1467⟩. If Class 1 or Class 2 solvents are present and LOD is less than 0.5%, then identification of Class 3 solvents is not required and only Class 1 and Class 2 solvents should be identified and quantified. See *Figure 5*.

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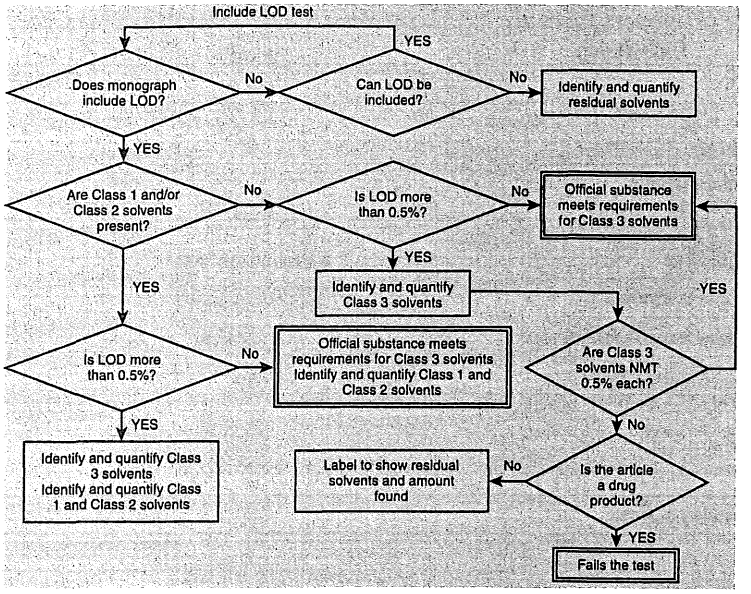


Figure 5. Decision tree for the control of Class 3 residual solvents. LOD: Loss on drying.

GLOSSARY

Genotoxic carcinogens: Carcinogens that produce cancer by affecting genes or chromosomes.

Likely to be present (LTBP): Those solvents that are 1) used or produced in the final manufacturing step; 2) used or produced in earlier manufacturing steps but they are not consistently removed by a validated process; 3) the solvents properly declared by a validated supplier of a drug substance, excipient, or dietary ingredient.

Lowest-observed-effect level (LOEL): The lowest dose of a substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in exposed humans or animals.

Modifying factor: A factor determined by professional judgment of a toxicologist and applied to bioassay data so that the data can be safely related to humans.

Neurotoxicity: The ability of a substance to cause adverse effects on the nervous system.

No-observed-effect level (NOEL): The highest dose of a substance at which there are no biologically significant increases in frequency or severity of any effects in exposed humans or animals.

Permitted daily exposure (PDE): The maximum acceptable intake per day of a residual solvent in pharmaceutical products or dietary supplements

Reversible toxicity: The occurrence of harmful effects that are caused by a substance and then disappear after the exposure to the substance ends.

Strongly suspected human carcinogen: A substance for which there is no epidemiological evidence of carcinogenesis but for which there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.

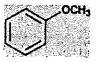
Teratogenicity: The occurrence of structural malformations in a developing fetus when a substance is administered during pregnancy.

APPENDICES

Appendix 1: Residual Solvents Included in This General Chapter

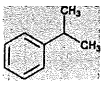
See *Table A-1* for a list of residual solvents included in this chapter.

Table A-1. List of Residual Solvents Included in this General Chapter

Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH_3COOH	Class 3
Acetone	2-Propanone; Propan-2-one	CH_3COCH_3	Class 3
Acetonitrile	AcN	CH_3CN	Class 2
Anisole	Methoxybenzene		Class 3

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Table A-1. List of Residual Solvents Included in this General Chapter *(continued)*

Solvent	Other Names	Structure	Class
Benzene	Benzol		Class 1
1-Butanol	*n*-Butyl alcohol; Butan-1-ol	$CH_3(CH_2)_3OH$	Class 3
2-Butanol	*sec*-Butyl alcohol; Butan-2-ol	$CH_3CH_2CH(OH)CH_3$	Class 3
Butyl acetate	Acetic acid butyl ester	$CH_3COO(CH_2)_3CH_3$	Class 3
tert-Butylmethyl ether	2-Methoxy-2-methylpropane	$(CH_3)_3COCH_3$	Class 3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class 1
Chlorobenzene			Class 2
Chloroform	Trichloromethane	$CHCl_3$	Class 2
Cumene	Isopropylbenzene; (1-Methylethyl)benzene		Class 2
Cyclohexane	Hexamethylene		Class 2
1,2-Dichloroethane	*sym*-Dichloroethane; Ethylene dichloride; Ethylene chloride	CH_2ClCH_2Cl	Class 1
1,1-Dichloroethene	1,1-Dichloroethylene; Vinylidene chloride	$H_2C=CCl_2$	Class 1
1,2-Dichloroethene	1,2-Dichloroethylene; Acetylene dichloride; DCE	$ClHC=CHCl$	Class 2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether; Monoglyme; Dimethyl cellosolve	$H_3COCH_2CH_2OCH_3$	Class 2
N,N-Dimethylacetamide	DMA	$CH_3CON(CH_3)_2$	Class 2
N,N-Dimethylformamide	Dimethylformamide	$HCON(CH_3)_2$	Class 2
Dimethyl sulfoxide	Methylsulfinylmethane; Methyl sulfoxide; Dimethyl sulfoxide	$(CH_3)_2SO$	Class 3
1,4-Dioxane	*p*-Dioxane; [1,4]Dioxane		Class 2
Ethanol	Ethyl alcohol	CH_3CH_2OH	Class 3
2-Ethoxyethanol	Cellosolve	$CH_3CH_2OCH_2CH_2OH$	Class 2
Ethyl acetate	Acetic acid ethyl ester	$CH_3COOCH_2CH_3$	Class 3
Ethylene glycol	1,2-Dihydroxyethane; 1,2-Ethanediol	$HOCH_2CH_2OH$	Class 2
Ethyl ether	Diethyl ether; Ethoxyethane; 1,1′-Oxybisethane	$CH_3CH_2OCH_2CH_3$	Class 3
Ethyl formate	Formic acid ethyl ester	$HCOOCH_2CH_3$	Class 3
Formamide	Methanamide	$HCONH_2$	Class 2
Formic acid		$HCOOH$	Class 3
Heptane	*n*-Heptane	$CH_3(CH_2)_5CH_3$	Class 3

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Table A-1. List of Residual Solvents Included in this General Chapter *(continued)*

Solvent	Other Names	Structure	Class
Hexane	*n*-Hexane	$CH_3(CH_2)_4CH_3$	Class 2
Isobutyl acetate	Acetic acid isobutyl ester	$CH_3COOCH_2CH(CH_3)_2$	Class 3
Isopropyl acetate	Acetic acid isopropyl ester	$CH_3COOCH(CH_3)_2$	Class 3
Methanol	Methyl alcohol	CH_3OH	Class 2
2-Methoxyethanol	Methyl cellosolve	$CH_3OCH_2CH_2OH$	Class 2
Methyl acetate	Acetic acid methyl ester	CH_3COOCH_3	Class 3
3-Methyl-1-butanol	Isoamyl alcohol; Isopentyl alcohol; 3-Methylbutan-1-ol	$(CH_3)_2CHCH_2CH_2OH$	Class 3
Methylbutylketone	2-Hexanone; Hexan-2-one	$CH_3(CH_2)_3COCH_3$	Class 2
Methylcyclohexane	Cyclohexylmethane		Class 2
Methylene chloride	Dichloromethane; MC	CH_2Cl_2	Class 2
Methylethylketone	2-Butanone; MEK; Butan-2-one	$CH_3CH_2COCH_3$	Class 3
Methyl isobutyl ketone	4-Methylpentan-2-one; 4-Methyl-2-pentanone; MIBK	$CH_3COCH_2CH(CH_3)_2$	Class 3
2-Methyl-1-propanol	Isobutyl alcohol; 2-Methylpropan-1-ol	$(CH_3)_2CHCH_2OH$	Class 3
N-Methylpyrrolidone	1-Methylpyrrolidin-2-one; 1-Methyl-2-pyrrolidinone		Class 2
Nitromethane		CH_3NO_2	Class 2
Pentane	*n*-Pentane	$CH_3(CH_2)_3CH_3$	Class 3
1-Pentanol	Amyl alcohol; Pentan-1-ol; Pentyl alcohol	$CH_3(CH_2)_3CH_2OH$	Class 3
1-Propanol	Propan-1-ol; Propyl alcohol	$CH_3CH_2CH_2OH$	Class 3
2-Propanol	Propan-2-ol; Isopropyl alcohol	$(CH_3)_2CHOH$	Class 3
Propyl acetate	Acetic acid propyl ester	$CH_3COOCH_2CH_2CH_3$	Class 3
Pyridine			Class 2
Sulfolane	Tetrahydrothiophene 1,1-dioxide		Class 2
Tetrahydrofuran	Tetramethylene oxide; Oxacyclopentane		Class 2
Tetralin	1,2,3,4-Tetrahydronaphthalene		Class 2

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Table A-1. List of Residual Solvents Included in this General Chapter *(continued)*

Solvent	Other Names	Structure	Class
Toluene	Methylbenzene		Class 2
1,1,1-Trichloroethane	Methylchloroform; 1,1,1-TCE	CH_3CCl_3	Class 1
Trichloroethylene	1,1,2-Trichloroethene	$HClC{=}CCl_2$	Class 2
Xylene[a]	Dimethylbenzene; Xylol		Class 2

[a] Usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene.

Appendix 2: Additional Background

ENVIRONMENTAL REGULATION OF ORGANIC VOLATILE SOLVENTS

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in *Environmental Health Criteria* (EHC) monographs and in the *Integrated Risk Information System* (IRIS) and other similar reference material. The approach to risk assessment described therein relates primarily to long-term or lifetime exposure of the general population via the ambient environment (i.e., inhaled air, food, drinking water, and other sources). The objectives of such groups as the International Programme on Chemical Safety (IPCS), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA) include the determination of acceptable exposure levels. The goal is maintenance of environmental integrity and protection of human health against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The procedures involved in the estimation of maximum safe exposure limits are usually based on long-term studies. When long-term study data are unavailable, shorter-term study data can be used with modification of the approach, such as use of larger safety factors.

RESIDUAL SOLVENTS IN PHARMACEUTICALS

Exposure limits in this chapter were established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, the following specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits.

1. Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.
2. The assumption of lifetime patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.
3. Residual solvents are unavoidable components in pharmaceutical production and will often be a part of pharmaceutical drug products.
4. Residual solvents should not exceed recommended levels except in exceptional circumstances.
5. Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described by the Organization for Economic Cooperation and Development (OECD), the EPA, and the FDA *Redbook* or derived from alternative sources also considered to be adequate to support PDE limit setting.

Appendix 3: Procedures for Establishing Exposure Limits

The Gaylor–Kodell method of risk assessment (Gaylor, DW, Kodell, RL. Linear interpolation algorithm for low dose risk assessment of toxic substances. *J Environ Pathol, Toxicol*, 1980;4:305–312) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 residual solvents could be determined with the use of a large safety factor (i.e., 10,000–100,000) with respect to the no-observed-effect level (NOEL). Detection and quantification of these residual solvents should be performed using state-of-the-art analytical techniques.

Acceptable exposure levels in this chapter for Class 2 residual solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (page 5748 of *Pharmacopeial Forum* [Nov.–Dec. 1989]), and the method adopted by IPCS for *Assessing Human Health Risks of Chemicals* (*Environmental Health Criteria 170*, WHO, 1994), detailed calculations are published in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997. These procedures are similar to those used by the U.S. EPA (IRIS) and the U.S. FDA (*Redbook*) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values presented in *Table 3* of this document.

PDE is derived from the NOEL, or the lowest-observed-effect level (LOEL), in the most relevant animal study as follows:

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$$PDE = (NOEL \times Weight\ Adjustment)/(F1 \times F2 \times F3 \times F4 \times F5)$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in *Environmental Health Criteria* (*Environmental Health Criteria 170*, WHO, 1994) and "modifying factors" or "safety factors" used in *Pharmacopeial Forum*. The assumption of 100% systemic exposure is used in all calculations regardless of the route of administration.

The modifying factors are as follows:

F1 = A factor to account for extrapolation between species.

- F1 = 5 for extrapolation from rats to humans
- F1 = 12 for extrapolation from mice to humans
- F1 = 2 for extrapolation from dogs to humans
- F1 = 2.5 for extrapolation from rabbits to humans
- F1 = 3 for extrapolation from monkeys to humans
- F1 = 10 for extrapolation from other animals to humans

F1 takes into account the comparative surface area to body weight ratios for the species concerned and for humans. Surface area (S) is calculated as:

$$S = kM^{0.67}$$

k = a constant that has been taken to be 10
M = body weight

The body weights used in the equation are those shown below in *Table A-2*:

F2 = A factor of 10 to account for variability between individuals.

A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this chapter.

F3 = A variable factor to account for toxicity studies of short-term exposure

- F3 = 1 for studies that last at least one half lifetime (1 year for rodents or rabbits; 7 years for cats, dogs, and monkeys)
- F3 = 1 for reproductive studies in which the whole period of organogenesis is covered
- F3 = 2 for a 6-month study in rodents, or a 3.5-year study in non-rodents
- F3 = 5 for a 3-month study in rodents, or a 2-year study in non-rodents
- F3 = 10 for studies of a shorter duration

In all cases, the higher factor has been used for study durations between the time points (e.g., a factor of 2 for a 9-month rodent study).

F4 = A factor that may be applied in cases of severe toxicity, e.g., nongenotoxic carcinogenicity, neurotoxicity, or teratogenicity. In studies of reproductive toxicity, the following factors are used:

- F4 = 1 for fetal toxicity associated with maternal toxicity
- F4 = 5 for fetal toxicity without maternal toxicity
- F4 = 5 for a teratogenic effect with maternal toxicity
- F4 = 10 for a teratogenic effect without maternal toxicity

F5 = A variable factor that may be applied if the no-effect level was not established.

When only a LOEL is available, a factor of up to 10 can be used, depending on the severity of the toxicity. The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kg. This relatively low weight provides an additional safety factor against the standard weights of 60 or 70 kg that are often used in this type of calculation. It is recognized that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for pediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider a toxicity study of acetonitrile in mice that is summarized in *Pharmeuropa*, 1997;9(1): S24. The NOEL is calculated to be 50.7 mg kg^{-1} day^{-1}. The PDE for acetonitrile in this study is calculated as follows:

$$PDE = (50.7\ mg\ kg^{-1}\ day^{-1} \times 50\ kg)/(12 \times 10 \times 5 \times 1 \times 1) = 4.22\ mg\ day^{-1}$$

In this example,

F1 = 12 to account for the extrapolation from mice to humans
F2 = 10 to account for differences between individual humans
F3 = 5 because the duration of the study was only 13 weeks
F4 = 1 because no severe toxicity was encountered
F5 = 1 because the no-effect level was determined

Table A-2. Values Used in the Calculations in This Chapter

Rat body weight	425 g
Pregnant rat body weight	330 g

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Table A-2. Values Used in the Calculations in This Chapter *(continued)*

Mouse body weight	28 g
Pregnant mouse body weight	30 g
Guinea-pig body weight	500 g
Rhesus monkey body weight	2.5 kg
Rabbit body weight (pregnant or not)	4 kg
Beagle dog body weight	11.5 kg
Rat respiratory volume	290 L/day
Mouse respiratory volume	43 L/day
Rabbit respiratory volume	1440 L/day
Guinea-pig respiratory volume	430 L/day
Human respiratory volume	28,800 L/day
Dog respiratory volume	9000 L/day
Monkey respiratory volume	1150 L/day
Mouse water consumption	5 mL/day
Rat water consumption	30 mL/day
Rat food consumption	30 g/day

The equation for an ideal gas, $PV = nRT$, is used to convert concentrations of gases used in inhalation studies from units of ppm to units of mg/L or mg/m³. Consider as an example the rat reproductive toxicity study by inhalation of carbon tetrachloride (molecular weight, 153.84) summarized in *Pharmeuropa*, 1997:9(1):S9.

$$\frac{n}{V} = \frac{P}{RT} = \frac{300 \times 10^{-6}\,\text{atm} \times 153{,}840\ \text{mg mol}^{-1}}{0.082\,\text{L at mK}^{-1}\text{mol}^{-1} \times 298\ \text{K}} = \frac{46.15\ \text{mg}}{24.45\ \text{L}} = 1.89\ \text{mg/L}$$

The relationship 1000 L = 1 m³ is used to convert to mg/m³.

USP Reference Standards ⟨11⟩

USP 1-Butanol RS
USP 2-Butanol RS
USP 2-Methyl-1-Propanol RS
USP 3-Methyl-1-butanol RS
USP 1-Pentanol RS
USP 1-Propanol RS
USP 2-Propanol RS
USP Acetone RS
USP Alcohol Determination—Alcohol RS
USP Anisole RS
USP Butyl Acetate RS
USP *tert*-Butylmethyl Ether RS
USP Dimethyl Sulfoxide RS
USP Ethyl Acetate RS
USP Ethyl Formate RS
USP Formic Acid RS
USP Glacial Acetic Acid RS
USP Heptane RS
USP Isobutyl Acetate RS
USP Isopropyl Acetate RS
USP Methyl Acetate RS
USP Methyl Ethyl Ketone RS
USP Methyl Isobutyl Ketone RS
USP Pentane RS
USP Propyl Acetate RS
USP Residual Solvents Mixture—Class 1 RS
USP Residual Solvent Class 1—Benzene RS
USP Residual Solvent Class 1—Carbon Tetrachloride RS
USP Residual Solvent Class 1—1,2-Dichloroethane RS
USP Residual Solvent Class 1—1,1-Dichloroethene RS
USP Residual Solvent Class 1—1,1,1-Trichloroethane RS
USP Residual Solvents Class 2—Mixture A RS

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USP Residual Solvents Class 2—Mixture B RS
USP Residual Solvent Class 2—Mixture C RS
USP Residual Solvent Class 2—Acetonitrile RS
USP Residual Solvent Class 2—Chlorobenzene RS
USP Residual Solvent Class 2—Chloroform RS
USP Residual Solvent Class 2—Cumene RS
USP Residual Solvent Class 2—Cyclohexane RS
USP Residual Solvent Class 2—1,2-Dichloroethene RS
USP Residual Solvent Class 2—1,2-Dimethoxyethane RS
USP Residual Solvent Class 2—*N*,*N*-Dimethylacetamide RS
USP Residual Solvent Class 2—*N*,*N*-Dimethylformamide RS
USP Residual Solvent Class 2—1,4-Dioxane RS
USP Residual Solvent Class 2—2-Ethoxyethanol RS
USP Residual Solvent Class 2—Ethylene Glycol RS
USP Residual Solvent Class 2—Hexane RS
USP Residual Solvent Class 2—Methanol RS
USP Residual Solvent Class 2—2-Methoxyethanol RS
USP Residual Solvent Class 2—Methylbutylketone RS
USP Residual Solvent Class 2—Methylcyclohexane RS
USP Residual Solvent Class 2—Methylene Chloride RS
USP Residual Solvent Class 2—*N*-Methylpyrrolidone RS
USP Residual Solvent Class 2—Nitromethane RS
USP Residual Solvent Class 2—Pyridine RS
USP Residual Solvent Class 2—Tetrahydrofuran RS
USP Residual Solvent Class 2—Tetralin RS
USP Residual Solvent Class 2—Toluene RS
USP Residual Solvent Class 2—Trichloroethylene RS
USP Residual Solvent Class 2—Xylenes RS▲ 2S (USP41)

⟨469⟩ ETHYLENE GLYCOL, DIETHYLENE GLYCOL, AND TRIETHYLENE GLYCOL IN ETHOXYLATED SUBSTANCES

The following procedure is used to determine the concentration of residual ethylene glycol, diethylene glycol, and triethylene glycol in ethoxylated products. Ethoxylated products may contain residual ethylene glycol, diethylene glycol, and triethylene glycol as a result of the manufacturing process. The procedure is suitable for the following substances:

1. Polyethylene glycol 200
2. Polyethylene glycol 300
3. Polyethylene glycol 400
4. Polyethylene glycol 600
5. Polyethylene glycol 1000
6. Polysorbate 20
7. Polysorbate 40
8. Polysorbate 60
9. Polysorbate 80
10. Polyethylene glycol monomethyl ether 350
11. Polyethylene glycol monomethyl ether 550
12. Polyoxyl 35 castor oil
13. Polyoxyl 15 hydroxystearate
14. Polyoxyl 20 cetostearyl ether
15. Polyoxyl 8 stearate
16. Octoxynol 9
17. Nonoxynol 9

IMPURITIES

• **PROCEDURE**

Diluent: Acetone

Standard solution: 25 µg/mL of USP Ethylene Glycol RS, 40 µg/mL of USP Diethylene Glycol RS, 40 µg/mL of USP Triethylene Glycol RS, and 40 µg/mL of USP Butane-1,3-diol RS (internal standard) in *Diluent*

Sample solution: 40 mg/mL of the test substance and 40 µg/mL of USP Butane-1,3-diol RS (internal standard) in *Diluent*

Chromatographic system

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

Mode: GC

Detector: Flame ionization

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Column: 0.53-mm × 30-m capillary column bonded with a 1.0-µm layer of phase G3
Temperatures
Detector: 290°
Injection port: 270°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	10	60	5
60	10	170	0
170	15	280	0, 60[a]

[a] Hold time was 0 min for the *Standard solution* and 60 min for the *Sample solution* and *Diluent*.

Carrier gas: Helium
Flow rate: 5.0 mL/min
Injection volume: 1.0 µL
Injection type: Split injection, split ratio is 2:1
Liner: General-purpose split/splitless, tapered, glass wool, deactivated
Run time: 26 min for the *Standard solution*; 86 min for the *Sample solution* and *Diluent*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]

Table 2

Name	Relative Retention Time
Ethylene glycol	0.45
Butane-1,3-diol (internal standard)	1.00
Diethylene glycol	1.25
Triethylene glycol	1.70

System suitability requirements
Resolution: NLT 20 between ethylene glycol and butane-1,3-diol; NLT 20 between butane-1,3-diol and diethylene glycol; NLT 20 between diethylene glycol and triethylene glycol
Tailing factor: 0.8–1.8 for each of the four peaks assigned to ethylene glycol, butane-1,3-diol, diethylene glycol, and triethylene glycol
Relative standard deviation: NMT 5.0% for the peak response ratio of the respective glycol (ethylene glycol, diethylene glycol, or triethylene glycol) to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the ethylene glycol, diethylene glycol, and triethylene glycol peaks in the *Sample solution* by comparison with those in the *Standard solution*.
Calculate the content of ethylene glycol, diethylene glycol, or triethylene glycol, in µg/g, in the portion of test substance taken:

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

R_U = peak response ratio of the respective glycol to the internal standard (peak response of the respective glycol/peak response of the internal standard) from the *Sample solution*
R_S = peak response ratio of the respective glycol to the internal standard (peak response of the respective glycol/peak response of the internal standard) from the *Standard solution*
C_S = concentration of the respective glycol (ethylene glycol, diethylene glycol, or triethylene glycol) in the *Standard solution* (µg/mL)
C_U = concentration of the test substance in the *Sample solution* (mg/mL)
F = conversion factor (1000 mg/g)

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butane-1,3-diol RS
USP Diethylene Glycol RS

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USP Ethylene Glycol RS
USP Triethylene Glycol RS

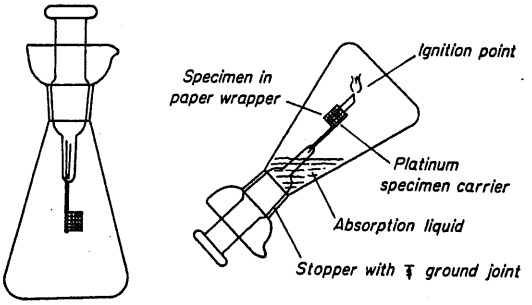
⟨471⟩ OXYGEN FLASK COMBUSTION

The oxygen flask combustion procedure is provided as the preparatory step in the determination of bromine, chlorine, iodine, selenium, and sulfur in some Pharmacopeial articles. Combustion of the material under test (usually organic) yields water-soluble inorganic products, which are analyzed for specific elements as directed in the individual monograph or general chapter.

The caution statement given for *Procedure* covers minimum safety precautions only, and serves to emphasize the need for exceptional care throughout.

APPARATUS

The apparatus[1] consists of a heavy-walled conical, deeply lipped or cupped 500-mL flask (unless a larger flask is specified), fitted with a ground-glass stopper to which is fused a test specimen carrier consisting of heavy-gauge platinum wire and a piece of welded platinum gauze measuring about 1.5 × 2 cm.



Apparatus for Oxygen Flask Combustion

PROCEDURE

[**CAUTION**—Wear safety glasses and use a suitable safety shield between yourself and the apparatus. Exercise care to ensure that the flask is scrupulously clean and free from even traces of organic solvents.]

Weigh the substance, if a solid, on a piece of halide-free filter paper measuring about 4 cm square, and fold the paper to enclose it. Liquid substances are weighed in tared capsules, polycarbonate capsules[1] being used for liquids in volumes not exceeding 200 µL, and gelatin capsules being satisfactory for use for larger volumes. [NOTE—Gelatin capsules may contain significant amounts of combined halide or sulfur. If such capsules are used, perform a blank determination, and make any necessary correction.] Place the specimen, together with a filter paper fuse-strip, in the platinum gauze specimen holder. Place the absorbing liquid specified in the individual monograph or general chapter in the flask, moisten the joint of the stopper with water, and flush the air from the flask with a stream of rapidly flowing oxygen, swirling the liquid to favor its taking up oxygen. [NOTE—Saturation of the liquid with oxygen is essential for the successful performance of the combustion procedure.] Ignite the fuse-strip by suitable means. If the strip is ignited outside the flask, immediately plunge the specimen holder into the flask, invert the flask so that the absorption solution makes a seal around the stopper, and hold the stopper firmly in place. If the ignition is carried out in a closed system, the inversion of the flask may be omitted. After combustion is complete, shake the flask vigorously, and allow to stand for not less than 10 minutes with intermittent shaking. Then proceed as directed in the individual monograph or general chapter.

⟨481⟩ RIBOFLAVIN ASSAY

ASSAY

• **CHEMICAL METHODS, PROCEDURE 1**

The following procedure is suitable for preparations in which riboflavin is a constituent of a mixture of several ingredients. In using the procedure, keep the pH of solutions below 7.0, and protect the solutions from direct sunlight at all stages.

[1] A suitable apparatus [Catalog Nos. 6513-C20 (500-ml capacity) and 6513-C30 (1000-ml capacity)] and suitable capsules [Catalog No. 6513-84 (1000 capsules)] are obtainable from Thomas Scientific, 99 High Hill Road, Swedesboro, NJ 08085.

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Standard riboflavin stock solution: To 50.0 mg of USP Riboflavin RS, previously dried and stored protected from light in a desiccator over phosphorus pentoxide, add about 300 mL of 0.02 N acetic acid, and heat the mixture on a steam bath with frequent agitation until the riboflavin has dissolved, then cool. To this solution add 0.02 N acetic acid to make 500 mL; then mix. Store the solution under toluene in a refrigerator.

Dilute an accurately measured portion of this solution by using 0.02 N acetic acid to a concentration of 10.0 µg/mL of the dried USP Riboflavin RS to obtain the *Standard riboflavin stock solution*. Store the solution under toluene in a refrigerator.

Standard solution: Dilute with water 10.0 mL of *Standard riboflavin stock solution* in a 100-mL volumetric flask to volume, and mix. Each mL represents 1.0 µg of USP Riboflavin RS. Prepare a fresh *Standard solution* for each assay.

Sample solution: Place an amount of the material to be assayed in a flask of suitable size, and add a volume of 0.1 N hydrochloric acid equal in mL to NLT 10 times the dry weight of the material in grams, but the resulting solution will contain NMT 100 µg/mL of riboflavin. If the material is not readily soluble, comminute the material so that it may be evenly dispersed in the liquid. Agitate vigorously, and wash down the sides of the flask with 0.1 N hydrochloric acid.

Heat the mixture in an autoclave at 121°–123° for 30 min, and cool. If clumping occurs, agitate the mixture until the particles are evenly dispersed. Adjust the mixture, with vigorous agitation, with sodium hydroxide solution[1] to a pH of 6.0–6.5, then add hydrochloric acid solution[1] immediately until no further precipitation occurs (usually at a pH of approximately 4.5, which is the isoelectric point of many of the proteins present). Dilute the mixture with water to make a measured volume that contains about 0.11 µg of riboflavin in each mL, and filter through paper known not to adsorb riboflavin. To an aliquot of the filtrate add, with vigorous agitation, sodium hydroxide solution[1] to produce a pH of 6.6–6.8, dilute the solution with water to make a final measured volume that contains approximately 0.1 µg of riboflavin in each mL, and if cloudiness occurs, filter again.

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 444 nm

Emission wavelength: 530 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

To each of four or more tubes (or reaction vessels) add 10.0 mL of the *Sample solution*. To each of two or more of these tubes add 1.0 mL of the *Standard solution*, and mix; to each of two or more of the remaining tubes add 1.0 mL of water, and mix. To each tube add 1.0 mL of glacial acetic acid, mix, then add, with mixing, 0.50 mL of potassium permanganate solution (1 in 25), and allow to stand for 2 min. To each tube add, with mixing, 0.50 mL of hydrogen peroxide solution, whereupon the permanganate color is destroyed within 10 s. Shake the tubes vigorously until excess oxygen is expelled. Remove any gas bubbles remaining on the sides of the tubes after foaming has ceased by tipping the tubes so that the solution flows slowly from end to end.

Measure the fluorescence of all tubes, designating the average reading from the tubes containing only the *Sample solution* as I_U, and designating the average from the tubes containing both the *Sample solution* and the *Standard solution* as I_S. Then to each of one or more tubes of each kind add, with mixing, 20 mg of sodium hydrosulfite, and within 5 s again measure the fluorescence, designating the average reading as I_B.

Calculate the quantity, in mg, of riboflavin ($C_{17}H_{20}N_4O_6$) in each mL of the *Sample solution* taken:

$$\text{Result} = [0.0001 \times (I_U - I_B)]/(I_S - I_U)$$

I_U = average reading of the tubes containing only the *Sample solution*

I_B = average reading of fluorescence of the tubes after adding 20 mg of sodium hydrosulfite

I_S = average reading of the tubes containing both the *Sample solution* and *Standard solution*

Calculate the quantity, in mg, of riboflavin ($C_{17}H_{20}N_4O_6$) in each capsule or tablet.

• **CHEMICAL METHODS, PROCEDURE 2**

This procedure is suitable for the determination of riboflavin as a dietary ingredient or active pharmaceutical ingredient.

[NOTE—Conduct the entire *Analysis* without exposure to direct sunlight.]

Standard solution: Transfer 50 mg of USP Riboflavin RS to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it within the operating sensitivity of the fluorometer used.

Sample solution: Transfer 50 mg of Riboflavin to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it to the same concentration as that of the *Standard solution*.

Blank: Prepare as directed for the *Sample solution*, except omit the test specimen.

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 444 nm

Emission wavelength: 530 nm

[1] The concentrations of the hydrochloric acid and sodium hydroxide solutions used are not stated in each instance, because these concentrations may be varied depending upon the amount of material taken for assay, volume of test solution, and buffering effect of material.

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Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Measure the fluorescence intensity of the *Standard solution*. Immediately after the reading, add to the solution 10 mg of sodium hydrosulfite, stirring with a glass rod until dissolved, and at once measure the fluorescence again. [NOTE—Depending on the final concentration of riboflavin in the solution, it may be necessary to increase the amount of sodium hydrosulfite to suppress the fluorescence activity completely.] The difference between the two readings represents the fluorescence intensity (I_S) due to the *Standard solution*. Similarly, measure the fluorescence intensity (I_U) due to the *Sample solution*. Perform the blank determination, and make any necessary correction.

Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence of the *Sample solution*
I_S = fluorescence of the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of riboflavin in the *Sample solution* (µg/mL)

The following liquid chromatographic procedures are provided for the determination of riboflavin as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in the dietary supplements or pharmaceutical dosage forms. Use the appropriate USP Reference Standards.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware.

Chromatographic Methods, Procedure 1

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: Acetonitrile, glacial acetic acid, and water (5:1:94)

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL

Standard solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric flask, and add 180 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking, or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

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System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and appropriate *Sample solution*
Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of sample taken:

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

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

• **CHROMATOGRAPHIC METHODS, PROCEDURE 2**
This procedure can be used to determine riboflavin in:
- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by the *Extraction solvent*, heat, and mechanical shaking.
Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.
Extraction solvent: Transfer 1 mL of glacial acetic acid and 2.5 g of edetate disodium to a 100-mL volumetric flask. Dissolve in and dilute with water to volume. Mix the resulting solution with methanol (3:1).
Solution A: 6.8 g of sodium acetate per 1000 mL of water
Mobile phase: Prepare a mixture of *Solution A* and methanol (13:7). Add 2 mL of triethylamine per L of the mixture, and adjust with glacial acetic acid to a pH of 5.2.
Standard stock solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric flask, and add 180 mL of *Extraction solvent*. Immerse the flask for 5 min in a water bath maintained at 65°–75°. Mix well, and repeat if necessary until dissolved. Chill rapidly in a cold water bath to room temperature, and dilute with *Extraction solvent* to volume.
Standard solution: Dilute 5.0 mL of the *Standard stock solution* with *Extraction solvent* to 25.0 mL.
Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without loss of shell material, and transfer the contents to a beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air. Weigh the empty capsule shells in the tared weighing bottle, and calculate the net weight of the capsule contents. Transfer a portion of the capsule contents, equivalent to 2 mg of riboflavin, to a 200-mL volumetric flask. Add 100.0 mL of *Extraction solvent*, and mix for 20 min using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, cool to room temperature, and filter. Use the clear filtrate.
Sample solution for tablets: Finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 2 mg of riboflavin, to a 200-mL volumetric flask. Add 100.0 mL of *Extraction solvent*, and mix for 20 min using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, cool to room temperature, and filter. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and appropriate *Sample solution*
Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of sample taken:

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

r_U = peak response of riboflavin from the appropriate *Sample solution*

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r_S = peak response of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

• **CHROMATOGRAPHIC METHODS, PROCEDURE 3**

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of riboflavin from the formulation by mixtures of organic solvents, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: 25 mg/mL of edetate disodium in water

Mobile phase: Transfer 0.4 mL of triethylamine, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask. Dilute with 0.008 M sodium 1-hexanesulfonate to volume.

Standard stock solution: 0.08 mg/mL of USP Riboflavin RS in *Diluent*, with heating if necessary

Standard solution for capsules/tablets: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Standard solution for oral solution: 8 µg/mL of USP Riboflavin RS in *Diluent*, diluted from the *Standard stock solution*

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 0.4 mg of riboflavin, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Sample solution for oral solution: Equivalent to 8 µg/mL of riboflavin from oral solution in the *Diluent*

Sample solution for tablets: Weigh and finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 0.4 mg of riboflavin, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

For capsules and tablets, calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of sample taken:

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

r_U = peak response of riboflavin from the appropriate *Sample solution*
r_S = peak response of riboflavin from the *Standard solution for capsules/tablets*
C_S = concentration of USP Riboflavin RS in the *Standard solution for capsules/tablets* (mg/mL)
C_U = nominal concentration of riboflavin in the appropriate *Sample solution* (mg/mL)

For oral solution, calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of sample taken:

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

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

• **CHROMATOGRAPHIC METHODS, PROCEDURE 4**

This procedure can be used to determine riboflavin in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is a newly added procedure as part of the USP monograph modernization efforts. The procedure uses hydrophilic interaction liquid chromatography (HILIC), and the sample preparation involves the extraction of riboflavin from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: Methanol, glacial acetic acid, and water (50:1:49)
Solution A: 50 mM ammonium formate; adjust with ammonium hydroxide to a pH of 9.0.
Solution B: Acetonitrile
Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	11	89
8	17	83
15	23	77
20	30	70
21	50	50
24	50	50
25	11	89
30	11	89

Standard solution: Transfer 20 mg of USP Riboflavin RS to a 200-mL volumetric flask, and add 160 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 68°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate.

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of riboflavin, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 267 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L68
Flow rate: 1.2 mL/min

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Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and appropriate *Sample solution*
Calculate the percentage of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of sample taken:

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

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

ADDITIONAL REQUIREMENTS
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Riboflavin RS

⟨501⟩ SALTS OF ORGANIC NITROGENOUS BASES

STANDARD PREPARATION

Unless otherwise directed, prepare a solution in dilute sulfuric acid (1 in 70) containing, in each mL, about 500 µg of the specified USP Reference Standard, calculated on the anhydrous basis, and accurately weighed.

ASSAY PREPARATION

If the dosage form is a tablet, weigh and finely powder not less than 20 tablets, weigh accurately a portion of the powder, equivalent to about 25 mg of the active ingredient, and transfer to a 125-mL separator; or, if the dosage form is a liquid, transfer a volume of it, equivalent to about 25 mg of the active ingredient and accurately measured, to a 125-mL separator. Then to the separator add 20 mL of dilute sulfuric acid (1 in 350), and shake vigorously for 5 minutes. Add 20 mL of ether, shake carefully, and filter the acid phase into a second 125-mL separator. Shake the ether phase with two 10-mL portions of dilute sulfuric acid (1 in 350), filter each portion of acid into the second separator, and discard the ether. To the acid extract add 10 mL of sodium hydroxide TS and 50 mL of ether, shake carefully, and transfer the aqueous phase to a third 125-mL separator containing 50 mL of ether. Shake the third separator carefully, and discard the aqueous phase. Wash the two ether solutions, in succession, with a single 20-mL portion of water, and discard the water. Extract each of the two ether solutions with 20-, 20-, and 5-mL portions of dilute sulfuric acid (1 in 70), in the order listed, but each time extract first the ether solution in the third separator and then that in the second separator. Combine the acid extracts in a 50-mL volumetric flask, dilute with the acid to volume, and mix.

[NOTE—Hexane or heptane may be substituted for ether if the distribution ratio of the nitrogenous base between water and hexane, or between water and heptane, favors complete extraction by the organic phase.]

PROCEDURE

Unless otherwise directed, dilute 5.0 mL each of the *Standard Preparation* and the *Assay Preparation* with dilute sulfuric acid (1 in 70) to 100.0 mL, and determine the absorbance of each solution at the specified wavelength, using dilute sulfuric acid (1 in 70) as the blank. Designate the absorbance of the solution from the *Standard Preparation* as A_S and that from the *Assay Preparation* as A_U, and calculate the result of the assay as directed in the individual monograph.

⟨503⟩ ACETIC ACID IN PEPTIDES

INTRODUCTION
This chapter provides procedures to be used to determine the amount of acetic acid in peptides. Acetic acid/acetate is a common counter ion in peptide preparations.

• **METHOD 1**
Strong sodium hydroxide solution: Dissolve 42 g of sodium hydroxide in water, and dilute with water to 100 mL.
Solution A: Add 0.7 mL of phosphoric acid to 1000 mL of water, and adjust with *Strong sodium hydroxide solution* to a pH of 3.0.
Solution B: Methanol

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Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
10	50	50
20	50	50
22	95	5

Diluent: Prepare a mixture of *Solution A* and *Solution B* (95:5).
Standard solution: Dissolve an accurately weighed quantity of USP Glacial Acetic Acid RS in *Diluent* to obtain a solution having a known concentration of about 0.1 mg/mL. [NOTE—The concentration can be adjusted, depending on the amount of acetate or acetic acid expected to be present in the test material.]
Sample solution: Prepare as directed in the individual monograph. If no direction is given in the individual monograph, the sample concentration can be adjusted so that the mid-range of the amount of acetic acid stated in the specification for the test material corresponds to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Retention time of acetic acid: 3–4 min
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid in the portion of test material taken:

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

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

• **METHOD 2**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** See *Trifluoroacetic Acid (TFA) in Peptides* ⟨503.1⟩.
Standard solution: 0.7 mg/mL of USP Sodium Acetate Trihydrate RS in *Diluent*
Calculate the concentration of acetic acid in the *Standard solution* (C_S), in mg/mL, taken:

$$C_S = 0.441 \times C$$

0.441 = molecular weight conversion factor (60.05/136.08)
C = concentration of USP Sodium Acetate Trihydrate RS in the *Standard solution* (mg/mL)

Sample solution: About 4 mg/mL of the test sample in *Diluent*. [NOTE—The sample concentration can be adjusted so that the mid-range of the amount of acetic acid stated in the specification for the test material corresponds to that of the *Standard solution*.]
[NOTE—Alternatively, the *Standard solution* and *Sample solution* can be prepared as described in *Method 1*.]
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis: Proceed as described in *Method 1*.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Glacial Acetic Acid RS
USP Sodium Acetate Trihydrate RS

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⟨503.1⟩ TRIFLUOROACETIC ACID (TFA) IN PEPTIDES

INTRODUCTION

The following procedures are to be used to determine the amount of trifluoroacetic acid (TFA) in peptides. TFA/Trifluoroacetate is a common residual process impurity in the preparation of peptides or a counter ion in active pharmaceutical ingredients (API).

• PROCEDURE

Solution A: Add 7.0 mL of phosphoric acid and 5.0 mL of ammonium hydroxide to 900 mL of water. Mix and dilute with water to 1000 mL. [NOTE—The pH of the solution is approximately 2.5.] Pass through a filter of 0.45-µm pore size and degas. Add 20 mL of methanol, mix, and degas for an additional 2 min.

Solution B: Acetonitrile and water (50:50). Mix and degas.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
6	0	100
14	0	100
15	100	0
25	100	0

Diluent: 0.5% phosphoric acid in water (v/v)

TFA stock solution (option 1): 12 mg/mL of USP Sodium Trifluoroacetate RS in water

Calculate the concentration (C_S), in mg/mL, of TFA in the *TFA stock solution* taken:

$$C_S = 0.838 \times C$$

0.838 = molecular weight conversion factor (114.02/136.01)
C = concentration of USP Sodium Trifluoroacetate RS in the *TFA stock solution* (mg/mL)

TFA stock solution (option 2): 10 mg/mL of TFA in water prepared as follows. Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Transfer 670 µL of TFA to the flask, stopper immediately, and weigh. Dilute with water to volume.

System suitability solution: 0.025 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*

Standard solution (when TFA content is tested as a process impurity): 0.01 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*

Standard solution (when TFA content is tested as a counter ion in the API): 0.25 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*. [NOTE—The concentration can be adjusted depending on the amount of TFA expected to be present in the test material.]

Sample solution: NLT 4 mg/mL of test sample in *Diluent*. [NOTE—The sample concentration can be adjusted so that the limit (when TFA content is tested as a process impurity) or the mid-range (when TFA content is tested as a counter ion) of the amount of TFA stated in the specification for the test material corresponds to that of the *Standard solution*.]

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1. [NOTE—A guard column of 4-mm × 2-cm; 5-µm packing L1 can be used.]

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time is about 3 min for TFA.]

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of TFA in the portion of sample taken:

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

r_U = TFA peak responses from the *Sample solution*
r_S = TFA peak responses from the *Standard solution*

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C_S = concentration of TFA in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Trifluoroacetate RS

⟨507⟩ PROTEIN DETERMINATION PROCEDURES

INTRODUCTION

Several procedures exist to determine the total protein in pharmaceutical drug substances and products. Procedures may differ based on the physical characteristic of the protein, which serves as the basis of the measurement principle (e.g., absorbance of ultraviolet light by aromatic amino acid residues or by dye binding). Understanding the protein structure and sample matrix is important when selecting a total protein quantitation procedure. Users must verify that the chosen validated method below is suitable for their specific purposes (see *Verification of Compendial Procedures* ⟨1226⟩).

PROCEDURE

Two options for Method I are presented: *Method IA*, using denaturing conditions; and *Method IB*, using native conditions. Depending on the protein structure and the nature of the protein sample (e.g., denaturing conditions may be more suitable for a strong coiled structure with most aromatic amino acids in the interior of the protein), one method may prove superior to the other, and users should select which is the most suitable method based on their protein and verify, as appropriate. At a minimum, both methods are dependent on the following conditions being met: 1) the amino acid sequence and molecular weight of the protein must be known; and 2) the protein must contain tyrosines or tryptophans, and no other chromophores (other than cystines) that absorb near 280 nm (including nucleic acids and diluent components) should be present.

- **METHOD IA. ULTRAVIOLET LIGHT ABSORBANCE UNDER DENATURING CONDITIONS**

Sample buffer A: Prepare 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium dihydrogen phosphate in about 80 mL of water. Adjust the pH with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Sample buffer B: Prepare 7 M guanidine hydrochloride and 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium dihydrogen phosphate and 66.87 g of guanidine hydrochloride in about 50 mL of water. Adjust with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Reference solution: Prepare 6 M guanidine hydrochloride in *Sample buffer A* by mixing 330 µL of *Sample buffer A* and 2000 µL of *Sample buffer B*.

Sample preparation A: Dilute the test sample by adding *Sample buffer A* and *Sample buffer B* to achieve a final concentration of 6 M guanidine hydrochloride and an absorbance of about 0.4–0.6. [NOTE—Gravimetric dilutions can be used and sometimes improve accuracy.] Prepare at least three replicates per test sample.

Analysis

Samples: *Sample buffer A*, *Sample buffer B*, *Reference solution*, and *Sample preparation A*

Determination of the protein concentration in denaturing buffer: Using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩), measure the absorbance of *Sample preparation A* in 1-cm cuvettes at an optical density of 280 nm against the *Reference solution*, and subtract the absorbance value at 330 nm to obtain $A_{corrected}$. Calculate the protein concentration (c) in the test sample:

$$c = A_{corrected} \times DF \times MW/(\varepsilon_{280\,nm} \times 1\ cm)$$

$A_{corrected}$ = absorbance at 280 nm minus the absorbance at 330 nm
DF = dilution factor
MW = calculated molecular weight of the protein
$\varepsilon_{280\,nm}$ = extinction coefficient of the test sample at 280 nm

$$\varepsilon_{280\,nm} = (n_{Trp} \times 5690 + n_{Tyr} \times 1280 + n_{Cys} \times 120)\ M^{-1} \times cm^{-1}$$

n_{Trp} = number of tryptophans
n_{Tyr} = number of tyrosines
n_{Cys} = number of cystines

System suitability

The test sample UV spectra must show a maximum between 270 and 285 nm and must show a minimum between 243 and 257 nm. The measured absorbance at 280 nm must be NLT 0.4 and NMT 0.6. The relative standard deviation (RSD) of the calculated concentration of all replicates must be NMT 3.0%.

- **METHOD IB. ULTRAVIOLET LIGHT ABSORBANCE UNDER NATIVE CONDITIONS**

Sample buffer A: Prepare 20 mM sodium phosphate, pH 6.5 by dissolving 0.24 g of sodium dihydrogen phosphate in about 80 mL of water. Adjust the pH with sodium hydroxide solution (10% w/w), and fill with water to 100 mL. Filter.

Sample preparation A: Dilute the test sample by adding *Sample buffer A* to achieve an absorbance of about 0.4–0.6. [NOTE—Gravimetric dilutions can be used and sometimes improve accuracy.] Prepare at least three replicates per test sample.

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Analysis

Samples: *Sample buffer A* and *Sample preparation A*

Determination of the protein concentration in native buffer: Using a suitable spectrophotometer (see ⟨857⟩), measure the absorbance of *Sample preparation A* in 1-cm cuvettes at 280 nm against *Sample buffer A* and subtract the absorbance value at 330 nm to obtain $A_{corrected}$.

Calculate the protein concentration (c) of the test sample:

$$c = A_{corrected} \times DF \times MW/(\varepsilon_{280\ nm\text{-}native2} \times 1\ cm)$$

$A_{corrected}$ = absorbance at 280 nm minus the absorbance at 330 nm
DF = dilution factor
MW = calculated molecular weight of the protein
$\varepsilon_{280\ nm\text{-}native2}$ = extinction coefficient of the test sample at 280 nm

where the extinction coefficient $\varepsilon_{280\ nm\text{-}native2}$ is calculated as:

$$\varepsilon_{280\ nm\text{-}native2} = (n_{Trp} \times 5500 + n_{Tyr} \times 1490 + n_{Cys} \times 125)\ M^{-1} \times cm^{-1}$$

n_{Trp} = number of tryptophans
n_{Tyr} = number of tyrosines
n_{Cys} = number of cystines

System suitability

The test sample UV spectra must show a maximum between 270 and 285 nm and must show a minimum between 243 and 257 nm. The measured absorbance at 280 nm must be NLT 0.4 and NMT 0.6. The RSD of the calculated concentration of all replicates must be NMT 3.0%.

• **METHOD II. BICINCHONINIC ACID METHOD**

BCA reagent solution:[1] Prepare a solution of 10 g/L of bicinchoninic acid (BCA), 20 g/L of sodium carbonate monohydrate, 1.6 g/L of sodium tartrate, 4 g/L of sodium hydroxide, and 9.5 g/L of sodium bicarbonate in water to a pH of 11.25.

Copper sulfate reagent solution: Prepare a solution of 40 g/L of cupric sulfate pentahydrate in water.

BCA working reagent solution: Mix 50 volumes of *BCA reagent solution* with 1 volume of *Copper sulfate reagent solution*. Mix until the green solution is clear.

Sample dilution buffer: Water or another suitable, non-interfering buffer

Standard solutions: Ideally, use the USP Reference Standard for the protein under test. If the protein of interest is unknown, is a mixture, or is a specific USP Reference Standard that is unavailable, use USP BSA for Protein Quantitation RS. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions* in triplicate. The selected *Standard solution* concentrations should result in a linear curve, typically in the range of 25–1000 µg/mL of protein.

Suitability standard solution: Prepare an independent dilution of the same protein used to make the *Standard solutions* above using *Sample dilution buffer* to obtain a *Suitability standard solution* with a concentration near the midpoint of the standard curve. Prepare the *Suitability standard solution* in triplicate.

Sample solution: Prepare a *Sample solution* with an expected concentration within the standard curve by diluting with the *Sample dilution buffer*. Prepare triplicate samples.

Analysis

Samples: *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution*

Accurately transfer 100 µL of *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* to individual test tubes. [NOTE—96-well, plate-based, and automated methods can also be used with suitable volume adjustments to the procedure below.] Add 2.0 mL of *BCA working reagent solution* to each tube, and mix well.

Cover and incubate the solutions at 37 ± 2° for 30 ± 1 min, then allow the samples to cool to room temperature for NLT 5 min and NMT 60 min. Determine the absorbances of the solutions at 562 nm with a suitable spectrophotometer (see ⟨857⟩). Autozero the instrument with the *Sample dilution buffer* sample.

Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 10 min.

Calculations: Plot the absorbances of the *Standard solutions* versus the protein concentrations of the *Standard solutions*, and prepare a standard curve using the linear regression method. Use this standard curve and the absorbance of the *Sample solution* to determine the concentration of protein in each *Suitability standard solution* and *Sample solution*.

System suitability

Samples: *Standard solutions* and *Suitability standard solution*

Suitability requirements

Linearity: The coefficient of determination (r^2) for a linear fit of all *Standard solutions* is NLT 0.99.

Relative standard deviation: NMT 5% for triplicate results of the *Suitability standard solution*

Accuracy: The calculated concentration of the *Suitability standard solution* must be within 90%–110% of the theoretical concentration.

Acceptance criteria

Relative standard deviation: NMT 5% for triplicate *Sample solution* results

• **METHOD III. BRADFORD METHOD**

Sample dilution buffer: Water or another suitable, non-interfering buffer

[1] Suitable BCA reagents are available from Thermo Scientific catalog #23225 or equivalent.

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Standard solutions: Use the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS if the protein of interest is unknown, is a mixture, or is a specific USP Reference Standard that is unavailable. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions* in triplicate. Two standard curve ranges are provided for use: either 0.1–1.0 mg/mL of protein (see *Analysis* procedure *A*, below) or 5–25 µg/mL of protein (see *Analysis* procedure *B*, below).

Suitability standard solution: Dilute the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS with *Sample dilution buffer* to obtain a *Suitability standard solution* having a concentration near the midpoint of the standard curve. Prepare the *Suitability standard solution* in triplicate.

Sample solution: Prepare a *Sample solution* with an expected concentration within one of the two concentration ranges in options *A* or *B* below by diluting with the *Sample dilution buffer*. Prepare triplicate samples.

Coomassie reagent solution: Dissolve 100 mg of brilliant blue G[2] in 50 mL of alcohol. Add 100 mL of phosphoric acid, dilute with water to 1 L, and mix. Pass the solution through filter paper (Whatman No. 1 or equivalent), and store the filtered reagent in an amber bottle at room temperature. [NOTE—Slow precipitation of the dye will occur during storage of the reagent. Filter the reagent before use.]

Analysis: Select one of the two concentration ranges listed below that is suitable for the expected concentrations of the *Sample solutions*.

A. Protein concentration range: 0.1–1.0 mg/mL

Samples: *Sample dilution buffer*, *Standard solutions*, and *Sample solution*

Add 1 mL of the *Coomassie reagent solution* to 20 µL of each *Sample dilution buffer*, *Standard solution*, and *Sample solution* sample. Mix by inversion, avoiding foaming. Incubate 10–30 min. By using a suitable spectrophotometer (see ⟨857⟩) [NOTE—Do not use quartz (silica) cuvettes.] , use the *Sample dilution buffer* to set the instrument to zero at 595 nm. Autozero the instrument with the *Sample dilution buffer* sample.

Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 60 min.

B. Protein concentration range: 5–25 µg/mL

Samples: *Sample dilution buffer*, *Standard solutions*, and *Sample solution*

Add 0.5 mL of the *Coomassie reagent solution* to 0.5 mL each of *Sample dilution buffer*, *Standard solution*, and *Sample solution* sample. Mix by inversion, avoiding foaming. Incubate 10–30 min. By using a suitable spectrophotometer (see ⟨857⟩) [NOTE—Do not use quartz (silica) cuvettes.] , use the *Sample dilution buffer* to set the instrument to zero at 595 nm. Autozero the instrument with the *Sample dilution buffer* sample. Measure the absorbance of all *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples within 60 min.

Calculations: [NOTE—The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Plot the absorbances of the *Standard solutions* versus the protein concentrations, and determine the standard curve best fitting the plotted points. From this standard curve and the absorbance of the *Sample solution*, determine the concentration of protein in the *Sample solution*.

System suitability

Samples: *Standard solutions* and *Suitability standard solutions*

Suitability requirements

Linearity: r^2 for a fit of all *Standard solutions* is NLT 0.99.

Relative standard deviation: NMT 15% for triplicate results of the *Suitability standard solution*

Recovery: The calculated concentration of the *Suitability standard solution* must be within 90%–110% of the theoretical concentration.

Acceptance criteria: %CV of triplicates of the *Sample solution* is NMT 15%.

• **METHOD IV. LOWRY METHOD**

SDS solution: If required, prepare a solution of 50 g/L of sodium dodecyl sulfate (SDS).

Lowry reagent A: Prepare a solution of 10 g/L of sodium hydroxide in water, and prepare a solution of 50 g/L of sodium carbonate anhydrous in water. Mix equal volumes (2 volumes and 2 volumes) of each solution, and dilute with water or *SDS solution*, if required, to 5 volumes. [NOTE—SDS may be added to *Lowry reagent A* when the test protein contains detergents or lipids or is poorly soluble after treatment for interfering substances.]

Lowry reagent B: Prepare a fresh solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate pentahydrate in water. Mix equal volumes (2 volumes and 2 volumes) of each solution, and dilute with water to 5 volumes.

Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*. [NOTE—Prepare fresh and protect from light.]

Sample dilution buffer: Purified water or other non-interfering buffer

Standard solutions: Use the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS if the protein of interest is unknown, is a mixture, or is a specific USP Reference Standard that is unavailable. Dilute portions of this solution with the *Sample dilution buffer* to obtain NLT five evenly spaced *Standard solutions*. The standard concentrations should be selected to result in a linear curve, typically in the range of 10–200 µg/mL of protein.

Suitability standard solution: Dilute the USP Reference Standard for the protein under test or USP BSA for Protein Quantitation RS with *Sample dilution buffer* to obtain a *Suitability standard solution* having a concentration near the midpoint of the standard curve. Prepare and analyze the *Suitability standard solution* in triplicate.

Sample solution: Prepare a *Sample solution* with an expected concentration within the standard curve by diluting with the *Sample dilution buffer*. Prepare and analyze samples in triplicate.

Diluted Folin–Ciocalteu's phenol reagent: Dilute Folin–Ciocalteu's phenol reagent with water (1:1). Alternative dilutions may be used provided that the pH of the samples (i.e., *Standard solutions* and *Sample solutions* after addition of *Lowry reagent C* and the *Diluted Folin–Ciocalteu's phenol reagent*) is 10.0–10.6.

[2] Suitable brilliant blue G dye is available from Sigma–Aldrich, catalog #B0770.

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Analysis

Samples: *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution*

Accurately transfer 1.0 mL of *Sample dilution buffer*, *Standard solutions*, *Suitability standard solution*, and *Sample solution* samples to individual test tubes. To each test tube add 5.0 mL of *Lowry reagent C*. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin–Ciocalteu's phenol reagent* to each test tube, mix immediately, and incubate at room temperature for 30 min. Determine the absorbance of each solution at 750 nm with a suitable spectrophotometer (see ⟨857⟩). Autozero the instrument with the *Sample dilution buffer*.

Calculations: Plot the absorbances of the *Standard solutions* versus the protein concentrations of the *Standard solutions*, and prepare a standard curve using the linear regression method. Use this standard curve and the absorbances of the *Sample solutions* to determine the concentration of protein in each of the *Sample solution* samples and *Suitability standard solution*.

System suitability

Sample: *Suitability standard solution*

Suitability requirements

Linearity: r^2 for a linear fit of all standards is NLT 0.995.

Relative standard deviation: NMT 5% for triplicate results of the *Suitability standard solution*

Recovery: The calculated concentration of the *Suitability standard solution* must be within 90%–110% of the theoretical concentration.

Acceptance criteria

Relative standard deviation: NMT 5% for triplicate sample results

• **METHOD V. AMINO ACID ANALYSIS**

Separation and determination of hydrolyzed amino acids by ion-exchange chromatography, followed by postcolumn ninhydrin derivatization, is described for protein samples containing a protein of known molecular weight and amino acid composition. [NOTE—For additional information, see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, which may be a helpful but not mandatory resource.]

Hydrolysis solution: 6 N hydrochloric acid

Sample hydrolysate preparations: Prepare a protein sample such that the content of amino acids is within the established linear working range of the procedure. [NOTE—Glassware used for hydrolysis must be clean.] Place suitable volumes of the sample and *Hydrolysis solution* in a hydrolysis tube. [NOTE—Common volumes range from 100 to 500 µL for the sample and from 1 to 2 mL for the *Hydrolysis solution* such that complete protein digestion is demonstrated within the hydrolysis incubation time.] Cool the mixture until solidification, and evacuate until the pressure is <0.2 bar. Flame-seal the hydrolysis tube in vacuum by melting the neck of the tube. [NOTE—Alternative commercially available hydrolysis vials also can be used.] Hydrolyze at 110° for 22 h. Allow the tube to cool, and remove the solvent by centrifugal vacuum concentrator.

Chromatography

Solution A: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, 0.2% thiodiglycol, and 2.0% propan-2-ol. Adjust with concentrated hydrochloric acid to a pH of 3.2.

Solution B: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate and 0.1% phenol. Adjust with concentrated hydrochloric acid to a pH of 4.25.

Solution C: Sodium citrate buffer (1.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, and 5.8% sodium chloride. Adjust with concentrated hydrochloric acid to a pH of 6.45.

Solution D: 0.4 M sodium hydroxide containing 0.1% ethylenediaminetetraacetic acid

Mobile phase: See *Table 1*. Equilibrate the system before each run.

Table 1

Time (min)	Solution (100%)	Column Temperature	Flow, Mobile Phase (mL/h)	Flow, Postcolumn Reagent (mL/h)
0	A	49°	35	25
8.5	A	49°	35	25
8.51	B	51°	35	25
23.0	B	51°	35	25
23.01	C	51°	35	25
24.0	C	51°	35	25
24.01	C	95°	35	25
41.5	C	95°	35	25
41.51	D	95°	35	25
45.5	D	95°	35	25
45.51	A	49°	35	25
49.0	A	49°	35	25
49.01	50% Isopropanol	49°	0	0
51.0	50% Isopropanol	49°	0	0

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Table 1 *(continued)*

Time (min)	Solution (100%)	Column Temperature	Flow, Mobile Phase (mL/h)	Flow, Postcolumn Reagent (mL/h)
51.01	A	49°	44	0
62.0	A	49°	44	0
62.01	A	49°	35	25
64.0	A	49°	35	25

Loading buffer: Sodium citrate buffer (0.2 N in sodium), containing 2.0% trisodium citrate dihydrate, 0.1% phenol, and 2.0% thiodiglycol. Adjust with concentrated hydrochloric acid to a pH of 2.2.

Postcolumn reagent

Solution 1: Solution containing 7.6% acetic acid, 47.7% ethylene glycol, 28.7% water, and 16.0% potassium acetate

Solution 2: Solution containing 9% methanol, 8% ninhydrin, 46.4% diethylene glycolmethyl ether, 36% ethylene glycol, and 0.6% hydrindantin. Prepare *Postcolumn reagent* by mixing 1.75 L of *Solution 1* and 0.25 L of *Solution 2*. Use nitrogen sparging during preparation and mixing. Keep the solution under nitrogen pressure.

Standard solution: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. [NOTE—Suitable concentrations are 250 and 125 nmol/mL, respectively.]

Sample solution: Reconstitute the *Sample hydrolysate preparations* in a suitable volume of *Loading buffer.*

Blank: *Hydrolysis solution* that has been taken through analytical steps including hydrolysis

Chromatographic system

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

Mode: LC

Detector: 440 and 570 nm

Columns

Prewash: 4.6-mm × 10-cm; 20-µm sodium form, packing L58

Analytical: 4.6-mm × 20-cm; 8-µm sodium form, packing L58

Injection volume: 40 µL, at 1 min with baseline reset

Postcolumn reaction: As the amino acids are eluted from the column, *Postcolumn reagent* is added at a rate of 25 mL/h. After mixing, the column effluent and the *Postcolumn reagent* are passed through a reaction coil of approximately 10-m × 0.3-mm PTFE tubing at a temperature of approximately 135°, where a purple color is developed.

Reaction coil temperature: 135°

System suitability

Sample: *Standard solution*

Suitability requirements: All 17 amino acid peaks must be visible in the *Standard solution.*

Resolution: NLT 1.2 between the pairs of L-threonine and L-serine, L-cystine and L-valine, and L-isoleucine and L-leucine

Tailing factor: 0.8–1.5 for the peak of L-aspartic acid

Relative standard deviation: NMT 2.0% determined from the L-aspartic acid peak

Analysis

Samples: *Blank, Standard solution,* and *Sample solution*

Record and measure the responses for each amino acid peak in the *Standard solution* and *Sample solution.* Use the responses obtained from the *Standard solution* to calculate the content of each amino acid in the sample. Analyze the *Blank* to confirm purity of the reagent and diluent.

Calculations: Use well-recovered amino acids to quantify the protein. [NOTE—For additional information, see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, *Protein Hydrolysis,* which may be a helpful but not mandatory resource.] Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. Discard protein content values that deviate >5% from the mean. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP BSA for Protein Quantitation RS

Add the following:

▲⟨509⟩ RESIDUAL DNA TESTING

INTRODUCTION

The following method is suitable for measurement of residual host cell DNA in recombinant therapeutic products produced in either *Escherichia coli* (*E. coli*) or Chinese hamster ovary (CHO) cell lines. Also provided is an extraction procedure that is suitable to be used prior to a quantitative polymerase chain reaction (qPCR)-based method for the measurement of residual host cell DNA. For discussion of the principles and best practices for this type of testing, see *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)* ⟨1130⟩, which may be a helpful resource.

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PROCEDURE

• **SAMPLE PREPARATION**

There are several procedures for isolating DNA that may be appropriate for biopharmaceutical sample testing. One such procedure is described in detail below and validated for starting DNA concentrations ranging from 0.01 to 50 pg/µL.

Resuspension solution: Dissolve tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and ethylenediaminetetraacetic acid (EDTA) to obtain a solution of 10 mM and 1.0 mM, respectively. Add hydrochloric acid or sodium hydroxide to adjust to a pH of 8.0.

DNA standard stock solution: Dilute either the USP CHO Genomic DNA RS or the USP *E. coli* Genomic DNA RS, as appropriate, to a concentration of 1 µg/mL in *Resuspension solution*.

Sample solutions: Samples for testing may require dilution or reconstitution to: 1) overcome matrix interference affecting the DNA recovery, 2) yield an appropriate starting volume, or 3) bring the analyte concentration within the quantitative range of the qPCR method. *Sample solutions* may be diluted in water or in *Resuspension solution* if necessary. For drug substance samples, *Sample solutions* should contain sufficient starting material to allow determination of the residual DNA content, if present at the specification limit.

Positive control solution: Prepare by spiking *DNA standard stock solution* to *Sample solutions* at a concentration appropriate for the assay (specification, or otherwise justified).

Negative control solution: Water or *Resuspension solution* is used in place of *Sample solutions* in the extraction procedures and will be extracted with any samples (if extraction is necessary). The *Negative control solution* is tested using the qPCR-based method to determine the DNA content contributed by the background and to demonstrate that there is no potential cross-contamination during the assay.

70% Ethanol: Prepare by adding water to ethanol to give a final concentration of 70% (v/v).

Proteinase K solution: Dissolve Tris-HCl, EDTA, and sodium dodecyl sulfate (SDS) to obtain solutions of 100 mM (pH 8.0), 5 mM, and 100 mg/mL, respectively. Add proteinase K[1] to this solution to a final concentration of 10 mg/mL.

Sodium iodide solution: 6 M sodium iodide, 15 mM EDTA, 0.25% sodium sulfite, 0.5% sodium *N*-lauroyl sarcosinate, 25 mM Tris-HCl pH 8.0, and 35 µg/mL linear acrylamide. For 100 mL (scale as appropriate), while mixing with a stir bar on a magnetic plate, add in the following order: 50 mL of nuclease-free water, 0.25 g of sodium sulfite, 3.0 mL of 0.5 M EDTA solution, and 2.5 mL of 1 M Tris-HCl pH 8.0; then slowly add 89.93 g of sodium iodide. Add nuclease-free water to a final volume of 100 mL. Filter the solution using a 0.2-µm nylon membrane. [NOTE—This stock solution can be stored in the dark at 4° for up to 1 year.] On the day of use, add linear acrylamide and sodium *N*-lauroyl sarcosinate to final concentrations of 35 µg/mL and 0.5% (w/v), respectively. [NOTE—Addition of sodium *N*-lauroyl sarcosinate to sodium iodide solution may result in turbidity. If so, warming to 50–55° for 2–3 min is helpful before using for extraction.]

Extraction: Add 50 µL of *Proteinase K solution* to 450 µL of triplicate samples each of *Sample solutions* and *Positive control solution*, and at least one sample of *Negative control solution*, in a 2.0-mL centrifuge tube. Mix and incubate at 60 ± 2° for 1–24 h. Add 500 µL of *Sodium iodide solution* to each tube. Mix and incubate at 40° for 15 min. Add 900 µL of 100% ethanol, mix well, and incubate at room temperature for 15 min. Centrifuge at 10,000 × g for 15 min to pellet the DNA. [NOTE—Centrifugation at 2°–8° may be helpful to improve DNA recovery.] Remove and discard the supernatant. Wash the pellet by adding 1 mL of 70% ethanol containing 3 µg/mL linear acrylamide, centrifuge again, and discard the supernatant. Air dry the pellet for 5–10 min until no visible liquid is observed. Resuspend the pellet and record the volume of water or *Resuspension solution* used, which may be needed when reporting the final results in *Calculations*.

• **QPCR ANALYSIS**

2X Master mix: A suitable buffer containing magnesium chloride, deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, deoxyuridine triphosphate, deoxythymidine triphosphate, and highly purified *Thermus aquaticus* DNA polymerase.[2] Mix well immediately before use.

DNA stock primers and probes: According to the DNA of the species tested, prepare individual 10-µM solutions of the following primer pairs and probe, using DNAse-free water (see *Table 1*).

Table 1

E. coli	Forward primer	5′-CCTTACGACCAGGGCTACACA-3′
	Reverse primer	5′-CTCGCGAGGTCGCTTCTC-3′
	Probe[a]	5′-CGTGCTACAATGGCGCATACA-3′
CHO	Forward primer	5′-CCTGAGTTCAATTCCCAGCAA-3′
	Reverse primer	5′-ACATTCTGCTTCCATGTATATCTGCA-3′
	Probe[a]	5′-TGGCTCACAACCATCCGTTATGAGACCT-3′

[a] The probe can be 5′-labeled with 6-carboxyfluorescein and 3′-labeled with 6-carboxytetramethylrhodamine or a suitable alternative.

DNA probe solution: Dilute *DNA stock probe* to 2.5 µM with DNAse-free water.

Standard solutions: Dilute the *DNA standard stock solution* to obtain five or more suitable standards within the concentration range of 0.001–100 pg/µL.

Analysis

Samples: *Sample solutions*, *Positive control solution*, *Negative control solution*, and *Standard solutions*

[NOTE—If samples are extracted, then extracted *Sample solutions* and extracted *Control solutions* will be used.]

[1] Sigma catalog #P2308 or a suitable alternative.
[2] ThermoFisher catalog #4440038 or a suitable alternative.

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Transfer 25 µL of the *2X Master mix* to each well of a 96-well qPCR plate. Add 5 µL each of the *DNA stock forward primer*, the *DNA stock reverse primer*, and the *DNA probe solution* of the appropriate species to each well. Add 10 µL of either (extracted) *Sample solutions*, *Standard solutions*, (extracted) *Negative control solution*, or (extracted) *Positive control solution* to their respective wells. [NOTE—The qPCR reaction volume may be scaled as appropriate to accommodate different instruments.] Mix, seal the plate tightly, and centrifuge for 1 min at 1000 × g. Place the plate in a suitable qPCR thermal cycler. Incubate for 2 min at 50°, then for 10 min at 95°, followed by 40 cycles, with each cycle consisting of 95° for 15 s and 60° for 1 min. [NOTE—Some instruments and reagents require a preincubation step. Carefully follow specific instrument/reagent recommendations.] Monitor the signal of the labeled probe using a suitable fluorescent detector. Determine the threshold value using the instrument-specific recommendations. Record the cycle thresholds (C_t) for each sample.

Calculations

Plot the log quantity of DNA of the *Standard solutions* versus the C_t.

Calculate the slope and the intercept.

Using these values and the following equation, calculate the quantity of DNA in each well:

$$\text{Result} = 10^{\left(\frac{C_t - b}{m}\right)}$$

C_t = cycle threshold of the *Sample solutions*
b = intercept of the line for the *Standard solutions*
m = slope of the line for the *Standard solutions*

Calculate the quantity of DNA in each of the *Sample solutions*. Correct for any dilution or concentration of the sample.

System suitability

Samples: *Negative control solution* and *Standard solutions*

Suitability requirements

Negative control solution: The C_t corresponding to the *Negative control solution*, if any, is NLT the C_t of all of the lowest concentrations of the *Standard solutions*.

Sensitivity: The C_t corresponding to the lowest concentration of the *Standard solutions* is NMT 39.

Linearity: The regression coefficient associated with the *Standard solutions* is NLT 0.98. The slope is between −3.1 and −3.8.

Acceptance criteria

Any measurable sample must fall within the standard curve.

Accuracy: The mean spike recovery of three replicates of *Positive control solution* is between 50% and 150%. [NOTE—If needed due to the extraction procedure, correct for the sample dilution.]

Relative standard deviation: NMT 30% for three replicates of the *Sample solutions*; NMT 30% for three replicates of *Positive control solution*

The limit of residual DNA is defined in the product monograph.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP CHO Genomic DNA RS

USP *E. coli* Genomic DNA RS▲ (USP 1-Dec-2019)

⟨511⟩ SINGLE-STEROID ASSAY

In the following procedure, the steroid to be assayed is separated from related foreign steroids and excipients by thin-layer chromatography and determined following recovery from the chromatogram.

PREPARATION OF THE PLATE

Prepare a slurry from 30 g of chromatographic silica gel with a suitable fluorescing substance by the gradual addition, with mixing, of about 65 mL of a mixture of water and alcohol (5:2). Transfer the slurry to a clean, 20- × 20-cm plate, spread to make a uniform layer 250 µm thick, and allow to dry at room temperature for 15 minutes. Heat the plate at 105° for 1 hour, and store in a desiccator.

SOLVENT A

Mix methylene chloride with methanol (180:16).

SOLVENT B

Mix chloroform with acetone (4:1).

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STANDARD PREPARATION

Dissolve in a mixture of equal volumes of chloroform and alcohol a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried as directed (see *USP Reference Standards* ⟨11⟩) and accurately weighed, to obtain a solution having a known concentration of about 2 mg per mL.

ASSAY PREPARATION

Prepare as directed in the individual monograph.

PROCEDURE

Divide the area of the chromatographic plate into three equal sections, the left and right sections to be used for the *Assay Preparation* and the *Standard Preparation,* respectively, and the center section for the blank. Apply 200 µL each of the *Assay Preparation* and the *Standard Preparation* as streaks 2.5 cm from the bottom of the appropriate section of the plate. Dry the solution as it is being applied, with the aid of a stream of air. Using the *Solvent* specified in the individual monograph, develop the chromatogram in a suitable chamber, previously equilibrated and lined with absorbent paper, until the solvent front has moved 15 cm above the initial streaks.

Remove the plate, evaporate the solvent, and locate the principal band occupied by the *Standard Preparation* by viewing under UV light. Mark this band, as well as corresponding bands in the *Assay Preparation* and blank sections of the plate. Remove the silica gel from each band separately, either by scraping onto glazed weighing papers or by using a suitable vacuum collecting device, and transfer it to a glass-stoppered, 50-mL centrifuge tube. To each tube add 25.0 mL of alcohol, and shake for not less than 2 minutes. Centrifuge the tubes for 5 minutes, pipet 20 mL of the supernatant from each tube into a glass-stoppered, 50-mL conical flask, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Proceed as directed for *Procedure* under *Assay for Steroids* ⟨351⟩, beginning with "Then to each flask."

⟨525⟩ SULFUR DIOXIDE

The following methods are provided for the determination of sulfur dioxide in pharmaceutical excipients.

METHOD I

Procedure

Mix 20 g of the test specimen, accurately weighed, with 200 mL of an appropriate solvent as indicated in each individual monograph, and stir until a smooth suspension is obtained. Allow the test specimen mixture to remain undisturbed until most of the test specimen has settled, and filter the aqueous portion through paper (Whatman No. 1 or equivalent). To 100 mL of the clear filtrate add an additional solvent as indicated in each individual monograph, add 3 mL of starch TS, and titrate with 0.01 N iodine solution VS to the first permanent blue or purple color. Each 1.0 mL of 0.01 N iodine solution VS consumed corresponds to 0.003% of sulfur dioxide found.

METHOD II

Procedure

Transfer about 50 to 100 g of the substance to be tested, accurately weighed, to a 250-mL conical flask, add 100 to 150 mL of water, and mix. Cool to between 5° and 10°. While stirring with a magnetic stirrer, add 10 mL of cold 1.5 N sodium hydroxide (at a temperature between 5° and 10°). Stir for an additional 20 seconds, and add 10 mL of starch indicator solution, prepared as follows: mix 10 g of soluble starch with 50 mL of cold water, transfer to 1000 mL of boiling water, stir until completely dissolved, cool, and add 1 g of salicylic acid preservative.

[NOTE—Discard the solution after 1 month.]

Add 10 mL of 2.0 N sulfuric acid (at a temperature between 5° and 10°), and titrate immediately with 0.005 N iodine VS until a light blue color persists for 1 minute (see *Titrimetry* ⟨541⟩). Perform a blank determination, using 200 mL of water treated similarly to the solution under test, and make any necessary correction. Each mL of 0.005 N iodine is equivalent to 0.16 mg of SO_2.

METHOD III

Procedure

Dissolve 20.0 g of the test specimen in 150 mL of hot water in a flask having a round bottom and a long neck, add 5 mL of phosphoric acid and 1 g of sodium bicarbonate, and at once connect the flask to a condenser.

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[NOTE—Excessive foaming can be alleviated by the addition of a few drops of a suitable antifoaming agent.] Distill 50 mL, receiving the distillate under the surface of 50 mL of 0.1 N iodine. Acidify the distillate with a few drops of hydrochloric acid, add 2 mL of barium chloride TS, and heat on a steam bath until the liquid is nearly colorless. The precipitate of barium sulfate, if any, when filtered, washed, and ignited, weighs not more than 3 mg, corresponding to not more than 0.004% of sulfur dioxide, correction being made for any sulfate that may be present in 50 mL of the 0.1 N iodine.

METHOD IV

In this test, sulfur dioxide is released from the test specimen in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in a dilute hydrogen peroxide solution, in which the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali, using a pH meter to control the pH value and titration. This test is performed under conditions such that the requirements specified in the system suitability test are met.

Special Reagents

CARBON DIOXIDE

Use carbon dioxide with a flow regulator that will maintain a flow of 100 ± 10 mL per minute.

HYDROGEN PEROXIDE SOLUTION

Dilute 30% hydrogen peroxide with water to obtain a 3% solution. Neutralize the 3% hydrogen peroxide solution with 0.01 N sodium hydroxide to a pH of 4.1 determined potentiometrically.

POTASSIUM METABISULFITE SOLUTION

Transfer 0.87 g of potassium metabisulfite ($K_2S_2O_5$) and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Dilute with water to volume before mixing. [NOTE—Edetate disodium is used to protect sulfite ion from oxidation.]

Apparatus

A suitable apparatus for sulfur dioxide determination is shown in the accompanying diagram (*Figure 1*).

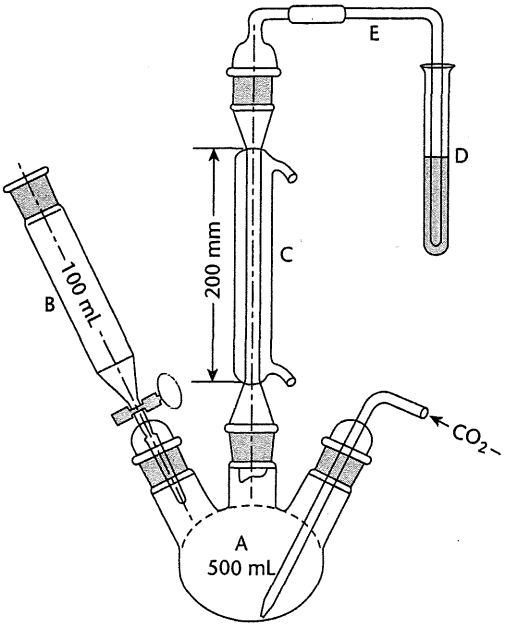


Figure 1. Apparatus for Method IV.

The apparatus consists of a 500-mL three-neck, round-bottom boiling flask, *A;* a separatory funnel, *B,* having a capacity of 100 mL or greater; a gas inlet tube of sufficient length to permit introduction of the carbon dioxide within 2.5 cm of the bottom of the boiling flask; a reflux condenser, *C,* having a jacket length of 200 mm; and a delivery tube, *E,* connecting the upper end of the reflux condenser to the bottom of a receiving test tube, *D.* Apply a thin film of stopcock grease to the sealing surfaces of all joints except the joint between the separatory funnel and the boiling flask, and clamp the joints to ensure tightness.

General Chapters

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System Suitability Test

TEST A

Using the *Potassium Metabisulfite Solution* as the standard, proceed as directed for *Procedure*, except replace the 25.0 g of test substance with 20 mL of *Potassium Metabisulfite Solution*. Calculate the content, in µg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* taken by the formula:

$$1000(32.03)VN/V_P$$

in which the factor 1000 converts mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and V_P is the volume, in mL, of the *Potassium Metabisulfite Solution* taken for the test.

TEST B

In a 100-mL conical flask, add 20 mL of 0.02 N iodine solution and 5 mL of 2 N hydrochloric acid. Add 1 mL of starch TS, and titrate with the *Potassium Metabisulfite Solution* until the first discoloration is observed. Calculate the content, in µg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* by the formula:

$$1000(32.03)V_IN_I/V_P$$

in which 1000 and 32.03 are defined above; V_I is the volume, in mL, of the iodine solution used in the test; N_I is the normality of the iodine solution; and V_P is the volume, in mL, of the *Potassium Metabisulfite Solution* consumed.

The difference between the sulfur dioxide contents obtained from *Test A* and *Test B* is not more than 5% of their mean value. *Test B* shall be performed within 15 minutes after completion of *Test A*. [NOTE—This avoids a potential variation of the sulfur dioxide content in the *Potassium Metabisulfite Solution* when stored at room temperature.]

Procedure

Add 150 mL of water to the boiling flask (*A*). Close the stopcock of the separatory funnel, and begin the flow of carbon dioxide at a rate of 100 ± 5 mL per minute through the apparatus. Start the condenser coolant flow. Place 10 mL of *Hydrogen Peroxide Solution* in the receiving test tube (*D*). After 15 minutes, without interrupting the flow of carbon dioxide, remove the separatory funnel (*B*) from the boiling flask, and transfer 25.0 g of the test specimen to the boiling flask with the aid of 100 mL of water. Apply stopcock grease to the outer joint of the separatory funnel, and replace the separatory funnel in the boiling flask. Close the stopcock of the separatory funnel, and add 80 mL of 2 N hydrochloric acid to the separatory funnel. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the boiling flask, guarding against escape of sulfur dioxide into the separatory funnel by closing the stopcock before the last few mL of hydrochloric acid drain out. Boil the mixture for 1 hour. Open the stopcock of the funnel, stop the flow of carbon dioxide, discontinue heating the flask, and turn off the cooling water in the condenser. Remove the receiving test tube, and transfer its contents to a 200-mL wide-necked, conical flask. Rinse the receiving test tube with a small portion of water, add the rinsing to the 200-mL conical flask, and mix. Heat on a water bath for 15 minutes, and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate the contents with 0.1 N sodium hydroxide VS until the color changes from yellow to violet-blue, with the color change lasting for at least 20 seconds. Perform a blank determination and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the content, in µg per g, of sulfur dioxide in the test specimen taken by the formula:

$$1000(32.03)VN/W$$

in which the factor 1000 converts mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and W is the weight, in g, of the test specimen taken.

METHOD V

In this method, similar to *Method IV*, sulfur dioxide is released from the test specimen in a boiling acid medium and is removed by a stream of nitrogen. The separated gas is collected in a dilute hydrogen peroxide solution, in which the sulfur dioxide is oxidized to sulfuric acid and titrated with standard alkali, using methyl red as an indicator. This test is performed under conditions such that the requirements specified in the system suitability test are met.

Special Reagents

HYDROGEN PEROXIDE SOLUTION

Dilute a portion of 30 percent hydrogen peroxide with water to obtain a 3% solution. Just before use, add 3 drops of methyl red TS, and neutralize to a yellow endpoint with 0.01 N sodium hydroxide. Do not exceed the endpoint.

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NITROGEN

Use high-purity nitrogen with a flow regulator that will maintain a flow of 200 ± 10 mL per minute. Guard against the presence of oxygen by passing the nitrogen through a scrubber, such as alkaline pyrogallol, prepared as follows: add 4.5 g of pyrogallol to a gas-washing bottle, purge the bottle with nitrogen for 3 minutes, and add a solution containing 85 mL of water and 65 g of potassium hydroxide while maintaining an atmosphere of nitrogen in the bottle.

POTASSIUM METABISULFITE SOLUTION

Transfer 0.87 g of potassium metabisulfite ($K_2S_2O_5$) and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Dilute with water to volume before mixing. [NOTE—Edetate disodium is used to protect sulfite ion from oxidation.]

Apparatus

The apparatus (see *Figure 2*) is designed to effect the selective transfer of sulfur dioxide from the specimen in boiling aqueous hydrochloric acid to the *Hydrogen Peroxide Solution* in vessel *G*. The backpressure is limited to the unavoidable pressure due to the height of the *Hydrogen Peroxide Solution* above the tip of the bubbler, *F*. Keeping the backpressure as low as possible reduces the likelihood that sulfur dioxide will be lost through leaks. Preboil vinyl and silicone tubing. Apply a thin film of stopcock grease to the sealing surfaces of all joints, except the joint between the separatory funnel and the flask, and clamp the joints to ensure tightness. The separatory funnel, *B*, has a capacity of 100 mL or greater. The inlet adapter, *A*, with a hose connector, provides a means of applying headpressure over the solution.

[NOTE—A pressure-equalizing dropping funnel is not recommended because condensate, which may contain sulfur dioxide, is deposited in the funnel and the side arm.]

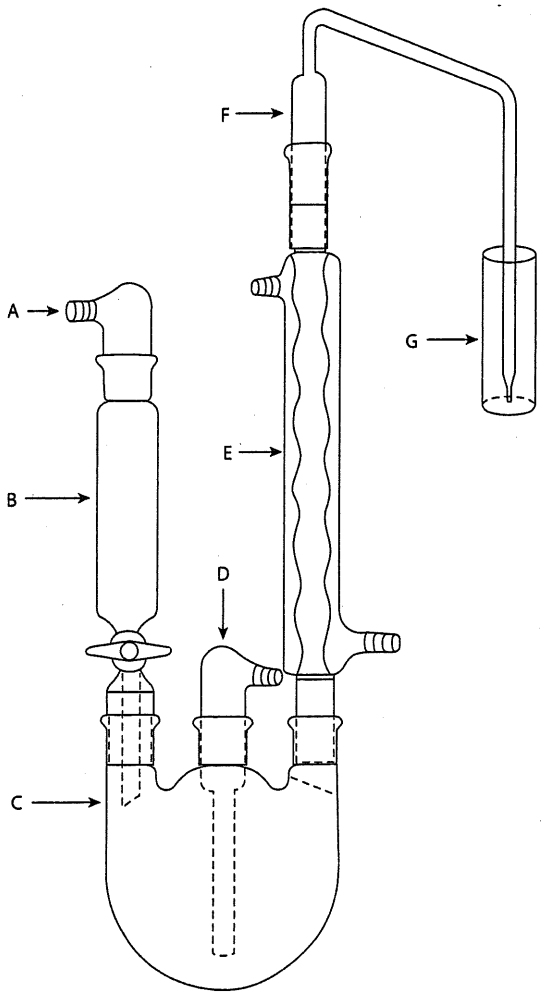


Figure 2. Apparatus for Method V.

The round-bottom flask, *C*, is a 1000-mL flask with three 24/40 tapered joints. The gas inlet tube, *D*, is long enough to permit introduction of the nitrogen to within 2.5 cm of the bottom of the flask. The Allihn condenser, *E*, has a jacket length of 300 mm.

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The bubbler, *F* (see *Figure 3*), is fabricated from glass according to the dimensions given in *Figure 3*. The *Hydrogen Peroxide Solution* is contained in the vessel, *G*, having an inside diameter of about 2.5 cm and a depth of about 18 cm. Circulate coolant, such as a mixture of water and methanol (4:1) maintained at 5°, to chill the condenser.

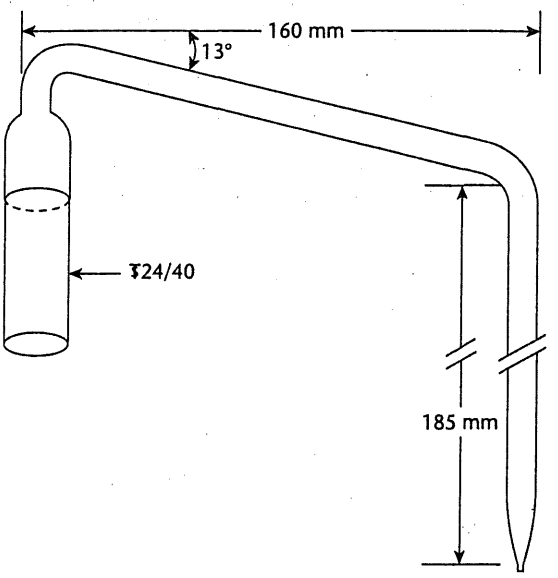


Figure 3. Bubbler (F) for apparatus in Method V.

System Suitability Test

TEST A

Using the *Potassium Metabisulfite Solution* as the standard, proceed as directed for *Procedure*, except replace the 50.0 g of test substance with 20 mL of *Potassium Metabisulfite Solution*. Calculate the content, in µg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* taken by the formula:

$$1000(32.03)VN/V_P$$

in which the factor 1000 converts mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and V_P is the volume, in mL, of *Potassium Metabisulfite Solution* taken for the test.

TEST B

In a 100-mL conical flask, add 20 mL of 0.02 N iodine solution and 5 mL of 2 N hydrochloric acid. Add 1 mL of starch TS, and titrate with the *Potassium Metabisulfite Solution* until the first discoloration is observed. Calculate the content, in µg per mL, of sulfur dioxide in the *Potassium Metabisulfite Solution* by the formula:

$$1000(32.03)V_IN_I/V_P$$

in which 1000 and 32.03 are defined above; V_I is the volume, in mL, of iodine solution used in the test; N_I is the normality of the iodine solution; and V_P is the volume, in mL, of *Potassium Metabisulfite Solution* consumed.

The difference between the sulfur dioxide contents obtained from *Test A* and *Test B* is not more than 5% of their mean value. *Test B* shall be performed within 15 minutes after completion of *Test A*. [NOTE—This avoids a potential variation of the sulfur dioxide content in the *Potassium Metabisulfite Solution* when stored at room temperature.]

Procedure

Position the apparatus in a heating mantle controlled by a power-regulating device. Add 400 mL of water to the flask. Close the stopcock of the separatory funnel, and add 90 mL of 4 N hydrochloric acid to the separatory funnel. Begin the flow of nitrogen at a rate of 200 ± 10 mL per minute. Start the condenser coolant flow. Add 30 mL of *Hydrogen Peroxide Solution* to the vessel *(G)*. After 15 minutes, remove the separatory funnel, and transfer a mixture of 50.0 g of the test specimen, accurately weighed, and 100 mL of alcohol solution (5 in 100) to the flask. Apply stopcock grease to the outer joint of the separatory funnel, return the separatory funnel to the tapered joint flask, and concomitantly resume the nitrogen flow. Apply headpressure above the hydrochloric acid solution in the separatory funnel with a rubber bulb equipped with a valve. Open the stopcock of the separatory funnel to permit the hydrochloric acid solution to flow into the flask. Continue to maintain sufficient pressure above the hydrochloric acid solution to force it into the flask.

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[NOTE—The stopcock may be temporarily closed, if necessary, to increase the pressure.]
To guard against escape of sulfur dioxide into the separatory funnel, close the stopcock before the last few mL of hydrochloric acid drain out. Apply power to the heating mantle sufficient to cause about 85 drops of reflux per minute. After refluxing for 1.75 hours, remove the vessel *(G)*, add 3 drops of methyl red TS, and titrate the contents with 0.01 N sodium hydroxide VS, using a 10-mL buret with an overflow tube and a hose connection to a carbon dioxide-absorbing tube, to a yellow endpoint that persists for at least 20 seconds. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Calculate the quantity, in µg, of SO_2 in each g of the test specimen taken by the formula:

$$1000(32.03)VN/W$$

in which the factor 1000 converts mg to µg; 32.03 is the milliequivalent weight of sulfur dioxide; V is the volume, in mL, of titrant consumed; N is the normality of the titrant; and W is the weight, in g, of the test specimen taken.

⟨531⟩ THIAMINE ASSAY

ASSAY

• CHEMICAL METHODS, PROCEDURE 1

The following procedure is provided for the determination of thiamine as an ingredient of pharmacopeial preparations containing other active constituents. The procedure involves thiamine reacting with potassium ferricyanide and subsequently being determined by fluorescence detection. Throughout the procedure, protect solutions containing and derived from the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of low-actinic glassware.

Potassium ferricyanide solution: Dissolve 1.0 g of potassium ferricyanide in water to make 100 mL. Prepare fresh on the day of use.

Oxidizing reagent: Mix 4.0 mL of *Potassium ferricyanide solution* with a sufficient amount of 3.5 N sodium hydroxide to make 100 mL. Use this solution within 4 h.

Quinine sulfate stock solution: Dissolve 10 mg of quinine sulfate in 0.1 N sulfuric acid to make 1000 mL. Preserve this solution, protected from light, in a refrigerator.

Quinine sulfate standard solution: Dilute 0.1 N sulfuric acid with *Quinine sulfate stock solution* (39:1). This solution fluoresces to approximately the same degree as the thiochrome obtained from 1 µg of thiamine hydrochloride and is used to correct the fluorometer at frequent intervals for variation in sensitivity from reading to reading within an assay. Prepare this solution fresh on the day of use.

Standard thiamine hydrochloride stock solution: Transfer about 25 mg of USP Thiamine Hydrochloride RS, accurately weighed, to a 1000-mL volumetric flask. Dissolve the weighed Standard in about 300 mL of dilute alcohol solution (1 in 5) adjusted with 3 N hydrochloric acid to a pH of 4.0, and add the acidified, dilute alcohol to volume. Store in a refrigerator in a light-resistant container. Prepare this stock solution fresh each month.

Standard solution: Dilute a portion of *Standard thiamine hydrochloride stock solution* quantitatively and stepwise with 0.2 N hydrochloric acid to obtain the *Standard solution*, each mL of which represents 0.2 µg of USP Thiamine Hydrochloride RS.

Sample solution: Place in a suitable volumetric flask a sufficient amount of the material to be assayed, accurately weighed or measured by volume as directed, such that when diluted with 0.2 N hydrochloric acid to volume, the resulting solution will contain about 100 µg of thiamine hydrochloride (or mononitrate) per mL. If the sample is difficult to dissolve, the solution may be heated on a steam bath, and then cooled and diluted with the acid to volume. Dilute 5 mL of this solution, quantitatively and stepwise, using 0.2 N hydrochloric acid, to an estimated concentration of 0.2 µg of thiamine hydrochloride (or mononitrate) per mL.

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 365 nm

Emission wavelength: 435 nm

Analysis: To each of three or more tubes (or other suitable vessels) of about 40-mL capacity, pipet 5 mL of *Standard solution*. To each of two of these tubes, add rapidly (within 1 to 2 s), with mixing, 3.0 mL of *Oxidizing reagent*, and within 30 s add 20.0 mL of isobutyl alcohol, then mix vigorously for 90 s by shaking the capped tubes manually, or by bubbling a stream of air through the mixture. Prepare a blank in the remaining tube of the standard by substituting for the *Oxidizing reagent* with an equal volume of 3.5 N sodium hydroxide and proceed in the same manner. Into each of three or more similar tubes, pipet 5 mL of the *Sample solution*. Treat these tubes in the same manner as directed for the tubes containing the *Standard solution*. Into each of the six tubes, pipet 2 mL of dehydrated alcohol, swirl for a few seconds, allow the phases to separate, and decant or draw off about 10 mL of the clear, supernatant isobutyl alcohol solution into the standardized cells; then measure the fluorescence in a suitable fluorometer, having an input filter of narrow transmittance range with a maximum at about 365 nm and an output filter of narrow transmittance range with a maximum at about 435 nm.

Calculate the quantity, in µg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in each 5 mL of the *Sample solution*:

$$\text{Result} = (A - b)/(S - d)$$

A = average fluorometer readings of the portions of the *Sample solution* treated with the *Oxidizing reagent*
b = reading for the blank of the *Sample solution*
S = average fluorometer readings of the portions of the *Standard solution* treated with the *Oxidizing reagent*

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d = reading for the blank of the *Standard solution*

Calculate the quantity, in mg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the assay material on the basis of the aliquots taken. Where indicated, the quantity, in mg, of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) may be calculated by multiplying the quantity of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) found by 0.9706.

The following liquid chromatographic procedures are provided for the determination of thiamine as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in dietary supplements or pharmaceutical dosage forms. Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware.

• **CHROMATOGRAPHIC METHODS, PROCEDURE 1**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: Acetonitrile, glacial acetic acid, and water (5:1:94)

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 180 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

r_U = peak response of thiamine from the appropriate *Sample solution*

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

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

C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

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For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

• **CHROMATOGRAPHIC METHODS, PROCEDURE 2**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by dilute hydrochloric acid, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Solution A: 1.88 g/L of sodium 1-hexanesulfonate in 0.1% phosphoric acid

Mobile phase: *Solution A* and acetonitrile (46:9)

Standard stock solution: 0.1 mg/mL of USP Thiamine Hydrochloride RS in 0.2 N hydrochloric acid

Standard solution: 0.02 mg/mL of USP Thiamine Hydrochloride RS from *Standard stock solution* diluted with 0.2 N hydrochloric acid

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Mix a portion of the capsule contents with a volume of 0.2 N hydrochloric acid to obtain a concentration of 0.02 mg/mL of thiamine. Shake the solution for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Sample solution for tablets: Weigh and finely powder NLT 20 tablets. Mix a portion of the powder with a volume of 0.2 N hydrochloric acid to obtain a concentration of 0.02 mg/mL of thiamine. Shake for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

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$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

• **Chromatographic Methods, Procedure 3**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by mixtures of organic solvents, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: 25 mg/mL of edetate disodium in water

Mobile phase: Transfer 0.4 mL of triethylamine, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask. Dilute with 0.008 M sodium 1-hexanesulfonate to volume.

Standard stock solution: 0.24 mg/mL of USP Thiamine Hydrochloride RS in *Diluent*, with heating if necessary

Standard solution for capsules/tablets: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Standard solution for oral solution: 24 µg/mL of USP Thiamine Hydrochloride RS, diluted from the *Standard stock solution* with *Diluent*

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 1.2 mg of thiamine hydrochloride, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Sample solution for oral solution: Equivalent to 24 µg/mL of thiamine hydrochloride or thiamine mononitrate from oral solution in *Diluent*

Sample solution for tablets: Weigh and finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 1.2 mg of thiamine, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

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

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the appropriate *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the appropriate *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

• **CHROMATOGRAPHIC METHODS, PROCEDURE 4**

This procedure can be used to determine thiamine in:

- An active pharmaceutical ingredient
- A dietary ingredient

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as active pharmaceutical or dietary supplement ingredients.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: 0.005 M sodium 1-octanesulfonate in dilute glacial acetic acid (1 in 100)
Solution B: Methanol and acetonitrile (3:2)
Mobile phase: *Solution A* and *Solution B* (60:40)
Internal standard solution: 2% (v/v) of methylbenzoate in methanol
Standard solution: Prepare a 1-mg/mL solution of USP Thiamine Hydrochloride RS in *Mobile phase*. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. The *Standard solution* contains 400 µg/mL of thiamine hydrochloride.
Sample solution: Prepare a 2-mg/mL solution of thiamine hydrochloride or thiamine mononitrate in *Mobile phase*. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted to obtain a retention time of about 12 min for thiamine.]
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between the thiamine and methylbenzoate peaks
Tailing factor: NMT 2.0 for the thiamine peak
Column efficiency: NLT 1500 theoretical plates for thiamine
Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the internal standard peak response
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

R_U = peak response ratio of thiamine to methylbenzoate from the *Sample solution*
R_S = peak response ratio of thiamine to methylbenzoate from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of thiamine hydrochloride in the *Sample solution* (mg/mL)

If the active pharmaceutical or dietary supplement ingredient is thiamine mononitrate, calculate the percentage of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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R_U = peak response ratio of thiamine to methylbenzoate from the *Sample solution*
R_S = peak response ratio of thiamine to methylbenzoate from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

• **Chromatographic Methods, Procedure 5**

This procedure can be used to determine thiamine in:

- *Thiamine Hydrochloride Injection*
- *Thiamine Hydrochloride Oral Solution*
- *Thiamine Mononitrate Oral Solution*

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as an active ingredient in the formulations such as those used in *Thiamine Hydrochloride Injection*, *Thiamine Hydrochloride Oral Solution*, and *Thiamine Mononitrate Oral Solution*.

Unless specified in the individual monographs, the *Standard solution*, *Internal standard solution*, and *Sample solution* are prepared as follows.

Mobile phase: Methanol and 0.04 M aqueous monobasic potassium phosphate (45:55)
Internal standard solution: 100 µg/mL of methylparaben in *Mobile phase*
Standard stock solution: 500 µg/mL of USP Thiamine Hydrochloride RS in *Mobile phase*
Standard solution: Dilute a mixture of equal volumes of the *Standard stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of USP Thiamine Hydrochloride RS of about 50 µg/mL.
Sample stock solution: Equivalent to 500 µg/mL of thiamine hydrochloride or thiamine mononitrate in *Mobile phase* from an accurately measured volume of oral solution or injection
Sample solution: Dilute a mixture of equal volumes of the *Internal standard solution* and *Sample stock solution* with *Mobile phase* to obtain a concentration of thiamine hydrochloride or thiamine mononitrate of about 50 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for thiamine and methylparaben are about 0.35 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between thiamine and methylparaben
Column efficiency: NLT 1500 theoretical plates for thiamine
Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the methylparaben peak response
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

R_U = peak response ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak response ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine hydrochloride in the *Sample solution* (mg/mL)

If the products contain thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak response ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

• **Chromatographic Methods, Procedure 6**

This procedure can be used to determine thiamine in the following:

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- *Thiamine Hydrochloride Tablets*

This is the procedure that involves the dissolution of the sample directly into water. The procedure is applicable to the performance (dissolution) test for *Thiamine Hydrochloride Tablets*.

Unless specified in the individual monographs, the *Standard solution* and *Sample solution* are prepared as follows.

Medium: Water, 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL
Standard solution: A known concentration of USP Thiamine Hydrochloride RS in *Medium* (water)
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* (water) if necessary
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

r_U = peak response of thiamine from the *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled amount of thiamine hydrochloride (mg/Tablet)

• **CHROMATOGRAPHIC METHODS, PROCEDURE 7**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is a newly added procedure as part of the USP monograph modernization efforts. The procedure uses hydrophilic interaction liquid chromatography (HILIC), and the sample preparation involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: Methanol, glacial acetic acid, and water (50:1:49)
Solution A: 50 mM ammonium formate. Adjust with ammonium hydroxide to a pH of 9.0.
Solution B: Acetonitrile
Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	11	89
8	17	83
15	23	77

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Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
20	30	70
21	50	50
24	50	50
25	11	89
30	11	89

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 160 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 68°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate.

Sample solution for oral solution: Equivalent to 0.1 mg/mL of thiamine hydrochloride in *Diluent*, from an accurately measured volume of oral solution. In an appropriate volumetric flask, dissolve the sample with *Diluent* to about 80% of the total volume, immerse the flask in a water bath maintained at 68° for 10 min, chill rapidly in a cold water bath to room temperature, and dilute with *Diluent*. Mix well, and filter a portion of the solution; finally, use the clear filtrate.

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

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

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L68

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

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

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

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36

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M_{r2} = molecular weight of thiamine hydrochloride, 337.27

ADDITIONAL REQUIREMENTS
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Thiamine Hydrochloride RS

⟨541⟩ TITRIMETRY

DIRECT TITRATIONS

Direct titration is the treatment of a soluble substance, contained in solution in a suitable vessel (the titrate), with an appropriate standardized solution (the titrant), the endpoint being determined instrumentally or visually with the aid of a suitable indicator.

The titrant is added from a suitable buret and is so chosen, with respect to its strength (normality), that the volume added is between 30% and 100% of the rated capacity of the buret.

[NOTE—Where less than 10 mL of titrant is required, a suitable microburet is to be used.]

The endpoint is approached directly but cautiously, and finally the titrant is added dropwise from the buret in order that the final drop added will not overrun the endpoint. The quantity of the substance being titrated may be calculated from the volume and the normality or molarity factor of the titrant and the equivalence factor for the substance given in the individual monograph.

RESIDUAL TITRATIONS

Some Pharmacopeial assays require the addition of a measured volume of a volumetric solution, in excess of the amount actually needed to react with the substance being assayed, the excess of this solution then being titrated with a second volumetric solution. This constitutes a residual titration and is known also as a "back titration." The quantity of the substance being titrated may be calculated from the difference between the volume of the volumetric solution originally added, corrected by means of a blank titration, and that consumed by the titrant in the back titration, due allowance being made for the respective normality or molarity factors of the two solutions, and the equivalence factor for the substance given in the individual monograph.

COMPLEXOMETRIC TITRATIONS

Successful complexometric titrations depend on several factors. The equilibrium constant for formation of the titrant-analyte complex must be sufficiently large that, at the endpoint, very close to 100% of the analyte has been complexed. The final complex must be formed rapidly enough that the analysis time is practical. When the analytical reaction is not rapid, a residual titration may sometimes be successful.

In general, complexometric indicators are themselves complexing agents. The reaction between metal ion and indicator must be rapid and reversible. The equilibrium constant for formation of the metal-indicator complex should be large enough to produce a sharp color change but must be less than that for the metal-titrant complex. Indicator choice is also restricted by the pH range within which the complexation reaction must be carried out and by interference of other ions arising from the sample or the buffer. Interfering ions may often be masked or "screened" via addition of another complexing agent. (The masking technique is also applicable to redox titrations.)

OXIDATION-REDUCTION (REDOX) TITRATIONS

Determinations may often be carried out conveniently by the use of a reagent that brings about oxidation or reduction of the analyte. Many redox titration curves are not symmetric about the equivalence point, and thus graphical determination of the endpoint is not possible; but indicators are available for many determinations, and a redox reagent can often serve as its own indicator. As in any type of titration, the ideal indicator changes color at an endpoint that is as close as possible to the equivalence point. Accordingly, when the titrant serves as its own indicator, the difference between the endpoint and the equivalence point is determined only by the analyst's ability to detect the color change. A common example is the use of permanganate ion as an oxidizing titrant since a slight excess can easily be detected by its pink color. Other titrants that may serve as their own indicators are iodine, cerium (IV) salts, and potassium dichromate. In most cases, however, the use of an appropriate redox indicator will yield a much sharper endpoint.

It may be necessary to adjust the oxidation state of the analyte prior to titration through use of an appropriate oxidizing or reducing agent; the excess reagent must then be removed, e.g., through precipitation. This is nearly always the practice in the determination of oxidizing agents since most volumetric solutions of reducing agents are slowly oxidized by atmospheric oxygen.

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TITRATIONS IN NONAQUEOUS SOLVENTS

Acids and bases have long been defined as substances that furnish, when dissolved in water, hydrogen and hydroxyl ions, respectively. This definition, introduced by Arrhenius, fails to recognize the fact that properties characteristic of acids or bases may be developed also in other solvents. A more generalized definition is that of Brönsted, who defined an acid as a substance that furnishes protons, and a base as a substance that combines with protons. Even broader is the definition of Lewis, who defined an acid as any material that will accept an electron pair, a base as any material that will donate an electron pair, and neutralization as the formation of a coordination bond between an acid and a base.

The apparent strength of an acid or a base is determined by the extent of its reaction with a solvent. In water solution all strong acids appear equally strong because they react with the solvent to undergo almost complete conversion to oxonium ion and the acid anion (leveling effect). In a weakly protophilic solvent such as acetic acid the extent of formation of the acetate acidium ion shows that the order of decreasing strength for acids is perchloric, hydrobromic, sulfuric, hydrochloric, and nitric (differentiating effect).

Acetic acid reacts incompletely with water to form oxonium ion and is, therefore, a weak acid. In contrast, it dissolves in a base such as ethylenediamine, and reacts so completely with the solvent that it behaves as a strong acid. The same holds for perchloric acid.

This leveling effect is observed also for bases. In sulfuric acid almost all bases appear to be of the same strength. As the acid properties of the solvent decrease in the series sulfuric acid, acetic acid, phenol, water, pyridine, and butylamine, the bases become progressively weaker until all but the strongest have lost their basic properties. In order of decreasing strength, the strong bases are sodium 2-aminoethoxide, potassium methoxide, sodium methoxide, and lithium methoxide.

Many water-insoluble compounds acquire enhanced acidic or basic properties when dissolved in organic solvents. Thus the choice of the appropriate solvent permits the determination of a variety of such materials by nonaqueous titration. Furthermore, depending upon which part of a compound is the physiologically active moiety, it is often possible to titrate that part by proper selection of solvent and titrant. Pure compounds can be titrated directly, but it is often necessary to isolate the active ingredient in pharmaceutical preparations from interfering excipients and carriers.

The types of compounds that may be titrated as acids include acid halides, acid anhydrides, carboxylic acids, amino acids, enols such as barbiturates and xanthines, imides, phenols, pyrroles, and sulfonamides. The types of compounds that may be titrated as bases include amines, nitrogen-containing heterocyclic compounds, oxazolines, quaternary ammonium compounds, alkali salts of organic acids, alkali salts of weak inorganic acids, and some salts of amines. Many salts of halogen acids may be titrated in acetic acid or acetic anhydride after the addition of mercuric acetate, which removes halide ion as the unionized mercuric halide complex and introduces the acetate ion.

For the titration of a basic compound, a volumetric solution of perchloric acid in glacial acetic acid is preferred, although perchloric acid in dioxane is used in special cases. The calomel-glass electrode system is useful in this case. In acetic acid solvent, this electrode system functions as predicted by theory.

For the titration of an acidic compound, two classes of titrant are available: the alkali metal alkoxides and the tetraalkylammonium hydroxides. A volumetric solution of sodium methoxide in a mixture of methanol and toluene is used frequently, although lithium methoxide in methanol-benzene solvent is used for those compounds yielding a gelatinous precipitate on titration with sodium methoxide.

The alkali error limits the use of the glass electrode as an indicating electrode in conjunction with alkali metal alkoxide titrants, particularly in basic solvents. Thus, the antimony-indicating electrode, though somewhat erratic, is used in such titrations. The use of quaternary ammonium hydroxide compounds, e.g., tetra-*n*-butylammonium hydroxide and trimethylhexadecylammonium hydroxide (in benzene-methanol or isopropyl alcohol), has two advantages over the other titrants in that (a) the tetraalkylammonium salt of the titrated acid is soluble in the titration medium, and (b) the convenient and well-behaved calomel-glass electrode pair may be used to conduct potentiometric titrations.

Because of interference by carbon dioxide, solvents for acidic compounds need to be protected from excessive exposure to the atmosphere by a suitable cover or by an inert atmosphere during the titration. Absorption of carbon dioxide may be determined by performing a blank titration. The blank should not exceed 0.01 mL of 0.1 N sodium methoxide VS per mL of solvent.

The endpoint may be determined visually by color change, or potentiometrically, as indicated in the individual monograph. If the calomel reference electrode is used, it is advantageous to replace the aqueous potassium chloride salt bridge with 0.1 N lithium perchlorate in glacial acetic acid for titrations in acidic solvents or potassium chloride in methanol for titrations in basic solvents.

Where these or other mixtures are specified in individual monographs, the calomel reference electrode is modified by first removing the aqueous potassium chloride solution and residual potassium chloride, if any, by rinsing with water, then eliminating residual water by rinsing with the required nonaqueous solvent, and finally filling the electrode with the designated nonaqueous mixture.

In nearly all cases, except those where silver ion might interfere, a silver-silver chloride reference electrode may be substituted for the calomel electrode. The silver-silver chloride electrode is more rugged, and its use helps to eliminate toxic mercury salts from the laboratory. Generally, a salt bridge may be used to circumvent interference by silver ion.

The more useful systems for titration in nonaqueous solvents are listed in *Table 1*.

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Table 1. Systems for Nonaqueous Titrations

Type of Solvent	Acidic (for titration of bases and their salts)	Relatively Neutral (for differential titration of bases)	Basic (for titration of acids)	Relatively Neutral (for differential titration of acids)
Solvent[1]	Glacial Acetic Acid	Acetonitrile	Dimethylformamide	Acetone
	Acetic Anhydride	Alcohols	*n*-Butylamine	Acetonitrile
	Formic Acid	Chloroform	Pyridine	Methyl Ethyl Ketone
	Propionic Acid	Benzene	Ethylenediamine	Methyl Isobutyl Ketone
	Sulfuryl Chloride	Toluene	Morpholine	*tert*-Butyl Alcohol
		Chlorobenzene		
		Ethyl Acetate		
		Dioxane		
Indicator	Crystal Violet	Methyl Red	Thymol Blue	Azo Violet
	Quinaldine Red	Methyl Orange	Thymolphthalein	Bromothylmol Blue
	p-Naphtholbenzein	*p*-Naphtholbenzein	Azo Violet	*p*-Hydroxyazobenzene
	Alphezurine 2-G		*o*-Nitroaniline	Thymol Blue
	Malachite Green		*p*-Hydroxyazobenzene	
Electrodes	Glass–calomel	Glass–calomel	Antimony–calomel	Antimony–calomel
	Glass–silver–silver chloride	Calomel–silver–silver chloride	Antimony–glass	Glass–calomel
	Mercury–mercuric acetate		Antimony–antimony[2]	Glass–platinum[2]
			Platinum–calomel	
			Glass–calomel	

[1] Relatively neutral solvents of low dielectric constant such as benzene, toluene, chloroform, or dioxane may be used in conjunction with any acidic or basic solvent in order to increase the sensitivity of the titration end-points.
[2] In titrant.

INDICATOR AND POTENTIOMETRIC ENDPOINT DETECTION

The simplest and most convenient method by which the equivalence point, i.e., the point at which the stoichiometric analytical reaction is complete, may be determined is with the use of indicators. These chemical substances, usually colored, respond to changes in solution conditions before and after the equivalence point by exhibiting color changes that may be taken visually as the endpoint, a reliable estimate of the equivalence point.

A useful method of endpoint determination results from the use of electrochemical measurements. If an indicator electrode, sensitive to the concentration of the species undergoing titrimetric reaction, and a reference electrode, whose potential is insensitive to any dissolved species, are immersed in the titrate to form a galvanic cell, the potential difference between the electrodes may be sensed by a pH meter and used to follow the course of the reaction. Where such a series of measurements is plotted correctly (i.e., for an acid-base titration, pH versus mL of titrant added; for a precipitimetric, complexometric, or oxidation-reduction titration, mV versus mL of titrant added), a sigmoid curve results with a rapidly changing portion (the "break") in the vicinity of the equivalence point. The midpoint of this linear vertical portion or the inflection point may be taken as the endpoint. The equivalence point may also be determined mathematically without plotting a curve. However, it should be noted that in asymmetrical reactions, which are reactions in which the number of anions reacting is not the same as the number of cations reacting, the endpoint as defined by the inflection of the titration curve does not occur exactly at the stoichiometric equivalence point. Thus, potentiometric endpoint detection by this method is not suitable in the case of asymmetric reactions, examples of which are the precipitation reaction,

$$2Ag^{+} + CrO_4^{-2}$$

and the oxidation-reduction reaction,

$$5Fe^{+2} + MnO_4^{-}.$$

All acid-base reactions, however, are symmetrical. Thus, potentiometric endpoint detection may be employed in acid-base titrations and in other titrations involving symmetrical reversible reactions where an indicator is specified, unless otherwise directed in the individual monograph.

Two types of automatic electrometric titrators are available. The first is one that carries out titrant addition automatically and records the electrode potential differences during the course of titration as the expected sigmoid curve. In the second type, titrant addition is performed automatically until a preset potential or pH, representing the endpoint, is reached, at which point the titrant addition ceases.

Several acceptable electrode systems for potentiometric titrations are summarized in *Table 2*.

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Table 2. Potentiometric Titration Electrode Systems

Titration	Indicating Electrode	Equation[1]	Reference Electrode	Applicability[2]
Acid-base	Glass	$E = k + 0.0591\ pH$	Calomel or silver–silver chloride	Titration of acids and bases
Precipitimetric (silver)	Silver	$E = E^\circ + 0.0591 \log [Ag^+]$	Calomel (with potassium nitrate salt bridge)	Titration with or of silver involving halides or thiocyanate
Complexometric	Mercury–mercury(II)	$E = E^\circ + 0.0296(\log k' - pM)$	Calomel	Titration of various metals (M), e.g., Mg^{+2}, Ca^{+2} Al^{+3}, Bi^{+3}, with EDTA
Oxidation–reduction	Platinum	$E = E^\circ + (0.0591/n) \times \log [ox]/[red]$	Calomel or silver–silver chloride	Titrations with arsenite, bromine, cerate, dichromate, exacyonoferrate(III), iodate, nitrite, permanganate, thiosulfate

1 Appropriate form of Nernst equation describing the indicating electrode system: k = glass electrode constant; k′ = constant derived from Hg–Hg(II)–EDTA equilibrium; M = any metal undergoing EDTA titration; [ox] and [red] from the equation,
$ox + ne \rightleftharpoons red$.
2 Listing is representative but not exhaustive.

BLANK CORRECTIONS

As previously noted, the endpoint determined in a titrimetric assay is an estimate of the reaction equivalence point. The validity of this estimate depends upon, among other factors, the nature of the titrate constituents and the concentration of the titrant. An appropriate *blank correction* is employed in titrimetric assays to enhance the reliability of the endpoint determination. Such a blank correction is usually obtained by means of a *residual blank titration*, wherein the required procedure is repeated in every detail except that the substance being assayed is omitted. In such instances, the actual volume of titrant equivalent to the substance being assayed is the difference between the volume consumed in the residual blank titration and that consumed in the titration with the substance present. The corrected volume so obtained is used in calculating the quantity of the substance being titrated, in the same manner as prescribed under *Residual Titrations*. Where potentiometric endpoint detection is employed, the blank correction is usually negligible.

⟨551⟩ VITAMIN E ASSAY

INTRODUCTION

The following liquid chromatographic procedures are provided for the determination of vitamin E as an active pharmaceutical ingredient, as a dietary supplement ingredient, or as a component in compendial dosage forms in the forms of alpha tocopherol ($C_{29}H_{50}O_2$), alpha tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$).

Throughout this assay, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.

Where vitamin E (alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard.

ASSAY

• **PROCEDURE 1**

- This procedure can be used to determine vitamin E in:
  - Oil-Soluble Vitamins Tablets
  - Oil-Soluble Vitamins Capsules
  - Oil-Soluble Vitamins with Minerals Tablets
  - Oil-Soluble Vitamins with Minerals Capsules
  - Oil- and Water-Soluble Vitamins Tablets
  - Oil- and Water-Soluble Vitamins Capsules
  - Oil- and Water-Soluble Vitamins with Minerals Tablets
  - Oil- and Water-Soluble Vitamins with Minerals Capsules
- This is a neutral procedure that involves the use of dimethyl sulfoxide to dissolve the excipients in the sample, followed by a liquid–liquid extraction of vitamin E with hexane. The hexane extract is then evaporated in vacuum to dryness, and the residue is reconstituted in methanol prior injection into the chromatograph.
- Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Solution A: Phosphoric acid solution (1 in 100) in water
Mobile phase: Methanol and *Solution A* (19:1)

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System suitability solution: Prepare a 0.65-mg/mL solution of USP Ergocalciferol RS in methanol. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing 100 mg of USP Alpha Tocopheryl Acetate RS. Dissolve in 30 mL of methanol, with the aid of sonication if necessary, and dilute with methanol to volume. Store this solution in a refrigerator.

Standard solution: 2 mg/mL of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the powder, to a centrifuge tube having a polytef-lined screw cap. Add about 2 mL of dimethyl sulfoxide per each g of powdered Tablets, and about 3 mL of *n*-hexane each per g of powdered Tablets, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. [NOTE—Volumetric flask size: NLT 20 mL.] Add 3 mL of *n*-hexane per each g of powdered Tablets to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Transfer NLT 20 mL of this solution to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate.

Sample solution for Capsules: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the mixture, to a centrifuge tube having a polytef-lined screw cap. [NOTE—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the mixture to a centrifuge tube having a polytef-lined screw cap.] Add about 2 mL of dimethyl sulfoxide per each g of Capsule contents, and about 3 mL of *n*-hexane per each g of Capsule contents, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. [NOTE—Volumetric flask size: NLT 20 mL.] Add 3 mL of *n*-hexane per each g of Capsule contents to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Transfer NLT 20 mL of this solution to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 8-mm × 10-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

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

[NOTE—The relative retention times for ergocalciferol and alpha tocopheryl acetate are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 12 between ergocalciferol and alpha tocopheryl acetate, *System suitability solution*

Tailing factor: 0.8–1.2, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate in the portion of the sample taken:

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

r_U = peak response of the relevant vitamin E form from the *Sample solution*

r_S = peak response of the relevant vitamin E form from the *Standard solution*

C_S = concentration of the relevant vitamin E form of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding form of vitamin E in the *Sample solution* (mg/mL)

- **PROCEDURE 2**
  - This procedure can be used for the formulations containing vitamins A, D, and E. Application includes:
    - Oil-Soluble Vitamins Tablets
    - Oil-Soluble Vitamins Capsules
    - Oil-Soluble Vitamins with Minerals Tablets
    - Oil-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Tablets

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- Oil- and Water-Soluble Vitamins Capsules
- Oil- and Water-Soluble Vitamins with Minerals Tablets
- Oil- and Water-Soluble Vitamins with Minerals Capsules

- It involves the treatment of sample with methanolic sulfuric acid, followed by extraction with 2,2,4-trimethylpentane.
- Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: Mix 240 mL of methanol with 10 mL of water, followed by 0.5 mL of 50% phosphoric acid, and dilute with acetonitrile to 1000 mL.

3 N methanolic sulfuric acid solution: Cautiously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.

Sodium ascorbate–pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.

Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane

System suitability solution: 2 mg/mL each of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, and USP Alpha Tocopheryl Acid Succinate RS in methanol

Standard solution: 2 mg/mL of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol

Sample solution for Tablets: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Finely powder NLT 20 Tablets. Use a portion of the powder nominally equivalent to an amount 90 mg of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. Transfer a volume of the supernatant 2,2,4-trimethylpentane layer to a suitable volumetric flask, the volume of the specimen withdrawn from the 2,2,4-trimethylpentane layer and the size of the volumetric flask being such that the final concentration of the *Sample solution* is equivalent to that of the *Standard solution*. Evaporate nearly to dryness, add several mL of methanol, and evaporate the remaining 2,2,4-trimethylpentane. Dilute with methanol to volume.

Sample solution for Capsules: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 55 mg of vitamin E, to a container having a polytef-lined screw cap. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant layer. Transfer a volume of the supernatant 2,2,4-trimethylpentane layer to a suitable volumetric flask, the volume of the specimen withdrawn from the 2,2,4-trimethylpentane layer and the size of the volumetric flask being such that the final concentration of the *Sample solution* is equivalent to that of the *Standard solution*. Evaporate nearly to dryness, add several mL of methanol, and evaporate the remaining 2,2,4-trimethylpentane. Dilute with methanol to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

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

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

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

[NOTE—The relative retention times for alpha tocopheryl acid succinate, alpha tocopherol, and alpha tocopheryl acetate are about 0.6, 0.8, and 1.0, respectively]

Suitability requirements

Resolution: NLT 4.0 between alpha tocopheryl acid succinate and alpha tocopherol; NLT 3.0 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alpha tocopherol, alpha tocopheryl acetate, alpha tocopheryl acid succinate in the portion of the sample taken:

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

r_U = peak response of the relevant vitamin E form from the *Sample solution*
r_S = peak response of the relevant vitamin E form from the *Standard solution*
C_S = concentration of the relevant vitamin E form of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding form of vitamin E in the *Sample solution* (mg/mL)

[NOTE—Account for the initial extraction volume of 26.5 mL of 2,2,4-trimethylpentane and the dilution factor to exchange the solvent from 2,2,4-trimethylpentane to methanol to calculate the nominal concentration.]

- **PROCEDURE 3**
  - This procedure can be used for the determination of vitamin E in:
    - Oil-Soluble Vitamins Tablets
    - Oil-Soluble Vitamins Capsules
    - Oil-Soluble Vitamins with Minerals Tablets
    - Oil-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Tablets
    - Oil- and Water-Soluble Vitamins Capsules
    - Oil- and Water-Soluble Vitamins with Minerals Tablets
    - Oil- and Water-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Oral Solution
    - Oil- and Water-Soluble Vitamins with Minerals Oral Solution
  - It involves the saponification of the sample, followed by a liquid–liquid extraction of vitamin E from the sample with *n*-hexane. Evaporate the hexane extract to dryness, and reconstitute the residue in a mixture of acetonitrile and ethyl acetate (1:1).
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: Methanol, acetonitrile, and *n*-hexane (46.5: 46.5: 7.0)
Diluent: Acetonitrile and ethyl acetate (1:1)
Potassium hydroxide solution: [NOTE—Used for the Oral Solution sample.] Transfer 90 g of potassium hydroxide pellets to a 100-mL volumetric flask containing 60 mL of water. Mix to dissolve, cool, and dilute with water to volume.
Standard solution: 0.3 mg/mL of USP Alpha Tocopherol RS in *Diluent*
Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 8 mg of alpha tocopherol, to a 125-mL flask fitted with a ground-glass joint. Add 25.0 mL of water, 25.0 mL of dehydrated alcohol, and 3.5 g of potassium hydroxide pellets. Shake for 1 h in a water bath maintained at 55°. Cool, and transfer with the aid of a minimum volume of water to a 125-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 60 s, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and add the rinsing to the hexane solution in the flask. Place the flask in a water bath maintained at 50°, and evaporate the hexane solution with the aid of a rotary evaporator to dryness. Immediately add 25.0 mL of *Diluent*, and swirl to dissolve the residue.
Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to an amount of 8.0 mg of alpha tocopherol, to a glass-stoppered conical flask. Add 25.0 mL of water, 25.0 mL of dehydrated alcohol, and 3.5 g of potassium hydroxide pellets. Shake for 1 h in a water bath maintained at 55°. Cool, and transfer with the aid of a minimum volume of water to a 125-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 60 s, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and add the rinsing to the hexane solution in the flask. Place the flask in a water bath maintained at 50°, and evaporate the hexane solution with the aid of a rotary evaporator to dryness. Immediately add 25.0 mL of *Diluent*, and swirl to dissolve the residue.
Sample solution for Oral Solution: Transfer an amount of Oral Solution equivalent to 1.5 mg of alpha tocopherol to a 125-mL conical flask fitted with a ground-glass joint, and add 25.0 mL of dehydrated alcohol. Attach a reflux condenser, and reflux in a boiling water bath for 1 min. Cautiously add 3 mL of *Potassium hydroxide solution* through the condenser, and continue to reflux for 30 min. Remove the flask from the bath, and rinse the condenser with about 15 mL of water. Cool, and transfer with a minimum volume of water to a 250-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsings to the separatory funnel. Insert the stopper, shake vigorously for 1 min, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of

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n-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with *n*-hexane, and add the rinsing to the hexane solution in the flask. Evaporate the hexane solution to dryness with the aid of a rotary evaporator over a water bath maintained at about 50°. Immediately add 5.0 mL of *Diluent*, and swirl to dissolve the residue.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 291 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 3 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of vitamin E, as alpha tocopherol, in the portion of the sample taken:

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

r_U = peak response of alpha tocopherol from the *Sample solution*
r_S = peak response of alpha tocopherol from the *Standard solution*
C_S = concentration of USP Alpha Tocopherol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of vitamin E, as alpha tocopherol, in the *Sample solution* (mg/mL)

[NOTE—Calculate the alpha tocopherol equivalent of alpha tocopheryl acetate, or alpha tocopheryl acid succinate by multiplying their contents by the factors 0.91 or 0.81, respectively.]

• **PROCEDURE 4**

- This gas chromatographic procedure is provided for the determination of vitamin E as an active pharmaceutical ingredient, as a dietary supplement ingredient, or as a component in compendial dosage forms. It can be used for:
  - Vitamin E
  - Vitamin E Capsules
  - Vitamin E Preparation
- Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared and used appropriately as follows.

Internal standard solution: 10 mg/mL of squalane in cyclohexane
System suitability solution: 0.1 mg/mL each of USP Alpha Tocopherol RS and USP Alpha Tocopheryl Acetate RS in cyclohexane
Standard solution 1: 10 mg/mL of USP Alpha Tocopherol RS in *Internal standard solution*
Standard solution 2: 10 mg/mL of USP Alpha Tocopheryl Acetate RS in *Internal standard solution*
Standard solution 3: Transfer 30.0 mg of USP Alpha Tocopheryl Acid Succinate RS into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solutions for Active Pharmaceutical Ingredient
Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate): 10 mg/mL of Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) in *Internal standard solution*
Sample solution 2 (vitamin E as alpha tocopheryl acid succinate): Transfer 30.0 mg of Vitamin E (*d*- or *dl*-alpha tocopheryl acid succinate) into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solutions for Vitamin E Preparation
Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate in liquid form): Dissolve a portion of Preparation in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with nominal concentration of 10 mg/mL.
Sample solution 2 (vitamin E as alpha tocopheryl acid succinate in liquid form): Transfer a portion of Preparation, equivalent to 30.0 mg of Vitamin E (*d*- or *dl*-alpha tocopheryl acid succinate), into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.
Sample solution 3 (vitamin E as alpha tocopherol or alpha tocopheryl acetate in solid form): Transfer a portion of Preparation, equivalent to 50 mg of alpha tocopherol or alpha tocopheryl acetate, into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool,

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and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and with the aid of a stream of nitrogen evaporate the ether solution on a water bath, controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains, and complete the evaporation without the application of heat. Dissolve the residue in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with nominal concentration of 10 mg/mL.

Sample solution 4 (vitamin E as alpha tocopheryl acid succinate in solid form): Transfer a portion of Preparation, equivalent to 30 mg of Vitamin E (*d*- or *dl*-alpha tocopheryl acid succinate), into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool, and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and with the aid of a stream of nitrogen evaporate the ether solution on a water bath, controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains. Quantitatively transfer the remains into a 20-mL vial, and complete the evaporation without the application of heat. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solutions for Vitamin E Capsules

Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate): Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule. Dissolve a portion of the combined Capsule contents in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with a nominal concentration of 10 mg/mL.

Sample solution 2 (vitamin E as alpha tocopheryl acid succinate): Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the combined Capsule contents, equivalent to 30.0 mg of Vitamin E (*d*- or *dl*-alpha tocopheryl acid succinate), into a 20-mL vial. Add 2.0 mL of methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of hydrochloric acid to the vial. Cap tightly, and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of *Internal standard solution*, and mix on a vortex mixer to dissolve.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused-silica capillary, bonded with a 0.25-µm film of phase G2

Temperatures

Column: 280°

Injection port: 290°

Detector: 290°

Carrier gas: Helium

Flow rate: 1 mL/min

Split ratio: 100:1

Injection volume: 1 µL

System suitability

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

Suitability requirements

Resolution: NLT 3.5 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

Relative standard deviation: NMT 2.0% for ratios of relevant vitamin E form to internal standard peak responses from replicate injections, appropriate *Standard solution*

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

Calculate the percentage of vitamin E in terms of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate in the portion of the sample taken:

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

R_U = internal standard ratio (peak response of relevant vitamin E form/peak response of the internal standard) from the appropriate *Sample solution*

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R_S = internal standard ratio (peak response of relevant vitamin E form/peak response of the internal standard) from the appropriate *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the appropriate *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding form of vitamin E in the appropriate *Sample solution* (mg/mL)

• **PROCEDURE 5**

- This gas chromatographic procedure is provided for the determination of Vitamin E (as *d*- or *dl*-alpha tocopherol) in:
  - Vitamin E Polyethylene Glycol Succinate (Vitamin E polyethylene glycol succinate is a mixture formed by the esterification of *d*-alpha tocopheryl acid succinate and polyethylene glycol).
- Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Solvent: 0.25 mL of phenolphthalein TS in 1 L of alcohol
Internal standard solution: 12 mg/mL of ethyl arachidate in isooctane
Standard solution: Transfer 32.5 mg of USP Alpha Tocopherol RS to a suitable reaction flask. Add 2 mL of pyridine and 0.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane, and heat the flask at 100° for 10 min. Cool the flask, add 5.0 mL of *Internal standard solution* followed by 20 mL of isooctane, and shake.
Sample solution: Transfer a quantity equivalent to 0.100–0.160 g of Vitamin E Polyethylene Glycol Succinate molten at 60° to a culture tube (about 20 cm long and 2.5 cm in diameter) equipped with a screw cap. Add 40–50 mg of ascorbic acid and a few boiling chips, followed by 20 mL of *Solvent*. [NOTE—Reflux the solution gently without emission of contents. Place the tube in a heating block set at 100°–150°.] When the sample is fully dissolved, add 0.25 g of potassium hydroxide, and continue to reflux for 30 min. Remove the tube from heat, and while contents are still hot, add 1–2 mL of hydrochloric acid dropwise until the pink coloration disappears. [**CAUTION**—Exothermic reaction. Allow the acid to trickle down the inside of the tube to prevent splashing.] Cool the tube, then wash the sides of the tube with 20 mL of water. Add 5.0 mL of *Internal standard solution*, cap, and shake to ensure thorough mixing. Allow the tube to stand until two distinct layers are formed. Transfer 2.5–3.5 mL of the upper layer into a suitable reaction flask, and add 2.0 mL of pyridine followed by 2.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane. Heat the flask at 100° for 10 min. Cool, and then add 12 mL of isooctane.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused-silica capillary, bonded with a 0.25-µm film of phase G27
Temperatures
Injection port: 280°
Detector: 345°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
260	20	340	1

Carrier gas: Helium
Flow rate: 1.5 mL/min
Split ratio: 200:1
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the alpha tocopherol peak
Relative standard deviation: NMT 2.0% for the ratio of the alpha tocopherol peak response to the internal standard peak response from replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vitamin E as *d*-alpha tocopherol in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = internal standard ratio (peak response of alpha tocopherol/peak response of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of alpha tocopherol/peak response of the internal standard) from the *Standard solution*
W_S = weight of USP Alpha Tocopherol RS used to prepare the *Standard solution* (mg)
W_U = weight of Vitamin E Polyethylene Glycol Succinate taken to prepare the *Sample solution* (mg)

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ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Alpha Tocopherol RS
USP Alpha Tocopheryl Acetate RS
USP Alpha Tocopheryl Acid Succinate RS
USP Ergocalciferol RS

⟨561⟩ ARTICLES OF BOTANICAL ORIGIN

SAMPLING

To reduce the effect of sampling bias in qualitative and quantitative results, it is necessary to ensure that the composition of the sample used be representative of the batch of drugs being examined. The following sampling procedures are the minimum considered applicable to vegetable drugs. Some articles, or some tests, may require more rigorous procedures involving more containers being sampled or more samples per container.

Gross Sample

Where external examination of containers, markings, and labels indicates that the batch can be considered to be homogeneous, take individual samples from the number of randomly selected containers indicated below. Where the batch cannot be considered to be homogeneous, divide it into sub-batches that are as homogeneous as possible, and then sample each one as a homogeneous batch. It is recommended to include samples from the first, middle, and last containers where the *No. of Containers in Batch* (*N*) is 11 or more, and each container in the batch is numbered or lettered in order.

No. of Containers in Batch (*N*)	No. of Containers to Be Sampled (*n*)
1–10	All
11–19	11
>19	$n = 10 + (N/10)$

(Rounding was calculated as "*n*" to next highest whole number.)

Samples are taken from the upper, middle, and lower sections of each container. If the crude material consists of component parts that are 1 cm or less in any dimension, and in the case of all powdered or ground materials, withdraw the sample by means of a sampling device that removes a core from the top to the bottom of the container, NLT two cores being taken from different angles. For materials with component parts >1 cm in any dimension, withdraw samples by hand. In the case of large bales or packs, samples should be taken from a depth of 10 cm, because the moisture content of the surface layer may be different from that of the inner layers.

Prepare the gross sample by combining and mixing the individual samples taken from each opened container, taking care not to increase the degree of fragmentation or significantly affect the moisture content.

For articles in containers holding <1 kg, mix the contents, and withdraw a quantity sufficient for the tests. For articles in containers holding 1–5 kg, withdraw equal portions from the upper, middle, and lower parts of the container, each of the samples being sufficient to carry out the tests. Thoroughly mix the samples, and withdraw an amount sufficient to carry out the tests. For containers holding more than 5 kg, withdraw three samples, each weighing NLT 250 g, from the upper, middle, and lower parts of the container. Thoroughly mix the samples, and withdraw a portion sufficient to carry out the tests.

Laboratory Sample

Prepare the *Laboratory Sample* by repeated quartering of the gross sample.

[NOTE—Quartering consists of placing the sample, adequately mixed, as an even and square-shaped heap and dividing it diagonally into four equal parts. The two opposite parts are then taken and carefully mixed. The process is repeated as necessary until the required quantity is obtained.]

The *Laboratory Sample* should be of a size sufficient for performing all the necessary tests.

Test Sample

Unless otherwise directed in the individual monograph or test procedure below, prepare the *Test Sample* as follows.

Decrease the size of the *Laboratory Sample* by quartering, taking care that each withdrawn portion remains representative. In the case of unground or unpowdered drugs, grind the withdrawn sample so that it will pass through a No. 20 standard-mesh sieve, and mix the resulting powder well. If the material cannot be ground, reduce it to as fine a state as possible, mix by rolling it on paper or sampling cloth, spread it out in a thin layer, and withdraw the portion for analysis.

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METHODS OF ANALYSIS

Foreign Organic Matter

TEST SAMPLE

Unless otherwise specified in the individual monograph, weigh the following quantities of the *Laboratory Sample*, taking care that the withdrawn portion is representative (quartering if necessary).

Roots, rhizomes, bark, and herbs	500 g
Leaves, flowers, seeds, and fruit	250 g
Cut vegetable drugs (average weight of the pieces is <0.5 g)	50 g

Spread the sample out in a thin layer, and separate the foreign organic matter by hand as completely as possible. Weigh it, and determine the percentage of foreign organic matter in the weight of drug taken.

Total Ash

Accurately weigh a quantity of the *Test Sample*, representing 2–4 g of the material, in a tared crucible and incinerate, gently at first, and gradually increase the temperature to 675 ± 25° until free from carbon, and determine the weight of the ash. [NOTE—For botanicals, a material in conformance with the *Loss on Drying* test in individual monographs should be used.] If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness, and heat the whole to a temperature of 675 ± 25°. If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15 mL of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a temperature of 675 ± 25°. Cool in a desiccator, weigh the ash, and calculate the percentage of total ash from the weight of the drug taken.

Acid-Insoluble Ash

Boil the ash obtained as directed in *Total Ash* with 25 mL of 3 N hydrochloric acid for 5 min, collect the insoluble matter on a tared filtering crucible or ashless filter, wash with hot water, ignite, and weigh. Determine the percentage of acid-insoluble ash calculated from the weight of drug taken.

Water-Soluble Ash

Boil the ash obtained as directed in *Total Ash* with 25 mL of water for 5 min. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water, and ignite for 15 min at a temperature not exceeding 450°. Subtract the weight of this residue, in mg, obtained in *Total Ash*, and calculate the percentage of water-soluble ash with reference to the weight of sample as determined in *Total Ash*.

Alcohol-Soluble Extractives

METHOD 1 (HOT EXTRACTION METHOD)

Transfer about 4 g of air-dried, coarsely powdered material, accurately weighed, to a glass-stoppered conical flask. Add 100 mL of alcohol, and weigh the flask. Shake, and allow to stand for 1 h. Attach a reflux condenser to the flask, boil gently for 1 h, cool, and weigh. Readjust to the original weight with alcohol. Shake, and filter rapidly through a dry filter. Transfer 25 mL of the filtrate to a tared flat-bottomed dish, and evaporate on a water bath to dryness. Dry at 105° for 6 h, cool in a desiccator for 30 min, and weigh without delay. Calculate the content, in mg/g, of alcohol-extractable matter in the test specimen.

METHOD 2 (COLD EXTRACTION METHOD)

Transfer about 4 g of air-dried, coarsely powdered material, accurately weighed, to a glass-stoppered conical flask. Add 100 mL of alcohol, insert a stopper into the flask, and macerate for 24 h, shaking frequently during the first 8 h, and then allowing to stand. Filter rapidly, taking precautions against loss of alcohol. Evaporate 25 mL of the filtrate to dryness in a tared, flat-bottomed, shallow dish, and dry at 105° to constant weight. Calculate the content, in mg/g, of alcohol-extractable matter in the test specimen.

Water-Soluble Extractives

METHOD 1 (HOT EXTRACTION METHOD)

Proceed as directed in *Method 1 (Hot Extraction Method)* in *Alcohol-Soluble Extractives*, except use water in place of alcohol.

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METHOD 2 (COLD EXTRACTION METHOD)

Proceed as directed in *Method 2 (Cold Extraction Method)* in *Alcohol-Soluble Extractives*, except use water in place of alcohol.

Crude Fiber

Exhaust a weighed quantity of the *Test Sample*, representing about 2 g of the material, with ether. Add 200 mL of boiling dilute sulfuric acid (1 in 78) to the ether-exhausted marc, in a 500-mL flask, and connect the flask to a reflux condenser. Reflux the mixture for 30 min, accurately timed, then pass through a linen or hardened-paper filter, and wash the residue on the filter with boiling water until the effluent washing is no longer acid. Rinse the residue back into the flask with 200 mL of boiling sodium hydroxide solution, adjusted to 1.25% by titration and free from sodium carbonate. Again reflux the mixture for 30 min, accurately timed, then rapidly pass through a tared filter, wash the residue with boiling water until the last washing is neutral, and dry it at 110° to constant weight. Incinerate the dried residue, ignite to constant weight, cool in a desiccator, and weigh the ash: the difference between the weight obtained by drying at 110° and that of the ash represents the weight of the crude fiber.

[NOTE—The boiling with acid and alkali should continue for 30 min, accurately timed, from the time that the liquid (which is cooled below the boiling point by being added to the cold flask) again boils. After the solution has been brought to boiling, the heat should be turned low enough just to maintain boiling. During the boiling, the flask should be gently rotated from time to time to wash down any particles that may adhere to the walls of the flask. A slow current of air introduced into the flask during the boiling operation aids in preventing excessive frothing.]

Starch Content

METHOD 1

The following is a general procedure for all reducing sugars and may be used to determine the starch content in botanical articles.

Malt extract: Use clean new barley malt of known efficacy, and grind just before use. Prepare malt extract just before use. For every 80 mL of malt extract needed, digest 5 g of ground malt with 100 mL of water at room temperature for 2 h. [NOTE—If an electric mixer is used, stir the mixture for 20 min.] Filter to obtain a clear extract, filtering again, if necessary, and mix the infusion well.

Test solution: Extract about 5 g of the finely ground test specimen with five 10-mL portions of ether, using a filter that will completely retain the smallest starch granule. Allow the ether to evaporate from the residue, and wash with 250 mL of aqueous alcohol solution (10 in 100). Carefully wash the residue from the paper into a 500-mL beaker with about 100 mL of water. Heat to about 60° (avoiding, if possible, gelatinizing starch), and allow to stand for about 1 h, stirring frequently to effect complete solution of sugars. Transfer to a wide-mouth bottle, rinse the beaker with a little warm water, and cool. Add an equal volume of alcohol, mix, and allow to stand for NLT 1 h.

Centrifuge until the precipitate is closely packed on the bottom of the bottle, and decant the supernatant. Wash the precipitate with successive 50-mL portions of alcohol solution (50 in 100) by centrifuging and decanting through a suitable filter until the washings are sugar free. [NOTE—To test for the presence of sugar, transfer a few drops of the washings to a test tube, and add 3 or 4 drops of a 20% solution of 1-naphthol in alcohol prepared by dissolving 200 mg of 1-naphthol in 1 mL of alcohol and 2 mL of water. Shake the test tube well to allow uniform mixing, allow 2–4 mL of sulfuric acid to flow down the sides of the test tube, and hold the test tube upright. If sugar is present, the interface of the two liquids is colored faint to deep violet, and on shaking, the whole solution becomes blue-violet.]

Transfer the residue from the bottle and hardened filter to a beaker with about 50 mL of water. Immerse the beaker in boiling water, and stir constantly for 15 min or until all of the starch is gelatinized. Cool the beaker to 55°, add 20 mL of *Malt extract*, and hold at this temperature for 1 h. Heat again to boiling for a few min, cool to 55°, add 20 mL of *Malt extract*, and hold at this temperature for 1 h or until the residue when treated with iodine TS shows no blue tinge upon microscopic examination. Cool, dilute with water to 250 mL, and filter.

General procedure: Transfer 200 mL of the *Test solution* to a flask fitted with a reflux condenser, add 20 mL of hydrochloric acid, and heat in a boiling water bath for 2.5 h. Cool, nearly neutralize with sodium hydroxide TS, complete neutralization with sodium carbonate TS, dilute with water to 500 mL, mix, and filter. The volume of aliquot taken depends on the starch content of the specimen under test (see *Table 1*). The aliquot should contain between 100 and 200 mg of dextrose. Transfer 50 mL of the filtrate to a 400-mL alkali-resistant glass beaker, add 50 mL of alkaline cupric tartrate TS, cover the beaker with a water glass, and heat. Adjust the flame in the burner so that the contents of the flask begin to boil in 4 min, and continue boiling for exactly 2 min. Pass the hot solution at once through a sintered-glass filter. Wash the precipitate of cuprous oxide thoroughly with water at about 60°, then with 10 mL of alcohol, and finally with 10 mL of ether.

Table 1. Determination of the Optimum Aliquot

Expected Starch Content (%)	Aliquot (mL)
60	25
50	35
40	50
30	50
20	50

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For solutions of reducing sugars of comparatively high purity, proceed as directed in *Method 1A* to determine the amount of reduced copper obtained by weighing the dried cuprous oxide. For solutions of reducing sugars containing large amounts of organic impurities, including sucrose, proceed as directed in *Method 1B* to determine the amount of reduced copper obtained by titration with sodium thiosulfate.

Method 1A: Dry the precipitate obtained in *General procedure* for 30 min in an oven at 110 ± 2°, cool to room temperature in a desiccator, and weigh. Refer to *Table 2* to find the quantity of dextrose, in mg, corresponding to the weight of cuprous oxide found. Determine the percentage of dextrose and then the content of starch by the following formula:

$$\text{Percentage of dextrose} = (\text{wt. of dextrose in mg} \times 0.1 \times 500)/(\text{wt. of sample in g} \times \text{aliquot in mL})$$

$$\text{Content of starch} = \%\ \text{dextrose} \times 0.9$$

Table 2. Calculating Dextrose (mg) [applicable when Cuprous Oxide (Cu_2O) is weighed directly]

Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)
10	4.0	90	38.9	170	75.1	250	112.8	330	152.2	410	193.7
12	4.9	92	39.8	172	76.0	252	113.7	332	153.2	412	194.7
14	5.7	94	40.6	174	76.9	254	114.7	334	154.2	414	195.8
16	6.6	96	41.5	176	77.8	256	115.7	336	155.2	416	196.8
18	7.5	98	42.4	178	78.8	258	116.6	338	156.3	418	197.9
20	8.3	100	43.3	180	79.7	260	117.6	340	157.3	420	199.0
22	9.2	102	44.2	182	80.6	262	118.6	342	158.3	422	200.1
24	10.0	104	45.1	184	81.5	264	119.5	344	159.3	424	201.1
26	10.9	106	46.0	186	82.5	266	120.5	346	160.3	426	202.2
28	11.8	108	46.9	188	83.4	268	121.5	348	161.4	428	203.3
30	12.6	110	47.8	190	84.3	270	122.5	350	162.4	430	204.4
32	13.5	112	48.7	192	85.3	272	123.4	352	163.4	432	205.5
34	14.3	114	49.6	194	86.2	274	124.4	354	164.4	434	206.5
36	15.2	116	50.5	196	87.1	276	125.4	356	165.4	436	207.6
38	16.1	118	51.4	198	88.1	278	126.4	358	166.5	438	208.7
40	16.9	120	52.3	200	89.0	280	127.3	360	167.5	440	209.8
42	17.8	122	53.2	202	89.9	282	128.3	362	168.5	442	210.9
44	18.7	124	54.1	204	90.9	284	129.3	364	169.6	444	212.0
46	19.6	126	55.0	206	91.8	286	130.3	366	170.6	446	213.1
48	20.4	128	55.9	208	92.8	288	131.3	368	171.6	448	214.1
50	21.3	130	56.8	210	93.7	290	132.3	370	172.7	450	215.2
52	22.2	132	57.7	212	94.6	292	133.2	372	173.7	452	216.3
54	23.0	134	58.6	214	95.6	294	134.2	374	174.7	454	217.4
56	23.9	136	59.5	216	96.5	296	135.2	376	175.8	456	218.5
58	24.8	138	60.4	218	97.5	298	136.2	378	176.8	458	219.6
60	25.6	140	61.3	220	98.4	300	137.2	380	177.9	460	220.7
62	26.5	142	62.2	222	99.4	302	138.2	382	178.9	462	221.8
64	27.4	144	63.1	224	100.3	304	139.2	384	180.0	464	222.9
66	28.3	146	64.0	226	101.3	306	140.2	386	181.0	466	224.0
68	29.2	148	65.0	228	102.2	308	141.2	388	182.0	468	225.1

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Table 2. Calculating Dextrose (mg) [applicable when Cuprous Oxide (Cu_2O) is weighed directly] *(continued)*

Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)
70	30.0	150	65.9	230	103.2	310	142.2	390	183.1	470	226.2
72	30.9	152	66.8	232	104.1	312	143.2	392	184.1	472	227.4
74	31.8	154	67.7	234	105.1	314	144.2	394	185.2	474	228.3
76	32.7	156	68.6	236	106.0	316	145.2	396	186.2	476	229.6
78	33.6	158	69.5	238	107.0	318	146.2	398	187.3	478	230.7
80	34.4	160	70.4	240	108.0	320	147.2	400	188.4	480	231.8
82	35.3	162	71.4	242	108.9	322	148.2	402	189.4	482	232.9
84	36.2	164	72.3	244	109.9	324	149.2	404	190.5	484	234.1
86	37.1	166	73.2	246	110.8	326	150.2	406	191.5	486	235.2
88	38.0	168	74.1	248	111.8	328	151.2	408	192.6	488	236.3

Method 1B

SODIUM THIOSULFATE SOLUTION: Transfer 3.9 g of sodium thiosulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

POTASSIUM IODIDE SOLUTION: Dissolve 42 g of potassium iodide in 100 mL of water.

SODIUM ACETATE SOLUTION: Dissolve 5.74 g of sodium acetate in 10 mL of water.

COPPER SOLUTION: Transfer about 0.3 g of pure electrolytic copper, accurately weighed, to a 250-mL flask, add 5 mL of nitric acid to dissolve the copper, add about 25 mL of water, and boil to expel red fumes. Add about 5 mL of bromine TS, and boil until the bromine is completely removed. Cool, add 10 mL of *Sodium acetate solution* followed by 10 mL of *Potassium iodide solution*, and titrate with *Sodium thiosulfate solution* to a light yellow color. Add enough starch TS to produce a marked blue color, and continue the titration. As the endpoint nears, add 2 g of potassium thiocyanate, and stir until completely dissolved. Continue titration until the precipitate is completely white. One mL of *Sodium thiosulfate solution* is equivalent to about 10 mg of copper. [NOTE—It is essential that the concentration of *Potassium iodide solution* be carefully regulated. If the solution contains less than 320 mg of copper at the completion of titration, add 4.2–5 g of potassium iodide to make a total solution of 100 mL. If greater amounts of copper are present, add *Potassium iodide solution* slowly, with constant agitation, from the buret in amounts proportionately greater.]

PROCEDURE: Wash the precipitated cuprous oxide obtained in *General procedure* with water, cover this filter with a watch glass, and dissolve the cuprous oxide with 5 mL of nitric acid directed under the watch glass with a pipette. Collect the filtrate in a 250-mL flask, wash the watch glass, and the filter with water. Collect all the washings in the flask. Boil the contents of the flask to expel red fumes. Add about 5 mL of bromine TS, and boil until the bromine is completely removed. Cool, and proceed as directed in *Copper solution* beginning with "add 10 mL of *Sodium acetate solution*". From the volume of *Sodium thiosulfate solution* consumed, obtain the weight of copper, in mg, and multiply the weight of copper by 1.1259 to obtain the weight, in mg, of cuprous oxide. From *Table 2*, find the quantity of dextrose, in mg, corresponding to the weight of cuprous oxide. The content of starch is equivalent to the weight, in mg, of dextrose obtained times 0.9. Conduct a blank determination, using 50 mL of alkaline cupric tartrate TS and 50 mL of *Malt extract*. If the weight of the cuprous oxide so obtained exceeds 0.5 mg, correct the result of the determination accordingly. [NOTE—The alkaline cupric tartrate TS deteriorates on standing, and the quantity of cuprous oxide obtained in the blank determination increases.]

METHOD 2

The following method is specific for dextrose (glucose), and because of its extreme sensitivity it may account for differences noted between values obtained from the same specimen. Duplicate determinations do not vary more than 2%.

Glucoamylase solution: Prepare a solution of glucoamylase in water containing 30 International Units (IU)/mL. Use glucoamylase obtained preferably from *Rhizopus delemar*. The total glucoamylase activity of the test specimen being used should be NLT 150 IU.

Acetate buffer solution: Dissolve 16.4 g of sodium acetate in 100 mL of water, add 12.0 mL of glacial acetic acid, and mix. The pH of this solution is 4.8.

Phosphate buffer: Dissolve 3.63 g of tris(hydroxymethyl) aminomethane and 5.0 g of monobasic sodium phosphate in 50.0 mL of water. At 37°, adjust with phosphoric acid to a pH of 7.0, dilute with water to 100.0 mL, and mix. [NOTE—The pH of the buffer medium is sensitive to temperature and should be adjusted to the desired pH at the temperature to be used during incubation.]

Enzyme solution: Dissolve 30 mg of glucose oxidase (Type II from *Aspergillus niger*), 3 mg of peroxidase (Type I from horseradish), and 10 mg of potassium ferrocyanide in 100 mL of *Phosphate buffer*. [NOTE—This mixture can be stored in a refrigerator for up to 10 days.]

18 N sulfuric acid: Add slowly, while stirring, 54 mL of sulfuric acid to 102 mL of water, allow to cool to 25°, and mix.

Standard solutions: Dissolve an accurately weighed quantity of USP Dextrose RS in water to obtain a solution containing 1.0 mg/mL of USP Dextrose RS. Quantitatively dilute a known volume of this solution with water to obtain *Standard solutions A*, *B*, *C*, *D*, and *E*, having known concentrations of 10, 20, 25, 40, and 50 µg/mL of USP Dextrose RS, respectively. [NOTE—Allow 4 h for complete mutarotation before use.]

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Test solutions: Extract about 5 g of finely ground test specimen with five 25-mL portions of 80% alcohol, and filter. Remove all the alcohol from the residue by drying in an air oven at 105° for about 8 h. [NOTE—Any traces of alcohol remaining in the residue will inhibit glucoamylase.] Cool, and transfer the flask containing the dried test specimen to a desiccator. Transfer about 1 g, accurately weighed, of the test specimen to a previously tared flask, add 25 mL of water, and adjust with phosphoric acid to a pH of 5.0–7.0, if necessary. Boil the suspension for about 3 min, transfer the flask to an autoclave, and heat to 135° for 2 h. Remove the flask from the autoclave, maintain the temperature near 55°, and add 2.5 mL of *Acetate buffer solution* and sufficient water to adjust the total weight of the solution to 45 ± 1 g. Immerse the flask in a water bath maintained at 55 ± 1°, and add 5 mL of *Glucoamylase solution*. Continuously swirl the flask for 2 h to effect hydrolysis, pass through filter paper into a 250-mL volumetric flask, wash quantitatively with water, and collect all the washings in the flask. Dilute the contents of the flask with water to volume, and mix. Transfer 1 mL of an aliquot containing 20–60 µg of D-glucose to each of five test tubes. [NOTE—To obtain the range of concentration of glucose in the hydrolysate, quantitatively dilute, if necessary, with water to volume.] Add 2 mL of *Enzyme solution* to each of the five test tubes, and place the test tubes in the dark at 37 ± 1° for exactly 30 min to develop the color. At the end of 30 min, add 2 mL of *18 N sulfuric acid* to each of the test tubes to stop the reaction, and mix.

Control solution: Transfer an accurately weighed quantity of about 0.4 g of starch to a previously tared flask, and proceed as directed in *Test solutions* beginning with "add 25 mL of water, and adjust the pH with phosphoric acid".

Procedure: Concomitantly determine the absorbances of the *Standard solutions* and the *Test solutions* at the wavelength of maximum absorbance at about 540 nm, with a suitable spectrophotometer, using the *Control solution* as the blank to set the instrument. Plot the absorbance values of the *Standard solutions* versus concentration, in µg/mL, of dextrose, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration, *C*, in µg/mL, of dextrose in each of the *Test solutions*, and calculate the average concentration, in µg/mL, of the solution under test. The percentage of starch content in the weight of the test specimen taken is calculated by the formula:

$$(0.9C/10^6) \times V_1 \times (250/V_0)(100/E)(100/W) = 2.25CV_1/V_0EW$$

C = average concentration of dextrose in the *Test solutions* (µg/mL)
V_1 = volume of the *Test solution* if extra dilution is done (mL) (see *Note 2* in *Test solutions*)
V_0 = volume of the aliquot taken from the 250-mL volumetric flask (mL)
E = weight of the test specimen (g)
W = percentage of dry weight of the test specimen

[NOTE—V_0 is 1.0 when no extra dilution is done.]

Volatile Oil Determination

Set up a round-bottom, shortneck, 1-L flask in a heating mantle set over a magnetic stirrer. Insert an egg-shaped stirring bar magnet in the flask, and attach a cold-finger condenser and an appropriate volatile oil trap of the type illustrated (see *Figure 1*).

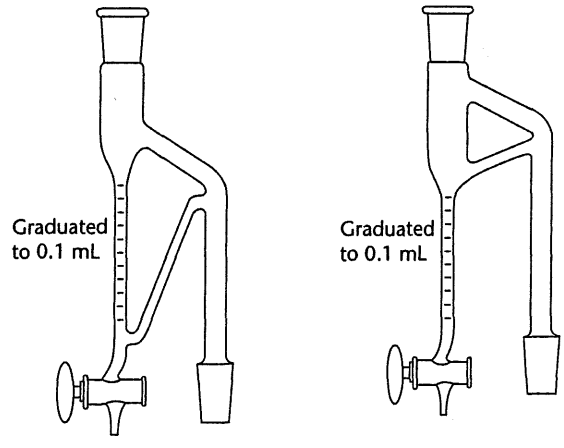


Figure 1. Traps for volatile oil apparatus.

Coarsely comminute a sufficient quantity of the material to yield from 1–3 mL of volatile oil. Small seeds, fruits, or broken leaves of herbs ordinarily do not need comminution. Very fine powders are to be avoided. If this is not possible, it may be necessary to mix them with purified sawdust or purified sand. Place a suitable quantity of the drug, accurately weighed, in the flask, and fill it one-half with water. Attach the condenser and the proper separator. Boil the contents of the flask, using a suitable amount of heat to maintain gentle boiling for 2 h, or until the volatile oil has been completely separated from the material and no longer collects in the graduated tube of the separator.

If a proper quantity of the volatile oil has been obtained in the graduated tube of the separator, it can be read to tenths of 1 mL, and the volume of volatile oil from each 100 g of material can be calculated from the weight of the drug taken. The

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graduations on the separator "for oils heavier than water" are so placed that oil remains below the aqueous condensate that automatically flows back into the flask.

Water Content

For unground or unpowdered materials, prepare about 10 g of the *Laboratory Sample* by cutting, granulating, or shredding, so that the parts are about 3 mm in thickness. Seeds or fruits smaller than 3 mm should be cracked. Avoid the use of high-speed mills in preparing the sample, and exercise care that no appreciable amount of moisture is lost during the preparation and that the portion taken is representative of the *Laboratory Sample*. Determine the water content as directed in *Water Determination* ⟨921⟩, *Method III (Gravimetric), Procedure for Articles of Botanical Origin*.

Change to read:

TEST FOR AFLATOXINS

[**CAUTION**—Aflatoxins are highly dangerous, and extreme care should be exercised in handling aflatoxin materials.]
Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for aflatoxin B_1 (AFB_1) and NMT 20 ppb for the sum of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2). The extent of testing may be determined using a risk-based approach that considers the likelihood of contamination. The presence of unexpected contamination with aflatoxins is to be considered in determining compliance. The following analytical procedures are provided for determining compliance. Unless otherwise specified in the individual monograph, use *Method I*. If system suitability fails, use either *Method II* or *Method III*.

Method I

This TLC test is provided to detect the possible presence of AFB_1, AFB_2, AFG_1, and AFG_2 in any material of plant origin.

ZINC ACETATE–ALUMINUM CHLORIDE REAGENT

Dissolve 20 g of zinc acetate and 5 g of aluminum chloride in sufficient water to make 100 mL.

SODIUM CHLORIDE SOLUTION

Dissolve 5 g of sodium chloride in 50 mL of water.

TEST SOLUTION 1

Grind about 200 g of plant material to a fine powder. Transfer about 50 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 mL of a mixture of methanol and water (17:3). Shake vigorously by mechanical means for NLT 30 min, and filter. [NOTE—If the solution has interfering plant pigments, proceed as directed for *Test Solution 2*.] Discard the first 50 mL of the filtrate, and collect the next 40-mL portion. Transfer the filtrate to a separatory funnel. Add 40 mL of *Sodium Chloride Solution* and 25 mL of solvent hexane, and shake for 1 min. Allow the layers to separate, and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 25 mL of methylene chloride, by shaking for 1 min. Allow the layers to separate each time, separate the lower organic layer, and collect the combined organic layers in a 125-mL conical flask. Evaporate the organic solvent on a water bath. Transfer the remaining extract to an appropriate sample tube, and evaporate to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup procedure* in *Test Solution 2*. Otherwise, dissolve the residue obtained above in 0.2 mL of a mixture of chloroform and acetonitrile (9.8: 0.2), and shake by mechanical means if necessary.

TEST SOLUTION 2

Collect 100 mL of the filtrate from the start of the flow, and transfer to a 250-mL beaker. Add 20 mL of *Zinc Acetate–Aluminum Chloride Reagent* and 80 mL of water. Stir, and allow to stand for 5 min. Add 5 g of a suitable filtering aid, such as diatomaceous earth, mix, and filter. Discard the first 50 mL of the filtrate, and collect the next 80-mL portion. Proceed as directed for *Test Solution 1*, beginning with "Transfer the filtrate to a separatory funnel".
Cleanup procedure: Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10-mm × 300-mm chromatographic tube. Prepare a slurry of 2 g of silica gel with a mixture of ethyl ether and solvent hexane (3:1), pour the slurry into the column, and wash with 5 mL of the same solvent mixture. Allow the absorbent to settle, and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate. Dissolve the residue obtained above in 3 mL of methylene chloride, and transfer it to the column. Rinse the flask twice with 1-mL portions of methylene chloride, transfer the rinses to the column, and elute at a rate NMT 1 mL/min. Add successively to the column 3 mL of solvent hexane, 3 mL of ethyl ether, and 3 mL of methylene chloride; elute at a rate NMT 3 mL/min; and discard the eluates. Add to the column 6 mL of a mixture of methylene chloride and acetone (9:1), and elute at a rate NMT 1 mL/min, preferably without the aid of vacuum. Collect this eluate in a small vial, add a boiling chip if necessary, and evaporate to dryness on a water bath. Dissolve the residue in 0.2 mL of a mixture of chloroform and acetonitrile (9.8: 0.2), and shake by mechanical means if necessary.

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TEST SOLUTION 3

If interferences still exist in the residue, proceed as directed for *Cleanup procedure with IAC* in *Test Solution* in *Method II*.

AFLATOXIN STANDARD SOLUTION

[**CAUTION**—Aflatoxins are highly toxic. Handle with care.]

Prepare an *Aflatoxin Standard Solution* containing AFB_1, AFB_2, AFG_1, and AFG_2 at 2.0, 0.50, 2.0, and 0.50 µg/mL, respectively, in acetonitrile as follows. First prepare individual stock solutions of aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2 in acetonitrile, each having a nominal concentration of 10 µg/mL.[1] Determine the concentration of each aflatoxin stock solution by measuring the absorbance (*A*) at a wavelength of maximum absorption close to 360 nm and using the equation:

$$\text{Aflatoxin (µg/mL)} = (A \times M_r \times 1000)/\varepsilon$$

A = absorbance
M_r = molecular weight
ε = ▲molar▲ (ERR 1-Jun-2019) absorptivity (see *Table 3*)

Table 3. Molecular Weights and Molar Absorptivities for Aflatoxins

Aflatoxin	Molecular Weight (M_r)	Solvent	Molar Absorptivity (ε)
AFB_1	312	Acetonitrile	20700
AFB_2	314	Acetonitrile	22500
AFG_1	328	Acetonitrile	17600
AFG_2	330	Acetonitrile	18900

Finally, to obtain the *Aflatoxin Standard Solution*, transfer an accurate volume of each aflatoxin ▲▲ (ERR 1-Jun-2019) stock solution to the same volumetric flask, and dilute with acetonitrile to volume. Calculate the concentration of each aflatoxin in the *Aflatoxin Standard Solution*:

$$C_{AF} = C'_{AF} \times V_{TS}/V_{AF}$$

C_{AF} = concentration of the relevant aflatoxin in the *Aflatoxin Standard Solution*
C'_{AF} = concentration of the relevant aflatoxin in the corresponding stock solution, as determined above
V_{TS} = volume of the volumetric flask used to prepare the final *Aflatoxin Standard Solution*
V_{AF} = volume of each individual aflatoxin stock solution transferred to the volumetric flask used to prepare the *Aflatoxin Standard Solution*

Store in a refrigerator. Equilibrate to room temperature before use.

AFLATOXIN SOLUTION

Dilute the *Aflatoxin Standard Solution* with acetonitrile to obtain a solution having a concentration of 0.4 µg/mL each of AFB_1 and AFG_1, and 0.1 µg/mL each of AFB_2 and AFG_2.

PROCEDURE

Separately apply 2.5, 5, 7.5, and 10 µL of the *Aflatoxin Solution* and three 10-µL applications of either *Test Solution 1*, *Test Solution 2*, or *Test Solution 3* to a suitable TLC plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Superimpose 5 µL of the *Aflatoxin Solution* on one of the three 10-µL applications of the *Test Solution*. Allow the spots to dry, and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone, and isopropyl alcohol (85:10:5) until the solvent front has moved NLT 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

SYSTEM SUITABILITY

The four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots. Observe any spot obtained from the *Test Solution* that coincides in hue and position with those of the *Aflatoxin Solution*. Any spot obtained from the *Test Solution* with the superimposed *Aflatoxin Solution* is not less intense than that of the corresponding *Aflatoxin Solution*.

[1] Suitable aflatoxin individual standards are available from Romer Labs, catalog #001012, 001013, 001014, 001015; and Sigma-Aldrich, catalog #A6636, A9887, A0138, A0263.

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ACCEPTANCE CRITERIA

No spot from any of the other applications of the *Test Solution* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution*. If any spot of aflatoxins is obtained in the *Test Solution*, match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxin spot, if present in the *Test Solution*, when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution*. Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

Method II

SODIUM CHLORIDE SOLUTION

See *Method I*.

PHOSPHATE BUFFERED SALINE SOLUTION

Prepare 10 mM phosphate buffer solution containing 0.138 M sodium chloride and 0.0027 M potassium chloride in water, and adjust with 2 M sodium hydroxide to a pH of 7.4.[2]

IMMUNOAFFINITY COLUMN

Before conditioning, adjust the immunoaffinity column (IAC) to room temperature. For conditioning, apply 10 mL of *Phosphate Buffered Saline Solution* onto the column and let it flow through the column by gravity force at a rate of 2–3 mL/min. Leave 0.5 mL of the *Phosphate Buffered Saline Solution* on top of the column until the *Test Solution* is applied.

TEST SOLUTION

Sample extraction: Transfer about 5 g of a representative powdered sample, accurately weighed, to a glass-stoppered flask. Add 20 mL of a mixture of methanol and water (17:3). Shake vigorously by mechanical means for NLT 30 min, and filter. Discard the first 5 mL of the filtrate, and collect the next 4-mL portion. Transfer the filtrate to a separatory funnel. Add 4 mL of *Sodium Chloride Solution* and 2.5 mL of hexane, and shake for 1 min. Allow the layers to separate, and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 2.5 mL of methylene chloride, by shaking for 1 min. Allow the layers to separate each time, separate the lower organic layer, and collect the combined organic layers in a 50-mL conical flask. Evaporate the organic solvent on a water bath. Transfer the remaining extract to an appropriate sample tube, and evaporate to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup procedure with IAC*. Otherwise, dissolve the residue obtained above in 200 µL of acetonitrile, and shake by mechanical means if necessary.
Cleanup procedure with IAC: The residue is dissolved in 5 mL of a mixture of methanol and water (6:4) and then diluted with 5 mL of water. This extract is applied onto a conditioned IAC. The IAC is rinsed twice with 10 mL of *Phosphate Buffered Saline Solution*, and the elution is performed slowly with 2 mL of methanol. Evaporate the eluate with nitrogen, and dissolve the residue in 200 µL of acetonitrile.

AFLATOXIN SOLUTION

[**Caution**—Aflatoxins are highly toxic. Handle with care.]
Dilute the *Aflatoxin Standard Solution* with acetonitrile to obtain a solution containing 0.04 µg/mL each of AFB_1 and AFG_1, and 0.01 µg/mL each of AFB_2 and AFG_2.

ANALYSIS

Separately apply 5, 7.5, and 10 µL of *Aflatoxin Solution* and three 10-µL applications of the *Test Solution* to a suitable HPTLC plate (see *Chromatography* ⟨621⟩) coated with a 200-µm layer of chromatographic silica gel mixture. Superimpose 5 µL of *Aflatoxin Solution* on one of the three 10-µL applications of the *Test Solution*. Allow the spots to dry, and develop the chromatogram in a saturated chamber containing a solvent system consisting of a mixture of chloroform, acetone, and water (140: 20: 0.3) until the solvent front has moved NLT 72 mm from the origin (80 mm from the lower edge of the plate). Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry for 5 min. Locate the spots on the plate by scanning fluorescence density (>400 nm) under UV light at 366 nm. Match the position of each fluorescent spot of the *Test Solution* with those of *Aflatoxin Solution* to identify the type of aflatoxin present. The concentration of aflatoxins in the *Test Solution* can be calculated from the calibration curve obtained from the scan data with *Aflatoxin Solution*.

SYSTEM SUITABILITY

The four applications of *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots. Observe any spot obtained from the *Test Solution* that coincides in hue and position with those of *Aflatoxin Solution*. Any spot obtained from the *Test*

[2] A suitable powder mixture is available from Sigma as PBS P-3813.

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Solution with the superimposed *Aflatoxin Solution* is not less intense than that of the corresponding *Aflatoxin Solution*. The mean recovery of spiked AFB_1 and AFG_1 is NLT 70%.

ACCEPTANCE CRITERIA

Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

Method III

This test method is provided as an example for the detection of the possible presence of AFB_1 and total aflatoxins (AF: sum of AFB_1, AFB_2, AFG_1, and AFG_2). It has been shown to be suitable for powdered ginseng and ginger. Its suitability to other articles of botanical origin must be demonstrated.

0.1 M PHOSPHATE BUFFER SOLUTION

Dissolve 8.69 g of anhydrous disodium phosphate and 4.66 g of anhydrous monosodium phosphate or 5.36 g of monosodium phosphate monohydrate in 800 mL of water, adjust with 2 M sodium hydroxide to a pH of 7.4, add 10 mL of polysorbate 20, and dilute with water to 1 L.

PHOSPHATE BUFFERED SALINE SOLUTION

Prepare as directed in *Method II*.

WORKING AFLATOXIN STANDARD SOLUTIONS

Prepare six solutions by diluting suitable volumes of *Aflatoxin Standard Solution* with methanol and water (1:1). The final aflatoxin concentrations in each *Working Aflatoxin Standard Solution* are shown in *Table 4*. Store in a refrigerator, and equilibrate to room temperature before use. Prepare the solutions daily.

Table 4. Preparation of Working Aflatoxin Standard Solutions

Working Aflatoxin Standard Solutions	Aflatoxin Standard Solution (µL)	Final Aflatoxin Concentration of Working Aflatoxin Standard Solution (ng/mL)				
		AFB_1	AFB_2	AFG_1	AFG_2	ΣAF
1	0	0	0	0	0	0
2	12.5	0.25	0.0625	0.25	0.0625	0.625
3	25	0.5	0.125	0.5	0.125	1.25
4	50	1	0.25	1	0.25	2.5
5	100	2	0.5	2	0.5	5
6	200	4	1	4	1	10

IMMUNOAFFINITY COLUMN[3]

Use an IAC that contains monoclonal antibodies cross reactive toward AFB_1, AFB_2, AFG_1, and AFG_2. The immunoaffinity columns have a minimum capacity of NLT 100 ng of total aflatoxin and give a recovery of NLT 80% for AFB_1, AFB_2, AFG_1, and AFG_2 when 5 ng of each AFB_1, AFB_2, AFG_1, and AFG_2 is applied in 10 mL of 10% methanol in *Phosphate Buffered Saline Solution* (v/v).

TEST SOLUTION

Extraction: Weigh 5 g of a representative test sample in a 50-mL centrifuge tube. Add 1 g of sodium chloride and 25 mL of a mixture of methanol and 0.5% sodium bicarbonate (7:3). Mix on a vortex mixer until sample particles and extract solvent are well mixed. Shake at 400 rpm for 10 min. Centrifuge for 10 min at 7000 rpm (*g* value = 5323 mm/s²) or at a speed that can result in a firm pellet of residues. Immediately pipette 7 mL into a 50-mL centrifuge tube, add 28 mL of *0.1 M Phosphate Buffer Solution*, mix, and filter through glass microfiber paper. Collect 25 mL of filtrate (equivalent to 1 g of test sample) into a 25-mL graduated cylinder, and proceed immediately with IAC chromatography.

IAC cleanup: [NOTE—For IAC cleanup, columns must be kept at room temperature for at least 15 min before use.] Remove the top cap from the column, and connect it with the reservoir. Remove the end cap from the column, and attach it to the column manifold (the fit must be tight). Let the liquid in the column pass through until the liquid is about 2–3 mm above the column bed. Pass 25 mL of filtrate into the reservoir. Let the filtrate flow through the column by gravity force. Let the column run dry. To start the flow again easily, remove the column from the manifold, add about 2 mL of *Phosphate Buffered Saline Solution* into the column, reattach the column to the reservoir, and wash the column with an additional 3 mL of *Phosphate Buffered Saline Solution* and then with 5 mL of water (the 5 mL of *Phosphate Buffered Saline Solution* can be added directly to

[3] AflaOchraTest column (G1017; Vicam, Watertown, MA, USA) or equivalent. Aflatoxin/OTA immunoaffinity columns are suitable.

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the column reservoir if other techniques are used to dislodge the air bubble at the end of the column and to start flow again easily). Let the column run dry, then force 3 mL of air through the column with a syringe. Elute with 1 mL of methanol, and collect the analytes in a 3-mL volumetric flask, letting the eluate drip freely. Let the column run dry. Let stand for 1 min, then elute with an additional 1 mL of methanol, and collect in the same volumetric flask. Let the column run dry, and force 10 mL of air through the column. Dilute the eluate with water to volume. Use this as the *Test Solution*, and perform the analysis of aflatoxins immediately.

SYSTEM SUITABILITY SOLUTION

Prepare a spiked sample by adding 5 mL of *Working Aflatoxin Standard Solution 5* to a 5-g sample and repeating the procedure for the *Test Solution*, using 20 mL instead of 25 mL of the mixture of methanol and 0.5% sodium bicarbonate (700:300).

CHROMATOGRAPHIC SYSTEM

Detector: Fluorescence, excitation wavelength (Ex) 362 nm and emission wavelength (Em) 440 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 0.8 mL/min
Mobile phase: Isocratic
For post-column derivatization with PHRED cell:[4] Water, methanol, and acetonitrile (60:25:15)
For post-column derivatization with Kobra cell:[5] A solution prepared by mixing 1 L of a mixture of water, methanol, and acetonitrile (60:25:15); 350 µL of 4 M nitric acid; and 120 mg of potassium bromide
Post-column derivatization (PCD) systems
PHRED cell: Post-column photochemical derivatization cell
Kobra cell: Electrochemical cell, post-column bromination derivatization cell

ANALYSIS

Post-column derivatization for aflatoxins: Use a UV or Kobra cell. Inject 50 µL of reagent blank *(Working Aflatoxin Standard Solution 1)*, *Working Aflatoxin Standard Solutions 2–6*, or the *Test Solution* into the LC column. Identify the aflatoxin peaks in the *Test Solution* by comparing the retention times with those of the working standards. The aflatoxins elute in the order AFG_2, AFG_1, AFB_2, and AFB_1. After passing through the PHRED or Kobra cell, the AFG_1 and AFB_1 have been derivatized to form AFG_{2a} (derivative of AFG_1) and AFB_{2a} (derivative of AFB_1). [NOTE—The chemical structures of the derivatives resulting from electrochemical bromination and photolysis are not the same. The structures of AFB_1 and AFG_1 photolysis products have not been established.] The retention times of AFG_2, AFG_{2a}, AFB_2, and AFB_{2a} are between about 14 and 27 min using the PHRED cell; retention times are shorter using the Kobra cell. The peaks should be baseline resolved. Construct standard curves for each aflatoxin. Determine the concentration of each aflatoxin in the *Test Solution* from the calibration curve.
Aflatoxins calibration curves: Calibration curves are prepared for each of the aflatoxins using the *Working Aflatoxin Standard Solutions* containing the four aflatoxins described. These solutions cover the range of 0.25–4 ng/mL for AFB_1 and AFG_1, and the range of 0.0625–1 ng/mL for AFB_2 and AFG_2. Make the calibration curves before analysis according to *Table 4*, and check the plot for linearity. If the test portion area response is outside (higher) the calibration range, then the *Test Solution* should be diluted with a mixture of methanol and water (1:1, v/v) and reinjected into the LC column.
Quantitation of aflatoxins: Quantitation of aflatoxins is performed by measuring peak areas at each aflatoxin retention time and comparing them with the corresponding calibration curve.

SYSTEM SUITABILITY

The mean recovery of spiked AFB_1 (2 µg/kg) and the total of aflatoxins (5 µg/kg) is NLT 68% and 70%, respectively. The relative standard deviation (RSD) is NMT 10% for AFB_1 and for the total of aflatoxins.

CALCULATIONS

Plot the peak area (response, *y*-axis) of each of the toxin standards against the concentration (ng/mL, *x*-axis) and determine the slope (S) and *y*-intercept (a). Calculate the level of toxin in the sample by the following formula:

$$\text{Toxin } (\mu g/kg) = \{[(R - a)/S] \times V/W\} \times F$$

R = peak area of the *Test Solution*
a = *y*-intercept of the calibration curve
S = slope of the calibration curve
V = final volume of the injected *Test Solution* (mL)
W = 1 g of test sample passed through the immunoaffinity column
F = dilution factor, 1 when $V = 3$ mL

The total of aflatoxins is the sum of AFG_2, AFG_1, AFB_2, and AFB_1.

[4] PHRED™ Photochemical Reactor (AURA Industries, New York, NY, USA) or equivalent. Avoid looking at the UV lamp.
[5] Kobra Cell™ (R-Biopharm Inc., Marshall, MI, USA) or equivalent. Set at 100 mA. Do not turn on the current until the LC pump is operating to avoid overheating the cell membrane.

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ACCEPTANCE CRITERIA

Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

PESTICIDE RESIDUE ANALYSIS

Definition

Where used in this Pharmacopeia, the designation "pesticide" applies to any substance or mixture of substances intended to prevent, destroy, or control any pest, unwanted species of plants, fungus, or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of pure articles. The designation includes substances intended for use as growth regulators, defoliants, or desiccants, and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.

Limits

Within the United States, many botanicals are treated as dietary supplements and are subject to the statutory provisions that govern foods but not drugs in the Federal Food, Drug, and Cosmetic Act. Limits for pesticides in foods are determined by the Environmental Protection Agency (EPA) as indicated in the Code of Federal Regulations (40 CFR §180) or the Federal Register (FR). In addition, the FDA establishes action levels for unavoidable pesticide residues (21 CFR §109 and 21 CFR §509). For pesticide chemicals without EPA-established tolerance levels or FDA action levels, the residues should be below the detection limit of the specified method. Results less than the EPA detection limits are considered zero values. The limits contained herein, therefore, are not applicable in the United States when articles of botanical origin are labeled for food purposes. The limits, however, may be applicable in other countries. Unless otherwise indicated in the monograph, the article to be examined complies with the limits given in *Table 5*. The limits for suspected pesticides that are not listed in *Table 5* must comply with the regulations of the EPA. For instances in which a pesticide is not listed in *Table 5* or in EPA regulations, calculate the limit by the formula:

$$\text{Limit (mg/kg)} = AM/100B$$

where A is the acceptable daily intake (ADI), as published by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), in mg/kg of body weight; M is body weight, in kilograms (60 kg); and B is the daily dose of the article, in kilograms.

If the article is intended for the preparation of extracts, tinctures, or other pharmaceutical forms of which the preparation method modifies the content of pesticides in the finished product, calculate the limits by the formula:

$$\text{Limit (mg/kg)} = AME/100B$$

where E is the extraction factor of pesticide in the preparation method, determined experimentally as the ratio between the original pesticide content in the plant material and the final pesticide content in the preparation; B is the daily dose of the preparation in kilograms; and A and M are as defined above.

A total or partial exemption from the test may be granted when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practices (GACP).

Table 5

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1
Bromide, inorganic (calculated as bromide ion)	125
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Bromopropylate	3
Chlordane (sum of *cis*-, *trans*-, and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2

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Table 5 *(continued)*

Substance	Limit (mg/kg)
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin (sum of)	0.1
λ-Cyhalothrin	1
Cypermethrin and isomers (sum of)	1
DDT (sum of *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-TDE, and *p,p'*-TDE)	1
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2
Endosulfan (sum of isomers and endosulfan sulphate)	3
Endrin	0.05
Ethion	2
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.03
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxon sulfone, and fensulfothion sulfone)	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon sulfone, fenthion-oxon sulfoxide, fenthion sulfone, and fenthion-sulfoxide)	0.05
Fenvalerate	1.5
Flucythrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide, and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ-, and ε-)	0.3
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
Parathion-ethyl and paraoxon-ethyl (sum of)	0.5
Parathion-methyl and paraoxon-methyl (sum of)	0.2
Pendimethalin	0.1
Pentachloranisole	0.01
Permethrin and isomers (sum of)	1
Phosalone	0.1

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Table 5 *(continued)*

Substance	Limit (mg/kg)
Phosmet	0.05
Piperonyl butoxide	3
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I, and pyrethrin II)	3
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline, and methyl pentachlorphenyl sulfide)	1
S-421	0.02
Tecnazene	0.05
Tetradifon	0.3
Vinclozolin	0.4

Qualitative and Quantitative Analysis of Pesticide Residues Use analytical procedures validated e.g., in accordance with the latest version of the EU guideline on analytical quality control and validation procedures for pesticide residue analysis [NOTE—Current version Document No. SANTE/11813/2017, https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf] or the EPA method validation principles (OPPTS 860.1340) that satisfy the following criteria. The method, especially with respect to its purification steps, is suitable for the combination of pesticide residue and substance under test, and is not susceptible to interference from co-extractives; the limit of quantification for each pesticide matrix combination to be analyzed is NMT the corresponding tolerance limit: the method is shown to recover between 70% and 120% of each pesticide with a repeatability NLT 20% RSD [NOTE—lower recoveries may be acceptable in certain cases as discussed in SANTE/11813/2017] ; and the concentrations of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

LIMITS OF ELEMENTAL IMPURITIES

The levels of elemental impurities should be restricted as shown in *Table 6* unless otherwise stated in the individual monograph. Specific monographs may provide different limits for articles that are typically used in large quantities.

Table 6. Limits of Elemental Impurities

Element	Limit (µg/g)
Arsenic (inorganic)[a]	2
Cadmium	0.5
Lead	5
Mercury (total)	1
Methylmercury (as Hg)[b]	0.2

[a] Arsenic may be measured using a nonspeciation procedure under the assumption that all arsenic contained in the supplement is in the inorganic form. Where the limit is exceeded using a nonspeciation procedure, compliance with the limit for inorganic arsenic shall be demonstrated on the basis of a speciation procedure.
[b] Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.

Articles are tested according to the procedures set forth in *Elemental Impurities—Procedures* ⟨233⟩. Where speciation is required, the procedures given in *Elemental Contaminants in Dietary Supplements* ⟨2232⟩ are used for testing.

USP REFERENCE STANDARDS ⟨11⟩

USP Dextrose RS

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⟨563⟩ IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN

INTRODUCTION

Identification of raw plant material intended for use in the manufacture of pharmaceuticals, excipients, or dietary supplements is carried out by examining the morphological and histological features of the article under test and by performing diagnostic chemical tests on the article. The botanical and chemical characteristics of the test article are then compared to the known botanical and chemical characteristics of the plant species. Reference articles may be specified to assist in the proper botanical and chemical identification of the plant and plant part. A reference article may be either a USP Authenticated Reference Material, which may be used for both botanical and chemical identification, or a USP Reference Standard, which is used for chemical identification only.

USP AUTHENTICATED REFERENCE MATERIALS

USP Authenticated Reference Materials are plant organs or tissues certified to have come from a plant that has been properly identified as belonging to the species listed on the label. The authentication is performed by botanical taxonomists, plant anatomists, phytochemists, or other plant scientists contracted by USP. A USP Authenticated Reference Material is typically a dried, pulverized plant organ or tissue, and it may be obtained from USP. Herbarium samples that may include roots, stems, leaves, flowers, fruits, and seeds of the authenticated plants are archived and made available for examination upon request. Standard herbarium samples usually consist of the entire mature plant. USP Authenticated Reference Materials undergo the same botanical and chemical diagnostic tests as those applied to test raw materials. A test article must have all botanical and chemical characteristics specified and found in the USP Authenticated Reference Material. To serve its intended purpose, each USP Authenticated Reference Material is properly stored, handled, and used. Generally, USP Authenticated Reference Materials are stored in their original containers under cool and dry conditions and protected from light and insect infestation. Where special storage conditions are necessary, directions are given on the label. Active principles and marker compounds typically degrade with time; therefore, expiration dates are assigned to USP Authenticated Reference Materials for their use in chemical identification. USP Authenticated Reference Materials are not intended for use in the manufacture of pharmaceuticals, excipients, or dietary supplements.

BOTANICAL IDENTIFICATION

The botanical identification of raw plant materials used in the manufacture of pharmaceuticals, excipients, or dietary supplements consists of ascertaining the macroscopic characteristics of the plant part, such as root, stem, leaf, flower, fruit, or seed, used in the manufacture of the article, as well as ascertaining its histological (microscopic) features. It may also include the inspection of organoleptic features of the botanical tissue such as the presence or absence of a characteristic odor. Individual compendial monographs may include botanical information on possible adulterant species to help ensure their absence in the raw material. For a proper identification of the plant, plant organ, or plant tissue, it is necessary to have a basic knowledge of plant anatomy.

Diagnostic Plant Morphology and Anatomy

This section exclusively addresses the diagnostic morphological and anatomical features of vascular plants and the various plant parts, such as roots, stems, leaves, flowers, fruits, and seeds, from which pharmaceuticals, excipients, or dietary supplements are derived. Vascular plants include pteridophytes (ferns and fern allies; for example, genera *Aspidium*, *Equisetum*, and *Lycopodium*), gymnosperms (seed plants, in which the seed is not enclosed within a fruit; for example, genera *Ephedra*,*Gingko*, and *Pinus*), and angiosperms (seed plants, in which the seed is enclosed within a fruit; for example, genera *Allium*, *Digitalis*, *Panax*, *Matricaria*, and *Rauwolfia*). Anatomical diagnostic features that are specified in an individual monograph (see *Botanic characteristics* in individual monographs) may include, but are not limited to, the presence of a particular tissue within an organ; the arrangement and type of cells within a tissue; the presence and type of secretory canal, oil, or resin duct or laticifers within an organ; the number of epithelial cells surrounding a secretory canal; and the presence and type of ergastic substances such as starch, inulin, fat globules, essential oils, calcium oxalate crystals, cystoliths, polyphenols, fluids, or other materials occurring in the cytoplasm, organelles, vacuoles, cavities, or cell wall.

ROOTS

The tissues present in young roots, starting with the most external tissue, include an epidermis with root hairs, cortex, endodermis, pericycle, phloem, xylem, and, in some species, pith. In some species, the outermost layer or layers of cortex are distinct from the inner layers, in which case they are referred to as a hypodermis. In species that undergo secondary growth in the roots, it is typical for all tissues external to the pericycle to be sloughed off. Roots that exhibit secondary growth have a periderm or bark, composed of a phellum (cork), phellogen (cork cambium), and phelloderm as the outermost tissue. Underneath the periderm, remnants of primary phloem, secondary phloem, vascular cambium, primary xylem, and secondary xylem can be found. Secondary vascular tissues have medullary rays separating clusters of the principal conducting cells of phloem (sieve elements or sieve cells) and the principal conducting cells of xylem (vessels and tracheids). Most species of plants that undergo secondary growth lack pith in the root. The type and arrangement of the principal conducting cells of the vascular tissues may be diagnostic of the species. Roots of many species develop into food storage organs. Abundant parenchyma and

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large amounts of starch or other polysaccharides characterize these types of roots. The presence, type, and arrangement of fibers, sclereids, and other tissues, and the presence and location of ergastic material, may also be diagnostic features. Morphologically, roots may be distinguished from rhizomes (the underground stems) primarily by the absence of nodes and internodes, which are present in rhizomes.

STEMS

Several external macroscopic features of stems that may be diagnostic of the species include the attributes of the nodes, internodes, leaf scars, vascular bundle scars, lenticles, and buds; the growth pattern of the buds; position and arrangement of the leaves along the stem; and the presence of tendrils, spines, thorns, or prickles. Starting with the outermost tissue, the internal arrangement of tissues in the young stems of most species is epidermis, cortex, a concentric ring of vascular bundles separated from each other by parenchymatous medullary rays, and pith. Depending on the species, stomata or trichomes or both structures may be present in the epidermis. The cortex of some species may include a hypodermis or an endodermis or both. In most monocotyledons, the vascular bundles are not arranged concentrically; instead, they are scattered throughout a mass of parenchyma tissue internal to the epidermis. Because of this arrangement, neither cortex, medullary rays, nor pith can be discerned. In woody plant stems that undergo secondary growth, it is typical for the epidermis to be sloughed off and replaced by a periderm composed of a phellum, phellogen, and phelloderm. Some species are characterized by having multiple periderms (rhytidome). Lenticles may be present in the periderm and their attributes may serve as diagnostic features. Underneath the periderm are the remnants of the cortex, primary phloem, secondary phloem, vascular cambium, secondary xylem, primary xylem, and pith. Medullary rays are also present. As in the root, the type and arrangement of the principal conducting cells of the vascular tissues; the presence, type, and arrangement of fibers, sclereids, and other tissues; and the presence and location of ergastic material may also be diagnostic features. Rhizomes may have some morphological characteristics similar to those of roots and therefore they may be mistaken for roots. However, rhizomes can be correctly identified as stems because they have distinct nodes and internodes.

LEAVES

Several macroscopic features of leaves that may be diagnostic of the species include the attributes of the leaf blade, petiole, and stipules and the phyllotaxy. The outermost tissue of a leaf blade is the epidermis, followed by mesophyll and vascular tissues. Microscopic diagnostic features of epidermal cells include the cuticle thickness and markings, the shape and arrangement of stomata and guard cells, the arrangement and size of subsidiary cells, stomatal number (number of stomata per unit area), and stomatal index (number of stomata per unit number of epidermal cells). Additional features useful in the identification of leaf material include types and arrangement of trichomes (plant hairs) present; type and arrangement of mesophyll and vascular tissues; palisade mesophyll ratio; presence and appearance of accessory tissues such as parenchymatous or sclerenchymatous bundle sheaths, paraveinal mesophyll, endodermis, and transfusion tissue; type and arrangement of the principal conducting cells of the vascular tissues; presence, type, and arrangement of fibers, sclereids, and other tissues; and presence, location, and physical appearance of ergastic material.

FLOWERS

Flowers are the best diagnostic morphological features of any flowering plant, and the floral structure is the principal criterion used in plant taxonomy. The diagnostic features of flowers include type of inflorescence; presence, number, and appearance of the primary floral parts (sepals, petals, stamens, and carpels); type of symmetry displayed by the floral parts; relative position of the ovaries in regard to the other parts of the flower; the number of ovules per ovary; type of placentation of the ovary; physical appearance of the pollen grains; presence of nectaries; presence of covering or glandular trichomes; and physical features of accessory structures such as the receptacle and bracts. The histological features and the presence of ergastic materials in the tissues of floral parts are also diagnostic of the species.

FRUITS

The identification of the species of plant from which a fruit was derived may be determined by observing several macroscopic criteria. These criteria include the number of pistils found in the fruit; the number of carpels within each pistil; the number of seeds within each carpel; the placentation of the fruit; and the determination of whether the fruit is dehiscent, indehiscent, or fleshy. Additional diagnostic features include the number of sutures in a dehiscent fruit, the determination of whether the seeds are fused to or free from the pericarp wall, physical features of the three layers of the pericarp of fleshy fruits (epicarp, mesocarp, and endocarp), and presence and physical appearance of accessory tissues such as the receptacle and bracts. Histological features of fruit tissues may aid in identification. The characteristics of the seeds within the fruit are also diagnostic features of the species.

SEEDS

The macroscopic features of seeds used in identification include the shape and size of the seed; appearance of the seed-coat surface; placement of the hilum and micropyle; and presence of accessory structures of the seed coat such as the arils, caruncle, or oil bodies. Physical features of the embryo, such as its size, shape, position, and the number and appearance of the cotyledons, as well as the presence and appearance of accessory nutritive tissues such as the remnants of a megagametophyte (in gymnosperms), perisperm (nucellus), or endosperm, are also diagnostic of the species. Histological features of the seed coat and other structures and tissues of the seed may also be used for species identification.

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Microtechnique

Histological analysis of botanical specimens can be performed on whole plant material or plant powder. The use of cytological stain or other reagents may be necessary to visualize certain histological features. Crossed polarizers can be used to detect structures that rotate plane-polarized light. These structures include starch grains, calcium oxalate crystals, some fibers, and grains of sand (present as a contaminant) that can be observed as bright objects against a dark background. One polarizer is commonly placed in the condenser or the light source, and the second polarizer is placed in the ocular. Light entering the slide from below is plane polarized, permitting only some light waves in a specific plane to pass through. When the two polarizers are aligned, the field becomes bright; when the two polarizers are crossed, the field becomes dark.

PROCEDURE FOR TEMPORARY MOUNTS AND POWDERED MATERIAL

General procedure: Plant samples are observed under the microscope by employing different mounting media, stains, or other solutions to assist in the correct identification of the test article. If a USP Authenticated Reference Material is available, prepare it with the same mounting media or reagent solutions used for the test article. Place 1 or 2 drops of water, *Glycerin–alcohol solution*, *Chloral hydrate solution*, or another reagent solution (see *Preparation and use of reagent solutions, optical devices, and mountants*) in the center of a clean slide. Transfer a small plant tissue section or a portion of plant powder into the mountant or reagent solution, and cover with a clean coverslip. (For specific preparation techniques, see *Preparation of temporary mounts and hand sections*, *Maceration*, or *Preparation of powdered materials*, as appropriate.) To prevent the formation of air bubbles, the coverslip may be carefully placed at an appropriate angle with its edge making the first contact with the slide and then pressed until it covers the specimen. Using a piece of filter paper, remove excess fluid from the margin of the coverslip. Air bubbles can be removed by placing the slide in a vacuum desiccator. When using chloral hydrate, air bubbles can be removed by gently boiling the sample over a small flame such as that from an alcohol lamp. To replace the mountant or reagent solution, place drops of the new mountant or reagent solution on one edge of the coverslip. Place a strip of filter paper at the opposite edge of the coverslip to remove the old mountant or reagent solution and to cause the new mountant or reagent solution to be drawn over the powdered material or tissue. Plant oils can be also washed away from the tissue in this manner when solvent hexane or acetone are washed through the slide followed by water and, if necessary, *Chloral hydrate solution*. Do not use *Chloral hydrate solution* immediately after treating the plant tissue with flammable solvents without thoroughly washing the tissue with water. This is to avoid setting fire to residual solvent when the microscope slide is later placed over a small flame to boil the tissue. Care must be taken when using reagent solutions that are volatile or corrosive to the microscope. To prevent drying of aqueous or chloral hydrate solutions during observation, add a small drop of glycerin to the slide. Observe the mounted sample under an optical microscope (see *Optical Microscopy* ⟨776⟩), and examine for histological features.

Preparation and use of reagent solutions, optical devices, and mountants: The following reagents, optical devices, and mounting media are used to assist in the identification of cells, tissues, structural features, and ergastic substances in the tissue or powdered material (see *Table 1* and *Table 2*).

Table 1. The Use of Reagent Solutions and Optical Devices

Detection	Reagent Solutions and Optical Devices
Calcium carbonate concretion	*Diluted acetic acid*
Calcium oxalate crystals	*Crossed polarizers*
Cellulose	*Carmine alum–methyl green solution* *Hydriodic acid* *Zinc chloride–iodine solution*
Cytoplasm	*Alcoholic picric acid solution*
1,8-Dihydroxyanthra-quinones	*1 M Potassium hydroxide solution*
Essential oils	*Osmium tetroxide solution* *Sudan III solution*
Inulin	*Naphthol–sulfuric acid solution*
Lignin	*Carmine alum–methyl green solution* *Phloroglucinol–hydrochloric acid solution* *Universal reagent*
Lipids (cutin, waxes, and suberin included)	*Carmine alum–methyl green solution* *Osmium tetroxide solution* *Sudan III solution* *Universal reagent*
Pectin and mucilage	*Ruthenium red solution* *Thionine solution* *Toluidine blue solution*
Phytoglycogen	*Ruthenium red solution*
Protein bodies	*Alcoholic picric acid solution* *Osmium tetroxide solution*

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Table 1. The Use of Reagent Solutions and Optical Devices *(continued)*

Detection	Reagent Solutions and Optical Devices
Saponin	*Blood–gelatin mixture* *Iodine–glycerin solution* (confirm by testing with *Blood–gelatin mixture*)
Starch	*Crossed polarizers* *Iodine solution* *Universal reagent*
Tannins and other polyphenols	*Ferric chloride solution* *Osmium tetroxide solution*

Table 2. Bleaching and Clarifying Agents and Mountants

Use	Mountants and Agents
Bleaching agents	*Sodium hypochlorite solution*
Clarifying agents	*Chloral hydrate solution* *Lactochloral solution* *Lactophenol solution*
Mountants	*Glycerin* *Glycerin–alcohol solution* *Glycerin–gelatin mixture* *Water*

Alcoholic picric acid solution—Prepare a 1% solution of picric acid in alcohol. Picric acid is useful to stain cells having dense cytoplasm such as aleurone cells in seeds. Place a small amount of powdered plant material in a test tube, and shake with about 1 mL of solvent hexane to remove plant oils, which would interfere with the reaction. Centrifuge, and discard solvent hexane. Soak the plant powder in *Alcoholic picric acid solution* for about 30 min. Transfer a portion of the powder to a microscope slide, and observe under a microscope. Cytoplasm and protein bodies turn bright yellow. [**CAUTION**—Picric acid is explosive when dry. Handle appropriately.]

Blood–gelatin mixture—Add 4.5 g of gelatin powder to 100 mL of a 0.9% sodium chloride solution, and allow to swell for 30 min. Heat the gel, while stirring, to about 80° in a water bath. Cool to 40°, and add 6 mL of defibrinated bovine blood. Heat to 45°–50°, and pour onto a microscope slide in a thin layer of about 1 mm while the slide is in a horizontal position. To prevent loss of blood-gelatin mixture from the sides, seal the microscope slide edge with a 1-cm wide adhesive tape to form a tray. After cooling and solidification, it is ready for use. [NOTE—Store in a humid chamber for NMT 1–2 days at 3°–4°.] To test for saponins, place small clusters of the powdered plant material on the blood–gelatin layer, spacing them a few mm apart from each other, transfer to a humidifier for a few h, and observe. Saponin-containing particles will cause light-transparent zones to appear in the blood–gelatin.

Carmine alum–methyl green solution—Boil 1.5 g of carmine for 30 min in a 15% solution of aluminum potassium sulfate. Cool, filter, and add 10 mL of a 0.75% methyl green solution while stirring. Add 1–2 drops to the plant material. Lignin and suberin turn green and cellulose turns red-violet.

Chloral hydrate solution—Use chloral hydrate TS. When using the solution as a clarifying agent, add a few drops to the plant material, and boil briefly over a small flame. Chloral hydrate dissolves cellular contents and intercellular substances and allows cell walls and shapes to be easily observed. It can be used to assist in the identification of cork, fibers, vessels, calcium oxalate crystals (with the aid of crossed polarizers), trichomes, stomata, and pollen.

Crossed polarizers—This optical device is used to detect calcium oxalate crystals and starch grains (amyloplasts). In polarized light, calcium oxalate crystals and starch grains appear as bright, birefringent objects on a dark background. Starch grains observed under polarized light will also have a Maltese-cross effect with the arms of the cross intersecting at the hilum. Calcium oxalate crystals are usually best viewed after the sample has been clarified with *Chloral hydrate solution* or another clarifying agent.

Diluted acetic acid—Add 1–2 drops to the plant material, and immediately observe under a microscope. Calcium carbonate deposits dissolve with effervescence.

Ferric chloride solution—Dilute 1 mL of ferric chloride TS with 9 mL of water. For the detection of phenol hydroxyl groups, such as tannins and flavonoids, from the side of the coverslip add the solution to the aqueous sample. Tannins and other polyphenols become blue-black to green.

Glycerin—Use as a mountant to prevent the drying of aqueous and chloral hydrate solutions.

Glycerin–alcohol solution—Mix equal volumes of glycerin and alcohol. Use as a mounting medium.

Glycerin–gelatin mixture—Add 10.0 g of powdered gelatin to 60 mL of water. Allow to stand for 2 h, and add 70 mL of glycerin containing 1.5 g of dissolved phenol. Heat in a water bath, and filter through a preheated funnel containing glass wool. The filtered mixture is liquefied before use, and it serves as a mounting medium. Add a few drops to the cut or powdered plant material, and cover with a heated coverslip. This preparation is used for long-term storage of specimen mounts. The margins of the coverslip may be sealed with Canada balsam after a few months of drying.

Hydriodic acid—Add 1–2 drops to plant material. Cellulosic cell walls become blue to blue-violet.

Iodine solution—Add 1–2 drops of 0.1 N iodine VS to the plant material. Starch particles become dark-blue to blue-violet; this reaction is reversible on heating. [NOTE—Proteins, lipids, and cellulose turn yellow to brown; guaiac powder particles become green to blue, but this reagent is not used for diagnostic identification of these features.]

Iodine–glycerin solution—Dissolve 0.3 g of iodine and 1.0 g of potassium iodide in a small quantity of water, and add 10 mL of a mixture of glycerin and water (1:1). Add 1–2 drops to the powdered plant material. Samples containing saponins form

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yellow lumps or aggregates. If a sample tests positive for saponin, the result has to be confirmed by testing the sample with *Blood–gelatin mixture* as well.

Lactochloral solution—Dissolve 50.0 g of chloral hydrate in 50 mL of lactic acid with gentle heating. Add a few drops to the plant material. Place the microscope slide in a small vacuum desiccator if it is necessary to eliminate air bubbles. *Chloral hydrate solution* and *Lactochloral solution* are used for the same type of identification, except that *Lactochloral solution* is a stronger clarifying agent and it is used for plant material that is more difficult to clarify.

Lactophenol solution—Mix 20 g of lactic acid, 40 g of glycerin, and 20 mL of water. Add 20 g of phenol, and mix. This is a strong clarifying agent suitable for the examination of pollen grains.

Naphthol–sulfuric acid solution—Prepare a 20% solution of 1-naphthol in alcohol. To the plant material add 1 drop of 1-napthol solution and 1 drop of sulfuric acid. Inulin crystals turn brownish red and then dissolve.

Osmium tetroxide solution—Dissolve 0.1 g of osmium tetroxide in 5 mL of distilled water. Add 1–2 drops of the solution so obtained to the plant material. Essential oils, fatty oils and other lipids, tannins, and protein bodies become brown to black.

Phloroglucinol–hydrochloric acid solution—This solution is used for the identification of lignin and other hydroxyphenylpropane derivatives; lignified tissues, such as sclereids, vessels, fibers, and stone cells; and lignified parenchyma. Moisten the powder or the cut sample with phloroglucinol TS, and allow to dry for 2–3 min before placing the coverslip. Add a few drops of a 25% hydrochloric acid solution, and cover with the coverslip. Lignified cell walls turn carmine red. [NOTE—This stain is not stable.] Cells with hydroxyphenylpropane derivatives, such as vanillin and ferulic acid, also turn red. Alternatively, hydroxyphenylpropane derivatives can be extracted from the plant material and the plant material then examined. To extract hydroxyphenylpropane derivatives repeatedly immerse the untreated material in alcohol, mix on a vortex mixer, centrifuge, and discard the alcohol between washings. Then treat the plant material as specified previously, beginning with the addition of phloroglucinol TS.

1 M Potassium hydroxide solution—Add 1 drop to plant material. Cells containing 1,8-dihydroxyanthraquinones will stain red.

Ruthenium red solution—Add a few drops of ammonium hydroxide to ruthenium red TS. [NOTE—Store the solution protected from light.] Add 1–2 drops to the plant material. Pectin-containing cell membranes, acidic mucilage, and phytoglycogen turn red.

Sodium hypochlorite solution—This solution is used to bleach deeply colored sections. Immerse the plant material in the solution for a few min until sufficiently bleached. Wash the tissue with water, and mount with a suitable mounting agent. [NOTE—Sodium hypochlorite will extract lignin; plant tissue so treated will test negative for lignin.]

Sudan III solution—Dissolve 0.5 g of sudan III in 50 mL of alcohol or isopropyl alcohol with reflux boiling. Cool, filter, and add 50 mL of glycerin. Add 1–2 drops of this solution to plant powder. Essential oils, waxes, cutin, suberin, and fatty oils and other lipids combine with this lipophilic colorant and become orange-red to red after a short time.

Thionine solution—Prepare a 0.2% thionine acetate solution in 25% alcohol. Immerse the dry sample in this solution. After about 15 min, wash out the excess of stain with 25% alcohol. Mucilage will have swollen into spherical globules and turned red-violet, while cellulose, pectin, and lignified septa will turn blue or blue-violet.

Toluidine blue solution—Using toluidine blue, proceed as directed for *Thionine solution*.

Universal reagent

SOLUTION A: Dilute 20 mL of a lactic acid-saturated solution of sudan III with 30 mL of lactic acid.

SOLUTION B: Dissolve 0.55 g of aniline sulfate in 35 mL of water.

SOLUTION C: Dissolve 0.55 g of potassium iodide and 0.05 g of iodine in 5 mL of water, and add 5 mL of alcohol.

PROCEDURE: Combine *Solution A*, *Solution B*, and *Solution C*, and add 2.5 mL of hydrochloric acid while stirring. [NOTE—The solution is used without filtering.] For identification, add 2–3 drops to the sample, and gently boil over a small flame. If necessary, small amounts of *Universal reagent* may be added during boiling. Cover with the coverslip. Lignified elements turn yellow, suberin turns red-brown, lipids turn red, and starch turns blue-violet.

Water—Use as a mounting medium. [NOTE—All grades of water are acceptable for this purpose.]

Zinc chloride–iodine solution—Dissolve 20.0 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water. Add 0.5 g of 0.1 N iodine VS, and shake for 15 min. Filter if necessary. Store in low-actinic glassware. Add 1–2 drops to the plant material, and allow to stand for a few min. Cellulosic cell walls are stained blue to blue-violet.

Preparation of temporary mounts and hand sections: When using the dry plant tissue, soak or gently boil in water until soft. Do not soften too much. Material can then be treated like fresh plant material. When appropriate, use the mountants or reagent solutions listed for use with plant powder to help visualize features of the tissue (see *Preparation and use of reagent solutions, optical devices, and mountants*).

To make an epidermal peel of the leaf, petal, sepal, bract, and other leaf-like appendages, roll the tissue into a cylinder, and nick with a sharp, polytef-coated razor blade that has been wetted with water. Grasp the nicked piece of tissue with forceps, and strip back removing a clear section of the epidermis. Mount in water on a microscope slide, place a coverslip over the tissue, and examine under a microscope.

If it is difficult to obtain an epidermal peel using the previous procedure, proceed as follows. Soak the tissue in a 40%–60% nitric acid solution at 60° for 3–4 min or until the epidermis can be easily peeled. The peel is then washed 3–5 times in water to remove the excess of nitric acid. Neutralize the tissue in a 1% potassium hydroxide solution or a 1% sodium hydroxide solution. Wash the tissue again with water, mount in water on a microscope slide, place a coverslip over the tissue, and examine under a microscope.

An alternative method of preparing leaf tissue for the examination of the epidermis is to heat a leaf fragment (about 5 mm × 5 mm) for 15 min in *Chloral hydrate solution* on a water bath. Transfer the tissue to a microscope slide, add a drop of water, and cover with a coverslip. These procedures can be used to determine the stomatal type, distribution, number, and index.

Stomatal number is determined by counting the number of stomata per unit area of a microscopic field. Determine the stomatal number on at least 10 different sites of the specimen, and calculate a mean value. Keep track of which leaf surface is being observed, abaxial or adaxial, as the stomatal number for different surfaces is frequently significantly different.

To calculate the stomatal index, the specimen is observed under a microscope at a low magnification. The size of the surface is determined with a calibrated micrometer ocular, and the number of stomata and the number of epidermal cells for that area are determined. The stomatal index is calculated:

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$$\text{Result} = (100 \times S)/(E + S)$$

S = number of stomata for a given area
E = number of epidermal cells of the same area

Determine the stomatal index on at least 10 different sites of the specimen, and calculate a mean value. Again, keep track of which leaf surface is being observed, abaxial or adaxial, as the stomatal indices for different surfaces is frequently significantly different.

To make a cross section of a leaf or thin roots, stems, or other thin appendages, lay the appendage to be sectioned on a microscope slide. Place another microscope slide over the appendage with a portion of the tissue exposed. Using a sharp, polytef-coated razor blade that has been wetted, cut straight down along the edge of upper slide. Without moving the upper slide, cut down again with the razor blade at an angle. Some practice may be necessary to be able to get sections thin enough so that when they are mounted and covered with a coverslip, these sections can be used to determine tissue arrangements (for instance, the number of palisade layers in leaf, thickness of cuticle, types of trichomes, types of vascular bundles, and the like). Because razor blades dull quickly, they have to be replaced frequently.

Use the cross section of leaf tissue so obtained to determine the palisade mesophyll ratio. Alternatively, boil leaf fragments of about 2 mm^2 in *Chloral hydrate solution*, mount, cover with a coverslip, and observe under a microscope. Identify groups of four adaxial epidermal cells, and count the palisade mesophyll cells that are lying below and are at least 50% covered by the epidermal cells. This value divided by 4 is the palisade mesophyll ratio. Determine the palisade mesophyll ratio of at least 10 groups of epidermal cells, and calculate a mean value. Palisade mesophyll ratio can also be determined on powdered leaf material.

To make a cross section of thick stems, roots, or other plant parts, including woody tissues, hold the tissue in one hand and using a sharp, polytef-coated razor blade that has been wetted with water, shave a cross section from the appendage. Mount in water, another medium, or reagent solution, place a coverslip over the material, and examine under a microscope. Sections thin enough to determine vascular tissue arrangement, ray type, parenchyma distribution, presence of crystals, and the like can usually be made with a little practice.

Maceration: For the proper identification of a plant material, it is sometimes necessary to macerate the tissue into its individual cells before microscopic examination. This can be an especially useful technique for woody or other hard tissues. The material is cut into small pieces of about 2-mm thickness and 5-mm length or sliced into pieces of about 1-mm thickness. Depending on the nature of the cell wall, one of the following methods is used. For hard or highly lignified tissues, use *Method I*. For tissues that are not extensively lignified, use *Method II*.

Method I

SOLUTION A: Use 4 N nitric acid solution.

SOLUTION B: Prepare a mixture of 1.2 M chromium trioxide solution and sulfuric acid (7:4).

PROCEDURE: Place the plant material in a test tube containing about 5 mL of a mixture of *Solution A* and *Solution B* (1:1). Heat in a water bath for 20 min. Wash the tissue repeatedly with water, and transfer to a microscope slide. Tease the tissue apart with a dissecting needle, add 1–2 drops of mountant, cover with a coverslip, and examine under a microscope. If necessary, cells can be further separated from each other by pressing down on the coverslip with a gentle, sliding motion. The macerated tissue will test negative for lignin.

Method II

PROCEDURE: Place the plant material in a test tube containing about 5 mL of 2 M potassium hydroxide solution. Heat in a water bath for 30 min. Wash the tissue repeatedly with water, and transfer to a microscope slide. Add 1–2 drops of mountant. Place a coverslip over the tissue, press down, squashing the tissue, and examine under a microscope. The macerated tissue will test negative for lignin.

Preparation of powdered materials: Place 1 or 2 drops of water, another mountant, or a reagent solution in the center of a clean slide. Moisten the tip of a dissecting needle with water, and dip into the powder under test. Transfer a small amount of material that adheres to the needle into the fluid on the slide, and stir thoroughly and carefully. Cover with a clean coverslip. Because the arrangement of the tissue structures within the plant tissue has been destroyed, the important features for observation of the powdered plant material are the chemical and physical features of tissues and cell types, as well as the presence and chemical and physical features of ergastic substances. The specific tissues, cells, and ergastic substances to be examined are specified in the individual monograph.

PROCEDURE FOR THIN, PERMANENT MOUNTS

When it is necessary to reveal detailed histological features of a plant specimen, thin tissue sections have to be obtained. The sections need to be thin enough to transmit light, and they have to be cut in such a plane that the desired features are exposed. The plant material is properly killed, fixed, dehydrated, and embedded in paraffin or other embedding media. The embedding medium is used as a solid-support matrix during tissue sectioning. After sectioning and mounting, staining of the specimen is frequently performed to aid in the differentiation of certain structures. [NOTE—The process of fixing, dehydration, embedding, and staining can be significantly expedited by utilizing a microwave oven specifically designed for histological work.]

Killing and fixation: As a first step in preparing plant material for sectioning, living cells are killed, and the tissue is preserved. This is most frequently done by employing a chemical fixative. A good general purpose fixative for plant material is a mixture of formaldehyde, acetic acid, and alcohol (FAA).

FAA solution—Mix 50 mL of alcohol, 5 mL of glacial acetic acid, 10 mL of formaldehyde solution, and 35 mL of water. [NOTE—Periodically prepare fresh solution, as it loses effectiveness with storage.]

Procedure—Completely immerse the plant material in the *FAA solution*. Allow the material to remain immersed for 18–24 h at room temperature. Plant material can be kept indefinitely in *FAA solution*, as long as it remains completely immersed and is not allowed to dry out. Certain plant tissues may require vacuum infiltration to facilitate the penetration of the fixative. Vacuum infiltration is required if the tissue has abundant air spaces or epidermal hairs or if it floats on top of the fixative solution. Place the tissue in a small vial containing the fixative. Place the uncapped vial into a bell jar or desiccator that is connected to a vacuum

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source, preferably an oil-sealed vacuum pump. The vacuum is vented into a fume hood to prevent fixative vapors from filling the room. Slowly turn on the vacuum. Do not use a strong vacuum because the fixative may start to boil and damage the tissue. As residual air is pulled from the tissue, it will rise to the surface. Turn the vacuum on and off through several cycles until the tissue stays at the bottom of the container during an "on" cycle.

Tissue dehydration: Paraffin and other embedding media are hydrophobic; thus, water must be removed from the plant tissue after fixation. This is accomplished by immersing the fixed tissue in dehydration solutions, which are a series of mixtures of alcohol and water with increasing alcohol concentration. The final solution in the series is dehydrated alcohol. Begin by washing the fixed tissue once or twice with fresh 50% alcohol to remove traces of FAA. Remove this solution, and subsequently remove any other dehydration solution, by decanting the solution or removing it with the aid of a glass pipet. Add the first dehydration solution (70% alcohol) to the vial, completely immersing the tissue. The graded alcohol–water series and the suggested times for tissue immersion are listed in *Table 3*.

Table 3

Dehydration Solution	Time (h)
50% alcohol	1–2
70% alcohol	1–2
90% alcohol	1–2
95% alcohol	1–2
Dehydrated alcohol containing 0.1% of safranin O	2–4
Dehydrated alcohol	1

Safranin O is added to the penultimate dehydration solution in the series to visualize the tissue when it has become embedded in paraffin. If the tissue to be sectioned is hard or woody, the time for each step in the series may need to be increased to up to 24 h. If necessary, the tissue can be stored for several days in 70% alcohol or in solutions of even higher alcohol concentrations.

Embedding

Preparation for embedding

ALCOHOL REMOVAL: Paraffin is the most common embedding medium, although other embedding media are available. After dehydration, alcohol is removed from the tissue by using a graded series of dehydrated alcohol–xylene solutions, because paraffin is not soluble in alcohol. The graded dehydrated alcohol–xylene series and the suggested times for tissue immersion are listed in *Table 4*.

Table 4

Alcohol Removal Solution	Time (h)
A mixture of dehydrated alcohol and xylene (3:1)	1
A mixture of dehydrated alcohol and xylene (1:1)	1
A mixture of dehydrated alcohol and xylene (1:3)	1
Xylene	1
Xylene	1

XYLENE REMOVAL: Once xylene has completely replaced alcohol, paraffin is added slowly to infiltrate the tissue and remove xylene. Proceed as follows:

1. For each mL of xylene add about 1 paraffin chip to the tissue vial, cap, and allow to stand at room temperature for 4 h. Add additional paraffin chips until no more chips dissolve.
2. Place the tissue in an oven maintained at 42°–45°. Add 2–3 paraffin chips every h until no more chips dissolve at that temperature.
3. Pour off one-third of the volume, and replace with an equal volume of melted paraffin. Do not cap, and transfer the vial to an oven maintained at 58°–60°.
4. After the paraffin remelts (about 4 h later) pour off one-half of the volume, and replace with an equal volume of melted paraffin. Transfer the vial to the oven maintained at 58°–60° if the paraffin begins to solidify.
5. Repeat the fourth step twice more, then pour off the entire volume of paraffin–xylene. Replace with pure melted paraffin. About 4 h later, pour off the paraffin, and replace with fresh pure melted paraffin. Repeat the pouring off and replacement 4 h later, and allow to stand overnight. [NOTE—Transfer the vial to the oven maintained at 58°–60° if the paraffin begins to solidify at any point.]

Embedding procedure—Pour the tissue with the paraffin to an embedding boat. Paraffin has to completely cover the tissue by about 3–5 mm. Place the embedding boat on top of a preheated warming platform that is designed for histology work. Adjust the tissue in the boat to its proper orientation for sectioning. Slowly cool the paraffin by sliding the boat down to the cool side of the platform until the paraffin has solidified. Immerse the paraffin block in ice water to rapidly cool the block and to prevent paraffin crystals from forming. Store the paraffin block at 4°.

Sectioning and mounting: Cut the paraffin block into pieces, each containing one tissue sample. Trim the paraffin block, as close to the tissue mass as possible, to form a rectangle or a slight trapezoid. Such trimming will prevent sectioning problems due to excess paraffin around the tissue. To make transverse sections, orient the tissue at a right angle to a wooden tissue block

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of which the face has been soaked in melted paraffin. Affix the paraffin block to the face of the tissue block. Add a small amount of melted paraffin to the base of the paraffin block to help form a tighter seal. Cool the block to 4°.

Properly mount and adjust the tissue and paraffin block in a microtome. Use a sharp stainless-steel microtome knife that has been properly honed. Set the microtome to cut sections 8–15 µm thick (10-µm thickness is optimal for most tissues). Cut individual or serial sections. Prepare a microscope slide as follows. An adhesive may be prepared as a solution containing 1% of gelatin and 0.5% of sodium benzoate that is heated to 30°–35° to dissolve the gelatin. Smear a thin film of the adhesive so obtained onto the slide, allow to dry, rinse with a 4% solution of formaldehyde TS, and add a small amount of water. Plate the cut sections upside down on the slide, so that they float on water, and flood with a 4% solution of formaldehyde TS. The sections will immediately spread out and wrinkles will disappear.

Place the slide on a warming platform, maintained at 42°, to relax the sections. Pipet, and blot the excess water and formaldehyde solution. Dry overnight in an oven at 42° to ensure adherence of the tissue section to the slide.

Staining

Preparation for staining—Immerse the microscope slide with the affixed tissue twice into xylene, each time for 10–15 min, to remove paraffin. Then immerse the slide into the following sequence of solutions, leaving it in each solution for 5 min and taking care not to dislodge the tissue: a mixture of dehydrated alcohol and xylene (1:1), dehydrated alcohol, alcohol, and a 70% alcohol solution. The tissue is bleached prior to staining if it is opaque because of the presence of tannins or other ergastic materials. To bleach, dip the slide into a 1% potassium permanganate solution for 1 min, rinse with water, dip into a 5% oxalic acid solution for 1 min, and rinse thoroughly with water. The material is ready for staining. One of the following two staining procedures is recommended for most botanical identification work. The first staining procedure uses safranin O counterstained with fast green. An alternative staining procedure uses safranin O counterstained with orange G.

Safranin O–fast green staining

SAFRANIN O STAINING SOLUTION: Prepare a mixture of methoxyethanol, dehydrated alcohol, water, and formaldehyde solution (50:25:25:2). Add a sufficient quantity of sodium acetate to obtain a solution containing 1% of sodium acetate, and mix. Add a sufficient quantity of safranin O to obtain a solution containing 1% of safranin O, and mix.

FAST GREEN STAINING SOLUTION: Prepare a mixture of methoxyethanol, dehydrated alcohol, and methyl salicylate (1:1:1) containing 0.05% of fast green FCF.

PROCEDURE: Once the tissue has been rehydrated to 70% alcohol as described in *Preparation for staining*, immerse for 2–24 h, depending on the tissue, in *Safranin O staining solution*. Remove excess stain by immersing the slide in water several times. Transfer the slide to an alcohol solution containing 0.5% of picric acid for 2–10 s to further remove excess stain from the section and to assist in differentiation of the tissue structures. To stop the action of the picric acid, transfer the slide for 10 s to 1 min to an alcohol solution containing 4 drops of ammonium hydroxide in each 100 mL of alcohol. Transfer the slide to dehydrated alcohol for 10 s. Visually inspect the stained tissue under a microscope to see if further destaining with picric acid is necessary. Counterstain for 10–15 s in *Fast green staining solution*. Transfer slide through two changes of a mixture of methyl salicylate, dehydrated alcohol, and xylene (2:1:1), each change lasting for 5–10 s. Then transfer the slide to a mixture of xylene and dehydrated alcohol (95:5) for 1 min. Transfer through two changes of xylene. Store in xylene until ready to mount the coverslip. Chromosomes, nuclei, and lignified, cutinized, or suberized cell walls will be stained red. Cytoplasm and cellulosic cell walls will be stained green to blue, depending on the pH of the tissue.

Safranin O–orange G staining

SAFRANIN O STAINING SOLUTION: Prepare a 0.004% solution of safranin O.

ORANGE G STAINING SOLUTION: Dissolve 2 g of orange G, 5 g of tannic acid, and 4 drops of hydrochloric acid in water, and dilute with water to 100 mL.

PROCEDURE: Once the tissue has been rehydrated to 70% alcohol as described in *Preparation for staining*, sequentially transfer the slide through the series of solutions in *Table 5*.

Table 5

Solution	Time
35% alcohol	5 min
A filtered 2% zinc chloride solution	1 min
Water	5 s
Safranin O staining solution	5 min
Water	5 s
Orange G staining solution	1 min
Water	5 s
A filtered 5% tannic acid solution	5 min
Water	3 s
A 1% ferric ammonium sulfate solution	2 min
Water	15 s
45% alcohol	10 s
90% alcohol	10 s
Dehydrated alcohol	10 s
A mixture of dehydrated alcohol and xylene (1:1)	1–2 min

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Finally, store in xylene until ready to mount the coverslip. Cellulosic cell walls will stain blue-black, nuclei will stain yellow, starch grains will stain black, and lignified cell walls will stain red.

Mounting the coverslip: The mounting of a coverslip over the tissue completes the preparation of the slide. Canada balsam, diluted with a small portion of xylene, can be used as an adhesive. Other mountants are also commercially available. Upon drying of the mountant, the slide can then be examined under a microscope. The entire process of making permanent microscope slides can be expected to take 5 or more days.

Scanning electron microscopy: Botanicals in commerce are often encountered in the form of powder or in pieces, thus making authentication by the routine method of cross-sectioning of the article difficult and often impossible. Structures such as xylem vessels and trachids may be broken into smaller bits, making detection of pitting and lignifications on the walls difficult if not impossible using an optical microscope. Structures that are resistant to these processes are most useful in identification. Scanning electron microscopy (SEM) is useful for characterizing the size and morphology of microscopic specimens. The more detailed differential characteristics in the structure of trichomes and peculiar elements in the epidermis, along with superficial granular material containing specific compounds, can be observed and identified with SEM, which assists in the identification of particular species. SEM has been used extensively to investigate surface topology of a wide variety of plant materials. It can play a vital role in authentication of an entire botanical, those in powder form, distinguishing between closely related species, and can be used to examine a mixture of powders.

Introduction and general information about SEM as applied to pharmacopeial articles can be found in general information chapter *Scanning Electron Microscopy* ⟨1181⟩.

SEM produces a higher resolution compared to that possible using an optical microscope, and the images obtained are three-dimensional. SEM has the advantage of providing images with a large depth of field, which allows a substantial thickness of the sample to be in focus at one time. It allows the analysis of specimens as large as 50 mm, making it possible to produce detailed topographical electron micrographs of an object clearly visible to the naked eye. The maximum resolution for SEM (minimum distance by which the two objects can be separated and observed as distinct objects) is 10–20 nm compared to 200–300 nm for optical microscopy. Typical SEM magnification ranges from ×10 to ×300,000. Commercial SEM instruments also are available with magnifications as low as ×5 and as high as ×2,000,000. In comparison, typical modern optical microscopes have a magnification range of ×10 to ×2000. At low magnification, images obtained with SEM provide more information than those of optical microscopy. SEM can produce images for which contrast is based on compositional variations of specimens.

Change to read:

CHEMICAL IDENTIFICATION

To help ensure the authenticity of the article, chemical identification is performed in conjunction with the botanical identification outlined previously. Chemical identification typically employs chromatographic procedures to detect the presence of marker compounds specified in the individual monograph. Spectroscopic or chromatographic profiles can be used to achieve chemical identification by fingerprint comparison against that of a reference sample or standard. Examples of spectroscopic methods include ultraviolet (UV), infrared (IR), and Fourier transformed IR (see ▲*Spectroscopic Identification Tests* ⟨197⟩▲ (CN 1-May-2020)). Examples of chromatographic methods include high-pressure liquid chromatography (HPLC), thin-layer chromatography (TLC), two-dimensional-TLC, and gas chromatography (GC) (see *Chromatography* ⟨621⟩). Analytical methods used for fingerprinting should be capable of detecting as many chemical constituents as possible. Multiple fingerprints, using a combination of analytical methods with different separation principles and test conditions, may be useful. In addition to the spectroscopic chromatographic methods, qualitative wet-chemistry methods may also be specified in the individual monograph.

Chemotaxonomy

Chemotaxonomy is the classification of the plants based on their chemical constituents and it may be useful in botanical articles identification. Metabolic compounds found within plant tissues can be divided into two broad categories based on their functions. The first category comprises primary metabolites—metabolites involved in the physiological plant processes that are absolutely necessary for life and ubiquitous throughout the plant kingdom. These processes include photosynthesis; respiration; and nucleic acid, protein, carbohydrate, and lipid metabolism. The second category comprises secondary metabolites—compounds that are thought not to be absolutely necessary for plant processes, although they may have important functions in the plant's interactions with other organisms, such as allelopathic interactions; in chemical defense against herbivores and plant pathogens; and in signaling to attract pollinating and seed-dispersing animals. Many secondary metabolites are known to have pharmacological activity. They are also the basis for the chemotaxonomy of plants. Secondary metabolites fall into several different chemical classes such as nonprotein amino acids, flavonoids, xanthones, coumarins, polyacetylenes, cyclic polyketides, monoterpenes, sesquiterpenes, iridoids, triterpenes, sterols, nitrogen-containing terpenes, and alkaloids. These chemical classes are not ubiquitous throughout the plant kingdom, but tend to be specific to certain botanical classes, orders, and families. Moreover, many chemical subclasses and individual secondary compounds are specific to certain subfamilies, genera, or species. It is these chemical subclasses and individual compounds that can be used as marker compounds to aid in the proper identification of plant material.

Active Principles and Marker Compounds

For chemical identification of botanical articles, extracts are prepared. Such extracts are usually complex mixtures of several chemical constituents. For a large majority of botanical extracts it is not known with certainty which of the various components is responsible for the reported pharmacological effect. It is generally believed that several constituents act synergistically to provide the reported effect. For articles for which compendial monographs are provided, certain chemical constituents of the

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article are chosen and quantitative test procedures for determining their content are provided. The choice of such constituents, known generally as marker compounds, is based on certain considerations. Currently, the following types of marker compounds are specified in compendial monographs and may be identified in raw materials:

ACTIVE PRINCIPLES: These are constituents that have proven clinical activity. A minimum content or range for the active principles is usually specified in the individual monograph. A quantitative determination of active principles during stability studies of botanical dosage forms provides necessary information for arriving at suitable expiration dates.

ACTIVE MARKERS: These are constituents that have known pharmacological activity contributing in some extent to efficacy. However, the clinical efficacy for these constituents may not be proven. A minimum content or range for active markers is usually specified in individual monographs. A quantitative determination of active markers during stability studies of botanical dosage forms provides necessary information for arriving at suitable expiration dates.

ANALYTICAL MARKERS: Where neither defined active principles nor active markers are known, other constituents of the botanical extract amenable to quantitative determination are chosen. These markers aid in the positive identification of the article under test. In addition, maintaining a minimum content or a specified range of the analytical markers helps to achieve standardization of the plant extract and to arrive at a suitable expiration date during stability studies.

NEGATIVE MARKERS: These are constituents that may have allergenic or toxic properties, rendering their presence in the botanical extract undesirable. For example, ginkgolic acids from ginkgo belong to this category. A stringent limit for these negative markers may be specified in individual monographs.

Use of USP Reference Articles

Reference articles are used to assist in the identification of marker compounds within the test article. Reference articles are either USP Authenticated Reference Materials or USP Reference Standards (see *USP Reference Standards* ⟨11⟩), whichever is specified in the individual monograph. USP Reference Standards used to identify marker compounds in the test articles may be a single purified chemical entity, a mixture of purified chemical entities, or a standardized extract prepared from the authenticated plant article. USP Reference Standards may also be used to quantitate marker compounds, as specified in the individual monograph.

A pulverized test article undergoes a specified extraction procedure (see *Methods of Extraction* in *Botanical Extracts* ⟨565⟩) and is prepared for chromatographic or wet-chemistry analysis. If a USP Authenticated Reference Material is available, then it undergoes the same extraction procedure as the test article. The test preparation and reference articles then undergo the same chromatographic or wet-chemistry procedure specified in the individual monograph. The response of the test preparation is compared to the response of reference articles to determine the presence of the marker compounds in the test article.

DNA-BASED METHODS FOR AUTHENTICATION OF ARTICLES OF BOTANICAL ORIGIN

Because morphological identification often is not possible when the original plant material consists of dried, cut and shifted, or processed plant parts or when the material consists only of a whole, single plant part containing no taxonomic characters, additional identification methods, such as DNA-based identification, often are required for these sample types. DNA-based methods have been shown to be efficient in distinguishing genuine plant materials from adulterants in complex botanical matrixes and can complement traditional botanical identification methods that rely on morphological features or chemistry. In addition, DNA-based methods often are more reliable than traditional methods, especially when applied to single-organ specimens that lack diagnostic taxonomic characters, to powdered materials in which the distinguishing characteristics are no longer visible, or when it is difficult to distinguish among closely related or morphologically similar species.

DNA Barcoding

DNA barcoding is a particular type of DNA sequence-based identification method that uses short sequences of specific nuclear or plastid DNA loci for identification of plant species. The assays rely on comparison of nucleotide sequences from a specific stretch of DNA (DNA sequences or DNA barcode) to perform DNA sequence-based identification. Further, DNA-based methods, such as next-generation sequencing (NGS) technologies, are able to identify multiple species in a mixture, including expected and unexpected species.

Botanical Identification Using DNA (Sanger) Sequencing

The process for botanical identification using DNA (Sanger) sequencing includes marker selection, DNA extraction, polymerase chain reaction (PCR) primers and amplification, DNA sequencing, and comparison with reference materials, as described in the following sections. See *Nucleic Acid-Based Techniques—Extraction, Detection, and Sequencing* ⟨1126⟩, *Nucleic Acid-Based Techniques—Amplification* ⟨1127⟩, and *Nucleic Acid-Based Techniques—Genotyping* ⟨1129⟩ for additional information.

MARKER SELECTION

The chosen sequence must be sufficiently specific to capture any potential primary and adulterant species in the sample but also sufficiently universal to avoid false-negative reactions for closely related species. For example, a species-specific primer is not appropriate for most identification procedures because adulterants cannot be detected and amplification failure can result because of either the absence of the species or degraded DNA. In many cases, a single marker may be sufficient for identification, but multiple markers from different parts of the genome (e.g., plastid or nuclear material) ensure that hybrids can be detected.

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Typically, regions used for DNA sequence identification range from 100–1500 base pairs in length. Smaller DNA fragments may be less susceptible to DNA degradation.

DNA EXTRACTION

Before amplification of the desired marker can be performed, the total genomic DNA must be extracted. The suitability of a procedure for genomic extraction depends on the starting material and the purity of the DNA required for downstream applications. The principal procedures are described as follows, and several commercial kits are available to accommodate different sample types and applications.

Total genomic DNA must be extracted from ground plant material. Plant materials can be homogenized manually using a mortar and pestle, mechanical grinder, or other apparatus depending on the nature of the material. Total genomic DNA extraction and purification can be challenging because of the abundance of secondary metabolites (polysaccharides, tannins, essential oils, phenolics, alkaloids, and waxes) in many medicinal plant species. Some of the secondary metabolites may coprecipitate with DNA during extraction and may inhibit further enzymatic reactions, including restriction digestion and PCR. In particular, large amounts of complex polysaccharides can make extraction of usable DNA impossible, rendering the aqueous portion of the extract too viscous to allow efficient separation of DNA from the contaminating polysaccharides. This type of contamination can lead to poor DNA yield and can prevent access by modifying enzymes.

Numerous commonly used DNA extraction methods are appropriate for a wide range of fresh and dried plant materials, including cetyl trimethylammonium bromide, silica-based methods, and a variety of commercially available kits that use silica columns or glass-coated magnetic beads. Although many of these methods work well on both fresh and dried materials and on any plant part, those that are degraded or contain significant levels of secondary compounds or other PCR inhibitors may require minor adjustments to standard extraction protocols.

PCR PRIMERS AND AMPLIFICATION

Typically, PCR primers are between 18 and 30 bases in length and amplify a region between 100 and 1500 base pairs in length. As noted, PCR primers may be universal, meaning they are capable of amplifying all potential organisms that are present in a test sample (including fungi, plants, and animals or an important and predicable subset), or they are taxon specific, meaning they have been designed to amplify only organisms in a targeted set (i.e., family, genus, species, or subspecies). A number of nuclear, mitochondrial, and plastid gene regions are used for universal amplification, including nrITS, nrITS1, nrITS2, *matK*, *rbcL*, *psbA–trnH* intergenic spacer, *cox3*, *COI* (also known as *cox1*), external transcribed spacer, 18S, 5S, *trnL–trnF* intergenic spacer, and *trnL* intron. Taxon-specific primers may be designed on the basis of proteins found in specific plant groups (e.g., the soy lectin gene found in soy) or by obtaining sequences of any variable DNA region for the target taxon and designing primers that specifically bind only to sequences from the taxon of interest.

DNA SEQUENCING

Most commonly, DNA sequencing is conducted using the Sanger protocol that has been modified to use fluorescent dye terminators on a capillary electrophoresis apparatus, although a number of emerging sequencing technologies [e.g., next-generation sequencing (NGS)] are now taking root. Once fluorescent dye has been incorporated into the amplified DNA, the bases are identified by their emission of light at different wavelengths. The resulting data are a chromatogram that can be visualized and analyzed by several sequence-analysis computer programs.

COMPARISON WITH REFERENCE MATERIALS

DNA sequences of test articles are compared to sequences obtained from multiple reference materials in an aligned matrix (commonly referred to as an alignment), which allows the sequences to be visually inspected to identify diagnostic nucleotide positions. Although numerous computer programs are able to automate comparisons between sequences from test articles and reference sequences, performance varies widely. Investigators should always manually check the results suggested by computer programs to confirm identity. Positive identifications are not possible when the sequences from test articles fall outside the range of known variation represented in the reference sequences. If a sufficiently large number of reference materials have been used to develop the assay, most test materials should be identified without ambiguity based on DNA sequence data.

⟨565⟩ BOTANICAL EXTRACTS

In the extraction practice for articles of botanical origin, the constituents of interest are completely or partially separated from other components with the aid of water, alcohol, alcohol-water mixtures, or other suitable solvents. This extraction process involves the removal of the desired constituents from the plant matter with suitable menstrua, the evaporation of all or nearly all of the solvent, and the adjustment of the residual fluids, masses, or powders to the prescribed standards. Suitable inert substances may be added as carriers or diluents to improve physical characteristics. Suitable antimicrobials and other preservatives may be added to preserve the integrity. Extracts may be subjected to processes that increase the content of characterized constituents, decrease the content of unwanted constituents, or both. Extracts with no added inert substances and no processing beyond the extraction are called native extracts. In some preparations, the plant matter may be pretreated by inactivation of enzymes and microbial contaminants, grinding, defatting, or a similar procedure.

Extracts may be defined as preparations with liquid, solid, or semisolid consistency. The products obtained by extraction are fluidextracts, powdered extracts, semisolid extracts, and tinctures.

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METHODS OF EXTRACTION

Percolation

In the manufacture of extracts, percolation is a commonly used method. The crude material being extracted is reduced to pieces of suitable size, if necessary, then mixed thoroughly with a portion of the specified solvent, and allowed to stand for about 15 minutes. The mixture is transferred to a percolator, sufficient amount of the specified solvent is added to cover the entire solid mass, and the mixture is allowed to percolate slowly (at a rate of not more than 1 mL per minute for 1000 g of material), the matter to be extracted being always covered with a layer of solvent. The residue may be pressed, and the obtained fluid is combined with the percolate. The entire percolates are concentrated, generally by distillation under reduced pressure, so as to subject the constituents of interest in the article under extraction to as little heat as possible.

Maceration

Unless otherwise specified, the crude material being extracted is reduced to pieces of suitable size, mixed thoroughly with the specified extracting solvent, and allowed to stand at room temperature in a closed container for an appropriate time, with frequent agitation until soluble matter is dissolved. The mixture is filtered, the insoluble material is washed with the same solvent used for maceration, and the filtrates are combined and concentrated, usually under reduced pressure, to the desired consistency.

PREPARATIONS

Fluidextracts

FLUIDEXTRACTS, also known as liquid extracts, are preparations of plant matter, containing alcohol as a solvent or as a preservative, or both, and are so made that each mL contains the extracted constituents of 1 g of the crude material that it represents, unless otherwise specified in the individual monograph. They may be prepared from suitable extracts and may contain suitable antimicrobial or other preservatives.

Pharmacopeial fluidextracts are made by percolation, often following a period of maceration. The required solvent is specified in the individual monograph. The common manufacturing procedure includes concentration of the more diluted portion of percolate by evaporation or distillation under vacuum at temperatures below 60°. The time of maceration and the rate of flow during percolation may be varied to adjust for the quantity and nature of the crude material under extraction, provided that the composition of the extracted constituents of interest is not adversely affected.

The rate of flow of the percolate can be slow, moderate, or rapid. With reference to the extraction of 1000 g of the starting material, at a slow rate, not more than 1 mL of percolate is produced per minute; at a moderate rate, between 1 and 3 mL per minute is produced; and at a rapid rate, between 3 and 5 mL per minute is produced. A fluidextract that tends to deposit sediment may be aged and filtered, or the clear portion may be decanted, provided that the resulting clarified liquid conforms to the Pharmacopeial standards.

Powdered Extracts

POWDERED EXTRACTS are solid preparations having a powdery consistency obtained by evaporation of the solvent used for extraction. They may contain suitable added substances such as excipients, stabilizers, and preservatives. Standardized powdered extracts are adjusted to the defined content of constituents, using suitable inert materials or a powdered extract of the plant matter used for preparation. Where applicable, a limit for the solvent used for extraction is specified in the individual monograph.

Semisolid Extracts

SEMISOLID EXTRACTS, also known as soft extracts or pillular extracts, are preparations having consistencies between those of fluidextracts and those of powdered extracts, and are obtained by partial evaporation of the solvent, water, alcohol, or hydroalcoholic mixtures being used as extracting solvents. They may contain suitable antimicrobial or other preservatives. A semisolid extract and a powdered extract obtained from the same material are interchangeable as drugs or as supplements, but each has its own advantages.

General Pharmacopeial Requirements

Unless otherwise specified in the individual monographs, Pharmacopeial requirements for the fluidextracts, powdered extracts, and semisolid extracts are as follows.

PACKAGING AND STORAGE—Store in tight, light-resistant containers. [NOTE—See *Packaging and Storage Requirements* ⟨659⟩, *General Definitions*.]

LABELING—Label it to indicate the name of the plant part used; the names of solvents, other than the hydroalcoholic solvents, used in preparation; the content, in percentage, of active principles or marker compounds identified in the individual monograph; and the name and concentration of any added antimicrobial or other preservative. Where active principles are

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unknown, the ratio of starting material to final product is stated. For semisolid extracts and powdered extracts, the identity and quantity of any added excipient is also indicated. In such cases the percentage of native extract may also be stated.

RESIDUE ON EVAPORATION—Transfer promptly about 2 mL, accurately measured, of Fluidextract, about 0.5 g of Powdered Extract, or about 2 g of Semisolid Extract to a suitable tared, round-bottom flask. Evaporate to dryness on a water bath, and dry the residue at 100° to 105° for 3 hours. Allow to cool in a desiccator over phosphorus pentoxide, and determine the weight of the residue obtained: not less than 95% of Powdered Extract specimen remains as residue; or not less than 70% of Semisolid Extract specimen remains as residue. [NOTE—Limits for Fluidextracts are specified in the individual monographs.]

RESIDUAL SOLVENTS—If prepared with solvents other than alcohol, water, or alcohol-water mixtures, it meets the requirements for *Residual Solvents* ⟨467⟩. [NOTE—See ICH document *Impurities: Residual Solvents* for related information.]

PESTICIDE RESIDUES—Botanical extracts, tinctures, or other pharmaceutical forms might contain pesticide residues at either enriched or reduced levels compared to their native form as plant materials. Unless otherwise indicated in the monograph, the limits for pesticides in extracts of botanical articles are calculated by the following formula:

$$\text{If } E \leq 10\text{: Limit (mg/kg)} = L \times E$$

$$\text{If } E > 10\text{: Limit (mg/kg)} = AM/100B$$

where L is the limit in the original article as listed in *Table 4* (see *Pesticide Residue Analysis* under *Articles of Botanical Origin* ⟨561⟩), or EPA tolerance or the FDA action level; E is the plant to extract ratio (i.e. the ratio between the quantity of botanical article used in the manufacture of the extract and the quantity of the extract obtained); A is the acceptable daily intake (ADI), as published by FAO-WHO, in mg/kg of body weight; M is body weight, in kg (60 kg); and B is the daily dose of the extract, in kg.

[NOTE—The higher pesticide limits for extracts of botanical ingredients may be justified if the suggested intake or dose of the extract is reduced by a factor which is higher than E.]

A total or partial exemption from the test may be granted when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).

ALCOHOL DETERMINATION, *Method II* ⟨611⟩ (IF PRESENT): Between 90% and 110% of the labeled amount of C_2H_5OH is found in Fluidextract and Semisolid Extract.

Tinctures

TINCTURES are liquid preparations usually prepared by extracting plant materials with alcohol or hydroalcoholic mixtures. Traditionally, tinctures of potent articles of botanical origin represent the activity of 10 g of the drug in each 100 mL of tincture, the strength being adjusted following the test for content of active principles or marker compounds. Most other plant tinctures represent 20 g of the respective plant material in each 100 mL of tincture.

Different tinctures are not always diluted to obtain the same ratio of starting plant material to final tincture. This ratio will depend on the requirements prescribed in the specific tests for content of active principles or marker compounds included in the individual monographs. As tinctures are being prepared, they are assayed in accordance with these content tests. Using the values obtained from such assays, the final concentration of a tincture is adjusted by adding more solvent or by evaporating part of the solvent.

Unless otherwise specified, tinctures are usually prepared from coarse powder or fine cuttings of plant materials either by a percolation process or a maceration process.

PERCOLATION PROCESS

Carefully mix the ground mixture of ingredients with a sufficient quantity of the prescribed extracting solvent to render it evenly and distinctly damp, allow it to stand for 15 minutes, transfer it to a suitable percolator, and pack the mass firmly. Pour on enough of the specified extracting solvent to saturate the drug, and cover the top of the percolator. When the liquid is about to drip from the percolator, close the lower orifice, and allow the drug to macerate for 24 hours or for the time specified in the monograph. If the test for content of active principles or marker compounds is not required in the individual monograph, allow the percolation to proceed slowly or at the specified rate (for definitions of flow rates, see under *Fluidextracts*), gradually adding sufficient quantity of extracting solvent to produce 1000 mL of tincture, and mix. If a test for content of active principles or marker compounds is required, collect only 950 mL of percolate, mix, and test a portion of it as directed in the individual monograph. Dilute the remainder of the percolate with as much of the prescribed extracting solvent as calculation from the content test indicates is necessary to produce a tincture that conforms to the requirements, and mix.

MACERATION PROCESS

Macerate the drug with 750 mL of the prescribed extracting solvent in a closed container, and put in a warm place. Agitate it frequently during 3 days or until the soluble matter is dissolved. Transfer the mixture to a filter. When most of the liquid has drained, wash the residue on the filter with a sufficient quantity of the prescribed extracting solvent, combining the filtrates, to produce 1000 mL of tincture, and mix.

GENERAL PHARMACOPEIAL REQUIREMENTS

Unless otherwise specified in the individual monographs, Pharmacopeial requirements for the tinctures are as follows.

Standards Prepared

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Packaging and Storage—Store in tight, light-resistant containers, and avoid exposure to direct sunlight and excessive heat. [NOTE—See *Packaging and Storage Requirements* ⟨659⟩, *General Definitions*.]
Labeling—Label it to indicate the name of the plant part used for preparation; the name of the solvent or solvent mixture used for extraction; and the content of the constituents of interest and the ratio of starting material to final product.

⟨571⟩ VITAMIN A ASSAY

ASSAY

Chemical Methods

• **PROCEDURE 1**

The following procedure is provided for the determination of vitamin A in dietary ingredients or pharmaceutical ingredients. It conforms to the procedure that was adopted in 1956 for international use by the International Union of Pure and Applied Chemistry.

Complete the assay promptly, and exercise care throughout the procedure to keep to a minimum the exposure to actinic light and to atmospheric oxygen and other oxidizing agents, preferably by the use of low-actinic glassware and an atmosphere of an inert gas.

For the test articles that contain tocopherol, an appropriate chromatographic method should be used.

Sample solution: Accurately weigh, count, or measure a portion of the test specimen expected to contain the equivalent of NLT 0.15 mg of retinol but containing NMT 1 g of fat. If in the form of capsules, tablets, or other solid, so that it cannot be saponified efficiently by the ensuing instructions, reflux the portion taken in 10 mL of water on a steam bath for about 10 min, crush the remaining solid with a blunt glass rod, and warm for about 5 min longer.

Transfer to a suitable borosilicate glass flask, and add 30 mL of alcohol, followed by 3 mL of potassium hydroxide solution (9 in 10). Reflux in an all-borosilicate glass apparatus for 30 min. Cool the solution, add 30 mL of water, and transfer to a conical separator. Add 4 g of finely powdered sodium sulfate decahydrate. Extract by shaking with one 150-mL portion of ether for 2 min, and then, if an emulsion forms, with three 25-mL portions of ether. Combine the ether extracts, if necessary, and wash by swirling gently with 50 mL of water. Repeat the washing more vigorously with three additional 50-mL portions of water. Transfer the washed ether extract to a 250-mL volumetric flask, add ether to volume, and mix.

Evaporate a 25.0-mL portion of the ether extract to about 5 mL. *Without applying heat and with the aid of a stream of inert gas or vacuum,* continue the evaporation to about 3 mL. Dissolve the residue in sufficient isopropyl alcohol to give an expected concentration of the equivalent of 3 µg–5 µg of vitamin A per mL or to give an absorbance in the range 0.5–0.8 at 325 nm.

Instrumental conditions

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

Mode: UV

Analytical wavelengths: 310, 325, and 334 nm

Cell: 1 cm

Blank: Isopropyl alcohol

Analysis

Sample: *Sample solution*

Determine the absorbances of the *Sample solution* at 310, 325, and 334 nm. Calculate the vitamin A, as retinol ($C_{20}H_{30}O$) content, in mg, in the portion of sample taken, using one of the following formulas:

$$\text{Result} = (0.549 \times A_{325})/(L \times C)$$

or

$$\text{Result} = (0.549 \times [A_{325}])/(L \times C)$$

L = length of the absorption cell (cm)
C = concentration in the final isopropyl alcohol solution of test specimen (g/100 mL) or capsules or tablets (units/100 mL)
$[A_{325}]$ = *corrected* absorbance at 325 nm, calculated:

$$\text{Result} = (6.815 \times A_{325}) - (2.555 \times A_{310}) - (4.260 \times A_{334})$$

Each mg of vitamin A, as retinol ($C_{20}H_{30}O$), represents 3333 USP Units of vitamin A.

Use the first formula when A_{325}, the observed absorbance at 325 nm, is between $[A_{325}]/1.030$ and $[A_{325}]/0.970$. Use the second formula when $[A_{325}]$ has a value less than $A_{325}/1.030$.

[NOTE—The range of the limits of error for this analytical procedure, indicating the extent of discrepancy to be expected in the results of different laboratories at $P = 0.05$, is approximately ±8%.]

• **PROCEDURE 2**

This procedure is used for dietary ingredients or pharmaceutical ingredients in the form of pure retinyl esters or prepared from pure retinyl esters into a vehicle excipient.

Sample solution: Dissolve 25–100 mg, accurately weighed, in 5 mL of pentane, and dilute with isopropyl alcohol to give an expected concentration of the equivalent of 3–4.5 µg/mL of retinol.

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Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 326 nm
Cell: 1 cm
Blank: Isopropyl alcohol

Analysis
Sample: *Sample solution*
Calculate the vitamin A, as retinol ($C_{20}H_{30}O$) content, in mg, in the portion of sample taken. Each mg of vitamin A, as retinol ($C_{20}H_{30}O$), represents 3333 USP Units of vitamin A.:

$$\text{Result} = (0.570 \times A_{326})/(L \times C)$$

A_{326} = absorbance at 326 nm
L = length of the absorption cell (cm)
C = concentration of the *Sample solution* (g/100 mL)

Chromatographic Methods
The following liquid chromatographic procedures are provided for the determination of vitamin A as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in the dietary supplements or pharmaceutical dosage forms.
Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.
Where an ester form of vitamin A (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard.

• **PROCEDURE 1**
This is a neutral procedure that involves either simply dissolving the sample directly into hexane and injecting into the liquid chromatograph or sample extraction by first dissolving the sample in dimethyl sulfoxide, followed by a liquid–liquid extraction of the vitamin A with hexane. Although its chromatographic system can separate the 13-*cis* and all-*trans*-isomers of vitamin A, only the all-*trans*-isomer peak is used for the quantitation of vitamin A. The procedure can be used to determine vitamin A in raw material, Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.
Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and *System suitability solution* are prepared as follows.
Mobile phase: *n*-Hexane
Standard solution 1: 15 µg/mL of retinol[1] from USP Retinyl Acetate RS in *n*-hexane
Standard solution 2: 15 µg/mL of retinol[2] from USP Retinyl Palmitate RS in *n*-hexane
System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.
Sample solution for raw materials: Transfer retinyl acetate or retinyl palmitate, accurately weighted, equivalent to 15 mg of retinol, to a 100-mL volumetric flask, dissolve in and dilute with *n*-hexane to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *n*-hexane to volume, and mix.
Sample solution for tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, not exceeding 7.5 g, equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytef-lined screw cap. Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of powdered Tablets, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of powdered Tablets to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution having a nominal concentration of 15 µg/mL of vitamin A, as retinol ($C_{20}H_{30}O$). [NOTE—Dilution may not be necessary.]
Sample solution for capsules: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, not exceeding 7.5 g equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytef-lined screw cap. [NOTE—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytef-lined screw cap.] Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of Capsule contents, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of Capsule contents to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution with a concentration of 15 µg/mL of vitamin A, as retinol ($C_{20}H_{30}O$). [NOTE—Dilution may not be necessary.]

[1] Use the value of 0.872 to convert retinyl acetate to its retinol equivalent.
[2] Use the value of 0.546 to convert retinyl palmitate to its retinol equivalent.

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Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 325 nm
Column: 4.6-mm × 15-cm; 3-µm packing L8
Flow rate: 1 mL/min
Injection volume: 40 µL
System suitability
Samples: *System suitability solution* and *Standard solution 1* or *Standard solution 2*
Suitability requirements
Resolution: NLT 10 between all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution 1* or *Standard solution 2*
Analysis
Samples: *Standard solution 1* or *Standard solution 2* and appropriate *Sample solution*
Calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in the portion of sample taken:

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

r_U = peak response of all-*trans* retinyl ester from the appropriate *Sample solution*
r_S = peak response of all-*trans* retinyl ester from *Standard solution 1* or *Standard solution 2*
C_S = concentration of retinol ($C_{20}H_{30}O$) in *Standard solution 1* or *Standard solution 2* (µg/mL)
C_U = concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* (µg/mL)

• **PROCEDURE 2**
This procedure involves the treatment of sample with methanolic sulfuric acid, followed by extraction with 2,2,4-trimethylpentane. Sample preparation can be used for the formulation containing vitamins A, D, and E. Application includes Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.
Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, *System suitability solution*, and reagent solutions are prepared as follows.
Mobile phase: *n*-Hexane and ethyl acetate (99.7: 0.3)
3 N methanolic sulfuric acid solution: Cautiously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.
Sodium ascorbate–pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.
Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane
Standard solution 1: 15 µg/mL of retinol[1] from USP Retinyl Acetate RS in 2,2,4-trimethylpentane
Standard solution 2: 15 µg/mL of retinol[2] from USP Retinyl Palmitate RS in 2,2,4-trimethylpentane
System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.
Sample solution for tablets: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Finely powder NLT 20 Tablets. Use a portion of the powder nominally equivalent to an amount between 0.4 mg and 2.5 mg of retinol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.
Sample solution for capsules: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of the labeled amount of vitamin A, as retinol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

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Detector: UV 325 nm
Column: 4.6-mm × 25-cm; 5-µm packing L24
Flow rate: 1.5 mL/min
Injection volume: 40 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 8.0 between all-*trans* retinyl acetate and all-*trans* retinyl palmitate
Relative standard deviation: NMT 3.0%

Analysis
Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*
Calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in the portion of sample taken:

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

r_U = peak response of all-*trans* retinyl ester from the *Sample solution*
r_S = peak response of the all-*trans* retinyl ester from *Standard solution 1* or *Standard solution 2*
C_S = concentration of retinol ($C_{20}H_{30}O$) in *Standard solution 1* or *Standard solution 2* (µg/mL)
C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* (µg/mL).
[NOTE—Use 26.5 mL as the final volume of the *Sample solution* to calculate the nominal concentration.]

• **PROCEDURE 3**

This procedure involves the saponification of both the standard and sample, followed by a liquid–liquid extraction of vitamin A from the sample with a mixture of *n*-hexane and methylene chloride (3:1). The 13-*cis*- and all-*trans*- isomers of vitamin A can be characterized and quantitated. The procedure can be used for Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: *n*-Hexane and isopropyl alcohol (92:8)
Extraction solvent: *n*-Hexane and methylene chloride (3:1)
Potassium hydroxide solution: 800 mg/mL of potassium hydroxide in water. [NOTE—Cautiously add potassium hydroxide in water. Mix, and cool.]
Diluent: 10 mg/mL of pyrogallol in alcohol
Standard solution: Dilute USP Retinyl Acetate RS or USP Retinyl Palmitate RS with *Diluent* to obtain a concentration of 8.5 µg/mL of retinol[1,2] ($C_{20}H_{30}O$). Transfer 10.0 mL of this solution to a stoppered 125-mL flask, and add 5 mL of water, 5 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. Withdraw a portion of the organic layer for injection into the chromatograph. This *Standard solution* contains 3.4 µg/mL of retinol.
Sample solution for capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 1.3 mg of retinol, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s or longer, if necessary, for complete extraction. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. [NOTE—Do not shake, because an emulsion may form.] Withdraw a portion of the organic layer, and dilute quantitatively and stepwise if necessary, with *Extraction solvent*, to obtain a concentration of 3.4 µg/mL of retinol.
Sample solution for tablets: Finely powder a counted number of Tablets. Transfer a portion of the powder, equivalent to 1.3 mg of retinol, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s or longer, if necessary, for complete extraction. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. [NOTE—Do not shake, because an emulsion may form.] Withdraw a portion of the organic layer, and dilute with *Extraction solvent* to obtain a concentration of 3.4 µg/mL of retinol.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 335 nm
Column: 6.2-mm × 8-cm; packing L3
Column temperature: 40°
Flow rate: 4 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*

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Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in the portion of sample taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times (C_S/C_U) \times 100$$

r_{T1} = sum of peak responses of all-*trans* retinyl ester and 13-*cis*-retinyl ester peaks from the *Sample solution*
r_{T2} = sum of peak responses of all-*trans* retinyl ester and 13-*cis*-retinyl ester peaks from the *Standard solution*
C_S = concentration of retinol ($C_{20}H_{30}O$) in the *Standard solution* (µg/mL)
C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* (µg/mL)

• **PROCEDURE 4**
This procedure involves a liquid–liquid extraction of vitamin A from the sample with hexane, followed by the evaporation of hexane and reconstitution of the residue in tetrahydrofuran and acetonitrile mixture (1:1). It can be used for the determination of vitamin A in Oil- and Water-Soluble Vitamins Oral Solution and Oil- and Water-Soluble Vitamins with Minerals Oral Solution.
Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and *Diluent* are prepared as follows.
Mobile phase: Methanol, acetonitrile, and *n*-hexane (46.5: 46.5: 7.0)
Diluent: Tetrahydrofuran and acetonitrile (1:1)
Standard solution: 0.33 mg/mL of retinol[1,2] ($C_{20}H_{30}O$) from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in *Diluent*
Sample solution for liquid dosage forms: Transfer an accurately measured volume of Oral Solution, equivalent to 3.3 mg of retinol, to a 500-mL separatory funnel containing 10 mL of water and 20 mL of dehydrated alcohol. Add 150 mL of solvent hexane, insert the stopper, and shake for 1 min. Add another 150 mL of solvent hexane, insert the stopper, shake, and allow the layers to separate. Discard the aqueous layer, and filter the solvent hexane extract through anhydrous sodium sulfate into a 500-mL, round-bottom flask. Evaporate the solution to dryness with the aid of a rotary evaporator over a water bath maintained at about 65°. Immediately add 10.0 mL of *Diluent*, swirl to dissolve the residue, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 50-cm (prepared from two concatenated 4.6-mm × 25-cm columns); packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in the portion of sample taken:

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

r_U = peak response of all-*trans* retinyl ester from the *Sample solution*
r_S = peak response of all-*trans* retinyl ester from the *Standard solution*
C_S = concentration of retinol ($C_{20}H_{30}O$) in the *Standard solution* (µg/mL)
C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* (µg/mL)

ADDITIONAL REQUIREMENTS
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Retinyl Acetate RS
USP Retinyl Palmitate RS

⟨580⟩ VITAMIN C ASSAY

INTRODUCTION
The following procedures are provided for analysis of different forms of vitamin C as ascorbic acid ($C_6H_8O_6$), sodium ascorbate ($C_6H_7NaO_6$), and calcium ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$) or their mixtures in finished dosage forms, as Capsules, Tablets, or Oral Solutions.
METHOD I—TITRIMETRIC METHOD
• **PROCEDURE**
Unless specified in the individual monographs, proceed as follows.

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Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 100 mg of ascorbic acid, to a 200-mL volumetric flask, and add 75 mL of metaphosphoric–acetic acids TS. Insert a stopper into the flask, and shake by mechanical means for 30 min. Dilute with water to volume.

Sample solution for Oral Solutions: Transfer a volume of Oral Solution equivalent to 50 mg of ascorbic acid, previously diluted with water if necessary, to a 100-mL volumetric flask. Add 20 mL of metaphosphoric–acetic acid TS, dilute with water to volume, and mix.

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent of 100 mg of ascorbic acid, to a 200-mL volumetric flask, and add 75 mL of metaphosphoric–acetic acids TS. Insert a stopper into the flask, and shake by mechanical means for 30 min. Dilute with water to volume.

Blank: A mixture of 5.5 mL of metaphosphoric–acetic acids TS and 15 mL of water.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: Standard dichlorophenol–indophenol solution VS

Endpoint detection: Visual

Analysis: Transfer a portion of the *Sample solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid into a 50-mL conical flask, and add 5 mL of metaphosphoric–acetic acids TS. Titrate with *Titrant* to a rose-pink color that persists for at least 5 s. Correct for the volume of *Titrant* consumed by the *Blank*.

Calculate the percentage of ascorbic acid ($C_6H_8O_6$) in the portion of sample taken:

$$\text{Result} = \{[(V_S - V_B) \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank*
F = concentration of *Titrant* in terms of its equivalent of ascorbic acid (mg/mL)
W = nominal amount of ascorbic acid taken for *Analysis* (mg)

METHOD II—CHROMATOGRAPHIC METHOD

• **PROCEDURE 1**

Unless specified in the individual monographs, the *Diluent*, *Standard solution*, and *Sample solutions* are prepared as follows.

[NOTE—Protect samples from air, light, and heat.]

Buffer: 2.04 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: *Buffer*

Diluent: 0.56 g of edetate disodium dihydrate and 2.04 g of monobasic potassium phosphate per 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

Standard solution: 0.25 mg/mL of USP Ascorbic Acid RS in *Diluent*

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to about 25 mg of ascorbic acid, into a 100-mL volumetric flask. Add 60 mL of *Diluent*, shake mechanically for 15 min, dilute with *Diluent* to volume, mix well, and pass through a membrane filter of 0.45-µm pore size, discarding the first 4 mL.

Sample solution for Oral Solutions: Dilute an accurately measured volume of Oral Solution with *Diluent* to obtain a solution with a concentration of 0.25 mg/mL of ascorbic acid. Mix carefully.

Sample solution for Tablets: Transfer a portion from NLT 20 finely powdered Tablets, nominally equivalent to about 25 mg of ascorbic acid, into a 100-mL volumetric flask. Add 60 mL of *Diluent*, shake mechanically for 15 min, dilute with *Diluent* to volume, mix well, and pass through a membrane filter of 0.45-µm pore size, discarding the first 4 mL.

Chromatographic system

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

Mode: LC

Detector: UV 245 nm

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

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vitamin C, as ascorbic acid ($C_6H_8O_6$), in the portion of sample taken:

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

r_U = peak area of ascorbic acid from the *Sample solution*
r_S = peak area of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

• **PROCEDURE 2**

Unless specified in the individual monographs, the *Diluent*, *Standard solution*, and *Sample solutions* are prepared as follows.

[NOTE—Protect samples from air, light, and heat. All prepared samples must be analyzed within 4 h.]

Buffer: 7.8 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: *Buffer* and methanol. See *Table 1* for gradient.

Table 1

Time (min)	Buffer (%)	Methanol (%)
0	100	0
3	100	0
5	0	100
6	50	50
7	100	0
10	100	0

Diluent: Dissolve 73 g of metaphosphoric acid in 800 mL of water, add 84 mL of glacial acetic acid, and dilute with water to 1000 mL.

Standard stock solution: 1 mg/mL of USP Ascorbic Acid RS in *Diluent*. [NOTE—Sonicate with intermittent shaking to help dissolve, if necessary. Prepare fresh every time.]

Standard solution: Dilute *Standard stock solution* with *Diluent* to obtain a solution containing 0.05 mg/mL of USP Ascorbic Acid RS.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to about 25 mg of ascorbic acid, into a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, sonicate for 15 min, and centrifuge at about 2000 rpm for 5 min. Quantitatively dilute the clear supernatant with *Diluent* to obtain a solution containing 0.05 mg/mL of ascorbic acid. Mix and pass through a membrane filter of 0.45-µm pore size.

Sample solution for Oral Solutions: Dilute an accurately measured volume of Oral Solution with *Diluent* to obtain a solution with a concentration of 0.05 mg/mL of ascorbic acid. Mix carefully.

Sample solution for Tablets: Transfer a portion from NLT 20 ground Tablets, nominally equivalent to about 25 mg of ascorbic acid, into a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, sonicate for 15 min, and centrifuge at about 2000 rpm for 5 min. Quantitatively dilute the clear supernatant with *Diluent* to obtain a solution containing 0.05 mg/mL of ascorbic acid. Mix and pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vitamin C, as ascorbic acid ($C_6H_8O_6$), in the portion of sample taken:

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

r_U = peak area of ascorbic acid from the *Sample solution*
r_S = peak area of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

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ADDITIONAL REQUIREMENTS
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Ascorbic Acid RS

⟨581⟩ VITAMIN D ASSAY

ASSAY

Change to read:

Chromatographic Methods

The following liquid chromatographic procedures are provided for the determination of vitamin D as an active pharmaceutical ingredient, as a dietary supplement ingredient, or as a component in compendial dosage forms.

Throughout this assay, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.

Where vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard.

- **PROCEDURE 1**

This procedure uses a sample preparation without pH adjustment and involves the use of dimethyl sulfoxide to dissolve the excipients in the sample, followed by a liquid-liquid extraction of the vitamin D with hexane. The chromatographic separation is achieved using normal phase on an L8 column. It can be used to determine vitamin D alone or in combination with other vitamins and minerals in compendial dosage forms.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and *System suitability solution* are prepared as follows.

Mobile phase: *n*-Hexane and isopropyl alcohol (99:1)

Standard solution: 2 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in *n*-hexane

System suitability solution: Heat a volume of the *Standard solution* at 60° for 1 h to partially isomerize vitamin D (cholecalciferol or ergocalciferol) to its corresponding precursor.

Sample solution for tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, not exceeding 7.5 g, equivalent to NLT 0.1 mg of vitamin D as cholecalciferol or ergocalciferol, to a centrifuge tube having a polytef-lined screw cap. Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of powdered Tablets, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly in order to achieve accurate recoveries.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of powdered Tablets to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution with a concentration of 2 µg/mL of cholecalciferol or ergocalciferol. [NOTE—Dilution may not be necessary.]

Sample solution for capsules: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, not exceeding 7.5 g equivalent to NLT 0.1 mg of vitamin D as cholecalciferol or ergocalciferol, to a centrifuge tube having a polytef-lined screw cap. [NOTE—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, equivalent to NLT 0.1 mg of vitamin D as cholecalciferol or ergocalciferol, to a centrifuge tube having a polytef-lined screw cap.] Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of Capsule contents, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly in order to achieve accurate recoveries.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of Capsule contents to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution with a concentration of 2 µg/mL of vitamin D as cholecalciferol or ergocalciferol. [NOTE—Dilution may not be necessary.]

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

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

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

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

Suitability requirements

Resolution: NLT 10 between the vitamin D form present and its corresponding precursor, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

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

r_U = peak response of cholecalciferol or ergocalciferol from the *Sample solution*
r_S = peak response of cholecalciferol or ergocalciferol from the *Standard solution*
C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)
F = correction factor to account for the average amount of previtamin D present in the *Sample solution*, 1.09

• **PROCEDURE 2**

This procedure involves the treatment of sample with sodium bicarbonate, an antioxidant acid solution generating gas evolution; lecithin as a surfactant; and dimethyl sulfoxide, followed by methanolic sulfuric acid to create a dispersion and efficiently extract vitamin D from the matrix components into the 2,2,4-trimethylpentane phase. Separation is achieved in normal-phase mode on an L24 column. The sample preparation and *Chromatographic system* used in this procedure also are suitable to determine vitamins A and E when present in the formulation; analysts should make corresponding adjustments in sample size and detection wavelength.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, *System suitability solution*, and reagent solutions are prepared as follows.

Mobile phase: *n*-Hexane and tertiary butyl alcohol (98.75: 1.25)

3 N methanolic sulfuric acid solution: Cautiously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.

Sodium ascorbate–pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.

Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane

Standard solution: 1 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in 2,2,4-trimethylpentane

System suitability solution: Heat a volume of the *Standard solution* at 60° for 1 h to partially isomerize vitamin D (cholecalciferol or ergocalciferol) to its corresponding precursor.

Sample solution for tablets: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E, when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Finely powder NLT 20 Tablets. Use a portion of the powder nominally equivalent to an amount NLT 0.1 mg of vitamin D. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.

Sample solution for capsules: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E, when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to NLT 0.1 mg of the labeled amount of vitamin D, as cholecalciferol or ergocalciferol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L24

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

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

Suitability requirements

Resolution: NLT 4.0 between the vitamin D form present and its corresponding precursor, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

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Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

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

r_U = peak response of cholecalciferol or ergocalciferol from the *Sample solution*
r_S = peak response of cholecalciferol or ergocalciferol from the *Standard solution*
C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

• **PROCEDURE 3**

This procedure is suitable for matrices with excipients that are soluble or degradable in alkaline conditions. It involves the saponification of the sample solution, followed by a liquid–liquid extraction with hexane and clean-up on a solid phase extraction (SPE) using a mixture of methylene chloride and isopropyl alcohol (99.8: 0.2) as eluent. The *Standard solution* undergoes similar treatment to compensate for losses in recovery due to isomerization. The eluate then is evaporated, and the residue is reconstituted in acetonitrile before injection into the chromatograph. Separation is achieved in reversed phase mode.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: Acetonitrile and methanol (91:9)

Diluted acetic acid: Glacial acetic acid solution (1 in 10) in water

Phenolphthalein solution: 10 mg/mL of phenolphthalein in alcohol

Potassium hydroxide solution: Slowly dissolve 14 g of potassium hydroxide in a mixture of 31 mL of dehydrated alcohol and 5 mL of water. Prepare fresh daily.

Extraction solvent: Methylene chloride and isopropyl alcohol (99.8: 0.2)

Extraction column: Silica packing with a sorbent mass-to-column volume ratio of 500 mg to 2.8 mL or equivalent. [NOTE—Condition the column by initially washing with 4.0 mL of a mixture of methylene chloride and isopropyl alcohol (4:1), followed by 5.0 mL of the *Extraction solvent*. Do not allow the column to dry.]

Standard stock solution: 0.2 mg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in dehydrated alcohol. [NOTE—Prepare fresh every 4 weeks. Store in a freezer.]

Standard solution: Dilute a volume of the *Standard stock solution* with dehydrated alcohol to obtain a concentration of 5 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS. Prepare this solution fresh daily. Transfer 2.0 mL of this solution to a stoppered 125-mL flask. Add 15.0 mL of water and 15.0 mL of *Potassium hydroxide solution*, insert the stopper, and shake for 30 min in a water bath maintained at 60°. Allow to cool to room temperature, and transfer the contents of the flask to a 250-mL separatory funnel. Add 15.0 mL of water to the flask, insert the stopper, shake vigorously, and transfer this solution to the separatory funnel. Rinse the flask with 60 mL of *n*-hexane, and transfer the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 90 s, and allow to stand for 15 min until the layers separate. Drain, and discard the aqueous layer. Add 15.0 mL of water to the hexane layer in the separatory funnel, insert the stopper, and shake vigorously. Allow to stand for 10 min until the layers separate, and discard the aqueous layer. Add 1 drop of *Phenolphthalein solution* and 15.0 mL of water to the separatory funnel. Add *Diluted acetic acid* dropwise, with shaking, until the washing is neutral. Allow to stand for 10 min until the layers separate. Drain, and discard the aqueous layer. Filter the hexane layer through anhydrous sodium sulfate supported by a small pledget of cotton into a 100-mL, round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and collect the rinsings in the same flask. Evaporate the hexane in the flask on a rotary evaporator at 50° to dryness. Immediately add 2.0 mL of the *Extraction solvent* to dissolve the residue. Transfer this solution to a freshly conditioned solid-phase *Extraction column*, rinse the round-bottom flask with 1.0 mL of the *Extraction solvent*, and transfer to the column. Elute the *Extraction column* with 2.0 mL of the *Extraction solvent*, and discard this fraction. Elute the column with 7.0 mL of the *Extraction solvent*, and collect the eluate in a suitable flask. Place the flask in a warm water bath maintained at 42°, and evaporate the solvent with the aid of a stream of nitrogen. Immediately add 2.0 mL of acetonitrile to the residue, and use the solution for injection into the chromatograph.

Sample solution for tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 10 µg of cholecalciferol or ergocalciferol, to a stoppered 125-mL flask. Add 15.0 mL of water and 15.0 mL of *Potassium hydroxide solution*, insert the stopper, and shake for 30 min in a water bath maintained at 60°. Allow to cool to room temperature, and transfer the contents of the flask to a 250-mL separatory funnel. Add 15.0 mL of water to the flask, insert the stopper, shake vigorously, and transfer this solution to the separatory funnel. Rinse the flask with 60 mL of *n*-hexane, and transfer the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 90 s, and allow to stand for 15 min until the layers separate. Drain and discard the aqueous layer. Add 15.0 mL of water to the hexane layer in the separatory funnel, insert the stopper, and shake vigorously. Allow to stand for 10 min until the layers separate, and discard the aqueous layer. Add 1 drop of *Phenolphthalein solution* and 15.0 mL of water to the separatory funnel. Add *Diluted acetic acid* dropwise, with shaking, until the washing is neutral. Allow to stand for 10 min until the layers separate. Drain, and discard the aqueous layer. Filter the hexane layer through anhydrous sodium sulfate supported by a small pledget of cotton into a 100-mL, round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and collect the rinsings in the same flask. Evaporate the hexane in the flask on a rotary evaporator at 50° to dryness. Immediately add 2.0 mL of the *Extraction solvent* to dissolve the residue. Transfer this solution to a freshly conditioned solid-phase *Extraction column*, rinse the round-bottom flask with 1.0 mL of the *Extraction solvent*, and transfer to the column. Elute the *Extraction column* with 2.0 mL of the *Extraction solvent*, and discard this fraction. Elute the column with 7.0 mL of the *Extraction solvent*, and collect the eluate in a suitable flask. Place the flask in a warm

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water bath maintained at 42°, and evaporate the solvent with the aid of a stream of nitrogen. Immediately add 2.0 mL of acetonitrile to the residue, and use the solution for injection into the chromatograph.

Sample solution for capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 10 µg of ergocalciferol or cholecalciferol, to a stoppered 125-mL flask. Add 15.0 mL of water and 15.0 mL of *Potassium hydroxide solution*, insert the stopper, and shake for 30 min in a water bath maintained at 60°. Allow to cool to room temperature, and transfer the contents of the flask to a 250-mL separatory funnel. Add 15.0 mL of water to the flask, insert the stopper, shake vigorously, and transfer this solution to the separatory funnel. Rinse the flask with 60 mL of *n*-hexane, and transfer the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 90 s, and allow to stand for 15 min until the layers separate. Drain, and discard the aqueous layer. Add 15.0 mL of water to the hexane layer in the separatory funnel, insert the stopper, and shake vigorously. Allow to stand for 10 min until the layers separate, and discard the aqueous layer. Add 1 drop of *Phenolphthalein solution* and 15.0 mL of water to the separatory funnel. Add *Diluted acetic acid* dropwise, with shaking, until the washing is neutral. Allow to stand for 10 min until the layers separate. Drain, and discard the aqueous layer. Filter the hexane layer through anhydrous sodium sulfate supported by a small pledget of cotton into a 100-mL, round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and collect the rinsings in the same flask. Evaporate the hexane in the flask on a rotary evaporator at 50° to dryness. Immediately add 2.0 mL of the *Extraction solvent* to dissolve the residue. Transfer this solution to a freshly conditioned solid-phase *Extraction column*, rinse the round-bottom flask with 1.0 mL of the *Extraction solvent*, and transfer to the column. Elute the *Extraction column* with 2.0 mL of the *Extraction solvent*, and discard this fraction. Elute the column with 7.0 mL of the *Extraction solvent*, and collect the eluate in a suitable flask. Place the flask in a warm water bath maintained at 42°, and evaporate the solvent with the aid of a stream of nitrogen. Immediately add 2.0 mL of acetonitrile to the residue, and use the solution for injection into the chromatograph.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

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

Column temperature: 27°

Flow rate: 0.7 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 4.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

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

r_U = peak response of cholecalciferol or ergocalciferol from the *Sample solution*

r_S = peak response of cholecalciferol or ergocalciferol from the *Standard solution*

C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

F = correction factor to account for the average amount of previtamin D present in the *Sample solution*, 1.09

• **PROCEDURE 4**

This procedure is applicable to liquid dosage forms. It involves a liquid-liquid extraction of the sample with hexane, followed by the evaporation of hexane, and reconstitution of the residue in a tetrahydrofuran and acetonitrile mixture (1:1). Separation is achieved in reversed-phase mode.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and *Diluent* are prepared as follows.

Mobile phase: Methanol, acetonitrile, and *n*-hexane (46.5: 46.5: 7.0)

Diluent: Tetrahydrofuran and acetonitrile (1:1)

Standard solution: 5 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in *Diluent*

Sample solution: Transfer an accurately measured volume of the sample, equivalent to 50 µg of cholecalciferol or ergocalciferol, to a 500-mL separatory funnel containing 10 mL of water and 20 mL of dehydrated alcohol. Add 150 mL of solvent hexane, insert the stopper, and shake for 1 min. Add another 150 mL of solvent hexane, insert the stopper, shake, and allow the layers to separate. Discard the aqueous layer. Drain the solvent hexane extract through anhydrous sodium sulfate into a 500-mL, round-bottom flask. Evaporate the solution to dryness with the aid of a rotary evaporator over a water bath maintained at about 65°. Immediately add 10.0 mL of *Diluent*, swirl to dissolve the residue, and filter.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: Two columns, 4.6-mm × 25-cm, connected in series; both with packing L1

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Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

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

r_U = peak response of cholecalciferol or ergocalciferol from the *Sample solution*
r_S = peak response of cholecalciferol or ergocalciferol from the *Standard solution*
C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)
F = correction factor to account for the average amount of previtamin D present in the *Sample solution*, 1.09

• **PROCEDURE 5**
This procedure involves the dissolution of vitamin D in toluene, and the solution is injected into the liquid chromatograph. It is applicable to simple matrices such as pure vitamins and ingredients that do not require saponification and that are soluble in toluene. Separation is achieved in normal-phase mode.
Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and *System suitability solution* are prepared as follows.
Dehydrated hexane: Prepare a chromatographic column by packing a chromatographic tube, 8-cm × 60-cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* ⟨621⟩, *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.
Mobile phase: *n*-Amyl alcohol in *Dehydrated hexane* (3 in 1000)
System suitability solution: 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol. For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]
Standard stock solution: 0.6 mg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in toluene
Standard solution: 120 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in *Mobile phase*, prepared from *Standard stock solution*
Sample stock solution: 0.6 mg/mL of cholecalciferol or ergocalciferol in toluene
Sample solution: 120 µg/mL of cholecalciferol or ergocalciferol in *Mobile phase*, prepared from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Column temperature: 40°
Flow rate: Can be varied to meet the *System suitability requirements*
Injection volume: 5–10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol
Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

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

r_U = peak response of cholecalciferol or ergocalciferol from the *Sample solution*
r_S = peak response of cholecalciferol or ergocalciferol from the *Standard solution*
C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

• **PROCEDURE 6**
The procedure involves the dissolution of the sample in *Mobile phase*, and the solution is injected into the liquid chromatograph. The application includes cholecalciferol and ergocalciferol dissolved in edible vegetable oil, polysorbate

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80, or propylene glycol, none of which will interfere with the corresponding precursor so that it can be quantitated. Separation is achieved in normal-phase mode on an L3 column, and *System suitability* requires separation of the *trans* forms from the vitamin D precursors.

Unless specified in the individual monographs, the *Standard solutions* and *Sample solution* are prepared as follows.

Mobile phase: Hexane and pentanol (997:3)

Standard stock solution: Dissolve USP Cholecalciferol RS or USP Ergocalciferol RS in toluene, and dilute with *Mobile phase* to 50 µg/mL. [NOTE—Prepare this solution fresh daily.]

Standard solution A: 5 µg/mL from the *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above 0°.]

Standard solution B: Transfer 5.0 mL of the *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Equivalent to 5 µg/mL of cholecalciferol or ergocalciferol in *Mobile phase* from an accurately measured volume of the sample

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 1–2 mL/min

Injection volume: 10–20 µL

System suitability

Sample: *Standard solution B* (USP Cholecalciferol RS or USP Ergocalciferol RS)

[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively. Those for pre-ergocalciferol and ergocalciferol are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak; NLT 1.0 between the pre-ergocalciferol peak and the ergocalciferol peak

Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol or ergocalciferol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Cholecalciferol or ergocalciferol response factor

Calculate the cholecalciferol or ergocalciferol response factor, F_C:

$$F_C = C_S/r_S$$

C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in *Standard solution A* (µg/mL)

r_S = peak area of cholecalciferol or ergocalciferol from *Standard solution A*

Precholecalciferol or pre-ergocalciferol response factor

Calculate the concentration, C'_S, in µg/mL, of cholecalciferol or ergocalciferol in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

F_C = response factor for cholecalciferol or ergocalciferol

r'_S = peak area of cholecalciferol or ergocalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of precholecalciferol or pre-ergocalciferol:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in *Standard solution A* (µg/mL)

C'_S = concentration of cholecalciferol or ergocalciferol in *Standard solution B* (µg/mL)

Calculate the response factor, F_{pre}, for precholecalciferol or pre-ergocalciferol:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of precholecalciferol or pre-ergocalciferol (µg/mL)

r_p = peak response of precholecalciferol or pre-ergocalciferol from *Standard solution B*

Content of vitamin D

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or as ergocalciferol ($C_{28}H_{44}O$) in the portion of the sample taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_C = response factor for cholecalciferol or ergocalciferol

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r_C = peak area of cholecalciferol or ergocalciferol from the *Sample solution*
F_{pre} = response factor for precholecalciferol or pre-ergocalciferol
r_{pre} = peak area of precholecalciferol or pre-ergocalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

• **PROCEDURE 7**

This procedure involves the saponification of the sample, followed by a liquid–liquid extraction with ether–solvent hexane, and by the evaporation of ether–hexane and reconstitution of the residue in a mixture of toluene and *Mobile phase* (1:4). It is applicable to solutions in oil and capsules containing vitamin D solutions in oil, in which the oil does not interfere with the corresponding precursor so that it can be quantitated. Separation is achieved in normal-phase mode on an L3 column, and *System suitability* requires separation of the *trans* forms from the vitamin D precursors.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solution*, and reagent solutions are prepared as follows.

Dehydrated hexane: Prepare a chromatographic column by packing a chromatographic tube, 8-cm × 60-cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* ⟨621⟩, *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.

Mobile phase: *n*-Amyl alcohol in *Dehydrated hexane* (3 in 1000)

Butylated hydroxytoluene solution: 10 mg/mL of butylated hydroxytoluene in chromatographic hexane

Aqueous potassium hydroxide solution: 1 g/mL of potassium hydroxide in freshly boiled water. [NOTE—Prepare this solution fresh daily.]

Alcoholic potassium hydroxide solution: 3 g of potassium hydroxide in 50 mL of freshly boiled water. Add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare this solution fresh daily.]

Sodium ascorbate solution: 175 mg/mL of ascorbic acid in 1 N sodium hydroxide. [NOTE—Prepare fresh daily.]

Sodium sulfide solution: 12 g of sodium sulfide in 20 mL of water. Dilute with glycerin to 100 mL.

Standard stock solution: 0.5 mg/mL of USP Ergocalciferol RS or USP Cholecalciferol RS in toluene. [NOTE—Prepare this solution fresh daily.]

Standard solution A: 20 µg/mL from the *Standard stock solution* in *Mobile phase*. [NOTE—Store this solution at a temperature not above 0°.]

Standard solution B: Pipet 4 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser, and add 2 or 3 crystals of butylated hydroxytoluene. Displace the air with nitrogen, and heat in a water bath maintained at a temperature of 90° in subdued light under a nitrogen atmosphere for 45 min to obtain a solution containing vitamin D and previtamin D. Cool, transfer with the aid of several portions of *Mobile phase* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

System suitability solution: Add 100 mg of USP Vitamin D Assay System Suitability RS to a 10-mL volumetric flask. Add a mixture (1 in 5) of toluene and *Mobile phase* to volume, and mix. Heat a portion of this solution under reflux, at 90° for 45 min, and cool.

Sample solution: Reflux NLT 10 Capsules with a mixture of 10 mL of *Sodium ascorbate solution* and 2 drops of *Sodium sulfide solution* on a steam bath for 10 min, crush any remaining solids with a blunt glass rod, and continue heating for 5 min. Cool, and add 25 mL of alcohol and 3 mL of *Aqueous potassium hydroxide solution*. Reflux the mixture on a steam bath for 30 min. Cool rapidly under running water, and transfer the saponified mixture to a conical separator, rinsing the saponification flask with two 15-mL portions of water, 10 mL of alcohol, and two 50-mL portions of ether. [NOTE—Use ether within 24 h after opening the container.] Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Transfer the aqueous phase to a second conical separator, add a mixture of 10 mL of alcohol and 50 mL of solvent hexane, and shake vigorously. Allow to separate, transfer the aqueous phase to a third conical separator, and transfer the solvent hexane phase to the first separator, rinsing the second separator with two 10-mL portions of solvent hexane and adding the rinsings to the first separator. Shake the aqueous phase in the third separator with 50 mL of solvent hexane, and add the solvent hexane phase to the first separator. Wash the combined ether–solvent hexane extracts by shaking vigorously with three 50-mL portions of *Alcoholic potassium hydroxide solution*, and wash with 50-mL portions of water vigorously until the last washing is neutral to phenolphthalein. Drain any remaining drops of water from the combined ether–solvent hexane extracts, add 2 sheets of 9-cm filter paper, in strips, to the separator, and shake. Transfer the washed ether–solvent hexane extracts to a round-bottom flask, rinsing the separator and paper with solvent hexane. Combine the solvent hexane rinsings with the ether–solvent hexane extracts, add 100 µL of *Butylated hydroxytoluene solution*, and mix. Evaporate under vacuum to dryness by swirling in a water bath maintained at a temperature not higher than 40°. Cool under running water, and introduce nitrogen sufficient to restore atmospheric pressure. Without delay, dissolve and dilute the residue in an accurately measured volume of a mixture (1 in 5) of toluene and *Mobile phase*, until the concentration of vitamin D is about 25 µg/mL.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 1–2 mL/min

Injection volume: 10–20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

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Suitability requirements

Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol

Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Cholecalciferol and ergocalciferol response factor

Calculate the cholecalciferol or ergocalciferol response factor, F_D:

$$F_D = C_S/r_S$$

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)
r_S = peak area of cholecalciferol or ergocalciferol from *Standard solution A*

Precholecalciferol and pre-ergocalciferol response factor

Calculate the concentration, C'_S, in µg/mL, of cholecalciferol or ergocalciferol in *Standard solution B*:

$$C'_S = F_D \times r'_S$$

F_D = response factor for cholecalciferol or ergocalciferol
r'_S = peak area of cholecalciferol or ergocalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of pre-ergolecalciferol:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of cholecalciferol or ergocalciferol in *Standard solution B* (µg/mL)

Calculate the response factor, F_{pre}, for precholecalciferol or pre-ergocalciferol:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of precholecalciferol or pre-ergocalciferol (µg/mL)
r_p = peak response of precholecalciferol or pre-ergocalciferol from *Standard solution B*

Content of vitamin D

Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$) or as cholecalciferol ($C_{27}H_{44}O$) in the portion of the sample taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r'_{pre})]/C_U\} \times 100$$

F_D = response factor for cholecalciferol or ergocalciferol
r_C = peak area of cholecalciferol or ergocalciferol from the *Sample solution*
F_{pre} = response factor for precholecalciferol or pre-ergocalciferol
r'_{pre} = peak area of precholecalciferol or pre-ergocalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

• **PROCEDURE 8**

This procedure is suitable for the determination of cholecalciferol in oils or fatty matrices. It involves two chromatographic systems, one for sample clean-up and the other for vitamin D determination. The standard, internal standard, and sample solutions are subjected to saponification, followed by a liquid–liquid extraction with a mixture of ether and hexane (1:1). The extract is evaporated and reconstituted in the *Butylated hydroxytoluene solution*.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, *Internal standard solution*, and reagent solutions are prepared as follows.

Solution A: *n*-Amyl alcohol and dehydrated hexane (3:997)

Solution B: Acetonitrile, water, and phosphoric acid (96: 3.8: 0.2)

Butylated hydroxytoluene solution: 10 mg/mL of butylated hydroxytoluene in chromatographic hexane

Aqueous potassium hydroxide solution: Dissolve 800 ▲g▲ (ERR 1-Oct-2018) of potassium hydroxide in 1000 mL of freshly boiled water, mix, and cool. [NOTE—Prepare this solution fresh daily.]

Alcoholic potassium hydroxide solution: Dissolve 3 g of potassium hydroxide in 50 mL of freshly boiled water, add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare this solution fresh daily.]

Ascorbic acid solution: 100 mg/mL of ascorbic acid in water. [NOTE—Prepare this solution fresh daily.]

Internal standard solution: 5 µg/mL of USP Ergocalciferol RS in alcohol

Standard stock solution: 5 µg/mL of USP Cholecalciferol RS in alcohol

Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution* to a round-bottom flask. Proceed as directed for *Sample solution 1* beginning with "Add 5 mL of...".

Sample solution 1: Transfer 4.00 g of oil to a round-bottom flask. Add 5 mL of the *Ascorbic acid solution*, 100 mL of alcohol, and 10 mL of the *Aqueous potassium hydroxide solution*, and mix. Reflux the mixture on a steam bath for 30 min.

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Add 100 mL of a 10-mg/mL sodium chloride solution. Cool rapidly under running water, and transfer the saponified mixture to a 500-mL separator, rinsing the saponification flask with 75 mL of a 10-mg/mL sodium chloride solution, and then with 150 mL of a mixture of ether and hexane (1:1). Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Discard the lower layer. Wash the ether–hexane extracts by shaking vigorously with 50 mL of the *Alcoholic potassium hydroxide solution*, and then washing with three 50-mL portions of a 10-mg/mL sodium chloride solution. Filter the upper layer through 5 g of anhydrous sodium sulfate on a fast filter paper into a 250-mL flask suitable for a rotary evaporator. Wash the filter with 10 mL of a mixture of ether and hexane (1:1), and combine with the extract. Evaporate the solvent at reduced pressure at a temperature not exceeding 30°, and fill with nitrogen when the evaporation is complete. Alternatively evaporate the solvent under a gentle stream of nitrogen at a temperature not exceeding 30°. Dissolve the residue in 1.5 mL of the *Butylated hydroxytoluene solution*. [NOTE—Gentle heating in an ultrasonic bath may be required. A large fraction of the white residue is cholesterol.]

Sample solution 2: To 4.00 g of oil add 2.0 mL of *Internal standard solution*, and proceed as directed for *Sample solution 1* beginning with "Add 5 mL of...".

Clean-up chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Mobile phase: *Solution A*
Clean-up column: 4.6-mm × 25-cm stainless steel; packing L10
▲Flow rate: 1.1 mL/min▲ (ERR 1-Aug-2017)
Injection volume: 350 µL

Analysis (clean-up)
Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2*
Collect separately the eluates from 2 min before to 2 min after the retention time of cholecalciferol in a glass tube containing 1 mL of *Butylated hydroxytoluene solution* and fitted with a hermetic closure. Evaporate each tube under a stream of nitrogen at a temperature not exceeding 30°. Dissolve each residue in 1.5 mL of acetonitrile, and inject into the analytical chromatographic system below.

Analytical chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Mobile phase: *Solution B*
Analytical column: 4.6-mm × 15-cm stainless steel; 5-µm packing L1
▲Flow rate: 1.0 mL/min▲ (ERR 1-Aug-2017)
Injection volume: 200 µL

System suitability
Sample: *Standard solution* (after the clean-up)
Suitability requirements
Resolution: NLT 1.4 between cholecalciferol and ergocalciferol
Relative standard deviation: NMT 2.0% for the cholecalciferol peak from replicate injections

Analysis
Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2* (after the clean-up)
Calculate the content of vitamin D, in µg/g, in the portion of the sample taken:

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

R_S = peak response of cholecalciferol relative to the internal standard in the *Standard solution*
C_S = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)
C_U = concentration of oil in *Sample solution 2* (g/mL)
R_U = peak response of cholecalciferol relative to the internal standard in the *Standard solution 2*, as calculated as follows:

[NOTE—If $r_{IS1} = 0$ due to no peak observed at the locus of the internal standard in the chromatogram of *Sample solution 1*, then $R_U = r_{U2}/r_{IS2}$.]

$$R_U = r_{U2}/[r_{IS2} - (r_{IS1} \times r_{U2}/r_{U1})]$$

r_{U2} = peak response of cholecalciferol from *Sample solution 2*
r_{IS2} = peak response of the internal standard from *Sample solution 2*
r_{IS1} = peak response of the internal standard from *Sample solution 1*
r_{U1} = peak response of cholecalciferol from the *Sample solution 1*

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cholecalciferol RS
  USP Ergocalciferol RS
  USP Vitamin D Assay System Suitability RS

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⟨591⟩ ZINC DETERMINATION

INTRODUCTION

Change to read:

▲A quantitative determination of zinc in drug substance and drug product monographs containing zinc is achieved by the *Dithizone Method*, *Ion Chromatographic Method*, or *Atomic Absorption Method*. Unless a specific method is indicated in the individual monograph, any of these methods can be used.▲ (USP 1-May-2020)

PROCEDURE

Change to read:

• DITHIZONE METHOD

Select all reagents for this test to have as low a content of heavy metals as practicable. If necessary, distill water and other solvents into a hard or borosilicate glass apparatus. Rinse thoroughly all glassware with warm diluted nitric acid (1 in 2) followed by water. Avoid using on the separator any lubricants that dissolve in chloroform.

Special solutions and solvents

Alkaline ammonium citrate solution: Dissolve 50 g of dibasic ammonium citrate in water to make 100 mL. Add 100 mL of ammonium hydroxide. Remove any heavy metals that may be present by extracting the solution with 20-mL portions of the *Dithizone extraction solution* (see *Lead* ⟨251⟩) until the *Dithizone* ▲*extraction*▲ (USP 1-May-2020) *solution* retains a clear green color, then extract any dithizone remaining in the citrate solution by shaking with chloroform.

Chloroform: Distill chloroform in a hard or borosilicate glass apparatus, receiving the distillate in sufficient dehydrated alcohol to make the final concentration 1 mL of alcohol for each 100 mL of distillate.

Dithizone solution: Use the *Standard dithizone solution* (see ⟨251⟩), prepared with the distilled *Chloroform*.

Standard zinc solution: Dissolve 625 mg of zinc oxide, accurately weighed and previously gently ignited to constant weight, in 10 mL of nitric acid, and add water to make 500.0 mL. This solution contains 1.0 mg/mL of zinc.

Diluted standard zinc solution: Dilute 1 mL of the *Standard zinc solution*, accurately measured, with 2 drops of nitric acid and sufficient water to make 100.0 mL. This solution contains 10 µg/mL of zinc. Use this solution within 2 weeks.

Trichloroacetic acid solution: Dissolve 100 g of trichloroacetic acid in water to make 1000 mL.

Procedure: Transfer 1–5 mL of the preparation to be tested, accurately measured, to a centrifuge tube graduated at 40 mL. If necessary, add 0.25 N hydrochloric acid, dropwise, to obtain a clear solution. Add 5 mL of the *Trichloroacetic acid solution* and sufficient water to make 40.0 mL. Mix and centrifuge.

Transfer to a hard-glass separator an accurately measured volume of the supernatant believed to contain 5–20 µg of zinc, and add water to make about 20 mL. Add 1.5 mL of the *Alkaline ammonium citrate solution* and 35 mL of the *Dithizone solution*. Shake vigorously 100 times. Allow the chloroform phase to separate. Insert a cotton plug in the stem of the separator to remove any water emulsified with the chloroform. Collect the chloroform extract (discarding the first portion that comes through) in a test tube, and determine the absorbance at 530 nm, with a suitable spectrophotometer.

Calculate the amount of zinc present by reference to a standard absorbance–concentration curve obtained by using 0.5, 1.0, 1.5 mL, and, if the zinc content of the sample extracted exceeds 15 µg, 2.0 mL of the *Diluted standard zinc solution*, corrected as indicated by a blank determination run concomitantly, using all of the reagents but no added zinc.

Change to read:

• ION CHROMATOGRAPHIC METHOD

The following ion chromatographic general procedure is provided for the determination of zinc in compendial articles. ▲▲ (USP 1-May-2020) See *Ion Chromatography* ⟨1065⟩ for discussion of the theory and principles of measurements using ion chromatography.

Use water with a resistivity of NLT 18 megohm-cm to prepare the solutions.

Diluent: 0.2% (w/v) hydrochloric acid

Mobile phase: 7.0 mM dipicolinic acid, 66.0 mM potassium hydroxide, 5.6 mM potassium sulfate, and 74.0 mM formic acid in water; adjust with 2 N potassium hydroxide TS to a pH of 4.2. Pass through a suitable filter of 0.2-µm pore size.

Post-column derivatization reagent: 0.5 mM 4-(2-pyridylazo)resorcinol monosodium salt, 1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, and 0.30 M sodium bicarbonate in water. Stir and sonicate until the solid is completely dissolved. Pass through a suitable filter of 0.2-µm pore size.

Standard stock solution: 1500 µg/mL of zinc from USP Zinc Oxide RS prepared as follows. Transfer an appropriate portion of USP Zinc Oxide RS to a suitable volumetric flask. Add 6 N hydrochloric acid to about 10% of the final flask volume to dissolve. Dilute with water to volume.

Standard solution: 15.0 µg/mL of zinc in *Diluent* from the *Standard stock solution*

Sample stock solution: Prepare as directed in the monograph.

Sample solution: Equivalent to 15.0 µg/mL of zinc in *Diluent* from the *Sample stock solution*, unless otherwise stated in the monograph

Chromatographic system

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

Mode: LC

Detector: Vis 530 nm

Columns

Guard: 4.0-mm × 5-cm; 9-µm packing L100

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Analytical: 4.0-mm × 25-cm; 9-µm packing L100
Column temperature: 30°
Flow rate: 1.2 mL/min
Flow rate of post-column reagent: 0.6 mL/min. Introduce using a pulseless flow of reagent through a 375-µL polymeric mixing coil or other suitable volume coil.[1] ▲(USP 1-May-2020)
Injection volume: 10 µL
Run time: NLT 2 times the retention time of zinc
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%, unless otherwise stated in the monograph
Analysis
Samples: *Standard solution* and *Sample solution*
Unless otherwise stated in the monograph, calculate the concentration of zinc in the portion of *Sample solution* taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of zinc in the *Standard solution* (µg/mL)

Add the following:

▲• ATOMIC ABSORPTION METHOD
The following atomic absorption general procedure is provided for the determination of zinc in compendial articles.
Diluent: 0.01 N hydrochloric acid
Zinc stock standard solution: Use a certified 1000-µg/mL standard.
Zinc working standard solution: Prepare a 10-µg/mL solution by pipetting 5.0 mL of the 1000-µg/mL *Zinc stock standard solution* into a 500-mL volumetric flask. Dilute with *Diluent* to volume.
Zinc calibration standard solutions: Prepare NLT 3 calibration standard solutions within the range of 0.2–1.6 µg/mL, or suitable concentrations to span anticipated zinc concentrations in the sample(s). Prepare these solutions by two-step dilutions of the *Zinc stock standard solution* with the *Diluent*.
Drug substance sample solution: Accurately weigh an amount of the sample, dissolve in *Diluent*, and dilute to volume in a volumetric flask. Dilute the solution such that the zinc concentration is within the concentration range of the prepared *Zinc calibration standard solutions*.
Drug product sample solution: If the sample is a suspension, resuspend and add 4–7 µL of 6 N hydrochloric acid, as needed to dissolve prior to dilution. Dilute the sample with *Diluent* to a zinc concentration within the concentration range of the prepared *Zinc calibration standard solutions*.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 213.9 nm
Lamp: Zinc hollow-cathode, 5–15 mA
Flame: Air–acetylene of suitable composition
Blank: 0.01 N hydrochloric acid
Analysis: Zero the instrument using the *Blank*. Determine the absorbance in triplicate, of the *Blank*, *Zinc working standard solution*, the *Zinc calibration standard solutions*, and the Sample solutions.
Calibration: Prepare a calibration curve from the mean of the readings of the absorbance of the *Blank* and the *Zinc calibration standard solutions* (linear or quadratic fit).
System suitability
Suitability requirements: The %RSD of the triplicate measurements for each aspiration of the *Zinc working standard solution* is NMT 2.5, and the correlation coefficient of the standard curve is NLT 0.997.
Calculations: Read the concentration of zinc in the sample solution using the calibration curve and calculate the concentration of zinc in the sample.
▲(USP 1-May-2020)

ADDITIONAL REQUIREMENTS

• USP REFERENCE STANDARDS ⟨11⟩
USP Zinc Oxide RS

[1] A knitted reaction coil, part number 043700, available from ThermoFisher Scientific (www.thermofisher.com), may be suitable.

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Physical Tests and Determinations

⟨601⟩ INHALATION AND NASAL DRUG PRODUCTS: AEROSOLS, SPRAYS, AND POWDERS—PERFORMANCE QUALITY TESTS

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INTRODUCTION

The major performance measures for nasal and inhalation aerosols, sprays, and powders relate to dose delivery to the patient, including delivered-dose uniformity and relevant measures of particle size (optical or aerodynamic) depending on the dosage form. Each of these is described in the following sections. A nomenclature table appears in *Inhalation and Nasal Drug Products —General Information and Product Quality Tests* ⟨5⟩, from which the descriptive terms for various dosage forms can be obtained.

A. DELIVERED-DOSE UNIFORMITY

A.1 Nasal Aerosols and Nasal Sprays

The following test is applicable to nasal aerosols and sprays formulated as nonaqueous or aqueous suspensions or solutions of drug, presented typically in multidose containers, and fitted with dose-metering valves or pumps. In all cases and for all tests, prepare and test the spray as directed in the labeling and the instructions for use.

A.1.1 DELIVERED-DOSE UNIFORMITY OF PRODUCT

Unless otherwise directed in the individual monograph, the drug content of the minimum delivered doses collected at the beginning of unit life, and also at the end of unit life, will be determined from each of 10 separate containers. This represents a total of 20 determinations. These measurements shall be made after priming as described in the labeling or instructions for use. The number of doses not to exceed 2 sprays (i.e., a total of 20 determinations).

A.1.1.1 Sampling the delivered dose from nasal sprays

Procedure—To ensure reproducible in vitro dose collection, it is recommended that a mechanical means of actuating the metering system or pump assembly be used to deliver doses for collection. The mechanical actuation procedure should have adequate controls for the critical mechanical actuation parameters (e.g., actuation force, actuation speed, stroke length, and rest periods). The test must be performed on units that have been thoroughly shaken and primed according to the patient-use instructions. The test unit should be actuated in a vertical or near-vertical, valve-up position. The dose collected at the beginning of each of the 10 test containers should be the dose immediately following priming, and the dose collected at the end of each container's life should correspond to the last label claim number of doses from the same container. The doses between the two sequential test samples should be disposed of appropriately.

For suspension products, the dose should be delivered into a suitable container (e.g., scintillation vial) in which quantitative transfer from the container under test can be accomplished. A validated analytical method is employed to determine the amount of drug in each delivered dose, and data are reported as a percent of label claim. For solution products, the delivered dose can be determined gravimetrically from the weight of the delivered dose and the concentration and density of the fill solution of the product under test.

A.1.1.2 Sampling the delivered dose from nasal aerosols: See *A.2.1.1 Sampling the Delivered Dose from Inhalation Aerosols and Inhalation Sprays.*

A.2 Inhalation Aerosols and Inhalation Sprays

The following tests are applicable to inhalation aerosols (commonly known as metered-dose inhalers) and inhalation sprays. Inhalation aerosols are formulated as suspensions or solutions of a drug substance in propellants and possibly other suitable excipients and are presented as multidose units. Inhalation sprays typically are aqueous-based liquid formulations packaged in a compact container–closure system containing an integral spray pump unit. Refer to ⟨5⟩ for additional information. The following test methods are specific to these products and may require modification when analysts test alternative inhalation technologies (for example, breath-actuated inhalation aerosols or inhalation sprays). However, pharmacopeial requirements for all dose-metered inhalation dosage forms require determination of the delivered dose and aerodynamic size distribution. In all cases and for all tests prepare and test the product as directed on the label and the instructions for use. When these directions are not provided by the product manufacturer, follow the precise dose-discharge directions included in the following tests.

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A.2.1 DELIVERED-DOSE UNIFORMITY OF PRODUCT

The test for *Delivered-Dose Uniformity* is required for inhalation aerosols and inhalation sprays containing drug formulation (e.g., solution or suspension) either in device-metered or in pre-metered unit presentations. The test for *Delivered-Dose Uniformity* includes dose uniformity over the entire unit life. (For products packaged in pre-metered dosage units, see also *Uniformity of Dosage Units* ⟨905⟩.) A dose in this test is defined as the minimum recommended number of sprays specified in the product labeling or instructions for use but not more than two sprays. For inhalation aerosols and inhalation sprays, the target-delivered dose is specified by the label claim, unless otherwise specified in the individual monograph. Its value reflects the expected mean drug content for a large number of delivered doses collected from many units of the product using the method specified in the monograph.

Unless otherwise directed in the individual monograph, the drug content of the delivered doses collected at the beginning of unit life (after priming as described on the label or instructions for use) and end of unit life of the label claim, will be determined from each of 10 separate containers (i.e., a total of 20 determinations).

A.2.1.1 Sampling the delivered dose from inhalation aerosols and inhalation sprays

Procedure—To determine the content of active ingredient in the discharged plume from an inhalation aerosol and inhalation spray, use the sampling *Apparatus A* (see *Figure 1*) described below. Prepare the product for use according to the label instructions for shaking, priming, and firing. Unless otherwise specified in the individual monograph, with the vacuum pump running, ensuring an airflow rate through the product device of 28.3 L of air per minute (±5%), discharge the minimum recommended actuations into the apparatus through the mouthpiece adapter by actuating the metering system for a duration sufficient to ensure that the dose has been completely discharged. The volume of air sampled should not exceed 2.0 L. The dose collected from each of the 10 test containers should be the dose immediately following priming, and the dose collected at the end of each container's life should be the last dose according to the label claim. Unless otherwise prescribed in the patient instructions, shake the product for 5 s, and collect the first actuation following priming. Wait for 5 s and collect the next actuation, if justified. The doses between the two sequential test samples (i.e., beginning and end of container life) should be disposed of appropriately. Note that for inhalation aerosols the rate of discharges (number of discharges per unit time) to waste should not cause excessive canister cooling. Following separate collections of the minimum number of actuations from each unit at each sequential test sample, detach the product from *Apparatus A* (see *Figure 1*), and disconnect the vacuum. Separately assay the contents of the apparatus for drug at the beginning and end sequential test samples after rinsing the filter and the interior of the apparatus with a suitable solvent.

APPARATUS A—The sampling apparatus (see *Figure 1*) consists of a filter support base with an open-mesh filter support such as a stainless steel screen, a collection tube that is clamped or screwed to the filter support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter that ensures that the opening of the product's mouthpiece is flush with the front face or 2.5-mm indented shoulder in the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source, flow regulator, and flowmeter. The source should be capable of pulling air through the complete assembly, including the filter and the product to be tested, at the desired flow rate. During tests of inhalation aerosols, air should be drawn continuously through the system to avoid loss of drug into the atmosphere. The filter support base is designed to accommodate 25-mm diameter filter disks. At the airflow used, the sample collection tube and the filter disk must be capable of quantitatively collecting the delivered dose. The filter disk and other materials used in the construction of the apparatus must be compatible with the drug and the solvents that are used to extract the drug from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection device passes through the product device.

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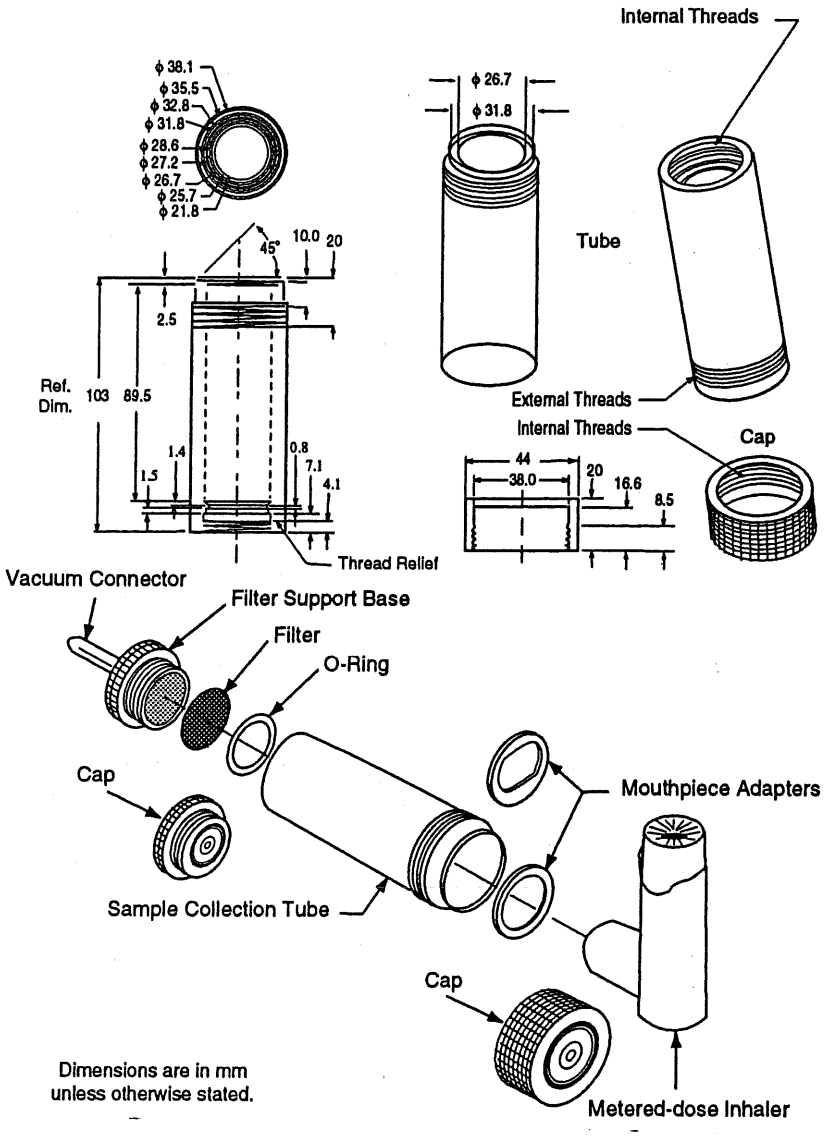


Figure 1. Sampling apparatus for inhalation aerosols and sprays.

A.3 Nasal Powders

The following test is applicable to nasal powders presented in pre-metered and device-metered units. In all cases and for all tests, prepare and test the powder as directed in the labeling and the instructions for use.

A.3.1 DELIVERED-DOSE UNIFORMITY OF PRODUCT

Unless otherwise directed in the individual monograph, the drug content of the minimum delivered doses from each of 10 separate containers will be determined at the beginning of unit life and again at the label claim number of metered doses. This represents a total of 20 determinations. For nasal powders packaged in single-dose units, *Delivered-Dose Uniformity* can be applied on 10 dosage units.

A.3.1.1 Sampling the delivered dose from nasal powders: To ensure reproducible in vitro dose collection, it is recommended that appropriate means of actuating the device assembly be used to deliver doses for collection. The test unit should be actuated in a vertical or near-vertical position. The two separate doses collected include the first dose and the dose corresponding to the last labeled dose from each of 10 units. The doses between the two sequential test samples for each unit should be disposed of appropriately. A validated analytical method is used to determine the amount of drug in each delivered dose, and data are reported as a percentage of label claim. Apparatus B (*Figure 2*) is recommended for nasal powders.

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A.4 Inhalation Powders

The following tests are applicable to inhalation powders (commonly known as dry powder inhalers) presented as pre-metered or device-metered units. Pharmacopeial requirements for all these drug products require determination of the delivered dose and aerodynamic size distribution. In all cases and for all tests, prepare and test the product as directed in the labeling and the instructions for use. When these directions are not provided by the product manufacturer, follow the precise dose-discharge directions included in the following tests.

A.4.1 DELIVERED-DOSE UNIFORMITY OF PRODUCT

The test for *Delivered-Dose Uniformity* is required for inhalation powders in device-metered and in pre-metered (including ordered multiple-dose) presentations as labeled for use with the specified delivery system. The test for *Delivered-Dose Uniformity* includes dose uniformity over the entire unit life. (For formulations packaged in pre-metered dosage units, see also ⟨905⟩.) Note that the target delivered dose is the expected mean drug content for a large number of delivered doses collected from many units under defined experimental conditions. In many cases, the target value may depend on the manner in which the test for delivered dose is performed. For inhalation powders, where the label claim usually is the pre-metered or metered dose of drug, the target delivered dose is specified in the individual monograph and usually is less than the label claim. Its value reflects the expected mean drug content for a large number of delivered doses collected from the product using the method specified in the monograph.

Unless otherwise directed in the individual monograph, the drug content of the minimum delivered dose from each of 10 separate units is determined in accordance with the procedure described as follows.

The test for *Delivered-Dose Uniformity* over the entire unit life is required for drug products packaged in device-metered or in ordered multiple-dose metering units of pre-metered dosage units that have a predetermined dose sequence.

Unless otherwise directed in the individual monograph, the drug content of the delivered doses will be collected at the beginning of unit life and again at the label claim number of doses. Two determinations will be obtained from each of 10 separate drug product units. This represents a total of 20 determinations.

For inhalation powders packaged in single dosage form units, *Delivered-Dose Uniformity* can be applied on 10 dosage units.

A.4.1.1 Sampling the delivered dose from inhalation powders: To determine the content of active ingredient emitted from the mouthpiece of an inhalation powder, use *Apparatus B* (see *Figure 2*). This apparatus is capable of sampling the emitted doses at a variety of airflow rates.

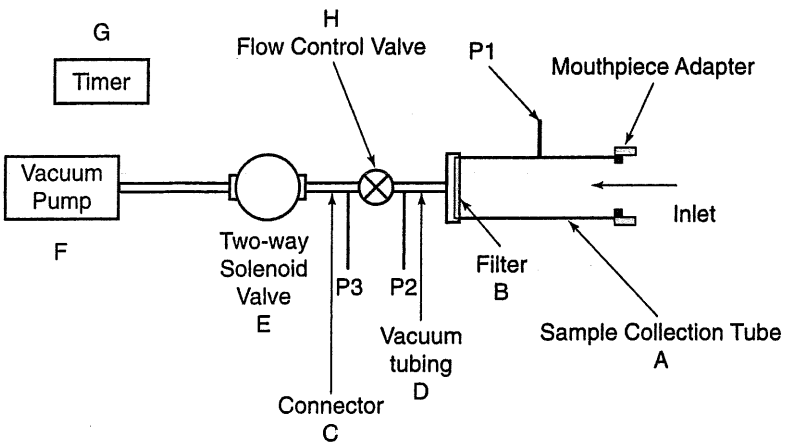


Figure 2. Apparatus B: Sampling apparatus for inhalation powders. (See *Table 1* for component specifications.)

Table 1. Component Specifications for Apparatus B (see *Figure 2*)

Code	Item	Description	Dimensions
A	Sample collection tube[a]	See *Figure 2*	34.85-mm ID × 12-cm length
B	Filter[b]	See *Figure 2*	47-mm glass fiber filter
C	Connector	(e.g., short metal coupling with low-diameter branch to P3)	≥8-mm ID
D	Vacuum tubing	(e.g., silicon tubing with an outside diameter of 14 mm and an internal diameter of 8 mm)	A length of suitable tubing ≥8-mm ID with an internal volume of 25 ± 5 mL
E	Two-way solenoid valve[c]	See *Figure 2*	2-way, 2-port solenoid valve having an ID ≥8 mm and an opening response time of ≤100 milliseconds.

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Table 1. Component Specifications for Apparatus B (see *Figure 2*) *(continued)*

Code	Item	Description	Dimensions
F	Vacuum pump[d]	See *Figure 2*	Pump must be capable of drawing the required flow rate through the assembled apparatus with the inhalation powder in the mouthpiece adapter. Connect the pump to the solenoid valve using short and wide (≥10-mm ID) vacuum tubing and connectors to minimize pump capacity requirements.
G	Timer[e]	See *Figure 2*	The timer switches current directly to the solenoid valve for the required duration.
P1	Pressure tap	See *Figure 2*	2.2-mm ID, 3.1-mm OD flush with the internal surface of the sample collection tube, centered and burr free, 59 mm from its inlet. The pressure taps P1, P2, and P3 must not be open to the atmosphere during dose collection.
P1, P2, P3	Pressure measurements[f]	—	—
H	Flow-control valve[g]	See *Figure 2*	Adjustable regulating valve with maximum $C_v \geq 1$[h]

[a] An example is a Millipore product number XX40 047 00 (Millipore Corporation, 80 Ashby Road, Bedford, MA 01732) modified so that the exit tube has an ID ≥8 mm, fitted with Gelman product number 61631.
[b] A/E (Gelman Sciences Inc., 600 South Wagner Road, Ann Arbor, MI 48106) or equivalent.
[c] ASCO product number 8030G13, Automatic Switch Company, 60 Hanover Road, Florham Park, NJ 07932.
[d] Gast product type 1023, 1423, or 2565 (Gast Manufacturing Inc., PO Box 97, Benton Harbor, MI 49022) or equivalent.
[e] Eaton Product number 45610-400 (Eaton Corporation, Automotive Products Division, 901 South 12th Street, Watertown, WI 53094) or equivalent.
[f] An example is a PDM 210 pressure meter (Air-Neotronics Ltd., Neotronics Technology plc, Parsonage Road, Takeley, Bishop's Stortford, CM22 6PU, UK) or equivalent.
[g] Parker Hannifin type 8FV12LNSS (Parker Hannifin plc., Riverside Road, Barnstable, Devon EX31 1NP, UK) or equivalent.
[h] Flow Coefficient, as defined by ISA S75.02 Control valve capacity test procedure in *Standards and Recommended Practices for Instrumentation and Control*, 10th ed., Vol. 2, 1989. Published by Instrument Society of America, 67 Alexander Drive, PO Box 1227, Research Triangle Park, NC 27709, USA.

Apparatus B—The apparatus is similar to that described in *Figure 1* for testing inhalation aerosols. In this case, however, the filter and collection tube have a larger internal diameter to accommodate 47-mm diameter filter disks. This feature enables dosage collection at higher airflow rates—up to 100 L of air/min—when necessary. A mouthpiece adapter ensures an airtight seal between the collection tube and the mouthpiece of the inhalation powder being tested. The mouthpiece adapter must ensure that the tip of the product's mouthpiece is flush with the open end of the sample collection tube. Tubing connectors, if they are used, should have an internal diameter ≥8 mm to preclude their own internal diameters from creating significant airflow resistance. A vacuum pump with excess capacity must be selected in order to draw air at the designated volumetric flow rate through both the sampling apparatus and the product simultaneously. A timer-controlled, low-resistance, solenoid-operated, two-way valve is interposed between the vacuum pump and the flow-control valve to control the duration of flow. This type of valve enables 4.0 L of air (±5%) to be withdrawn from the mouthpiece of the product at the designated flow rate. Flow control is achieved by ensuring that critical (sonic) flow occurs in the flow-control valve (absolute pressure ratio P3/P2 is ≤0.5 under conditions of steady-state flow).

Procedure—Operate the apparatus at an airflow rate that produces a pressure drop of 4 kPa (40.8 cm H_2O) over the product to be tested and for a period consistent with the withdrawal of 2.0 L of air from the mouthpiece of the product. [NOTE—If the flow rate and duration are defined otherwise in the monograph, adjust the system to within 5% of those values.] The volume of air sampled should not exceed 2.0 L. Determine the test flow rate using *Apparatus B* as follows. Insert the product into the mouthpiece adapter to ensure an airtight seal. In cases where the drug packaging modifies the product's resistance to airflow, use a loaded, drug-free device (with previously emptied packaging). In other cases, use an unloaded (drug-free) device. Connect one port of a differential pressure transducer to the pressure tap, P1, and leave the other open to the atmosphere. Switch on the pump, and open the two-way solenoid valve. Adjust the flow-control valve until the pressure drop across the product is 4.0 kPa (40.8 cm H_2O). Ensure that critical (sonic) flow occurs in the flow-control valve by determining the individual values for absolute pressure, P2 and P3, so that their ratio P3/P2 is ≤0.5. If this ratio of P3/P2 is not achieved, switch to a more powerful pump and remeasure the test flow rate. Critical (sonic) flow conditions in the flow-control valve are required in order to ensure that the volumetric airflow drawn from the mouthpiece is unaffected by pump fluctuations and changes in airflow resistance of the product. Remove the product from the mouthpiece adapter and, without disturbing the flow-control valve, measure the airflow rate drawn from the mouthpiece, Q_{out}, by connecting a flowmeter to the mouthpiece adaptor in an airtight fashion. Use a flowmeter calibrated for the volumetric flow leaving the meter in an airtight fashion to directly determine Q_{out} or, if such a meter is unobtainable, calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For example, for a meter calibrated for the entering volumetric flow (Q_{in}) calculate:

$$Q_{out} = Q_{in}P_0/(P_0 - \Delta P)$$

P_0 = atmospheric pressure

ΔP = pressure drop over the meter

If the flow rate is >100 L of air/min, adjust the flow-control valve until Q_{out} equals 100 L/min; otherwise, record the value of Q_{out}, and leave the flow-control valve undisturbed. Define the test flow duration, e.g., at 60 L/min $T = 120/Q_{out}$, in seconds, so that a volume of 2.0 L of air (±5%) is withdrawn from the product at the test flow rate Q_{out}, and adjust the timer controlling the operation of the two-way solenoid valve accordingly. Prime or load the device with powder for inhalation according to the labeled instructions. With the vacuum pump running and the solenoid valve closed, insert the product's mouthpiece horizontally into the mouthpiece adapter. Discharge the powder into the sampling apparatus by activating the timer controlling the solenoid valve and withdrawing 2.0 L of air from the product at the previously defined airflow rate. Repeat the whole operation $n - 1$ times beginning with the text, "Prime or load the device with powder," where n is the number of times defined in the labeling

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as the minimum recommended dose. Detach the inhalation powder device container from the sampling apparatus, and disconnect the vacuum tubing, D. Assay the contents of the apparatus for drug after rinsing the filter and the interior of the apparatus with a suitable solvent. Where specified in the individual monograph, perform this test under conditions of controlled temperature and humidity.

B. DROPLET/PARTICLE SIZE DISTRIBUTION—NASAL AEROSOLS, SPRAYS, AND POWDERS

For suspension and solution nasal aerosols, sprays, and powders, the emitted droplet/particle size distribution should be determined for the delivered plume subsequent to delivery under specified experimental conditions. If a laser diffraction method is used (for more detail, refer to the description of this method in the following section), droplet size distribution can be controlled in terms of ranges for the 10th (D_{10}), 50th (D_{50}), and 90th (D_{90}) percentiles of the cumulative volume (mass)-weighted size distribution, as well as the span of the distribution, expressed as $[(D_{90} - D_{10})/D_{50}]$, and the percentage of droplets less than 10 µm. Note that D_{50} is identical with the volume (mass) median diameter if the distribution is unimodal. Appropriate and validated or calibrated emitted droplet/particle size analytical procedures should be described in sufficient detail to allow accurate and reproducible assessment including the following:

- complete information about the apparatus and accessories
- theoretical model (Lorenz–Mie or Fraunhofer approximation)
- the application of a disabling option for one or more of the innermost detectors to mitigate beam steering effects (only applicable to nasal aerosols)
- software version
- sample placement with respect to the optical bench of the laser diffractometer
- measurement range
- beam width
- laser trigger condition in connection with the initiation and termination of the measurement sequence
- lower limit of detection (if laser triggering is not used)
- and obscuration limit (upper bound of detection range in terms of particle concentration).

B.1 Particle Size Measurement by Laser Diffraction

Nasal drug products intended for topical application to the nasal cavity produce liquid droplets that typically are much larger than the operating range for multistage inertial impactors. Thus, laser diffraction (sometimes referred to as low-angle laser light scattering) is an acceptable alternative for determining the size distribution because there is no need to relate the size scale to aerodynamic diameter.

The theory and operating principles for laser diffraction are well described in ISO 13320:2009. These systems use the light scattering pattern produced by passage of a cloud of droplets through the measurement zone to develop the volume-weighted size distribution in an iterative process in which a theoretical model (either Fraunhofer or Lorenz–Mie) is used to interpret the data. The process is summarized schematically in *Figure 3*. [NOTE—If the Lorenz–Mie model option is chosen, it will be necessary to input values for the real (refraction) and imaginary (absorption) components of refractive index for the liquid being studied. Manufacturers of laser diffraction equipment provide this information for commonly encountered liquid media either on their website or upon enquiry.]

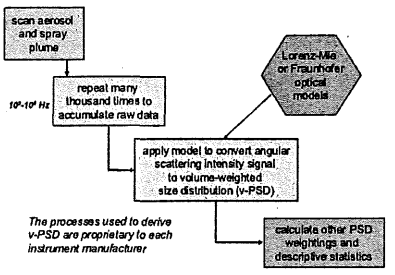


Figure 3. Conversion of light scattering data into a droplet size distribution by laser diffractometry.

An apparatus that has the capability to assess aerosols or sprays is required. [NOTE—not all commercially available laser diffractions possess this capability.] Set up the measurement system in accordance with the procedure outlined schematically in *Figure 4*.

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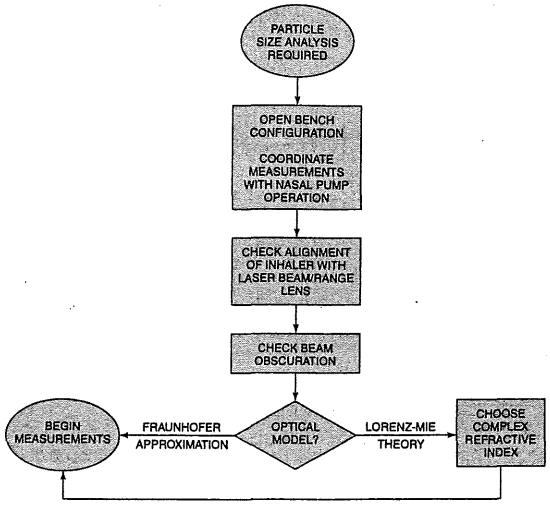


Figure 4. Setup for laser diffractometry with a nasal drug product.

Nasal drug product sprays generally are measured with an open-bench arrangement with an automated actuation station set-up so that reproducibility in inhaler operation from one actuation to the next can be optimized. This precaution is particularly important for the most accurate measurements with nasal spray pumps. Check the alignment of the inhaler with the optical configuration of the laser diffractometer using the tools provided by the manufacturer to optimize alignment of the optical bench. Check that the light beam obscuration is within the upper and lower limits specified by the manufacturer, consulting the operator's manual for the laser diffraction system being used. Vacuum extraction is required to efficiently remove droplets after they pass through the laser. An unrepresentative result may occur if this value is set too low (typically <5%). Unquantifiable error due to multiple scattering will happen if the obscuration is above the recommended upper limit. [NOTE—This limit varies from one manufacturer to another and may also vary from one laser diffractometer system to another from the same manufacturer.]

Make as many replicates as needed to achieve a representative data set. Inspect each size distribution presented, ideally in differential volume-weighted format or equivalent to assess whether or not it is unimodal and symmetrical. [NOTE—The following data analysis assumes that these criteria are met. If not, additional analysis taking into account the presence of more than one mode in the distribution or skewness may be required.]

Assuming that the density of the liquid under investigation is constant irrespective of droplet size, the volume-weighted size distributions may be treated as mass-weighted size distributions for the purpose of data interpretation. From the cumulative volume–size distribution data, calculate the mean and one standard deviation for the sizes that correspond to mass fractions less than 10%, 50%, and 90% of the distribution (D_{10}, D_{50}, and D_{90}).

C. AERODYNAMIC SIZE DISTRIBUTION—INHALATION AEROSOLS, SPRAYS, AND POWDERS

C.1 General Principles of Aerodynamic Particle Size Measurement

The particle or droplet size distribution in the plume discharged from inhalation aerosols and sprays and the particle size distribution in the cloud discharged from inhalation powders are important characteristics used in judging product performance. Although particle size measurement by microscopy can be used to evaluate the number of large particles, agglomerates, and foreign particulates in the emissions of inhalation aerosols and sprays, whenever possible this test should be replaced with a method to determine the aerodynamic size distribution of the drug aerosol leaving the product. The aerodynamic diameter of an aerosol particle is equal to the diameter of a sphere of unit density whose gravimetric settling velocity is the same. The aerodynamic size distribution defines the manner in which an aerosol deposits during inhalation. In use, many inhalers discharge drug in the form of large particles or droplets (the ballistic fraction) that leave the inhaler at high velocity and impact in and are captured by the moist surfaces in the mouth and throat. The remainder of the discharge from the inhaler is useful aerosol, a "nonballistic fraction" that is inhaled into the remainder of the respiratory tract.

Cascade impaction devices classify aerosol particles and droplets on the basis of aerodynamic diameters. The principle of their operation whereby they separate aerosol particles and droplets from a moving airstream on the basis of particle or droplet inertia is shown in *Figure 5*. Because the dimensions of the induction port used to connect products to the cascade impactors and impingers (shown in *Apparatus 1, 2, 3, 4, 5,* and *6*) also define the mass of drug that enters the aerodynamic sizing device, these are carefully defined and, where possible, are held constant between each apparatus (see *Figures 6, 7, 8, 9, 10,* and *11*).

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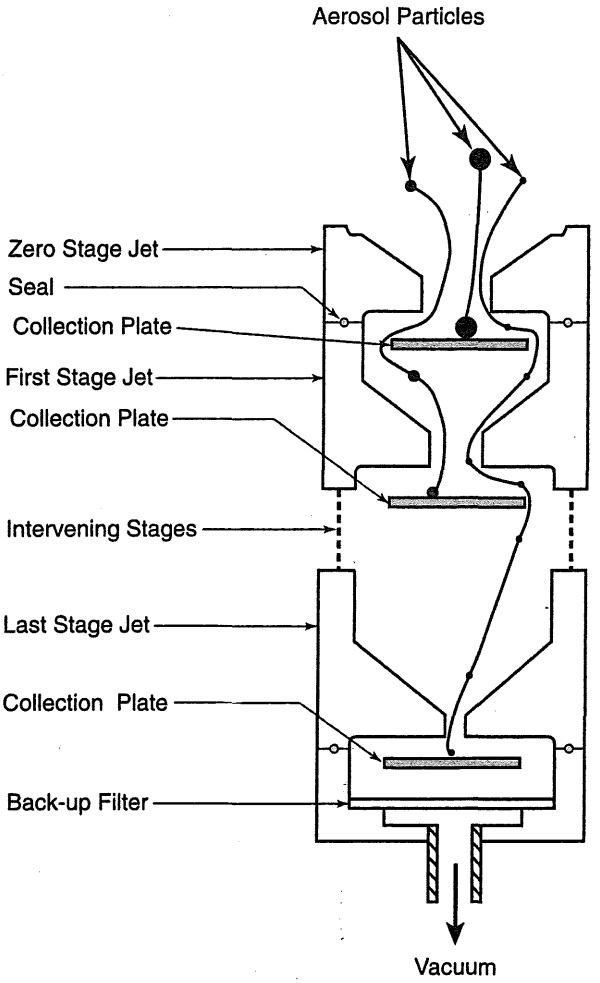


Figure 5. Schematic representation of the principle of operation of cascade impactors. (A single jet per impactor stage is shown. Impactors with multiple jets in each stage function in the same manner.)

C.1.1 STAGE MENSURATION

Manufacturers of cascade impaction devices provide a definitive calibration for the separation characteristics of each impaction stage in terms of the relationship between the stage collection efficiency and the aerodynamic diameter of particles and droplets passing through it as an aerosol. Calibration is a property of the jet dimensions, the spatial arrangement of the jet and its collection surface, and the airflow rate passing through it. Because jets can corrode and wear over time, the critical dimensions of each stage, which define that impaction stage's calibration, must be measured on a regular basis. This process, known as stage mensuration, replaces the need for repetitive calibration using standard aerosols and ensures that only devices that conform to specifications are used for testing product output. The process involves the measurement and adjustment of the critical dimensions of the instrument.

C.1.2 INTERSTAGE DRUG LOSSES (WALL LOSSES)

Where method variations are possible and there is no apparatus specified in the monograph, the selected procedure should ensure that NMT 5% of the product's total delivered drug mass (into the impactor) is subject to loss between the impaction device's sample collection surfaces. In the event that interstage drug losses are known to be >5%, either the procedure should be performed in such a way that wall losses are included along with the associated collection plate, or an alternative apparatus should be used. As an example, the following procedures described for *Apparatus 1* and *3* have been written to include wall losses along with the associated collection plate. Provided, however, that such losses are known to be ≤5% of the total delivered drug mass into the impactor and that there are no instructions to the contrary in an individual monograph, the procedure may be simplified by assaying only drug on the collection plates.

C.1.3 RE-ENTRAINMENT

Where method variations are possible, the selected method should seek to minimize particle re-entrainment (from an upper to a lower impaction stage) on stages that contribute to size fractions defined in the individual monograph, especially where

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this may affect the amounts of drug collected. Minimizing the number of sampled doses, using coated particle-collection surfaces, and proving that multiple-dose procedures produce results that are statistically similar to those from smaller numbers of doses are all methods that can be used for this purpose. In the event that re-entrainment cannot be avoided, the number of doses collected, the time interval between doses, and the total duration of airflow through the cascade impaction device should be standardized. Under these circumstances, the presentation of impaction data should not presume the validity of the impactor's calibration (i.e., aerodynamic diameter ranges should not be assigned to drug masses collected on specific stages).

By using appropriate assay methods and a suitable mensurated impaction device, analysts can determine aerodynamic particle size distributions for drugs leaving the mouthpieces of inhalation aerosols and sprays or inhalation powders. If temperature or humidity limits for use of the product are stated on the label, it may be necessary to control the temperature and humidity of the air surrounding and passing through the device to conform to those limits. Ambient conditions are presumed unless otherwise specified in individual monographs.

C.1.4 MASS BALANCE

In addition to the size distribution, good analytical practice dictates that a mass balance be performed in order to confirm that the amount of the drug discharged from the product and captured and measured in the induction port through the after-filter of the cascade impactor apparatus is within an acceptable range around the measured delivered dose. The result for the recovered mass can be expressed on a per-actuation basis as a percent of the labeled delivered dose, which represents the dose delivered from the mouthpiece.

The total mass of drug collected in all of the components (material balance) divided by the total number of minimum recommended doses discharged typically is NLT 85% and NMT 115% of the target delivered label claim. This is not a test of the product but serves to ensure that the test results are valid.

Procedure

Use one of the following multistage impaction devices, or an equivalent, to determine aerodynamic particle size distributions of drugs leaving the mouthpieces of inhalation aerosols and sprays or inhalation powders. *Apparatus 1* and *6* [*Figures 6* and *11* (without pre-separator), respectively] are intended for use with inhalation aerosols and sprays at a single airflow rate. *Apparatus 2, 3, 4,* and *5* (*Figures 8, 9, 10,* and *11*, respectively) are intended for use with inhalation powders at the appropriate airflow rate, Q_{out}, determined earlier, provided that the value of Q_{out} falls in the range 30–100 L/min. [NOTE—If Q_{out} is >100 L/min, testing should be performed with Q_{out} set at 100 L/min; if Q_{out} is less than 30 L/min, testing should be performed with Q_{out} at 30 L/min.]

Table 2 indicates the apparatus used to determine aerodynamic particle size and the products that they are used to evaluate.

Table 2. Aerodynamic Particle Size Apparatus: Their Description and the Products That They Can Be Used to Evaluate

Apparatus	Description	Product
1	Andersen (8-Stage Non-Viable) Impactor (without pre-separator)	Inhalation aerosols and sprays
2	Marple-Miller Impactor	Inhalation powders
3	Andersen (8-Stage Non-Viable) Impactor (with pre-separator)	Inhalation powders
4	Multistage Liquid Impinger	Inhalation powders
5	Next Generation Impactor (with pre-separator)	Inhalation powders
6	Next Generation Impactor (without pre-separator)	Inhalation aerosols and sprays

Table 3. Cut-off Values for All Impactors at Specified Flow Rates a. Cut-Points for Apparatus 1 and 3 with Standard Configuration for Operation at 28.3 L/min Compared with Specialized Configurations for Use at 60 L/min and 90 L/min

Stage	28.3 L/min	60 L/min	90 L/min
–2	—	—	8.0
–1	—	8.6	6.5
0[a]	9.0	6.5	5.2
1	5.8	4.4	3.5
2	4.7	3.2	2.6
3	3.3	1.9	1.7
4	2.1	1.2	1.0
5	1.1	0.55	0.22
6	0.7	0.26	—
7	0.4	—	—

[a] The version of stage 0 used at 60 and 90 L/min has external modification permitting another stage rather than the inlet adapter cone to be fitted above it. Its internal characteristics and performance are unaltered.

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b. Cut-points (µm) for Apparatus 2 at 60 and 90 L/min

Stage	Flow Rate (60 L/min)	Flow Rate (90 L/min)
1	10.0	8.1
2	5.0	4.0
3	2.5	2.0
4	1.25	1.0
5	0.63	0.5

c. Cut-points (µm) for Apparatus 4 at 60 L/min

Stage	Flow Rate (60 L/min)
1	13.0
2	6.8
3	3.1
4	1.7

d. Cut-points (µm) for Apparatus 5 and 6 at 30, 60, and 100 L/min

Stage	Flow Rate (30 L/min)	Flow Rate (60 L/min)	Flow Rate (100 L/min)
1	11.76	8.06	6.12
2	6.40	4.46	3.42
3	3.97	2.82	2.18
4	2.30	1.66	1.31
5	1.36	0.94	0.72
6	0.83	0.55	0.40
7	0.54	0.34	0.24
MOC[a]	0.36	0.14	0.07

[a] MOC = Micro-orifice collector. Sizes correspond to 80% collection efficiency for this back-up stage.

C.2 Apparatus 1 for Inhalation Aerosols and Sprays—Andersen Impactor (without pre-separator)

Use *Apparatus 1*, or an equivalent, at a flow rate of 28.3 L/min (±5%) as specified by the manufacturer of the cascade impactor.

C.2.1 DESIGN—APPARATUS 1

The design and assembly of this apparatus and the induction port to connect the impactor to a product are shown in *Figures 6*, *6a*, and *6b*.[1]

[1] A suitable cascade impactor is available as Model Mk II from Thermo-Electron, 27 Forge Parkway, Franklin, MA 02038. The impactor is used without the pre-separator. The product is connected to the impactor via the induction port atop the entrance cone shown in *Figure 6*. If an equivalent impactor is employed, the induction port in *Figure 6a* should be used, although the entrance cone (*Figure 6b*) should be replaced with one to fit the impactor in question. Note that the internal surfaces of the induction port (*Figure 6a*) are designed to fit flush with their counterparts in the entrance cone (*Figure 6b*). This design avoids aerosol capture at the junction of the two pipes.

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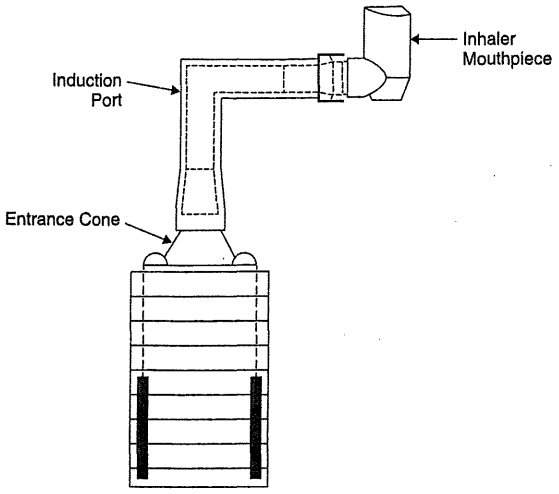


Figure 6. Apparatus 1: Assembly of induction port and entrance cone mounted on cascade impactor.

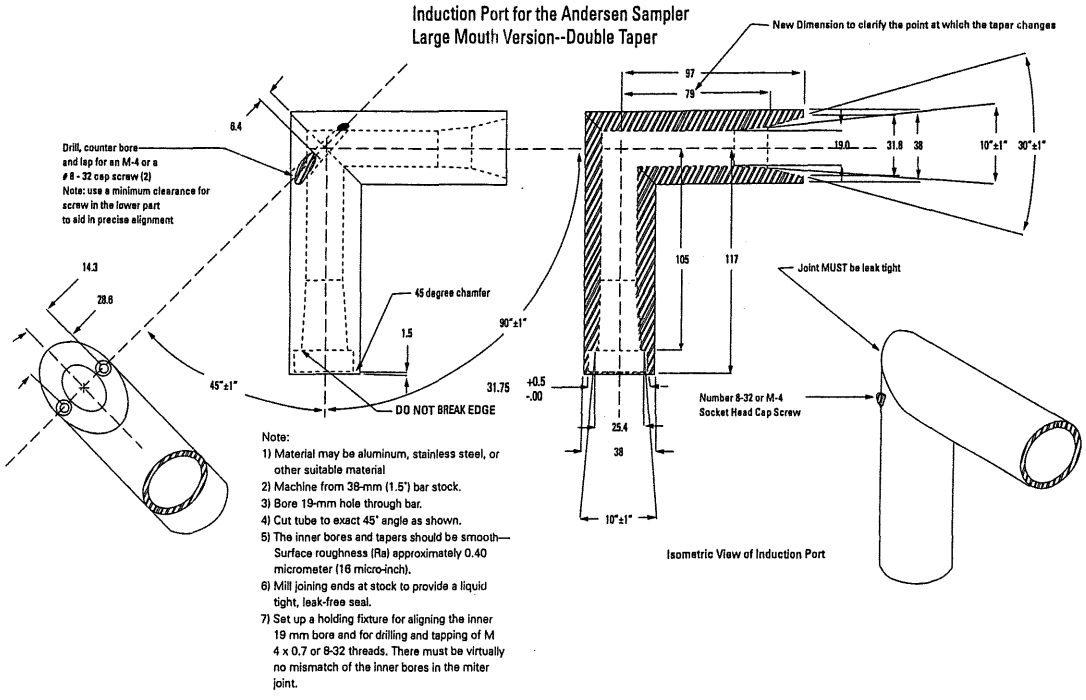


Figure 6a. Apparatus 1: Expanded view of induction port for use with inhalation aerosols and sprays and inhalation powders.

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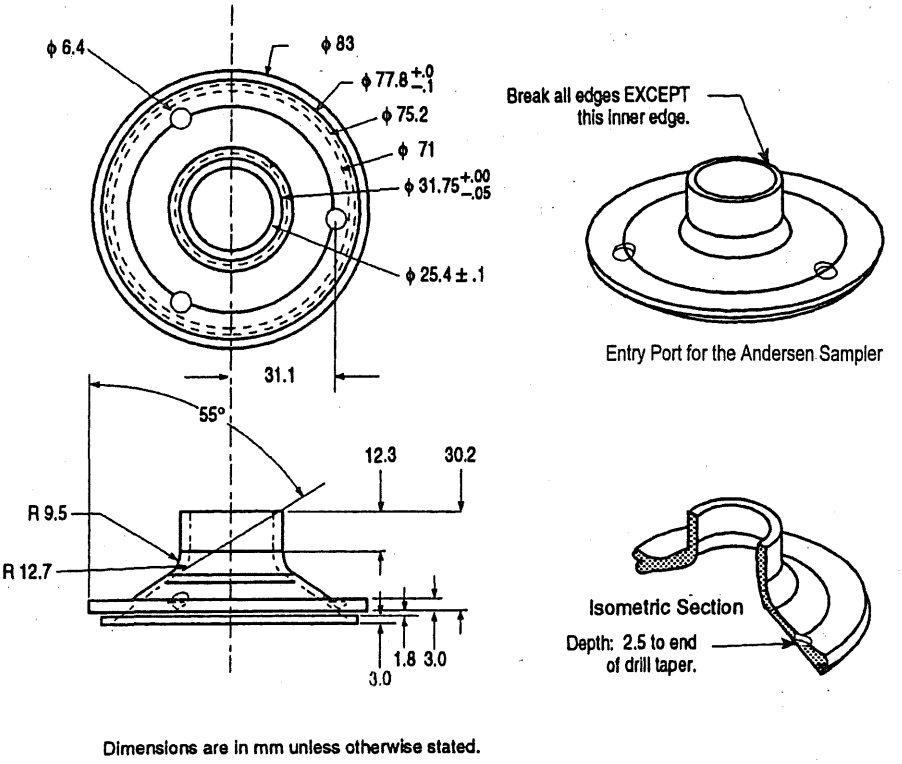


Figure 6b. Apparatus 1: Expanded view of the entrance cone for mounting induction port on the Andersen cascade impactor without pre-separator. Material can be aluminum, stainless steel, or other suitable material. Surface roughness (R_a) should be approximately 0.4 µm.

Manufacturers' critical engineering dimensions for the stages of *Apparatus 1* are provided in *Table 4*. During use, some occlusion and blockage of jet nozzles may occur, and therefore, in-use mensuration tolerances must be justified.

Table 4. Critical Dimensions for the Jet Nozzles of Apparatus 1

Stage Number	Number of Jets	Nozzle Diameter (mm)
0	96	2.55 ± 0.025
1	96	1.89 ± 0.025
2	400	0.914 ± 0.013
3	400	0.711 ± 0.013
4	400	0.533 ± 0.013
5	400	0.343 ± 0.013
6	400	0.254 ± 0.013
7	201	0.254 ± 0.013

C.2.2 PROCEDURE—APPARATUS 1

Set up the multistage cascade impactor as described in the manufacturer's literature with an after-filter below the final stage to capture any fine particles that otherwise would escape from the device. To ensure efficient particle capture, coat the particle collection surface of each stage with glycerol, silicone oil, or other suitable liquid typically deposited from a volatile solvent unless this has been demonstrated to be unnecessary. Attach the induction port and mouthpiece adapter to produce an airtight seal between the product mouthpiece and the induction port as shown in *Figure 6*. Use a mouthpiece adapter that ensures that the tip of the product's mouthpiece is flush with the open end of the induction port. Ensure that the various stages of the cascade impactor are connected with airtight seals to prevent leaks. Turn on the vacuum pump to draw air through the cascade impactor, and calibrate the airflow through the system with an appropriate flowmeter attached to the open end of the induction port. Adjust the flow-control valve on the vacuum pump to achieve steady flow through the system at the required rate, and ensure that the airflow through the system is within ±5% of the flow rate specified by the manufacturer. Unless otherwise prescribed in the patient instructions, shake the product for 5 s and discharge one delivery to waste. With the vacuum pump

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running, insert the mouthpiece into the mouthpiece adapter and immediately fire the minimum recommended dose into the cascade impactor. Keep the valve depressed for a duration sufficient to ensure that the dose has been completely discharged. If additional sprays are required for the sample, wait for 5 s before removing the product from the mouthpiece adapter, shake the product, reinsert it into the mouthpiece adapter, and immediately fire the next minimum recommended dose. Repeat until the required number of doses has been discharged. The number of minimum recommended doses discharged must be sufficient to ensure an accurate and precise determination of aerodynamic size distribution. [NOTE—The number of minimum recommended doses typically is not >10.] After the last dose has been discharged, remove the product from the mouthpiece adapter. Rinse the mouthpiece adapter and the induction port with a suitable solvent, and dilute quantitatively to an appropriate volume. Disassemble the cascade impactor, place each stage and its associated collection plate or filter in a separate container, and rinse the drug from each of them. [NOTE—If it has been determined that wall losses in the impactor are ≤5%, then only the collection plates need to be analyzed.] Dilute each quantitatively to an appropriate volume. Using the method of analysis specified in the individual monograph, determine the mass of drug collected in each of the components. To analyze the data, proceed as directed under *Data Analysis*.

C.3 Apparatus 2 for Inhalation Powders—Marple Miller Impactor

C.3.1 DESIGN—APPARATUS 2

The design and assembly of *Apparatus 2* and the induction port to connect the impactor to the product are shown in *Figure 8*.[2] [NOTE—The induction port is shown in detail in *Figure 6a*.] The impactor has five impaction stages and an after-filter. At a volumetric airflow rate of 60 L/min (the nominal flow rate, Q_n), the cutoff aerodynamic diameters $D_{50,Qn}$ of Stages 1–5 are 10, 5, 2.5, 1.25, and 0.625 µm, respectively. The after-filter effectively retains aerosolized drug in the particle size range up to 0.625 µm. Set up the multistage cascade impactor with the control system as specified in *Figure 7*. To ensure efficient particle capture, coat the particle collection surface of each stage with glycerol, silicone oil, or other suitable liquid typically deposited from a volatile solvent unless this has been demonstrated to be unnecessary. Assemble the impactor as described in the manufacturer's literature with an after-filter below the final stage to capture any fine particles that otherwise would escape from the device. Attach the induction port and mouthpiece adapter to produce an airtight seal between the product's mouthpiece and the induction port. Use a mouthpiece adapter that ensures that the tip of the product's mouthpiece is flush with the open end of the induction port. Ensure that the various stages of the cascade impactor are connected with airtight seals to prevent leaks.

Turn on the vacuum pump, open the solenoid valve, and calibrate the airflow through the system as follows. Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter to directly determine Q_{out}, or, if such a meter is unobtainable, calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For example, for a meter calibrated for the entering volumetric flow (Q_{in}), calculate:

$$Q_{out} = Q_{inP0} / (P_0 - \Delta P)$$

P_0 = atmospheric pressure

ΔP = pressure drop over the meter

Adjust the flow-control valve to achieve a steady flow through the system at the required rate, Q_{out}, so that Q_{out} is within ± 5% of the value determined during testing for *Delivered-Dose Uniformity*. Ensure that critical flow occurs in the flow-control valve at the airflow rate to be used during testing by using the following procedure. With the product in place, and the intended flow running, measure the absolute pressure on both sides of the flow-control valve (P2 and P3 in *Figure 7*). A ratio of P3/P2 ≤0.5 indicates critical flow. If this ratio of P3/P2 is not achieved, switch to a more powerful pump, and remeasure the test flow rate. Adjust the timer controlling the operation of the two-way solenoid valve so that it opens this valve for a duration such that the total volume sampled is at least 4 L. Prime or load the inhalation powder according to the labeled instructions. With the vacuum pump running and the two-way solenoid valve closed, insert the product's mouthpiece, held horizontally, into the induction port mouthpiece adapter. Discharge the powder into the apparatus by opening the two-way solenoid valve for a duration of T seconds. After the two-way solenoid valve has closed, remove the product from the mouthpiece adapter. If additional doses are required for the sample, reload the product according to the labeled instructions, reinsert the mouthpiece into the mouthpiece adapter, and repeat the operation until the required number of doses has been discharged. After discharge of the last dose, switch off the vacuum pump. Rinse the mouthpiece adapter and induction port with a suitable solvent, and quantitatively dilute to an appropriate volume. Disassemble the cascade impactor, and place the after-filter in a separate container. Rinse the drug from each of the stages and the filter, and quantitatively dilute each to an appropriate volume. Using the method of analysis specified in the individual monograph, determine the mass of drug collected in each of the components.

[2] The cascade impactor is available as Model 160 Marple Miller Impactor from MSP Corporation, Minneapolis, MN. The product should be connected to the impactor via the induction port shown in *Figure 6a*.

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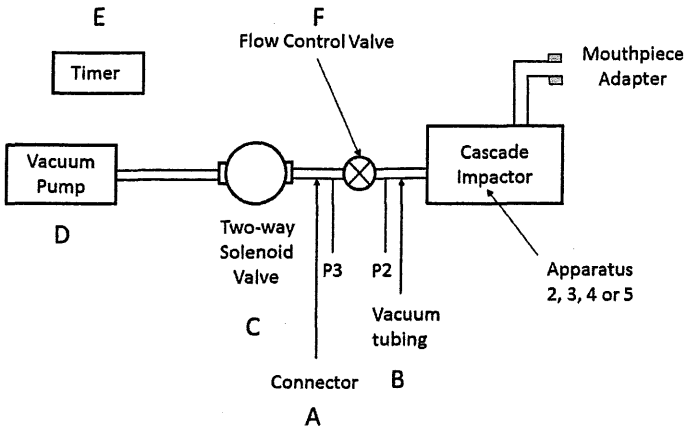


Figure 7. Apparatus 2, 3, 4, or 5: General control equipment. (See *Table 6* for component specifications.)

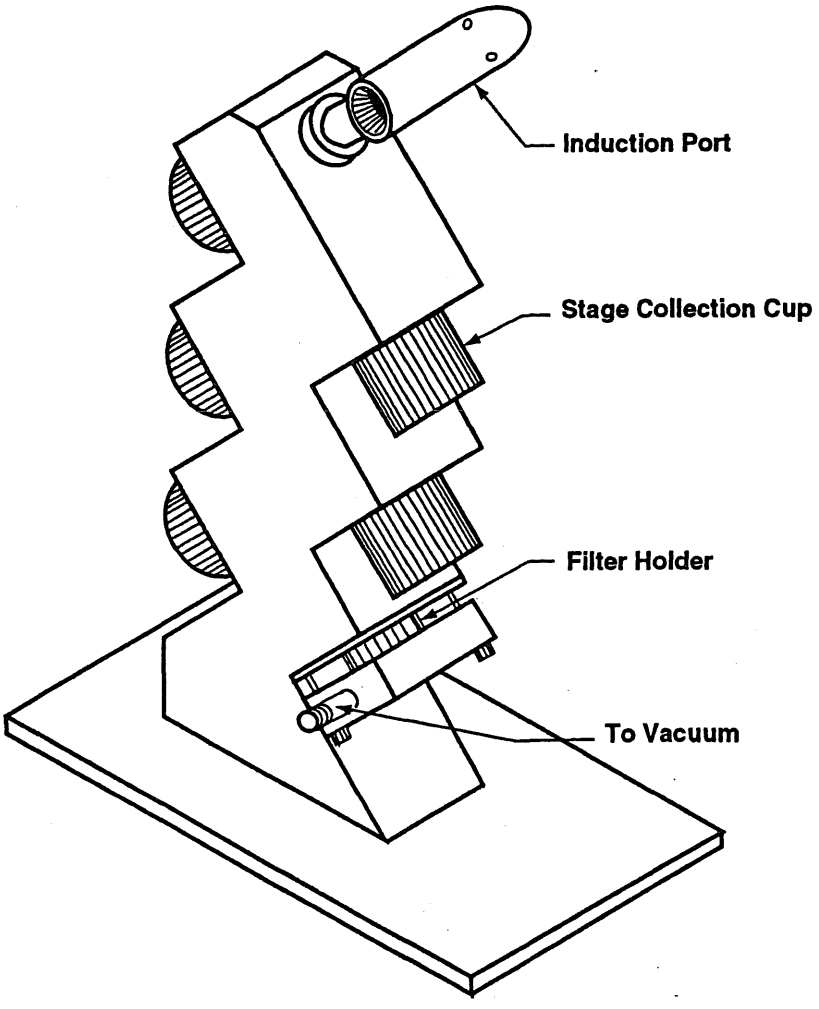


Figure 8. Apparatus 2: Assembly of induction port, stage collector, and filter holder. (Marple-Miller impactor, Model 160 with USP induction port.)

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Table 5. Critical Dimensions for the Jet Nozzles of Apparatus 2

Stage Number	Number of Jets	Nozzle Diameter (mm)
1	1	16.8 ± 0.05
2	20	3.40 ± 0.03
3	40	1.70 ± 0.01
4	80	0.84 ± 0.01
5	160	0.41 ± 0.01

C.3.2 PROCEDURE—APPARATUS 2

Perform the test using *Apparatus 2* at the airflow rate, Q_{out} determined earlier during testing for *Delivered-Dose Uniformity* provided Q_{out} is ≤100 L/min. [NOTE—If Q_{out} is >100 L/min, use an airflow rate of 100 L/min.] Connect the apparatus to a flow control system that is based on critical (sonic) flow as specified in *Figure 7* (see also *Table 6*).

Table 6. Component Specifications for *Figure 7*

Code	Item	Description	Dimensions
A	Connector	e.g., short metal coupling with low diameter branch to P3	≥8-mm ID
B	Vacuum tubing	e.g., silicon tubing with an outside diameter of 14 mm and an internal diameter of 8 mm	A length of suitable tubing ≥8 mm ID with an internal volume of 25 ± 5 mL
C	Two-way solenoid valve[a]	See *Figure 7*	2-way, 2-port solenoid valve having an ID ≥8 mm and an opening response time of ≤100 milliseconds
D	Vacuum pump[b]	See *Figure 7*	Pump must be capable of drawing the required flow rate through the assembled apparatus with the dry powder inhaler in the mouthpiece adapter. Connect the pump to the solenoid valve using short and wide (≥10-mm ID) vacuum tubing and connectors to minimize pump capacity requirements.
E	Timer[c]	See *Figure 7*	The timer switches current directly to the solenoid valve for the required duration.
P2, P3	Pressure measurements		Determined under steady-state flow conditions with an absolute pressure transducer
F	Flow control valve[d]	See *Figure 7*	Adjustable regulating valve with maximum $C_V \geq 1$

[a] An example is ASCO product number 8030G13 (Automatic Switch Company, 60 Hanover Road, Florham Park, NJ 07932) or equivalent. See also footnote *h* in *Table 1*.
[b] Gast product type 1023, 1423, or 2565 (Gast Manufacturing Inc., PO Box 97, Benton Harbor, MI 49022) or equivalent.
[c] An example is Eaton Product number 45610-400 (Eaton Corporation, Automotive Products Division, 901 South 12th Street, Watertown, WI 53094) or equivalent.
[d] Parker Hannifin type 8FV12LNSS or equivalent (Parker Hannifin plc, Riverside Road, Barnstable, Devon EX31 1NP, UK). See also footnote *h* in *Table 1*.

Under steady flow conditions at the appropriate volumetric airflow rate through the entire apparatus ensure that critical (sonic) flow occurs in the flow control valve by determining the individual values for absolute pressure, P2 and P3, so that the ratio P3/P2 is ≤0.5. If this ratio of P3/P2 is not achieved, switch to a more powerful pump, and remeasure the test flow rate. Coat the particle collection surface of each of the stages of the cascade impactor to ensure that particles that have impacted on a given stage are not re-entrained in the flowing airstream unless this has been shown to be unnecessary. Analyze the data as directed under *Data Analysis*.

C.4 Apparatus 3 for Inhalation Powders—Andersen Impactor (with pre-separator)

C.4.1 DESIGN—APPARATUS 3

Apparatus 3 is identical to *Apparatus 1* (*Figure 6*) except that the manufacturer's pre-separator is added atop Stage 0 to collect large masses of noninhalable powder prior to their entry into the impactor, and the outlet nipple used to connect to vacuum tubing B (*Figure 7*) is replaced with one having an internal diameter ≥8 mm. To connect the pre-separator of the impactor to the induction port (*Figure 6a*), use a specially designed top for the pre-separator. This is shown in *Figure 9*.[3] The impactor, therefore, has eight stages, a pre-separator (to collect large particulates), and an after-filter. A pre-separator may not be required for certain engineered powders, notably those of low bulk density. Connect the cascade impactor into the control system specified in *Figure 7*. Omit Stage 6 and Stage 7 from the impactor if the test flow rate, Q_{out}, used during testing for *Delivered-Dose Uniformity* was ≥60 L/min. To ensure efficient particle capture, coat the particle collection surface of each stage with glycerol, silicone oil, or other suitable liquid typically deposited from a volatile solvent unless this has been demonstrated to be unnecessary. Assemble the impactor as described in the manufacturer's literature with an after-filter below the final stage to

[3] The cascade impactor is available as Andersen 1ACFM Non-Viable Cascade Impactor (Mark II) from Thermo-Electron, 27 Forge Parkway, Franklin, MA 02038. The impactor is used with the pre-separator.

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capture any fine particles that otherwise would escape from the device. Place an appropriate volume (up to 10 mL) of an appropriate solvent into the pre-separator, or coat the particle collection surfaces of the pre-separator to prevent re-entrainment of impacted particles. [**CAUTION**—Some solvents form flammable vapor–air mixtures that may be ignited during passage through a vacuum pump. Take appropriate precautions (alternative solvents, use of vapor traps, minimal pump operating times, etc.) to ensure operator safety during testing.] Attach a molded mouthpiece adapter to the end of the induction port to produce an airtight seal between the product's mouthpiece and the induction port. Use a mouthpiece adapter that ensures that the tip of the product's mouthpiece is flush with the open end of the induction port. Ensure that the various stages of the cascade impactor are connected with airtight seals to prevent leaks.

Turn on the vacuum pump, open the two-way solenoid valve, and calibrate the airflow through the system as follows. Prime or load the inhalation powder according to the labeled instructions. With the vacuum pump running and the two-way solenoid valve closed, insert the product's mouthpiece, held horizontally, into the induction port mouthpiece adapter. Once the product is positioned, discharge the powder into the apparatus by activating the timer and opening the two-way solenoid valve for the required duration such that the total volume sampled is at least 4 L. After the two-way solenoid valve has closed, remove the product from the mouthpiece adapter. If additional doses are required for the sample, reload the inhalation powder according to the labeled instructions, reinsert the mouthpiece into the mouthpiece adapter, and repeat the operation until the required number of doses has been discharged. After discharge of the last dose, remove the product from the mouthpiece adapter, and switch off the vacuum pump.

Carefully disassemble the apparatus. Using a suitable solvent, rinse the drug from the mouthpiece adapter, induction port, and pre-separator, and quantitatively dilute to an appropriate volume. Rinse the drug from each stage and the impaction plate immediately below into appropriately sized flasks. Quantitatively dilute each flask to an appropriate volume. Using the method of analysis specified in the individual monograph, determine the mass of drug collected in each of the samples.

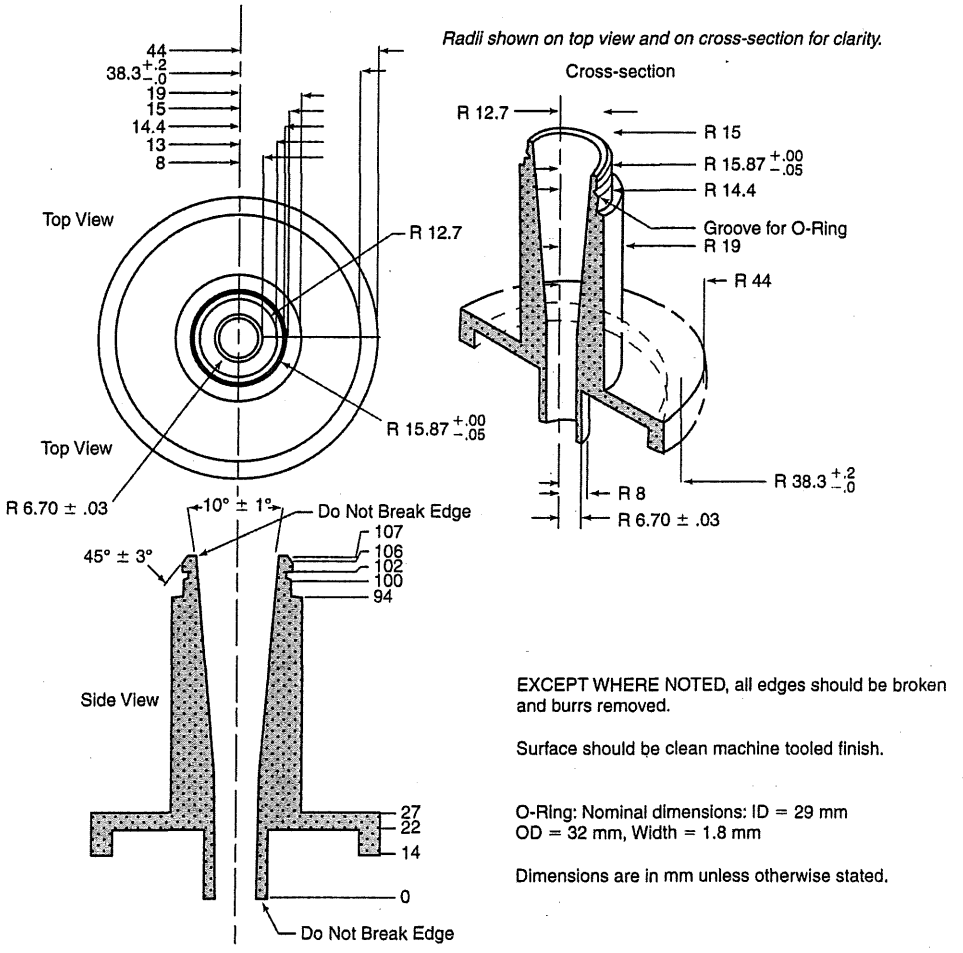


Figure 9. Apparatus 3: Expanded views of top for the Andersen pre-separator adapted to the USP induction port. Material may be aluminum, stainless steel, or other suitable material; interior bore should be polished to surface roughness (R_a) approximately 0.4 µm.

C.4.2 PROCEDURE—APPARATUS 3

Proceed as directed in the *Procedure* under *Apparatus 2*, except use *Apparatus 3*.

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C.5 Apparatus 4 for Inhalation Powders—Multistage Liquid Impinger

[NOTE—*Apparatus 4*, the multistage liquid impinger, has a small number of stages and is used extensively outside the United States. It is provided here for the benefit of users in countries other than the United States.]

C.5.1 DESIGN—APPARATUS 4

The design and assembly of *Apparatus 4* are shown in *Figures 10*, *10a*, and *10b*.[4] The induction port used to connect the impinger to a product is shown in *Figure 6a*. The device is a multistage liquid impinger consisting of impaction Stages 1, 2, 3, and 4 and an integral after-filter (Stage 5). The collection stages of the liquid impinger (see *Figure 10* and *Table 7*) are kept moist, unlike those of traditional impactors such as *Apparatus 1, 2, 3, 5,* and *6*. Wetting may produce an effect similar to coating the stages of *Apparatus 2, 3, 5,* and *6* at certain flow rates, although this should be confirmed by demonstrating control over re-entrainment as described earlier. An impaction stage comprises an upper horizontal metal partition wall (B) through which a metal inlet jet tube (A) with its impaction plate (D) is protruding; a glass cylinder (E) with sampling port (F), forming the vertical wall of the stage; and a lower horizontal metal partition wall (G) through which a jet tube (H) connects to the lower stage. The tube into Stage 4 (U) ends in a multi-jet arrangement. The impaction plate (D) is secured in a metal frame (J), which is fastened by two wires (K) to a sleeve (L) secured on the jet tube (C). For more detail of the jet tube and impaction plate, see *Figure 10a*. The horizontal plane of the collection plate is perpendicular to the axis of the jet tube and is centrally aligned. The upper surface of the impaction plate is slightly raised above the edge of the metal frame. A recess around the perimeter of the horizontal partition wall guides the position of the glass cylinder. The glass cylinders are sealed against the horizontal partition walls with gaskets (M) and are clamped together by six bolts (N). The sampling ports are sealed by stoppers. The bottom side of the lower partition wall of Stage 4 has a concentric protrusion fitted with a rubber O-ring (P) that seals against the edge of a filter placed in the filter holder. The filter holder (R) is a basin with a concentric recess in which a perforated filter support (S) is flush-fitted. The filter holder is designed for 76-mm diameter filters. The whole impaction stage assembly is clamped onto the filter holder by two snap locks (T). The impinger is equipped with an induction port (*Figure 6a*) that fits onto the Stage 1 inlet jet tube. A rubber O-ring on the jet tube provides an airtight connection to the induction port. An elastomeric mouthpiece adapter to fit the product being tested provides an airtight seal between the product's mouthpiece and the induction port.

Ensure that *Apparatus 4* is clean and free of drug solution from any previous tests. Place a 76-mm diameter filter in the filter stage, and assemble the apparatus. Use a low-pressure filter capable of quantitatively collecting the passing drug aerosol, which also allows a quantitative recovery of the collected drug. Set up *Apparatus 4* using the control system as specified in *Figure 7*. Attach the induction port (*Figure 6a*) and mouthpiece adapter to produce an airtight seal between the product mouthpiece and the induction port. Use a mouthpiece adapter that ensures that the tip of the product's mouthpiece is flush with the open end of the induction port. Ensure that the various stages of the apparatus are connected with airtight seals to prevent leaks. Turn on the vacuum pump, open the two-way solenoid valve, and calibrate the airflow through the system as follows. Connect a flowmeter calibrated for the volumetric flow rate leaving the meter to the induction port. Adjust the flow-control valve to achieve a steady flow through the system at the required rate, Q_{out}, so that Q_{out} is within ±5% of the value determined during testing for *Delivered-Dose Uniformity*. Ensure that critical flow occurs in the flow-control valve at the value of Q_{out} that will be used during testing, using the following procedure. With the product in place and the intended flow running, measure the absolute pressure on both sides of the flow-control valve (P2 and P3 in *Figure 7*). A ratio of P3/P2 ≤0.5 indicates critical flow. If this ratio of P3/P2 is not achieved, switch to a more powerful pump, and remeasure the test flow rate. Adjust the timer controlling the operation of the two-way solenoid valve so that it opens that valve for the same duration, *T*, as used during testing for *Delivered-Dose Uniformity*. Dispense 20 mL of a solvent that is capable of dissolving the drug into each of the four upper stages of *Apparatus 4*, and replace the stoppers. [**CAUTION**—Some solvents form flammable vapor–air mixtures that may be ignited during passage through a vacuum pump. Take appropriate precautions (alternative solvents, use of vapor traps, minimal pump operating times, etc.) to ensure operator safety during testing.] Tilt the apparatus to wet the stoppers, thereby neutralizing their electrostatic charge. Adjust the timer controlling the operation of the two-way solenoid valve so that it opens the valve for the duration such that the total volume sampled is at least 4 L. Prime or load the inhalation powder according to the labeled instructions. With the vacuum pump running and the two-way solenoid valve closed, insert the product's mouthpiece, held horizontally, into the induction port mouthpiece adapter. Discharge the powder into the apparatus by activating the timer and opening the two-way solenoid valve for the required duration, T ± 5%. After the two-way solenoid valve has closed, remove the product from the mouthpiece adapter. If additional doses are required for the sample, reload the inhalation powder according to the labeled instructions, reinsert the mouthpiece into the mouthpiece adapter, and repeat the operation until the required number of doses has been discharged. After discharge of the last dose, switch off the vacuum pump.

Dismantle the filter stage of *Apparatus 4*. Carefully remove the filter (*Figure 10b*), and extract the drug with solvent. Rinse the mouthpiece adapter and induction port with a suitable solvent, and quantitatively dilute to an appropriate volume. Rinse the inside of the inlet jet tube to Stage 1 (*Figure 10*), allowing the solvent to flow into the stage. Rinse the drug from the inner walls and the collection plate of each of the four upper stages of the apparatus into the solution in the respective stage by tilting and rotating the apparatus while ensuring that no liquid transfer occurs between the stages. Using the method of analysis specified in the individual monograph, determine the mass of drug collected in each of the six volumes of solvent. Ensure that the method corrects for possible evaporation of the solvent during the test. This may involve the use of an internal standard (of known original concentration in the solvent and assayed at the same time as the drug) or the quantitative transfer of the liquid contents from each of the stages, followed by dilution to a known volume. Using the method of analysis specified in the individual monograph, determine the mass of drug collected in each of the samples.

[4] The five-stage impinger is available from Copley Instruments, plc, Nottingham, UK. The product should be connected to the impactor via the induction port shown in *Figure 8* and *Figure 6a*.

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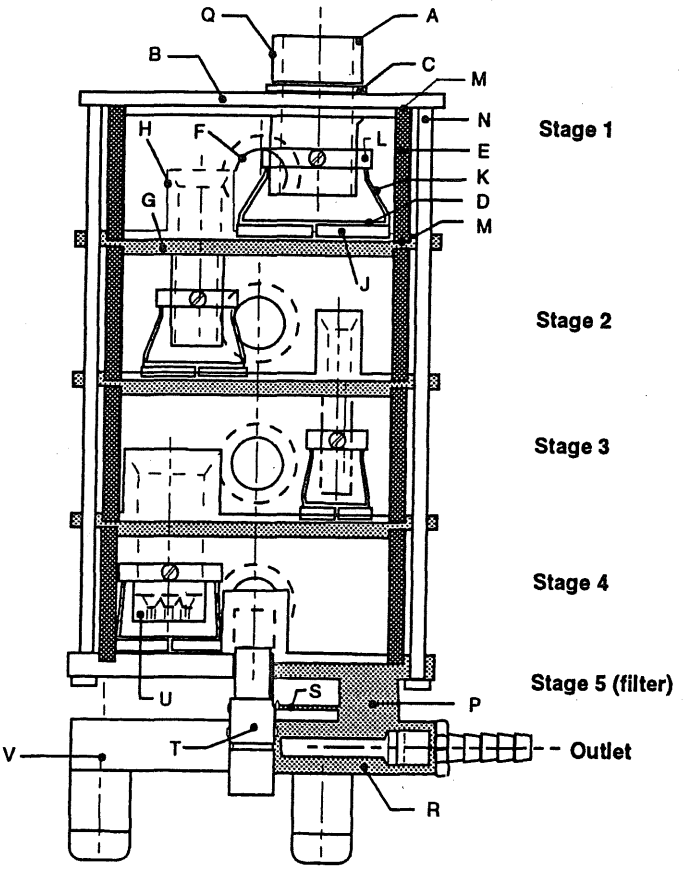


Figure 10. Apparatus 4: Schematic of multistage liquid impinger. (See *Table 7* for component specifications.)

Table 7. Component Units of Multistage Liquid Impinger (see *Figure 10*, *Figure 10a*, and *Figure 10b*)

Code	Item	Description	Dimensions (in mm unless otherwise stated)
A, H	Jet tube	Metal tube screwed onto partition wall sealed by gasket (C), polished inner surface	See *Figure 10a*
B, G	Partition wall	Circular metal plate, diameter	120
		Thickness	See *Figure 10a*
C	Gasket	e.g., PTFE	To fit jet tube
D	Impaction plate	Porosity O sintered-glass disk	
		Diameter	See *Figure 10a*
E	Glass cylinder	Plane polished cut glass tube	
		Height, including gaskets	46
		Outer diameter	100
		Wall thickness	3.5
		Sampling port (F) diameter	18
		Stopper in sampling port	ISO 24/25
J	Metal frame	L-Profiled circular frame with slit	
		Inner diameter	To fit impaction plate
		Height	4
		Thickness of horizontal section	0.5
		Thickness of vertical section	2

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Table 7. Component Units of Multistage Liquid Impinger (see *Figure 10*, *Figure 10a*, and *Figure 10b*) *(continued)*

Code	Item	Description	Dimensions (in mm unless otherwise stated)
K	Wire	Steel wire interconnecting metal frame and sleeve (two for each frame)	
		Diameter	1
L	Sleeve	Metal sleeve secured on jet tube by screw	
		Inner diameter	To fit jet tube
		Height	6
		Thickness	5
M	Gasket	e.g., silicone	To fit glass cylinder
N	Bolt	Metal bolt with nut (six pairs), length	205
		Diameter	4
P	O-ring	Rubber O-ring, diameter × thickness	66.34 × 2.62
Q	O-ring	Rubber O-ring, diameter × thickness	29.1 × 1.6
R	Filter holder	Metal housing with stand and outlet	See *Figure 10b*
S	Filter support	Perforated sheet metal, diameter	65
		Hole diameter	3
		Distance between holes (center-points)	4
T	Snap-locks		
U	Multi-jet tube	Jet tube (H) ending in multi-jet arrangement	See inserts *Figure 10a*
V	Outlet	Outlet and nozzle for connection to vacuum	Internal diameter ≥10 *(Figure 10b)*

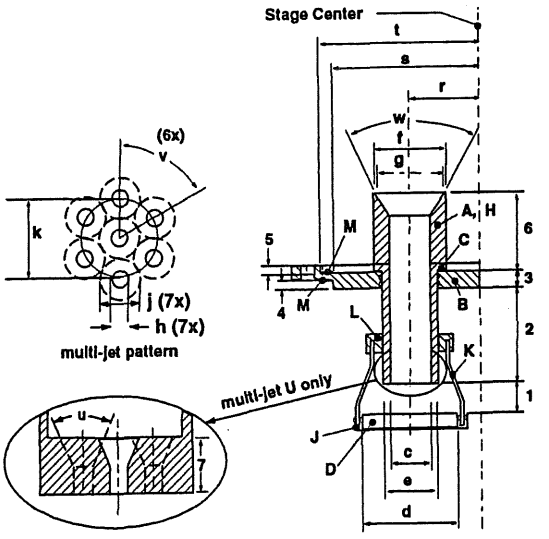


Figure 10a. Apparatus 4: Details of jet tube and impaction plate. Inserts show end of multi-jet tube (U) leading to Stage 4. (See *Table 8* for dimension specifications.)

Table 8. Apparatus 4—Dimensions of Multi-stage Liquid Impinger

Stage	Number of Nozzles	Diameter (mm)	
		Nominal Value	Tolerance (±)

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Table 8. Apparatus 4—Dimensions of Multi-stage Liquid Impinger *(continued)*

Stage		Number of Nozzles	Diameter (mm)	
1		1	25.0	0.1[a]
2		1	14.0	0.1[a]
3		1	8.0	0.1[a]
4	Inlet	7	6.3[a]	0.1[a]
	Outlet		2.7[a]	0.05[a]

[a] Courtesy of Copley Scientific Ltd,. Nottingham, UK.

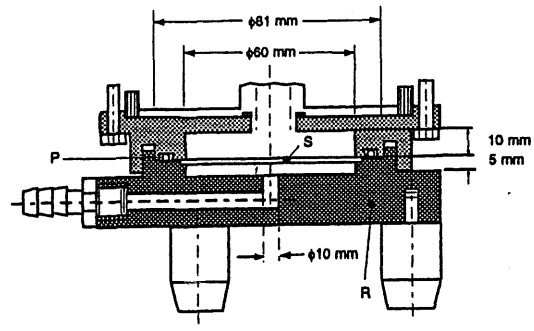


Figure 10b. Apparatus 4: Expanded view of Stage 5. (See *Table 7* for component specifications.)

C.5.2 PROCEDURE—APPARATUS 4

Proceed as directed in the *Procedure* under *Apparatus 2*, except to use *Apparatus 4*.

C.6 Apparatus 5 for Inhalation Powders—Next Generation Impactor (with pre-separator)

C.6.1 DESIGN—APPARATUS 5

The design and assembly of *Apparatus 5*[5] are shown in Figures *11*, *11a*, *11b*, *11c*, and *11d*. The induction port used to connect the impactor to a product is shown in *Figure 6a*. The device is a cascade impactor with seven stages and a micro-orifice collector (MOC). The collection efficiency curves for each stage are sharp and minimize overlap between stages. Material can be aluminum, stainless steel, or other suitable material.

The impactor layout has removable impaction cups with all the cups in one plane (*Figures 11–11c*). There are three main sections to the impactor: the bottom frame that holds the impaction cups, the seal body that holds the jets, and the lid that contains the interstage passageways (shown in *Figures 11–11b*). Multiple nozzles are used at all but the first stage (*11c*). The flow passes through the impactor in a saw-tooth pattern.

Stage mensuration is performed periodically together with confirmation of other dimensions critical to the effective operation of the impactor. Critical dimensions are provided in *Table 9*.

Table 9. Critical Dimensions for Apparatus 5 and 6

Description	Dimension (mm)
Pre-separator (see *Dimension a* in *Figure 11d*)	12.80 ± 0.05
Stage 1[a] nozzle diameter	14.30 ± 0.05
Stage 2[a] nozzle diameter	4.88 ± 0.04
Stage 3[a] nozzle diameter	2.185 ± 0.02
Stage 4[a] nozzle diameter	1.207 ± 0.01
Stage 5[a] nozzle diameter	0.608 ± 0.01

[5] The cascade impactor is available as Next Generation Pharmaceutical Impactor from MSP Corporation, Minneapolis, MN.

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Table 9. Critical Dimensions for Apparatus 5 and 6 *(continued)*

Stage 6[a] nozzle diameter	0.323 ± 0.01
Stage 7[a] nozzle diameter	0.206 ± 0.01
MOC[a]	Approximately 0.070
Optional cup depth (see *Dimension b* in *Figure 11b*)	14.625 ± 0.10
Collection cup surface roughness	0.5–2 µm
Stage 1 nozzle to seal body distance (see *Dimension c*)[b]	0 ± 1.18
Stage 2 nozzle to seal body distance (see *Dimension c*)[b]	5.236 ± 0.736
Stage 3 nozzle to seal body distance (see *Dimension c*)[b]	8.445 ± 0.410
Stage 4 nozzle to seal body distance (see *Dimension c*)[b]	11.379 ± 0.237
Stage 5 nozzle to seal body distance (see *Dimension c*)[b]	13.176 ± 0.341
Stage 6 nozzle to seal body distance (see *Dimension c*)[b]	13.999 ± 0.071
Stage 7 nozzle to seal body distance (see *Dimension c*)[b]	14.000 ± 0.071
Optional: MOC nozzle to seal body distance[b]	14.429–14.571

[a] See *Figure 11c*. MOC = Micro-orifice collector.
[b] See *Figure 11b*.

In routine operation, the seal body and lid are held together as a single assembly. The impaction cups are accessible when this assembly is opened at the end of a product test. The cups are held in a support tray so that all cups can be removed from the impactor simultaneously by lifting out the tray.

An induction port with internal dimensions identical to those defined in *Figure 6a* is connected to the impactor inlet. When necessary with inhalation powders, a pre-separator can be added to avoid overloading the first stage. This pre-separator connects between the induction port and the impactor. A suitable mouthpiece adapter is used to provide an airtight seal between the product's mouthpiece and the induction port.

The apparatus contains a terminal MOC that for most formulations may eliminate the need for a final filter if this capability is demonstrated by method validation. The MOC is an impactor nozzle plate and collection cup. The nozzle plate contains, nominally, 4032 jets, each approximately 70 µm in diameter. Most particles not captured on Stage 7 of the impactor will be captured on the cup surface below the MOC. There is an optional filter holder that can replace the MOC for formulations with a significant fraction of particles not captured by the MOC. Alternatively, an after-filter (glass fiber is often suitable) can be placed in a filter holder external to *Apparatus 5* and *6*, which is located downstream of the MOC.

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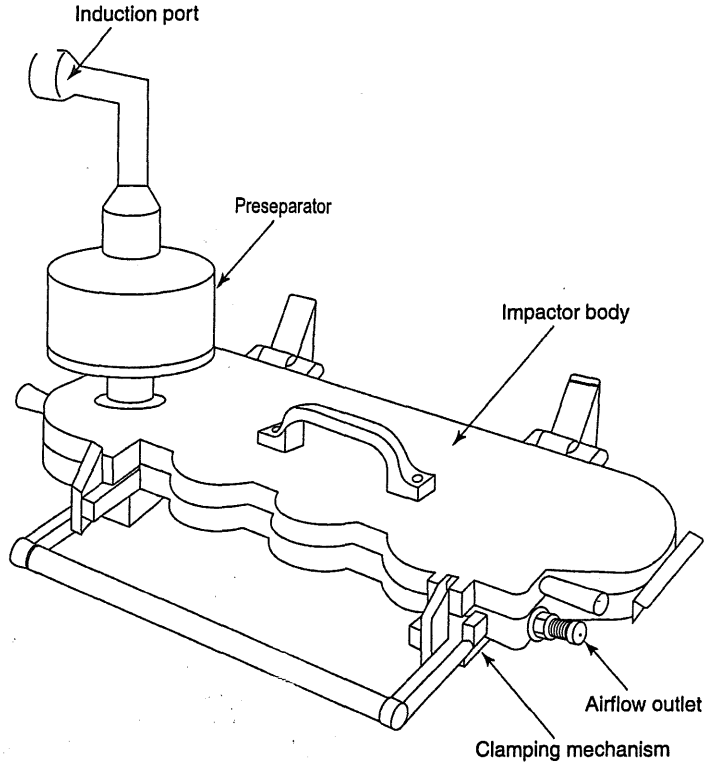


Figure 11. Apparatus 5 (shown with the pre-separator in place).

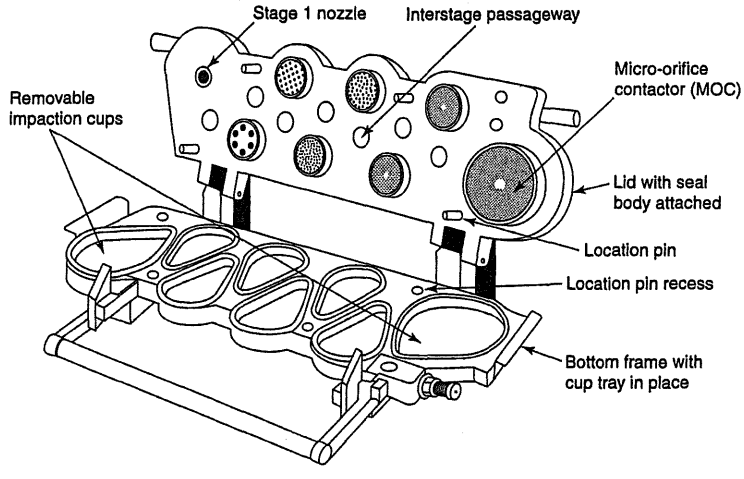


Figure 11a. Components of Apparatus 5.

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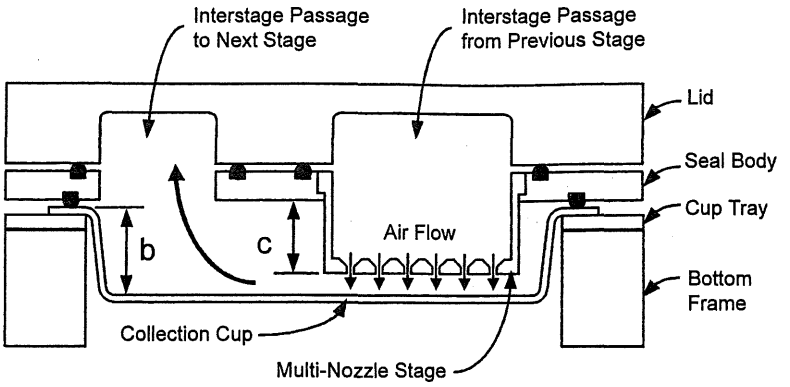


Figure 11b. Layout of interstage passageways of Apparatus 5.

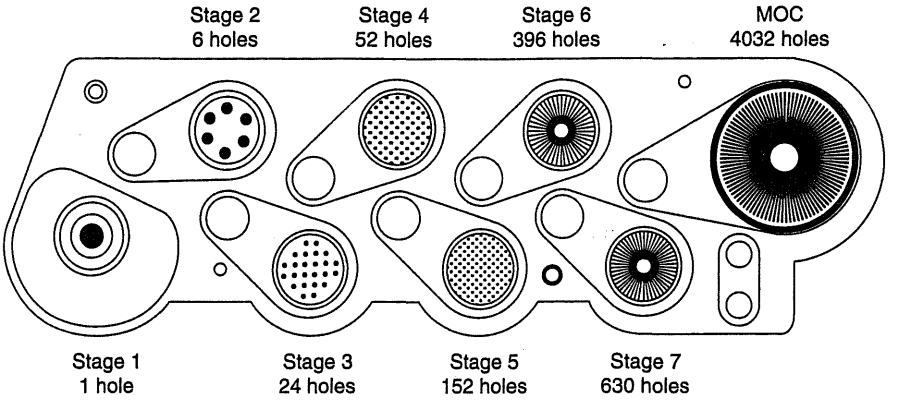


Figure 11c. Nozzle configuration of Apparatus 5.

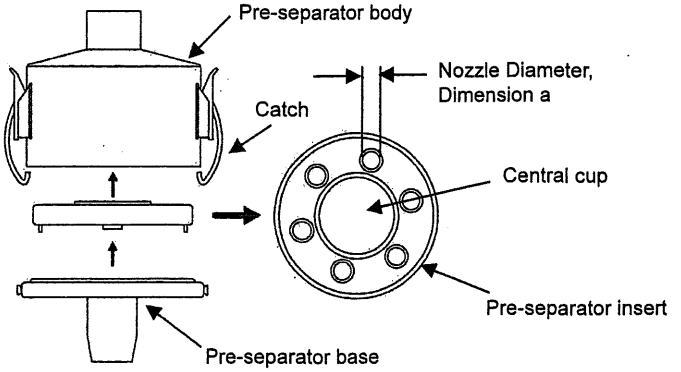


Figure 11d. Pre-separator layout for Apparatus 5.

C.6.2 PROCEDURE—APPARATUS 5

Assemble the apparatus with the pre-separator (*Figure 11d*) unless experiments have shown that its omission does not result in increased interstage drug losses (>5%) or particle re-entrainment, in which case the pre-separator can be omitted. Place cups into the apertures in the cup tray. To ensure efficient particle capture, coat the particle collection surface of each stage with glycerol, silicone oil, or other suitable liquid typically deposited from a volatile solvent, unless it has been demonstrated to be unnecessary. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached, and operate the handle to lock the impactor together so that the system is airtight.

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The pre-separator can be assembled as follows. Assemble the pre-separator insert into the pre-separator base; fit the pre-separator base to the impactor inlet; add up to about 15 mL of the solvent used for sample recovery to the central cup of the pre-separator insert; place the pre-separator body on top of this assembly; and close the two catches. [**CAUTION**—Some solvents form flammable vapor–air mixtures that can be ignited during passage through a vacuum pump. Take appropriate precautions (e.g., alternative solvents, use of vapor traps, minimal pump operating times, etc.) to ensure operator safety during testing.]

Connect an induction port with internal dimensions as defined in *Figure 6a* either to the impactor inlet or to the pre-separator inlet atop the cascade impactor (*Figure 11d*). Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the product, when inserted, lines up along the horizontal axis of the induction port. The front face of the product's mouthpiece is flush with the front face of the induction port, producing an airtight seal. When attached to the mouthpiece adapter, the product should be positioned in the same orientation as intended for use. Connect the apparatus to a flow system according to the scheme specified in *Figure 7*.

Unless otherwise prescribed, conduct the test at the flow rate used in the test for *Delivered-Dose Uniformity* drawing 4.0 L of air from the mouthpiece of the product and through the apparatus. Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), calculate:

$$Q_{out} = Q_{in}P_0/(P_0 - \Delta P)$$

P_0 = atmospheric pressure

ΔP = pressure drop over the meter

Adjust the flow-control valve to achieve steady flow through the system at the required rate, Q_{out} (±5%). Ensure that critical flow occurs in the flow-control valve by the *Procedure* described for *Apparatus 2*. Adjust the timer controlling the operation of the two-way solenoid valve so that it opens this valve for a duration such that the total volume sampled is at least 4 L.

Prime or load the inhalation powder according to the labeled instructions. With the vacuum pump running and the two-way solenoid valve closed, insert the product's mouthpiece, held horizontally, into the induction port mouthpiece adapter. Discharge the powder into the apparatus by activating the timer and opening the two-way solenoid valve for the required duration, T (±5%). After the two-way solenoid valve has closed, remove the product from the mouthpiece adapter. If additional doses are required for the sample, reload the inhalation powder according to the labeled instructions, reinsert the mouthpiece into the mouthpiece adapter, and repeat the operation until the required number of doses have been discharged. After discharge of the last dose, switch off the vacuum pump.

Dismantle the apparatus, and recover drug for analysis as follows. Remove the induction port and mouthpiece adapter from the pre-separator, and extract the drug into an aliquot of solvent; if used, remove the pre-separator from the impactor without spilling the solvent into the impactor; and recover the active ingredient from all inner surfaces. Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and recover the active ingredient from each cup into an aliquot of solvent. Using the method of analysis specified in the individual monograph, determine the mass of drug contained in each of the aliquots of solvent.

C.7 Apparatus 6 for Inhalation Aerosol and Sprays—Next Generation Impactor (without pre-separator)

C.7.1 DESIGN—APPARATUS 6

Apparatus 6 is identical to *Apparatus 5* (*Figures 11–11d*) except that the pre-separator is not used. Use this apparatus at a flow rate of 30 L/min (±5%) unless otherwise prescribed in the individual monograph.

C.7.2 PROCEDURE—APPARATUS 6

Assemble the apparatus without the pre-separator. Place cups into the apertures in the cup tray. To ensure efficient particle capture, coat the particle collection surface of each stage with glycerol, silicone oil, or other suitable liquid typically deposited from a volatile solvent, unless this has been demonstrated to be unnecessary. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached, and operate the handle to lock the impactor together so that the system is airtight. Connect an induction port with internal dimensions as defined in *Figure 6a* to the impactor inlet. Use a mouthpiece adapter that ensures that the tip of the product's mouthpiece is flush with the open end of the induction port. Turn on the vacuum pump to draw air through the cascade impactor, and calibrate the airflow through the system with an appropriate flowmeter attached to the open end of the induction port. Adjust the flow-control valve on the vacuum pump to achieve steady flow through the system at the required rate, and ensure that the airflow through the system is within ±5% of this flow rate. Unless otherwise prescribed in the patient instructions, shake the product for 5 s, and discharge one delivery to waste. With the vacuum pump running, insert the mouthpiece into the mouthpiece adapter, and immediately fire the minimum recommended dose into the cascade impactor. Keep the valve depressed for a duration sufficient to ensure that the dose has been completely discharged. If additional sprays are required for the sample, shake the product, reinsert it into the mouthpiece adapter, and immediately fire the next minimum recommended dose.

Repeat until the required number of doses has been discharged. The number of minimum recommended doses discharged must be sufficient to ensure an accurate and precise determination of *Aerodynamic Size Distribution*. [NOTE—The number of minimum recommended doses typically is not >10.] After the last dose has been discharged, remove the product from the mouthpiece adapter. Rinse the mouthpiece adapter and induction port with a suitable solvent, and dilute quantitatively to an appropriate volume.

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Dismantle the apparatus, and recover the drug for analysis as follows. Remove the induction port and mouthpiece adapter from the apparatus, and recover the deposited drug into an aliquot of solvent; open the impactor by releasing the handle and lifting the lid; remove the cup tray with the collection cups; and extract the active ingredient in each cup into an aliquot of solvent. Using the method of analysis specified in the individual monograph, determine the quantity of active ingredient contained in each of the aliquots of solvent.

⟨602⟩ PROPELLANTS

[**CAUTION**—Hydrocarbon propellants are highly flammable and explosive. Observe precautions, and perform sampling and analytical operations in a well-ventilated fume hood.]

GENERAL SAMPLING PROCEDURE

This procedure is used to obtain test specimens for those propellants that occur as gases at about 25° and that are stored in pressurized cylinders. Use a stainless steel sample cylinder equipped with a stainless steel valve with a capacity of NLT 200 mL and a pressure rating of 240 psi or more. Dry the cylinder with the valve open at 110° for 2 h, and evacuate the hot cylinder to less than 1 mm of mercury. Close the valve, cool, and weigh. Connect one end of a charging line tightly to the propellant container and the other end loosely to the sample cylinder. Carefully open the propellant container, and allow the propellant to flush out the charging line through the loose connection. Avoid excessive flushing, which causes moisture to freeze in the charging line and connections. Tighten the fitting on the sample cylinder, and open the sample cylinder valve, allowing the propellant to flow into the evacuated cylinder. Continue sampling until the desired amount of specimen is obtained, then close the propellant container valve, and finally close the sample cylinder valve.

[**CAUTION**—Do not overload the sample cylinder. Hydraulic expansion due to temperature change can cause overloaded cylinders to explode.]

Again weigh the charged sample cylinder, and calculate the weight of the specimen.

APPROXIMATE BOILING TEMPERATURE

Transfer a 100-mL specimen to a tared, pear-shaped, 100-mL centrifuge tube containing a few boiling stones, and weigh. Suspend a thermometer in the liquid, and place the tube in a medium maintained at a temperature of 32° above the expected boiling temperature. When the thermometer reading becomes constant, record as the boiling temperature the thermometer reading after at least 5% of the specimen has distilled. Retain the remainder of the specimen for the determination of high-boiling residues.

HIGH-BOILING RESIDUES, METHOD I

Allow 85 mL of the specimen to distill as directed in the test for *Approximate Boiling Temperature*, and transfer the centrifuge tube containing the remaining 15 mL of specimen to a medium maintained at a temperature 10° above the boiling temperature. After 30 min, remove the tube from the water bath, blot dry, and weigh. Calculate the weight of the residue.

HIGH-BOILING RESIDUES, METHOD II

Prepare a cooling coil from copper tubing (about 6 mm outside diameter × about 6.1 m long) to fit into a vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and acetone in a vacuum-jacketed flask, and connect one end of the tubing to the propellant sample cylinder. Carefully open the sample cylinder valve, flush the cooling coil with about 50 mL of the propellant, and discard this portion of liquefied propellant. Continue delivering liquefied propellant from the cooling coil, and collect it in a previously chilled 1000-mL sedimentation cone until the cone is filled to the 1000-mL mark. Allow the propellant to evaporate, using a warm water bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-mL portions of pentane, and combine the rinsings in a tared 150-mL evaporating dish. Transfer 100 mL of the pentane solvent to a second tared 150-mL evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 min. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-min periods until successive weighings are within 0.1 mg, and calculate the weight of the residue obtained from the propellant as the difference between the weights of the residues in the two evaporating dishes.

WATER CONTENT

Proceed as directed in *Water Determination* ⟨921⟩, with the following modifications: (a) Provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sampling cylinder. (b) Dilute the *Reagent* with anhydrous methanol to give a water equivalence factor of between 0.2 and 1.0 mg/mL, and age this diluted solution for NLT 16 h before standardization. (c) Obtain a 100-g specimen as directed under *General Sampling Procedure*, and introduce the specimen into the titration vessel through the gas dispersion tube at a rate of about 100 mL of gas per min. If necessary, heat the sample cylinder gently to maintain this flow rate.

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OTHER DETERMINATIONS

For those aerosols that use propellants, perform the tests specified in the individual *NF* propellant monographs.

⟨603⟩ TOPICAL AEROSOLS

INTRODUCTION

Topical aerosol products contain the drug in solution or in suspension, packaged under pressure, and released upon activation of an appropriate valve system. The topical aerosol products should follow the product quality test requirements described in general test chapters *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* ⟨5⟩, *Microbial Enumeration Tests* ⟨61⟩, *Tests for Specified Organisms* ⟨62⟩, *Minimum Fill* ⟨755⟩, and *Leak Rate* ⟨604⟩. The topical aerosols include dermatological foams and sprays. Topical aerosol foams should include a physical appearance of both the foam and also of the collapsed foam.

DELIVERY RATE AND DELIVERED AMOUNT

Perform these tests only on containers fitted with continuous valves.

Delivery Rate: Select NLT four aerosol containers; shake, if the label includes this directive; remove the caps and covers; and actuate each valve for 2–3 s. Weigh each container accurately, and immerse in a constant-temperature bath until the internal pressure is equilibrated at a temperature of 25° as determined by constancy of internal pressure, as directed under the *Pressure Test* below. Remove the containers from the bath; remove excess moisture by blotting with a paper towel; shake, if the label includes this directive; actuate each valve for 5.0 s (accurately timed by use of a stopwatch); and weigh each container again. Return the containers to the constant-temperature bath, and repeat the foregoing procedure three times for each container. Calculate the average *Delivery Rate*, in g/s, for each container.

Delivered Amount: Return the containers to the constant-temperature bath, continuing to deliver 5 s actuations to waste, until each container is exhausted. [NOTE—Ensure that sufficient time is allowed between each actuation to avoid significant canister cooling.] Calculate the total weight loss from each container. This is the *Delivered Amount*.

PRESSURE TEST

Perform this test only on topical aerosols fitted with continuous valves.

Select NLT four aerosol containers, remove the caps and covers, and immerse in a constant-temperature bath until the internal pressure is constant at a temperature of 25°. Remove the containers from the bath, shake, and remove the actuator and water, if any, from the valve stem. Place each container in an upright position, and determine the pressure in each container by placing a calibrated pressure gauge on the valve stem, holding firmly, and actuating the valve so that it is fully open. The gauge is of a calibration approximating the expected pressure and is fitted with an adapter appropriate for the particular valve stem dimensions. Read the pressure directly from the gauge.

MINIMUM FILL

Topical aerosols meet the requirements for aerosols in *Minimum Fill* ⟨755⟩.

LEAKAGE TEST

Proceed as directed in *Leak Rate* ⟨604⟩.

NUMBER OF DISCHARGES PER CONTAINER

Perform this test only on topical aerosols fitted with dose-metering valves, at the same time as, and on the same containers used for, the test for *Delivered-Dose Uniformity*. Determine the number of discharges or deliveries by counting the number of priming discharges plus those used in determining the spray contents, and continue to fire until the label claim number of discharges. The requirements are met if all the containers or inhalers tested contain NLT the number of discharges stated on the label.

DELIVERED-DOSE UNIFORMITY

The test for *Delivered-Dose Uniformity* is required for topical aerosols fitted with dose-metering valves. For collection of the minimum dose, proceed as directed in *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩, *A. Delivered-Dose Uniformity/A.2 Inhalation Aerosols and Inhalation Sprays*, and *A.4 Inhalation Powders*, as described

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in ⟨601⟩, except modify the dose sampling apparatus so that it is capable of quantitatively capturing the delivered dose from the preparation being tested.

⟨604⟩ LEAK RATE

Select 12 aerosol containers, and record the date and time to the nearest half hour. Weigh each container to the nearest mg, and record the weight, in mg, of each as W_1. Allow the containers to stand in an upright position at a temperature of 25.0 ± 2.0° for not less than 3 days, and again weigh each container, recording the weight, in mg, of each as W_2 and recording the date and time to the nearest half hour. Determine the time, T, in hours, during which the containers were under test. Calculate the leakage rate, in mg per year, of each container taken by the formula:

$(365)(24/T)(W_1 - W_2)$.

Where plastic-coated glass aerosol containers are tested, dry the containers in a desiccator for 12–18 h, and allow them to stand in a constant-humidity environment for 24 h prior to determining the initial weight as indicated above. Conduct the test under the same constant-humidity conditions. Empty the contents of each container tested by employing any safe technique (e.g., chill to reduce the internal pressure, remove the valve, and pour). Remove any residual contents by rinsing with suitable solvents, then rinse with a few portions of methanol. Retain as a unit the container, the valve, and all associated parts, and heat them at 100° for 5 min. Cool, weigh, record the weight as W_3, and determine the net fill weight ($W_1 - W_3$) for each container tested. [NOTE—If the average net fill weight has been determined previously, that value may be used in place of the value ($W_1 - W_3$) above.] The requirements are met if the average leakage rate per year for the 12 containers is not more than 3.5% of the net fill weight, and none of the containers leaks more than 5.0% of the net fill weight per year. If 1 container leaks more than 5.0% per year, and if none of the containers leaks more than 7.0% per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 5.0% of the net fill weight per year, and none of the 36 containers leaks more than 7.0% of the net fill weight per year. Where the net fill weight is less than 15 g and the label bears an expiration date, the requirements are met if the average leakage rate of the 12 containers is not more than 525 mg per year and none of the containers leaks more than 750 mg per year. If 1 container leaks more than 750 mg per year but not more than 1.1 g per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 750 mg per year, and none of the 36 containers leaks more than 1.1 g per year. This test is in addition to the customary in-line leak testing of each container.

⟨610⟩ ALTERNATIVE MICROBIOLOGICAL SAMPLING METHODS FOR NONSTERILE INHALED AND NASAL PRODUCTS

INTRODUCTION

Proper microbiological sampling of microbiologically susceptible nonsterile products can be difficult because these products are often filled into unique primary containers that are designed to protect the product from inadvertent contamination during storage and use. These unique designs may increase the difficulty of taking an aseptic sample of sufficient size or volume for microbiological testing. Unless special approaches are used, products such as inhaled, nasal liquid, or powder dosage forms can be difficult to sample without potential exposure to extraneous microbial contamination. This general test chapter provides these special approaches for sampling either low- or high-content inhaled or nasal dosage forms. Alternative sampling approaches may provide better ways to sample containers in an aseptic manner. Any alternative methodology should employ aseptic techniques and should be conducted under environmental and other conditions that are appropriate for aseptic sampling.

INHALED OR NASAL DOSAGE FORMS

Low-content inhaled and nasal drug products (low-content INDP) are products that have a target fill of less than 100 mg of powder or 1 mL of liquid formulation per unit (primary container). Examples are pre-metered inhalation powders, more commonly known as dry powder inhalers (DPIs), and single-dose nasal sprays.

High-content INDP are multidose drug products that have a target fill of more than 100 mg of powder or more than 1 mL of liquid formulation per unit. Examples are aerosols for inhalation and nasal delivery, known as metered-dose inhalers (MDIs); device-metered inhalation powders; and multidose nasal sprays.

The appropriate sample quantity or volume should be based on the test methodology, including any relevant general test chapters, such as ⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* and ⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*. Testing may be performed on the unpackaged bulk dry powder or liquid formulation or the finished product. If testing is performed on the bulk material alone, then the process leading from the bulk to the finished product should be validated for its ability to prevent microbial contamination. Testing should be performed on the finished product if this process is not validated.

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SAMPLE SIZE DETERMINATION

For each microbiological test, sample 10 drug product containers or units or a number of units that can provide a minimum of 1 gram of product that are representative of the batch. For batch sizes smaller than 200 units (e.g., batches used in clinical trials), sample size may be reduced to 1% of the units or 1 unit, whichever is greater. The contents of individual containers may be pooled for testing.

Bulk Testing for Low-Content INDP

Bulk lot testing may be preferable for low-content INDP in lieu of finished product testing to allow larger sample sizes that are representative of the batch, without unduly increasing the risk of inadvertent microbial contamination. Bulk testing can be performed on the bulk powder or liquid formulation just before filling. If bulk testing is performed in lieu of finished product testing, then manufacturing processes following bulk sampling (e.g., filling and packaging) must be validated in accordance with current good manufacturing practice (CGMP) for their ability to prevent microbial contamination. For microbial enumeration tests, at least 10 g or 10 mL of bulk material, or, for specified microorganisms tests, 1 g or 1 mL of bulk material may be sampled. For small batch sizes (i.e., less than 1000 g or 1000 mL), the recommended sample size is 1% of the batch for both microbial enumeration and specified microorganisms tests.

SAMPLING METHODS FOR HIGH-CONTENT INDP

Dry Powder Inhalers

DPIs have an internal reservoir that contains a sufficient quantity of formulation for multiple doses that are metered by the device itself during activation by the patient. For DPIs, appropriate validated procedures should be used to sample a nonsterile drug product container.

Inhalation Aerosols

Consider safety issues related to both inhalation of the drug substance and the potential of a flammability hazard. Avoid contamination of samples by employing aseptic techniques whenever necessary.

AUTOMATIC ACTUATION METHOD

The contents of the inhalation aerosol containers may be collected by automatically actuating each aerosol container and collecting the delivered formulation on a suitable sterile filter.

ROOM TEMPERATURE METHOD

Disinfect the outside of the test containers with an appropriate disinfectant, and allow the containers to dry in a controlled environment. Empty the contents of the aerosol container into a sterile vessel using a needle apparatus or similar device (e.g., icemaker water line tap). If it has been demonstrated that the propellant does not inhibit the growth of microorganisms, the contents of the sterile vessel may be added directly to the liquid media or buffer for the test. Otherwise, allow the propellant to evaporate from the vessel by leaving the vessel at room temperature for several minutes. Remove any residual gaseous propellant by tilting the vessel slightly or by allowing a slow stream of microbiologically inert sterile gas to pass over the surface. For some less volatile propellants such as chlorofluorocarbon (CFC) 11/12 combinations, the vessel may be heated slightly (to temperatures ≤ 45°) to assist with evaporation. After the propellant has evaporated, add the liquid media or buffer, and mix the contents to prepare for testing.

Direct expulsion into the broth media or buffer may be feasible if a needle apparatus that is thin and strong enough to puncture the container and to allow slow removal of the contents is available. In this case, the contents may be expelled into and mixed with the aqueous medium. Layering of the propellant and aqueous medium may occur, in which case a longer time period and slight heating (not to exceed 45°) may be required for propellant evaporation.

CHILLING METHOD

Place the disinfected aerosol containers in dry ice, or a dry ice slurry (ensure the microbial quality of the dry ice and slurry-forming liquid), or a cryofreezer for the period required to liquefy the contents. Disinfect the outside of the test containers with an appropriate disinfectant, and allow the containers to dry in an aseptic environment. Aseptically open the aerosol containers using an appropriate tool. Be aware that freezing can affect the viability of microorganisms. For CFC-based products, pour the contents of the containers into sterile vessels. Allow the propellant to escape, and combine residues with a diluent appropriate for the drug product. Other drug-specific procedures may also be employed. For hydrofluoroalkane-based products, pour the contents of the containers into sterile vessels partially immersed in larger vessels containing dry ice. Drive off the propellant, for example by allowing a slow stream of sterile, filtered, oil-free compressed air to evaporate the material to dryness. Combine residues with a diluent appropriate for the drug product. An alternative procedure when testing the entire contents of a previously chilled container is to pour the contents of the opened container onto a sterile membrane filtration unit, allow the propellant to escape, and then rinse with an appropriate amount of sterile diluent.

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Multidose Nasal Sprays

Multidose nasal spray containers usually have a cap that is screwed on, crimped on, or forms a snap-fit. The container can be opened by unscrewing the cap, cutting the seal, or using a decrimping tool, taking care to avoid microbial contamination during the process. Following cap removal, traditional sampling methods as described in general test chapters such as ⟨61⟩ *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* and ⟨62⟩ *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* are typically appropriate for use.

⟨611⟩ ALCOHOL DETERMINATION

PROCEDURES

Method I—Distillation Method

Method I is to be used for the determination of alcohol, unless otherwise specified in the individual monograph. It is suitable for examining most fluidextracts and tinctures, provided the capacity of the distilling flask is sufficient (commonly two to four times the volume of the liquid to be heated) and the rate of distillation is such that clear distillates are produced. Cloudy distillates may be clarified by agitation with talc, or with calcium carbonate, and filtered, after which the temperature of the filtrate is adjusted and the alcohol content determined from the specific gravity. During all manipulations, take precautions to minimize the loss of alcohol by evaporation.

Treat liquids that froth to a troublesome extent during distillation by rendering them strongly acidic with phosphoric, sulfuric, or tannic acid, or treat with a slight excess of calcium chloride solution or with a small amount of paraffin or silicone oil before starting the distillation.

Prevent bumping during distillation by adding porous chips of insoluble material such as silicon carbide, or beads.

For liquids presumed to contain 30% of alcohol or less: By means of a pipet, transfer to a suitable distilling apparatus not less than 25 mL of the liquid in which the alcohol is to be determined, and note the temperature at which the volume was measured. Add an equal volume of water, distill, and collect a volume of distillate about 2 mL less than the volume taken of the original test liquid, adjust to the temperature at which the original test liquid was measured, add sufficient water to measure exactly the original volume of the test liquid, and mix. The distillate is clear or not more than slightly cloudy, and does not contain more than traces of volatile substances other than alcohol and water. Determine the specific gravity of the liquid at 25°, as directed under *Specific Gravity* ⟨841⟩, using this result to ascertain the percentage, by volume, of C_2H_5OH contained in the liquid examined by reference to the *Alcoholometric Table* in the section *Reference Tables*.

For liquids presumed to contain more than 30% of alcohol: Proceed as directed in the foregoing paragraph, except to do the following: dilute the specimen with about twice its volume of water, collect a volume of distillate about 2 mL less than twice the volume of the original test liquid, bring to the temperature at which the original liquid was measured, add sufficient water to measure exactly twice the original volume of the test liquid, mix, and determine its specific gravity. The proportion of C_2H_5OH, by volume, in this distillate, as ascertained from its specific gravity, equals one-half that in the liquid examined.

Special Treatment

Volatile acids and bases: Render preparations containing volatile bases slightly acidic with diluted sulfuric acid before distilling. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS.

Glycerin: To liquids that contain glycerin add sufficient water so that the residue, after distillation, contains not less than 50% of water.

Iodine: Treat all solutions containing free iodine with powdered zinc before the distillation, or decolorize with just sufficient sodium thiosulfate solution (1 in 10), followed by a few drops of sodium hydroxide TS.

Other volatile substances: Spirits, elixirs, tinctures, and similar preparations that contain appreciable proportions of volatile materials other than alcohol and water, such as volatile oils, chloroform, ether, camphor, etc., require special treatment, as follows:

For liquids presumed to contain 50% of alcohol or less: Mix 25 mL of the specimen under examination, accurately measured, with about an equal volume of water in a separator. Saturate this mixture with sodium chloride, then add 25 mL of solvent hexane, and shake the mixture to extract the interfering volatile ingredients. Draw off the separated, lower layer into a second separator, and repeat the extraction twice with two further 25-mL portions of solvent hexane. Extract the combined solvent hexane solutions with three 10-mL portions of a saturated solution of sodium chloride. Combine the saline solutions, and distill in the usual manner, collecting a volume of distillate having a simple ratio to the volume of the original specimen.

For liquids presumed to contain more than 50% of alcohol: Adjust the specimen under examination to a concentration of approximately 25% of alcohol by diluting it with water, then proceed as directed in *For liquids presumed to contain 50% of alcohol or less,* beginning with "Saturate this mixture with sodium chloride."

In preparing *Collodion* or *Flexible Collodion* for distillation, use water in place of the saturated solution of sodium chloride directed above.

If volatile oils are present in small proportions only, and a cloudy distillate is obtained, the solvent hexane treatment not having been employed, the distillate may be clarified and rendered suitable for the specific gravity determination by shaking it with about one-fifth its volume of solvent hexane, or by filtering it through a thin layer of talc.

Method II—Gas Chromatographic Method

Use *Method IIa* when *Method II* is specified in the individual monograph. For a discussion of the principles upon which it is based, see *Gas Chromatography* under *Chromatography* ⟨621⟩.

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• **METHOD IIA**

Apparatus: Under typical conditions, use a gas chromatograph equipped with a flame-ionization detector and a 4-mm × 1.8-m glass column packed with 100- to 120-mesh chromatographic column packing support S3, using nitrogen or helium as the carrier. Prior to use, condition the column overnight at 235° with a slow flow of carrier gas. The column temperature is maintained at 120°, and the injection port and detector temperatures are maintained at 210°. Adjust the carrier flow and temperature so that acetonitrile, the internal standard, elutes in 5 to 10 minutes.

Solutions

Test stock preparation: Dilute the specimen under examination stepwise with water to obtain a solution containing approximately 2% (v/v) of alcohol.

Test preparation: Pipet 5 mL each of the *Test stock preparation* and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 50-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation: Pipet 5 mL each of the USP Alcohol Determination—Alcohol RS and the USP Alcohol Determination—Acetonitrile RS. [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure: Inject about 5 µL each of the *Test preparation* and the *Standard preparation,* in duplicate, into the gas chromatograph, record the chromatograms, and determine the peak response ratios. Calculate the percentage of alcohol (v/v) in the specimen under test according to the formula:

$$\text{Result} = CD(R_U/R_S)$$

C = labeled concentration of USP Alcohol Determination—Alcohol RS
D = dilution factor (the ratio of the volume of the *Test stock preparation* to the volume of the specimen taken)
R_U = peak response ratio obtained from the *Test preparation*
R_S = peak response ratio obtained from the *Standard preparation*

System suitability test: In a suitable chromatogram, the resolution factor, R, is not less than 2; the tailing factor of the alcohol peak is not greater than 2.0; and six replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0% in the ratio of the peak of alcohol to the peak of the internal standard.

• **METHOD IIB**

Apparatus: The gas chromatograph is equipped with a split injection port with a split ratio of 5:1, a flame-ionization detector, and a 0.53-mm × 30-m capillary column coated with a 3.0-µm film of phase G43. Helium is used as the carrier gas at a linear velocity of 34.0 cm per second. The chromatograph is programmed to maintain the column temperature at 50° for 5 minutes, then to increase the temperature at a rate of 10° per minute to 200°, and maintain at this temperature for 4 minutes. The injection port temperature is maintained at 210° and the detector temperature at 280°.

Solutions

Test stock preparation: Dilute the specimen under examination stepwise with water to obtain a solution containing approximately 2% (v/v) of alcohol.

Test preparation: Pipet 5 mL each of the *Test stock preparation* and the USP Alcohol Determination—Acetonitrile RS [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 25-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation: Pipet 5 mL each of the USP Alcohol Determination—Alcohol RS and the USP Alcohol Determination—Acetonitrile RS. [NOTE—Alternatively, a 2% aqueous solution of acetonitrile of suitable quality may be used as the internal standard solution] into a 25-mL volumetric flask, dilute with water to volume, and mix.

Procedure: Inject about 0.2 to 0.5 µL each of the *Test preparation* and the *Standard preparation,* in duplicate, into the gas chromatograph, record the chromatograms, and determine the peak response ratios. Calculate the percentage of alcohol (v/v) in the specimen under test according to the formula:

$$\text{Result} = CD(R_U/R_S)$$

C = labeled concentration of USP Alcohol Determination—Alcohol RS
D = dilution factor (the ratio of the volume of the *Test stock preparation* to the volume of the specimen taken)
R_U = peak response ratio obtained from the *Test preparation*
R_S = peak response ratio obtained from the *Standard preparation*

System suitability test: In a suitable chromatogram, the resolution factor, R, between alcohol and the internal standard is not less than 4; the tailing factor of the alcohol peak is not greater than 2.0; and six replicate injections of the *Standard preparation* show a relative standard deviation of not more than 4.0% in the ratio of the peak of alcohol to the peak of the internal standard.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Alcohol Determination—Acetonitrile RS
USP Alcohol Determination—Alcohol RS

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⟨616⟩ BULK DENSITY AND TAPPED DENSITY OF POWDERS

BULK DENSITY

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. •The portion that is not harmonized is marked with symbols (•,) to specify this fact.•

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per mL (g/mL) although the international unit is kilograms per cubic meter (1 g/mL = 1000 kg/m^3) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm^3). The bulking properties of a powder are dependent upon the preparation, treatment, and storage of the sample, i.e., how it was handled. The particles can be packed to have a range of bulk densities; however, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made. The bulk density of a powder is determined by measuring the volume of a known weight of powder sample, that may have been passed through a sieve, into a graduated cylinder (*Method I*), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (*Method II*) or a measuring vessel (*Method III*).

Method I and *Method III* are favored.

Method I—Measurement in a Graduated Cylinder

PROCEDURE

Pass a quantity of material sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated 250-mL cylinder (readable to 2 mL) introduce, without compacting, approximately 100 g of test sample, M, weighed with 0.1% accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in g/mL by the formula m/V_0. Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as the test sample, such that its untapped apparent volume is 150–250 mL (apparent volume greater than or equal to 60% of the total volume of the cylinder); the weight of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL, a 100-mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Method II—Measurement in a Volumeter

APPARATUS

The apparatus (*Figure 1*) consists of a top funnel fitted with a 1.0-mm sieve.[1] The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup of specified capacity mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or cubical (16.39 ± 0.20 mL volume with inside dimensions of 25.400 ± 0.076 mm).

[1] The apparatus (the Scott Volumeter) conforms to the dimensions in ASTMB329–06(2012).

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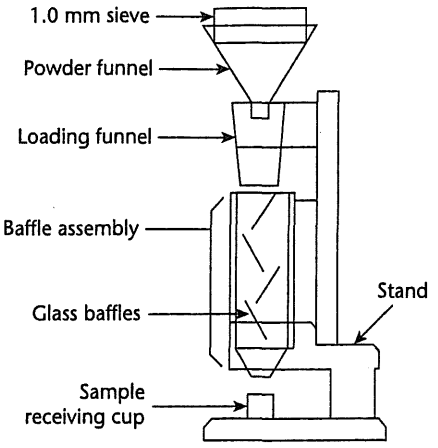


Figure 1.

PROCEDURE—Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm^3 of powder with the square cup and 35 cm^3 of powder with the cylindrical cup. Carefully scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the sides of the cup, and determine the weight, *M*, of the powder to the nearest 0.1%. Calculate the bulk density, in g/mL, by the formula:

$$(M)/(V_0)$$

in which V_0 is the volume, in mL, of the cup. Record the average of three determinations using three different powder samples.

Method III—Measurement in a Vessel

APPARATUS

The apparatus consists of a 100-mL cylindrical vessel of stainless steel with dimensions as specified in *Figure 2*.

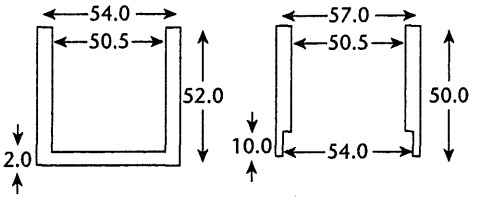


Figure 2.

PROCEDURE

Pass a quantity of powder sufficient to complete the test through a 1.0-mm sieve, if necessary, to break up agglomerates that may have formed during storage, and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for *Method II*. Determine the weight (M_0) of the powder to the nearest 0.1% by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula $M_0/100$, and record the average of three determinations using three different powder samples.

TAPPED DENSITY

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. Tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing a powder sample. After observing the initial powder volume or weight, the measuring cylinder or vessel is mechanically tapped, and volume or weight readings are taken until little further volume or weight change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop under its own weight a specified distance by either of three methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

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Method I

APPARATUS

The apparatus (*Figure 3*) consists of the following:

- A 250-mL graduated cylinder (readable to 2 mL with a mass of 220 ± 44 g)
- A settling apparatus capable of producing, in 1 min, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

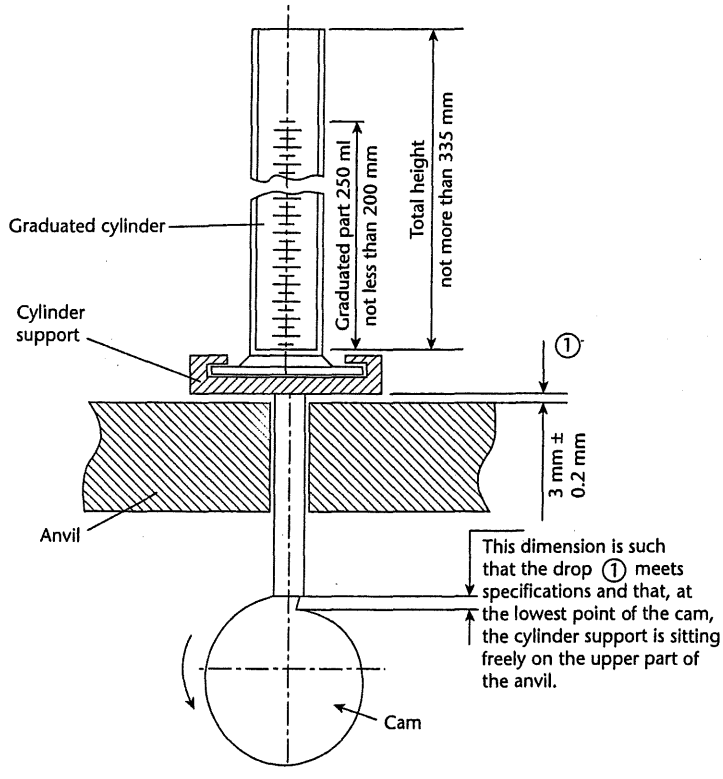


Figure 3.

PROCEDURE

Proceed as described above for the determination of the bulk volume (V_0). Secure the cylinder in the holder. Carry out 10, 500, and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500}, and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than or equal to 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_F, in which V_F is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results. If it is not possible to use a 100-g test sample, use a reduced amount and a suitable 100-mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. If the difference between V_{500} and V_{1250} is less than or equal to 1 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 1 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 1 mL. The modified test conditions are specified in the expression of the results.

Method II

APPARATUS and PROCEDURE

Proceed as directed under *Method I* except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per min.

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Method III

APPARATUS and PROCEDURE

Proceed as directed in *Method III—Measurement in a Vessel* for measuring bulk density using the measuring vessel equipped with the cap shown in *Figure 2*. The measuring vessel with the cap is lifted 50–60 times per min by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap, and carefully scrape excess powder from the top of the measuring vessel as described in *Method III—Measurement in a Vessel* for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the two masses obtained after 200 and 400 taps exceeds 2%, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2%. Calculate the tapped density (g/mL) using the formula $M_F/100$, where M_F is the mass of powder in the measuring vessel. Record the average of three determinations using three different powder samples. The test conditions including tapping height are specified in the expression of the results.

MEASURES OF POWDER COMPRESSIBILITY

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the *Compressibility Index* or the *Hausner Ratio* as described below.

The *Compressibility Index* and *Hausner Ratio* are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder's ability to settle, and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the *Compressibility Index* and the *Hausner Ratio*.

Compressibility Index

Calculate by the formula:

$$100(V_0 - V_F)/V_0$$

V_0 = unsettled apparent volume
V_F = final tapped volume

Hausner Ratio

$$V_0/V_F$$

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0. [NOTE—If V_{10} is used, it will be clearly stated in the results.]

⟨621⟩ CHROMATOGRAPHY

INTRODUCTION

Chromatographic separation techniques are multistage separation methods in which the components of a sample are distributed between two phases, of which one is stationary and the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, distributed as a film, or applied by other techniques. The mobile phase may be in a gaseous or liquid form, or a supercritical fluid. The separation may be based on adsorption, mass distribution (partition), or ion exchange; or it may be based on differences among the physicochemical properties of the molecules, such as size, mass, and volume. This chapter contains general procedures, definitions, and calculations of common parameters and describes general requirements for system suitability. The types of chromatography useful in qualitative and quantitative analyses employed in *USP* procedures are column, gas (GC), paper, thin-layer (TLC) [including high-performance thin-layer chromatography (HPTLC)], and pressurized liquid chromatography [commonly called high-pressure or high-performance liquid chromatography (HPLC)].

GENERAL PROCEDURES

This section describes the basic procedures used when a chromatographic method is described in a monograph. These procedures are followed unless otherwise indicated in the individual monograph.

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Paper Chromatography

STATIONARY PHASE

The stationary phase is a sheet of paper of suitable texture and thickness. Development may be ascending, in which the solvent is carried up the paper by capillary forces, or descending, in which the solvent flow is also assisted by gravitational force. The orientation of paper grain, with respect to solvent flow, is to be kept constant in a series of chromatograms. The machine direction is usually designated by the manufacturer.

APPARATUS

The essential equipment for paper chromatography consists of a vapor-tight chamber with inlets for the addition of solvent and a rack of corrosion-resistant material about 5 cm shorter than the inside height of the chamber. The rack serves as a support for solvent troughs and antisiphon rods that, in turn, hold up the chromatographic sheets. The bottom of the chamber is covered with the prescribed solvent system or mobile phase. Saturation of the chamber with solvent vapor is facilitated by lining the inside walls with paper wetted with the prescribed solvent system.

SPOTTING

The substance or substances analyzed are dissolved in a suitable solvent. Convenient volumes delivered from suitable micropipettes of the resulting solution, normally containing 1–20 µg of the compound, are placed in 6- to 10-mm spots NLT 3 cm apart.

DESCENDING PAPER CHROMATOGRAPHY PROCEDURE

1. A spotted chromatographic sheet is suspended in the apparatus, using the antisiphon rod to hold the upper end of the sheet in the solvent trough. [NOTE—Ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack, the chamber walls, or the fluid in the chamber.]
2. The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
3. After equilibration of the chamber, the prepared mobile phase is introduced into the trough through the inlet.
4. The inlet is closed, and the mobile solvent phase is allowed to travel the desired distance down the paper.
5. The sheet is removed from the chamber.
6. The location of the solvent front is quickly marked, and the sheet is dried.
7. The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated drug or drugs.

ASCENDING PAPER CHROMATOGRAPHY PROCEDURE

1. The mobile phase is added to the bottom of the chamber.
2. The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with the solvent vapor. Any excess pressure is released as necessary.
3. The lower edge of the stationary phase is dipped into the mobile phase to permit the mobile phase to rise on the chromatographic sheet by capillary action.
4. When the solvent front has reached the desired height, the chamber is opened, the sheet is removed, the location of the solvent front is quickly marked, and the sheet is dried.
5. The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated drug or drugs.

Thin-Layer Chromatography

STATIONARY PHASE

The stationary phase is a relatively thin, uniform layer of dry, finely powdered material applied to a glass, plastic, or metal sheet or plate (typically called the plate). The stationary phase of TLC plates has an average particle size of 10–15 µm, and that of HPTLC plates has an average particle size of 5 µm. Commercial plates with a preadsorbent zone can be used if they are specified in a monograph. The sample applied to the preadsorbent region develops into sharp, narrow bands at the preadsorbent–sorbent interface. The separations achieved may be based on adsorption, partition, or a combination of both effects, depending on the particular type of stationary phase.

APPARATUS

A chromatographic chamber made of inert, transparent material and having the following specifications is used: a flat-bottom or twin trough, a tightly fitted lid, and a size suitable for the plates. The chamber is lined on at least one wall with filter paper. Sufficient mobile phase or developing solvent is added to the chamber so that, after impregnation of the filter paper, a depth appropriate to the dimensions of the plate used is available. The chromatographic chamber is closed and allowed to equilibrate. [NOTE—Unless otherwise indicated, the chromatographic separations are performed in a saturated chamber.]

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DETECTION/VISUALIZATION

An ultraviolet (UV) light source suitable for observations under short- (254 nm) and long- (365 nm) wavelength UV light and a variety of other spray reagents, used to make spots visible, is often used.

SPOTTING

Solutions are spotted on the surface of the stationary phase (plate) at the prescribed volume in sufficiently small portions to obtain circular spots of 2–5 mm in diameter (1–2 mm on HPTLC plates) or bands of 10–20 mm × 1–2 mm (5–10 mm × 0.5–1 mm on HPTLC plates) at an appropriate distance from the lower edge and sides of the plate. [NOTE—During development, the application position must be at least 5 mm (TLC) or 3 mm (HPTLC) above the level of the mobile phase.] The solutions are applied on a line parallel to the lower edge of the plate with an interval of at least 10 mm (5 mm on HPTLC plates) between the centers of spots or 4 mm (2 mm on HPTLC plates) between the edges of bands, then allowed to dry.

PROCEDURE

1. Place the plate in the chamber, ensuring that the spots or bands are above the surface of the mobile phase.
2. Close the chamber.
3. Allow the mobile phase to ascend the plate until the solvent front has traveled three-quarters of the length of the plate, or the distance prescribed in the monograph.
4. Remove the plate, mark the solvent front with a pencil, and allow to dry.
5. Visualize the chromatograms as prescribed.
6. Determine the chromatographic *Retardation factor* (R_F) values for the principal spots or zones.
7. Presumptive identification can be made by observation of spots or zones of identical R_F value and about equal magnitude obtained, respectively, with an unknown and a standard chromatographed on the same plate. A visual comparison of the size or intensity of the spots or zones may serve for semiquantitative estimation. Quantitative measurements are possible by means of densitometry (absorbance or fluorescence measurements).

Column Chromatography

SOLID SUPPORT

Purified siliceous earth is used for normal-phase separation. Silanized chromatographic siliceous earth is used for reverse-phase partition chromatography.

STATIONARY PHASE

The solid support is modified by the addition of a stationary phase specified in the individual monograph. If a mixture of liquids is used as the stationary phase, mix the liquids before the introduction of the solid support.

MOBILE PHASE

The mobile phase is specified in the individual monograph. If the stationary phase is an aqueous solution, equilibrate with water. If the stationary phase is a polar organic fluid, equilibrate with that fluid.

APPARATUS

Unless otherwise specified in the individual monograph, the chromatographic tube is about 22 mm in its inside diameter and 200–300 mm long. Attached to it is a delivery tube, without stopcock, about 4 mm in its inside diameter and about 50 mm long.

Apparatus preparation: Pack a pledget of fine glass wool in the base of the tube. Combine the specified volume of stationary phase and the specified amount of solid support to produce a homogeneous, fluffy mixture. Transfer this mixture to the chromatographic tube, and tamp using gentle pressure to obtain a uniform mass. If the specified amount of solid support is >3 g, transfer the mixture to the column in portions of approximately 2 g, and tamp each portion. If the assay or test requires a multisegment column with a different stationary phase specified for each segment, tamp after the addition of each segment, and add each succeeding segment directly to the previous one. Pack a pledget of fine glass wool above the completed column packing. [NOTE—The mobile phase should flow through a properly packed column as a moderate stream or, if reverse-phase chromatography is applied, as a slow trickle.]

If a solution of the analyte is incorporated into the stationary phase, complete the quantitative transfer to the chromatographic tube by scrubbing the beaker used for the preparation of the test mixture with a mixture of about 1 g of solid support and several drops of the solvent used to prepare the sample solution before adding the final portion of glass wool.

PROCEDURE

1. Transfer the mobile phase to the column space above the column packing, and allow it to flow through the column under the influence of gravity.
2. Rinse the tip of the chromatographic column with about 1 mL of mobile phase before each change in composition of mobile phase and after completion of the elution.

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3. If the analyte is introduced into the column as a solution in the mobile phase, allow it to pass completely into the column packing, then add the mobile phase in several small portions, allowing each to drain completely, before adding the bulk of the mobile phase.
4. Where the procedure indicates the use of multiple chromatographic columns mounted in series and the addition of mobile phase in divided portions is specified, allow each portion to drain completely through each column, and rinse the tip of each with mobile phase before the addition of each succeeding portion.

Gas Chromatography

LIQUID STATIONARY PHASE

This type of phase is available in packed or capillary columns.

PACKED COLUMN GAS CHROMATOGRAPHY

The liquid stationary phase is deposited on a finely divided, inert solid support, such as diatomaceous earth, porous polymer, or graphitized carbon, which is packed into a column that is typically 2–4 mm in internal diameter and 1–3 m in length.

CAPILLARY COLUMN GAS CHROMATOGRAPHY

In capillary columns, which contain no packed solid support, the liquid stationary phase is deposited on the inner surface of the column and may be chemically bonded to it.

SOLID STATIONARY PHASE

This type of phase is available only in packed columns. In these columns the solid phase is an active adsorbent, such as alumina, silica, or carbon, packed into a column. Polyaromatic porous resins, which are sometimes used in packed columns, are not coated with a liquid phase. [NOTE—Packed and capillary columns must be conditioned before use until the baseline and other characteristics are stable. The column or packing material supplier provides instructions for the recommended conditioning procedure.]

APPARATUS

A gas chromatograph consists of a carrier gas source, injection port, column, detector, and recording device. The injection port, column, and detector are temperature controlled and may be varied as part of the analysis. The typical carrier gas is helium, nitrogen, or hydrogen, depending on the column and detector in use. The type of detector used depends on the nature of the compounds analyzed and is specified in the individual monograph. Detector output is recorded as a function of time, and the instrument response, measured as peak area or peak height, is a function of the amount present.

TEMPERATURE PROGRAM

The length and quality of a GC separation can be controlled by altering the temperature of the chromatographic column. When a temperature program is necessary, the individual monograph indicates the conditions in table format. The table indicates the initial temperature, rate of temperature change (ramp), final temperature, and hold time at the final temperature.

PROCEDURE

1. Equilibrate the column, injector, and detector with flowing carrier gas until a constant signal is received.
2. Inject a sample through the injector septum, or use an autosampler.
3. Begin the temperature program.
4. Record the chromatogram.
5. Analyze as indicated in the monograph.

Liquid Chromatography

LC, as used in the compendia, is synonymous with HPLC (both high-pressure and high-performance). LC is a separation technique based on a solid stationary phase and a liquid mobile phase.

STATIONARY PHASE

Separations are achieved by partition, adsorption, or ion-exchange processes, depending on the type of stationary phase used. The most commonly used stationary phases are modified silica or polymeric beads. The beads are modified by the addition of long-chain hydrocarbons. The specific type of packing needed to complete an analysis is indicated by the "L" designation in the individual monograph (see also *Chromatographic Columns*). The size of the beads is often described in the monograph as well. Changes in the packing type and size are covered in *System Suitability* in this chapter.

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CHROMATOGRAPHIC COLUMN

The term "column" includes stainless steel, lined stainless steel, and polymeric columns, packed with a stationary phase. The length and inner diameter of the column affects the separation, and therefore typical column dimensions are included in the individual monograph. Changes to column dimensions are discussed in *System Suitability*. Compendial monographs do not include the name of appropriate columns; this omission avoids the appearance of endorsement of a vendor's product and natural changes in the marketplace. See *Chromatographic Columns* for more information.

In LC procedures, a guard column may be used with the following requirements, unless otherwise indicated in the individual monograph: (a) the length of the guard column must be NMT 15% of the length of the analytical column, (b) the inner diameter must be the same or smaller than that of the analytical column, and (c) the packing material should be the same as the analytical column (e.g., silica) and contain the same bonded phase (e.g., C18). In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.

MOBILE PHASE

The mobile phase is a solvent or a mixture of solvents, as defined in the individual monograph.

APPARATUS

A liquid chromatograph consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device.

GRADIENT ELUTION

The technique of continuously changing the solvent composition during the chromatographic run is called gradient elution or solvent programming. The gradient elution profile is presented in the individual monograph as a gradient table, which lists the time and proportional composition of the mobile phase at the stated time.

PROCEDURE

1. Equilibrate the column and detector with mobile phase at the specified flow rate until a constant signal is received.
2. Inject a sample through the injector, or use an autosampler.
3. Begin the gradient program.
4. Record the chromatogram.
5. Analyze as directed in the monograph.

CHROMATOGRAPHIC COLUMNS

A complete list of packings (L), phases (G), and supports (S) used in *USP–NF* tests and assays is located in *USP–NF, Reagents, Indicators, and Solutions—Chromatographic Columns*. This list is intended to be a convenient reference for the chromatographer in identifying the pertinent chromatographic column specified in the individual monograph.

DEFINITIONS AND INTERPRETATION OF CHROMATOGRAMS

Chromatogram: A graphical representation of the detector response, concentration of analyte in the effluent, or other quantity used as a measure of effluent concentration versus effluent volume or time. In planar chromatography, chromatogram may refer to the paper or layer with the separated zones.

Figure 1 represents a typical chromatographic separation of two substances, 1 and 2. t_{R1} and t_{R2} are the respective retention times; h is the height, $h/2$ is the half-height, and $W_{h/2}$ is the width at half-height, for peak 1. W_1 and W_2 are the respective widths of peaks 1 and 2 at the baseline. Air peaks are a feature of gas chromatograms and correspond to the solvent front in LC. The retention time of these air peaks, or unretained components, is designated as t_M.

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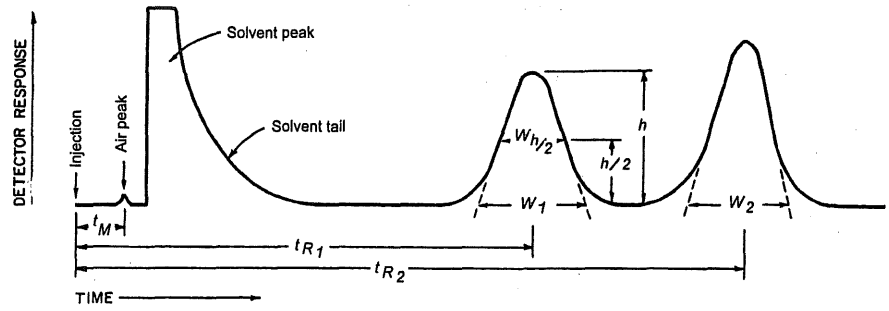


Figure 1. Chromatographic separation of two substances.

Dwell volume (*D*): Also known as "gradient delay volume", is the volume between the point at which the eluents meet and the top of the column.

Hold-up time (t_M): The time required for elution of an unretained component (see *Figure 1*, shown as an air or unretained solvent peak, with the baseline scale in minutes).

Hold-up volume (V_M): The volume of mobile phase required for elution of an unretained component. It may be calculated from the hold-up time and the flow rate, *F*, in mL/min:

$$V_M = t_M \times F$$

In size-exclusion chromatography, the symbol V_O is used.

Number of theoretical plates (*N*):[1] A measure of column efficiency. For Gaussian peaks, it is calculated by:

$$N = 16(t_R/W)^2$$

where t_R is the retention time of the substance, and *W* is the peak width at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline. The value of *N* depends upon the substance being chromatographed as well as the operating conditions, such as the flow rate and temperature of the mobile phase or carrier gas, the quality of the packing, the uniformity of the packing within the column, and, for capillary columns, the thickness of the stationary phase film and the internal diameter and length of the column.

Where electronic integrators are used, it may be convenient to determine the number of theoretical plates, by the equation:

$$N = 5.54\left(\frac{t_R}{W_{h/2}}\right)^2$$

where $W_{h/2}$ is the peak width at half-height. However, in the event of dispute, only equations based on peak width at baseline are to be used.

Peak: The portion of the chromatographic recording of the detector response when a single component is eluted from the column. If separation is incomplete, two or more components may be eluted as one unresolved peak.

Peak-to-valley ratio (*p/v*): *p/v* may be employed as a system suitability criterion in a test for related substances when baseline separation between two peaks is not achieved. *Figure 2* represents a partial separation of two substances, where H_p is the height above the extrapolated baseline of the minor peak and H_v is the height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks:

$$p/v = H_p/H_v$$

[1] The parameters *k*, *N*, *r*, and r_G were developed for isothermal GC separations and isocratic HPLC separations. Because these terms are thermodynamic parameters, they are only valid for separations made at a constant temperature, mobile phase composition, and flow rate. However, for separations made with a temperature program or solvent gradient, these parameters may be used simply as comparative means to ensure that adequate chromatographic conditions exist to perform the methods as intended in the monographs.

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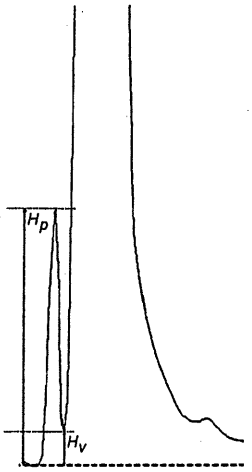


Figure 2. Peak-to-valley ratio determination.

Relative retardation (R_{ret}): The ratio of the distance traveled by the analyte to the distance simultaneously traveled by a reference compound (see *Figure 3*) and is used in planar chromatography.

$$R_{ret} = b/c$$

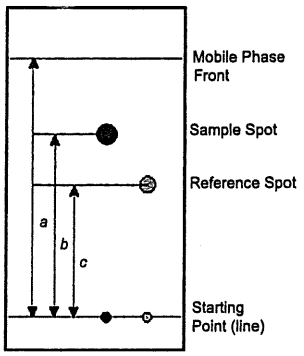


Figure 3. Typical planar chromatography.

Relative retention (r):[1] The ratio of the adjusted retention time of a component relative to that of another used as a reference, obtained under identical conditions:

$$r = (t_{R2} - t_M)/(t_{R1} - t_M)$$

where t_{R2} is the retention time measured from the point of injection of the compound of interest; t_{R1} is the retention time measured from the point of injection of the compound used as reference; and t_M is the retention time of a nonretained marker defined in the procedure, all determined under identical experimental conditions on the same column.

Relative retention time (RRT): Also known as the "unadjusted relative retention". Comparisons in *USP–NF* are normally made in terms of unadjusted relative retention, unless otherwise indicated.

$$\text{RRT} = t_{R2}/t_{R1}$$

The symbol r_G is also used to designate unadjusted relative retention values.

Relative standard deviation in percentage (%RSD):

$$\%\text{RSD} = \frac{100}{\bar{x}}\left(\frac{\sum_{i=1}^{N}\left(x_i - \bar{x}\right)^2}{N-1}\right)^{1/2}$$

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Resolution (R_S): The resolution is the separation of two components in a mixture, calculated by:

$$R_S = 2 \times (t_{R2} - t_{R1})/(W_1 + W_2)$$

where t_{R2} and t_{R1} are the retention times of the two components; and W_2 and W_1 are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Where electronic integrators are used, it may be convenient to determine the resolution, by the equation:

$$R_S = 1.18 \times (t_{R2} - t_{R1})/(W_{1,h/2} + W_{2,h/2})$$

Retardation factor (R_F): The ratio of the distance traveled by the center of the spot to the distance simultaneously traveled by the mobile phase and is used in planar chromatography. Using the symbols in *Figure 3*:

$$R_F = b/a$$

Retention factor (k):[1] Also known as the "capacity factor (k')". Defined as:

$$k = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in mobile phase}}$$

or

$$k = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}}$$

The k of a component may be determined from the chromatogram:

$$k = (t_R - t_M)/t_M$$

Retention time (t_R): In LC and GC, the retention time, t_R, is defined as the time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone. t_R may be used as a parameter for identification. Chromatographic retention times are characteristic of the compounds they represent but are not unique. The coincidence of retention times of a sample and a reference substance can be used as a partial criterion in construction of an identity profile, but may not be sufficient on its own to establish identity. Absolute retention times of a given compound may vary from one chromatogram to the next.

Retention volume (V_R): The volume of mobile phase required for elution of a component. It may be calculated from the retention time and the flow rate in mL/min:

$$V_R = t_R \times F$$

Separation factor (α): The relative retention calculated for two adjacent peaks (by convention, the value of the separation factor is always >1):

$$\alpha = k_2/k_1$$

Symmetry factor (A_S):[2] Also known as the "tailing factor", of a peak (see *Figure 4*) is calculated by:

$$A_S = W_{0.05}/2f$$

where $W_{0.05}$ is the width of the peak at 5% height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

[2] It is also a common practice to measure the "Asymmetry factor" as the ratio of the distance between the vertical line connecting the peak apex with the interpolated baseline and the peak front, and the distance between that line and the peak back measured at 10% of the peak height (see *Figure 4*), which would be $(W_{0.10} - f_{0.10})/f_{0.10}$. However, for the purposes of *USP*, only the formula (A_S) as presented here is valid.

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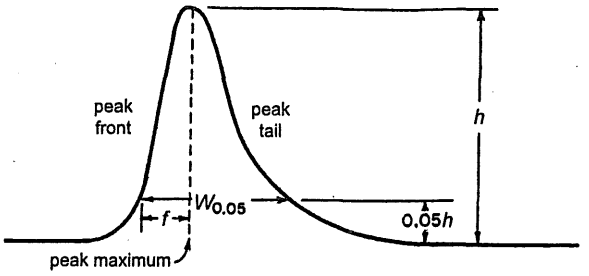


Figure 4. Asymmetrical chromatographic peak.

Tailing factor (*T*): See *Symmetry factor.*

SYSTEM SUITABILITY

System suitability tests are an integral part of GC and LC methods. These tests are used to verify that the chromatographic system is adequate for the intended analysis.

The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such.

Factors that may affect chromatographic behavior include the following:

- Composition, ionic strength, temperature, and apparent pH of the mobile phase
- Flow rate, column dimensions, column temperature, and pressure
- Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area
- Reverse-phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, and others)

R_S is a function of the number of theoretical plates, N (also referred to as efficiency), α, and k. [NOTE—All terms and symbols are defined in *Definitions and Interpretation of Chromatograms.*] For a given stationary phase and mobile phase, N may be specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug. This is a less reliable means to ensure resolution, as opposed to direct measurement. Column efficiency is, in part, a reflection of peak sharpness, which is important for the detection of trace components.

Replicate injections of a standard preparation or other standard solutions are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation (RSD), if the requirement is ≤2.0%; data from six replicate injections are used if the RSD requirement is >2.0%.

For the assay in a drug substance monograph, where the value is 100% for the pure substance, and no maximum RSD is stated, the maximum permitted %RSD is calculated for a series of injections of the reference solution:

$$\%\text{RSD} = KB\sqrt{n}/t_{90\%,n-1}$$

where K is a constant (0.349), obtained from the expression $K = (0.6/\sqrt{2}) \times (t_{90\%,5}/\sqrt{6})$, in which $0.6/\sqrt{2}$ represents the required %RSD after six injections for $B = 1.0$; B is the upper limit given in the definition of the individual monograph − 100%; n is the number of replicate injections of the reference solution ($3 \le n \le 6$); and $t_{90\%,n-1}$ is the Student's t at the 90% probability level (double sided) with $n-1$ degrees of freedom.

Unless otherwise prescribed, the maximum permitted RSD does not exceed the appropriate value given in *Table 1* of repeatability requirements. This requirement does not apply to tests for related substances.

Table 1. RSD Requirements

	Number of Individual Injections			
	3	4	5	6
B (%)	Maximum Permitted RSD			
2.0	0.41	0.59	0.73	0.85
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

A_S, a measure of peak symmetry, is unity for perfectly symmetrical peaks; its value increases as tailing becomes more pronounced (see *Figure 4*). In some cases, values less than unity may be observed. As peak symmetry moves away from values of 1, integration, and hence precision, become less reliable.

The signal-to-noise (S/N) ratio is a useful system suitability parameter. The S/N ratio is calculated as follows:

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$$S/N \text{ ratio} = 2H/h$$

where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance ≥5 times the peak width at its half-height; and h is the difference between the largest and smallest noise values observed over a distance ≥5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest (see *Figure 5*).

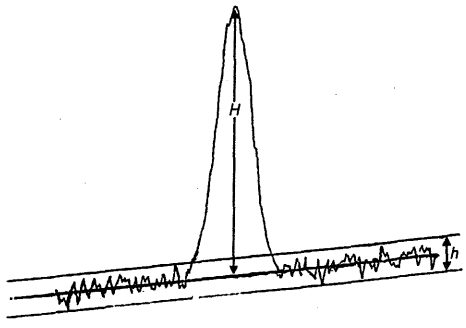


Figure 5. Noise and chromatographic peak, components of the S/N ratio.

These system suitability tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph.

The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions.

Adjustments to the specified chromatographic system may be necessary in order to meet system suitability requirements. Adjustments to chromatographic systems performed in order to comply with system suitability requirements are not to be made in order to compensate for column failure or system malfunction. Adjustments are permitted only when suitable standards (including Reference Standards) are available for all compounds used in the suitability test, and the adjustments or column change yields a chromatogram that meets all the system suitability requirements specified in the official procedure.

If adjustments of operating conditions are necessary in order to meet system suitability requirements, each of the items in the following list is the maximum variation that can be considered, unless otherwise directed in the monograph; these changes may require additional verification data. To verify the suitability of the method under the new conditions, assess the relevant analytical performance characteristics potentially affected by the change. Multiple adjustments can have a cumulative effect on the performance of the system and are to be considered carefully before implementation. In some circumstances, it may be desirable to use an HPLC column with different dimensions to those prescribed in the official procedure (different length, internal diameter, and/or particle size). In either case, changes in the chemical characteristics ("L" designation) of the stationary phase will be considered a modification to the method and will require full validation. Adjustments to the composition of the mobile phase in gradient elution may cause changes in selectivity and are not recommended. If adjustments are necessary, a change in column packing (maintaining the same chemistry), the duration of an initial isocratic hold (when prescribed), and/or the dwell volume are allowed. Additional allowances for gradient adjustment are noted in the text below.

pH of mobile phase (HPLC): The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 units of the value or range specified. Applies to both gradient and isocratic separations.

Concentration of salts in buffer (HPLC): The concentration of the salts used in the preparation of the aqueous buffer employed in the mobile phase can be adjusted to within ±10% if the permitted pH variation (see above) is met. Applies to both gradient and isocratic separations.

Ratio of components in mobile phase (HPLC): The following adjustment limits apply to minor components of the mobile phase (specified as ≤50%). The amounts of these components can be adjusted by ±30% relative. However, the change in any component cannot exceed ±10% absolute (i.e., in relation to the total mobile phase). Adjustment can be made to one minor component in a ternary mixture. Examples of adjustments for binary and ternary mixtures are given below.

Binary mixtures

SPECIFIED RATIO OF 50:50: 30% of 50 is 15% absolute, but this exceeds the maximum permitted change of ±10% absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40:60–60:40.

SPECIFIED RATIO OF 2:98: 30% of 2 is 0.6% absolute. Therefore, the maximum allowed adjustment is within the range of 1.4:98.6–2.6:97.4.

Ternary mixtures

SPECIFIED RATIO OF 60:35:5: For the second component, 30% of 35 is 10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore, the second component may be adjusted only within the range of 25%–45% absolute. For the third component, 30% of 5 is 1.5% absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100%. Therefore, mixture ranges of 50:45:5–70:25:5 or 58.5:35:6.5–61.5:35:3.5 would meet the requirement.

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Wavelength of UV-visible detector (HPLC): Deviations from the wavelengths specified in the procedure are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is used to verify that error in the detector wavelength is, at most, ±3 nm.

Stationary phase

COLUMN LENGTH (GC): Can be adjusted by as much as ±70%.

COLUMN LENGTH (HPLC): See *Particle size (HPLC)* below.

COLUMN INNER DIAMETER (HPLC): Can be adjusted if the linear velocity is kept constant. See *Flow rate (HPLC)*.

COLUMN INNER DIAMETER (GC): Can be adjusted by as much as ±50%.

FILM THICKNESS (CAPILLARY GC): Can be adjusted by as much as −50% to 100%.

Particle size (HPLC): For isocratic separations, the particle size and/or the length of the column may be modified provided that the ratio of the column length (*L*) to the particle size (*dp*) remains constant or into the range between −25% and 50% of the prescribed *L*/*dp* ratio. Alternatively (as for the application of particle-size adjustment to superficially porous particles), other combinations of *L* and *dp* can be used provided that the number of theoretical plates (*N*) is within −25% to 50%, relative to the prescribed column. Caution should be used when the adjustment results in a higher number of theoretical plates that generate smaller peak volumes, which may require adjustments to minimize extra-column band broadening by factors such as instrument plumbing, detector cell volume and sampling rate, and injection volume. For gradient separations, changes in length, column inner diameter, and particle size are not allowed.

Particle size (GC): Changing from a larger to a smaller or from a smaller to a larger particle size GC mesh support is acceptable if the chromatography meets the requirements of system suitability and the same particle size range ratio is maintained. The particle size range ratio is defined as the diameter of the largest particle divided by the diameter of the smallest particle.

Flow rate (GC): The flow rate can be adjusted by as much as ±50%. [NOTE—When the monograph specifies a linear velocity parameter, the allowed velocity adjustment is between +50% and −25%, provided the carrier gas system can be maintained under control at the desired set points.]

Flow rate (HPLC): When the particle size is changed, the flow rate may require adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). Flow rate changes for both a change in column diameter and particle size can be made by:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$$

where F_1 and F_2 are the flow rates for the original and modified conditions, respectively, dc_1 and dc_2 are the respective column diameters, and dp_1 and dp_2 are the particle sizes.

When a change is made from ≥3-µm to <3-µm particles in isocratic separations, an additional increase in linear velocity (by adjusting flow rate) may be justified, provided that the column efficiency does not drop by >20%. Similarly, a change from <3-µm to ≥3-µm particles may require additional reduction of linear velocity (flow rate) to avoid reduction in column efficiency by >20%. Changes in *F*, *dc*, and *dp* are not allowed for gradient separations.

Additionally, the flow rate can be adjusted by ±50% (isocratic only).

EXAMPLES: Adjustments in column length, internal diameter, particle size, and flow rate can be used in combination to give equivalent conditions (same *N*), but with differences in pressure and run time. *Table 2* lists some of the more popular column configurations to give equivalent efficiency (*N*), by adjusting these variables.

Table 2. Column Configurations

Length (*L*, mm)	Column Diameter (*dc*, mm)	Particle Size (*dp*, µm)	Relative Values				
			L/*dp*	*F*	*N*	Pressure	Run Time
250	4.6	10	25,000	0.5	0.8	0.2	3.3
150	4.6	5	30,000	1.0	1.0	1.0	1.0
150	2.1	5	30,000	0.2	1.0	1.0	1.0
100	4.6	3.5	28,600	1.4	1.0	1.9	0.5
100	2.1	3.5	28,600	0.3	1.0	1.9	0.5
75	4.6	2.5	30,000	2.0	1.0	4.0	0.3
75	2.1	2.5	30,000	0.4	1.0	4.0	0.3
50	4.6	1.7	29,400	2.9	1.0	8.5	0.1
50	2.1	1.7	29,400	0.6	1.0	8.5	0.1

For example, if a monograph specifies a 150-mm × 4.6-mm; 5-µm column operated at 1.5 mL/min, the same separation may be expected with a 75-mm × 2.1-mm; 2.5-µm column operated at 1.5 mL/min × 0.4 = 0.6 mL/min, along with a pressure increase of about four times and a reduction in run time to about 30% of the original.

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Injection volume (HPLC): The injection volume can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits. Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in *N* and resolution, which applies to both gradient and isocratic separations.

Injection volume and split volume (GC): The injection volume and split volume may be adjusted if detection and repeatability are satisfactory.

Column temperature (HPLC): The column temperature can be adjusted by as much as ±10°. Column thermostating is recommended to improve control and reproducibility of retention time, which applies to both gradient and isocratic separations.

Oven temperature (GC): The oven temperature can be adjusted by as much as ±10%.

Oven temperature program (GC): Adjustment of temperatures is permitted as stated above. When the specified temperature must be maintained or when the temperature must be changed from one value to another, an adjustment of up to ±20% is permitted.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

Measured values of R_r, R_F, or t_R for the sample substance do not deviate from the values obtained for the reference compound and mixture by more than the statistically determined reliability estimates from replicate assays of the reference compound. RRT may be provided in monographs for informational purposes only to aid in peak identification. There are no acceptance criteria applied to RRT.

Suitability testing is used to ascertain the effectiveness of the final operating system, which should be subjected to this testing. Make injections of the appropriate preparation(s), as required, in order to demonstrate adequate system suitability (as described in the *Chromatographic system* section of the method in a monograph) throughout the run.

The preparation can be a standard preparation or a solution containing a known amount of analyte and any additional materials (e.g., excipients or impurities) useful in controlling the analytical system. Whenever there is a significant change in the chromatographic system (equipment, mobile phase component, or other components) or in a critical reagent, system suitability is to be reestablished. No sample analysis is acceptable unless the suitability of the system has been demonstrated.

QUANTITATION

During quantitation, disregard peaks caused by solvents and reagents or arising from the mobile phase or the sample matrix.

In the linear range, peak areas and peak heights are usually proportional to the quantity of compound eluting. The peak areas and peak heights are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs. The components measured are separated from any interfering components. Peak tailing and fronting is minimized, and the measurement of peaks on tails of other peaks are avoided when possible.

Although comparison of impurity peaks with those in the chromatogram of a standard at a similar concentration is preferred, impurity tests may be based on the measurement of the peak response due to impurities and expressed as a percentage of the area of the drug peak. The standard may be the drug itself at a level corresponding to, for example, 0.5% impurity, assuming similar peak responses. When impurities must be determined with greater certainty, use a standard of the impurity itself or apply a correction factor based on the response of the impurity relative to that of the main component.

External Standard Method

The concentration of the component(s) quantified is determined by comparing the response(s) obtained with the sample solution to the response(s) obtained with a standard solution.

Internal Standard Method

Equal amounts of the internal standard are introduced into the sample solution and a standard solution. The internal standard is chosen so that it does not react with the test material and does not contain impurities with the same retention time as that of the analytes, and is stable and resolved from the component(s) quantified (analytes). The concentrations of the analytes are determined by comparing the ratios of their peak areas or peak heights and the internal standard in the sample solution with the ratios of their peak areas or peak heights and the internal standard in the standard solution.

Normalization Procedure

The percent content of a component of the test material is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix and those at or below the limit at which they can be disregarded.

Calibration Procedure

The relationship between the measured or evaluated signal *y* and the quantity (e.g., concentration or mass) of substance *x* is determined, and the calibration function is calculated. The analytical results are calculated from the measured signal or evaluated signal of the analyte and its position on the calibration curve.

In tests for impurities for both the external standard method, when a dilution of the sample solution is used for comparison, and the normalization procedure, any correction factors indicated in the monograph are applied (e.g., when the relative response factor is outside the range of 0.8–1.2).

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When the impurity test prescribes the total of impurities or there is a quantitative determination of an impurity, choice of an appropriate threshold setting and appropriate conditions for the integration of the peak areas is important. In such tests the limit at or below which a peak is disregarded is generally 0.05%. Thus, the threshold setting of the data collection system corresponds to at least half of this limit. Integrate the peak area of any impurity that is not completely separated from the principal peak, preferably by valley-to-valley extrapolation (tangential skim).

Add the following:

▲⟨630⟩ VISUAL COMPARISON

The purpose of this test is to provide the details for the visual comparison of the color and/or turbidance of sample solutions of certain concentration to a standard solution or a series of standard solutions of known concentration. Where a color or turbidity comparison is directed, follow the procedures and conditions outlined below for performing these tests.

Comparison vessels: Color-comparison tubes matched as closely as possible in internal diameter, in depth of sample solution, and in all other respects should be used.

Viewing conditions for turbidity comparison: Tubes should be viewed horizontally against a dark background with the aid of a light source directed from the sides of the tubes.

Viewing conditions for color comparison: Tubes should be viewed downward against a white background. Most of the time, common room lighting is sufficient to perform the assessment. A light source directed from beneath the bottoms of the tubes may be used if needed and if the practice is consistent between the materials under comparison. ▲ (USP 1-May-2019)

⟨631⟩ COLOR AND ACHROMICITY

DEFINITION

For the purposes of this chapter, color may be defined as the perception or subjective response by an observer to the objective stimulus of radiant energy in the visible spectrum extending over the range 400 nm to 700 nm in wavelength. Perceived color is a function of three variables: spectral properties of the object, both absorptive and reflective; spectral properties of the source of illumination; and visual characteristics of the observer.

Two objects are said to have a color match for a particular source of illumination when an observer cannot detect a color difference. Where a pair of objects exhibit a color match for one source of illumination and not another, they constitute a metameric pair. Color matches of two objects occur for all sources of illumination if the absorption and reflectance spectra of the two objects are identical.

Achromicity or colorlessness is one extreme of any color scale for transmission of light. It implies the complete absence of color, and therefore the visible spectrum of the object lacks absorbances. For practical purposes, the observer in this case perceives little if any absorption taking place in the visible spectrum.

COLOR ATTRIBUTES

Because the sensation of color has both a subjective and an objective part, color cannot be described solely in spectrophotometric terms. The common attributes of color therefore cannot be given a one-to-one correspondence with spectral terminology.

Three attributes are commonly used to identify a color: (1) hue, or the quality by which one color family is distinguished from another, such as red, yellow, blue, green, and intermediate terms; (2) value, or the quality that distinguishes a light color from a dark one; and (3) chroma, or the quality that distinguishes a strong color from a weak one, or the extent to which a color differs from a gray of the same value.

The three attributes of color may be used to define a three-dimensional color space in which any color is located by its coordinates. The color space chosen is a visually uniform one if the geometric distance between two colors in the color space is directly a measure of the color distance between them. Cylindrical coordinates are often conveniently chosen. Points along the long axis represent value from dark to light or black to white and have indeterminate hue and no chroma. Focusing on a cross-section perpendicular to the value axis, hue is determined by the angle about the long axis and chroma is determined by the distance from the long axis. Red, yellow, green, blue, purple, and intermediate hues are given by different angles. Colors along a radius of a cross-section have the same hue, which become more intense farther out. For example, colorless or achromic water has indeterminate hue, high value, and no chroma. If a colored solute is added, the water takes on a particular hue. As more is added, the color becomes darker, more intense, or deeper; i.e., the chroma generally increases and value decreases. If, however, the solute is a neutral color, i.e., gray, the value decreases, no increase in chroma is observed, and the hue remains indeterminate.

Laboratory spectroscopic measurements can be converted to measurements of the three color attributes. Spectroscopic results for three chosen lights or stimuli are weighted by three distribution functions to yield the tristimulus values, X, Y, Z (see *Color—Instrumental Measurement* ⟨1061⟩). The distribution functions were determined in color matching experiments with human subjects.

The tristimulus values are not coordinates in a visually uniform color space; however, several transformations have been proposed that are close to being uniform, one of which is given in the chapter cited ⟨1061⟩ *Color—Instrumental Measurement.*

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The value is often a function of only the Y value. Obtaining uniformity in the chroma-hue subspace has been less satisfactory. In a practical sense, this means in visual color comparison that if two objects differ significantly in hue, deciding which has a higher chroma becomes difficult. This points out the importance of matching standard to sample color as closely as possible, especially for the attributes of hue and chroma.

COLOR DETERMINATION AND STANDARDS

The perception of color and color matches is dependent on conditions of viewing and illumination. Determinations should be made using diffuse, uniform illumination under conditions that reduce shadows and nonspectral reflectance to a minimum. The surface of powders should be smoothed with gentle pressure so that a planar surface free from irregularities is presented. Liquids should be compared in matched color-comparison tubes, against a white background. If results are found to vary with illumination, those obtained in natural or artificial daylight are to be considered correct. Instead of visual determination, a suitable instrumental method may be used.

Colors of standards should be as close as possible to those of test specimens for quantifying color differences. Standards for opaque materials are available as sets of color chips that are arranged in a visually uniform space.* Standards identified by a letter for matching the colors of fluids can be prepared according to the accompanying table. To prepare the matching fluid required, pipet the prescribed volumes of the colorimetric test solutions [see under *Colorimetric Solutions* (CS) in the section *Reagents, Indicators, and Solutions*] and water into one of the matching containers, and mix the solution in the container. Make the comparison as directed in the individual monograph, under the viewing conditions previously described. The matching fluids, or other combinations of the colorimetric solutions, may be used in very low concentrations to measure deviation from achromicity.

Matching Fluids

Matching Fluid	Parts of Cobaltous Chloride CS	Parts of Ferric Chloride CS	Parts of Cupric Sulfate CS	Parts of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

⟨641⟩ COMPLETENESS OF SOLUTION

Place the quantity of the substance specified in the individual monograph in a meticulously cleansed, glass-stoppered, 10-mL glass cylinder approximately 13 mm × 125 mm in size. Using the solvent that is specified in the monograph or on the label of the product, fill the cylinder almost to the constriction at the neck. Shake gently to effect solution: the solution is not less clear than an equal volume of the same solvent contained in a similar vessel and examined similarly.

* Collections of color chips, arranged according to hue, value, and chroma in a visually uniform space and suitable for use in color designation of specimens by visual matching are available from GretagMacbeth LLC, 617 Little Britain Road, New Windsor, NY 12553-6148.

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⟨643⟩ TOTAL ORGANIC CARBON

Total organic carbon (TOC) is an indirect measure of organic molecules present in pharmaceutical waters measured as carbon. Organic molecules are introduced into the water from the source water, from purification and distribution system materials, from biofilm growing in the system, and from the packaging of sterile and nonsterile waters. TOC can also be used as a process control attribute to monitor the performance of unit operations comprising the purification and distribution system. A TOC measurement is not a replacement test for endotoxin or microbiological control. Although there can be a qualitative relationship between a food source (TOC) and microbiological activity, there is no direct numerical correlation.

A number of acceptable methods exist for analyzing TOC. This chapter does not endorse, limit, or prevent any technologies from being used, but this chapter provides guidance on how to qualify these analytical technologies for use as well as guidance on how to interpret instrument results for use as a limit test.

Apparatuses commonly used to determine TOC in water for pharmaceutical use have in common the objective of oxidizing the organic molecules in the water to produce carbon dioxide followed by the measurement of the amount of carbon dioxide produced. Then the amount of CO_2 produced is determined and used to calculate the organic carbon concentration in the water.

All technologies must discriminate between the inorganic carbon, which may be present in the water from sources such as dissolved CO_2 and bicarbonate, and the CO_2 generated from the oxidation of organic molecules in the sample. The discrimination may be accomplished either by determining the inorganic carbon and subtracting it from the total carbon (total carbon is the sum of organic carbon and inorganic carbon), or by purging inorganic carbon from the sample before oxidation. Although purging may entrain organic molecules, such purgeable organic carbon is present in negligible quantities in water for pharmaceutical use.

PROCEDURES

• BULK WATER

The following sections apply to tests for bulk *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and the condensate of *Pure Steam*.

Apparatus requirements: This test method is performed either as an on-line test or as an off-line laboratory test using a calibrated instrument. The suitability of the apparatus must be periodically demonstrated as described below. In addition, it must have a manufacturer's specified limit of detection of 0.05 mg/L (0.05 ppm) or lower of carbon.

When testing water for quality control purposes, ensure that the instrument and its data are under appropriate control and that the sampling approaches and locations of both on-line and off-line measurements are representative of the quality of the water used. The nature of the water production, distribution, and use should be considered when selecting either on-line or off-line measurement.

Reagent water: Use water having a TOC level of not more than 0.10 mg/L. [NOTE—A conductivity requirement may be necessary in order to ensure method reliability.]

Container preparation: Organic contamination of containers results in higher TOC values. Therefore, use labware and containers that have been scrupulously cleaned of organic residues. Any method that is effective in removing organic matter can be used (see *Cleaning Glass Apparatus* ⟨1051⟩). Use *Reagent water* for the final rinse.

Standard solution: Unless otherwise directed in the individual monograph, dissolve in the *Reagent water* an accurately weighed quantity of USP Sucrose RS to obtain a solution having a concentration of 1.19 mg/L of sucrose (0.50 mg/L of carbon).

System suitability solution: Dissolve in *Reagent water* an accurately weighed quantity of USP 1,4-Benzoquinone RS to obtain a solution having a concentration of 0.75 mg/L (0.50 mg/L of carbon).

Reagent water control: Use a suitable quantity of *Reagent water* obtained at the same time as that used in the preparation of the *Standard Solution* and the *System suitability solution*.

Water sample: Obtain an on-line or off-line sample that suitably reflects the quality of water used.

Other control solutions: Prepare appropriate reagent blank solutions or other specified solutions needed for establishing the apparatus baseline or for calibration adjustments following the manufacturer's instructions, and run the appropriate blanks to zero the instrument, if necessary.

System suitability: Test the *Reagent water control* in the apparatus, and record the response, r_W. Repeat the test using the *Standard solution*, and record the response, r_S. Calculate the corrected *Standard solution* response, which is also the limit response, by subtracting the *Reagent water control* response from the response of the *Standard solution*. The theoretical limit of 0.50 mg/L of carbon is equal to the corrected *Standard solution* response, $r_S - r_W$. Test the *System suitability solution* in the apparatus, and record the response, r_{SS}. Calculate the corrected *System suitability solution* response by subtracting the *Reagent water control* response from the response of the *System suitability solution*, $r_{SS} - r_W$. Calculate the percent response efficiency for the *System suitability solution*:

$$\% \text{ response efficiency} = 100[(r_{SS} - r_W)/(r_S - r_W)]$$

r_{SS} = instrument response to the *System suitability solution*
r_W = instrument response to the *Reagent water control*
r_S = instrument response to the *Standard solution*

The system is suitable if the percent response efficiency is not less than 85% and not more than 115%.

Procedure: Perform the test on the *Water Sample*, and record the response, r_U. The *Water Sample* meets the requirements if r_U is not more than the limit response, $r_S - r_W$. This method can be performed using on-line or off-line instrumentation that meets the *Apparatus requirements*.

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• **STERILE WATER**

The following sections apply to tests for *Sterile Water for Injection*, *Sterile Purified Water*, *Sterile Water for Irrigation*, and *Sterile Water for Inhalation*.

Follow the requirements in *Bulk Water*, with the following exceptions.

Apparatus requirements: In addition to the *Apparatus requirements* in *Bulk Water*, the apparatus must have a manufacturer's specified limit of detection of 0.10 mg/L (0.10 ppm) or lower of carbon.

Reagent water: Use water having a TOC level of not more than 0.50 mg/L. [NOTE—A conductivity requirement may be necessary in order to ensure method reliability.]

Standard solution: Unless otherwise directed in the individual monograph, dissolve in the *Reagent water* an accurately weighed quantity of USP Sucrose RS to obtain a solution having a concentration of 19.0 mg/L of sucrose (8.0 mg/L of carbon).

System suitability solution: Dissolve in *Reagent water* an accurately weighed quantity of USP 1,4-Benzoquinone RS to obtain a solution having a concentration of 12.0 mg/L (8.0 mg/L of carbon).

Water sample: Obtain a sample that suitably reflects the quality of water used. Before opening, vigorously agitate the package to homogenize the water sample. Several packages may be required in order to collect sufficient water for analysis.

System suitability: Test the *Reagent water control* in the apparatus, and record the response, r_W. Repeat the test using the *Standard solution*, and record the response, r_S. Calculate the corrected *Standard solution* response, which is also the limit response, by subtracting the *Reagent water control* response from the response of the *Standard solution*. The theoretical limit of 8.0 mg/L of carbon is equal to the corrected *Standard solution* response, $r_S - r_W$. Test the *System suitability solution* in the apparatus, and record the response, r_{SS}. Calculate the corrected *System suitability solution* response by subtracting the *Reagent water control* response from the response of the *System suitability solution*, $r_{SS} - r_W$. Calculate the percent response efficiency for the *System suitability solution*:

$$\% \text{ response efficiency} = 100[(r_{SS} - r_W)/(r_S - r_W)]$$

r_{SS} = instrument response to the *System suitability solution*
r_W = instrument response to the *Reagent water control*
r_S = instrument response to the *Standard solution*

The system is suitable if the percent response efficiency is not less than 85% and not more than 115%.

Procedure: Perform the test on the *Water Sample*, and record the response, r_U. The *Water sample* meets the requirements if r_U is not more than the limit response, $r_S - r_W$, determined in the *System suitability* requirements in *Sterile Water*.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 1,4-Benzoquinone RS
USP Sucrose RS

Add the following:

▲⟨644⟩ CONDUCTIVITY OF SOLUTIONS

Portions of the chapter text that are national *USP* text and are not part of the harmonized text, are marked with symbols (•,•) to specify this fact.

INTRODUCTION

This chapter provides information on how to apply electrical conductivity measurements (hereafter referred to as "conductivity") of fluid solutions, including pure fluids. ◆This chapter does not replace the official *Water Conductivity* ⟨645⟩ procedure, which is used to ensure the ionic purity of compendial waters such as *Water for Injection*, *Purified Water*, *Pure Steam* condensate, and *Sterile Water for Injection*, among others.◆ This chapter is intended for ◆other◆ fluid applications when conductivity is used to measure, monitor, or control chemical dispensing, chemical purity, ionic concentration, and other applications where the ionic character of the fluid needs to be known or controlled.

Applications include, but are not limited to, solutions that may be used in clean-in-place, chromatography detection, ionic solution preparations, endpoint detection, dosing, fermentation, and buffer production. In some cases, conductivity measurements can be extended to pure organic fluids such as alcohols and glycols where a weak conductivity signal exists, and the signal can be significantly increased if the organics become contaminated with water or salts. ◆ [NOTE—For additional background information, see *Theory and Practice of Electrical Conductivity Measurements of Solutions* ⟨1644⟩.] ◆

Conductivity is the measurement of the ability of a fluid to conduct electricity via its chemical ions. The ability of any ion to electrically conduct is directly related to its ion mobility. Conductivity is directly proportional to the concentrations of ions in the fluid, according to *Equation 1*:

$$\kappa = 1000 \sum_i^{\text{all ions}} C_i \lambda_i$$

κ = conductivity (S/cm)
C_i = concentration of chemical ion i (mol/L)

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λ_i = specific molar conductance of ion i ($S \cdot cm^2/mol$)

Although the SI unit S/m is the appropriate SI unit for conductivity, historically the unit S/cm has been selected by industry as the accepted unit.

On the basis of *Equation 1*, conductivity is not ion selective because it responds to all ions. Furthermore, the specific molar conductance of each ion is different. As a result, unless the percentage composition of ions of the solution is limited and known, the precise concentrations of ionic species cannot be determined from conductivity measurements. However, for examples such as a solution of a single salt or acid or base, such as a caustic solution used in cleaning, the precise concentration can be directly determined. Despite the lack of ionic specificity, conductivity is a valuable laboratory and process tool for measurement and control of total ionic content because it is proportional to the sum of the concentrations of all ionic species (anions and cations) for diluted solutions as described in *Equation 1*. At higher concentrations, conductivity measurements are not perfectly linear with concentration. Conductivity measurements cannot be applied to solids or gases, but they can be applied to the condensate of gases.

Another variable that influences conductivity measurements is the fluid temperature. As the fluid temperature increases, the ion conductance increases, making this physicochemical phenomenon the predominant reason for the temperature-compensation requirement when testing conductive fluids.

The conductivity, κ, is proportional to the conductance, G (S), of a fluid between two electrodes (*Equation 2*):

$$\kappa = G \times \left(\frac{d}{A}\right) = G \times K$$

κ = conductivity (S/cm)
G = conductance (S)
d = distance between the electrodes (cm)
A = area of the conducting electrodes (cm^2)
K = cell constant (cm^{-1}), which also equals the ratio of d/A

The resistivity ρ (Ω-cm) of the fluid is, by definition, the reciprocal of the conductivity (*Equation 3*):

$$\rho = 1/\kappa = 1 / (G \times K) = R/K$$

ρ = resistivity (Ω-cm)
κ = conductivity (S/cm)
G = conductance (S)
K = cell constant (cm^{-1})
R = resistance (Ω), which is the reciprocal of the conductance, G

APPARATUS

An electrical conductivity measurement consists of the determination of resistance of the fluid between and around the electrodes of the conductivity sensor. To achieve this measurement, the primary instrumentation is the resistance-measuring circuit and the conductivity sensor, and they are usually connected by a cable when the sensor and the user interface are separated.

The resistance measurement is made by applying an alternating current (AC, meaning the flow of electric charge periodically reverses direction) voltage (or current) to the electrodes, measuring the current (or voltage), and calculating the resistance according to Ohm's law. The alternating source is used to prevent the polarization (collection of ions) at the electrodes. Depending on the instrument, the measuring frequency of the measuring system adjusts automatically according to the measuring conditions of the instrument, and there may be multiple resistance-measuring circuits embedded in the measuring system. The resistance-measurement circuit may be embedded in the transmitter or in the sensor.

The conductivity sensor consists of at least two electrical conductors of a fixed size and geometry, separated by an electrical insulator. The electrodes, insulator, and any other wetted materials should be constructed of materials that are unreactive to fluids with which they may come into contact. Also, the sensor construction should withstand the environmental conditions (process or ambient temperature, pressure, cleaning applications) that it would be subjected to.

Most conductivity sensors have temperature devices such as a platinum resistance temperature device (RTD) or negative temperature coefficient (NTC) thermistor embedded inside the sensor, although external temperature measurement is possible. The purpose of the temperature measurement is for temperature compensation of the conductivity measurement.

CELL CONSTANT DETERMINATION

The purpose of the sensor's cell constant is to normalize the conductance (or resistance) measurement for the geometrical construction of the two electrodes.

The cell constant is determined by immersing the conductivity sensor in a solution of known conductivity. Solutions of known conductivity can be obtained by preparation of specific mixtures according to national authoritative sources or procurement of commercially available certified and traceable standard solutions. These recipes or certified solutions can range from 5 to 200,000 µS/cm, depending on the level of accuracy desired. Alternatively the cell constant is determined by comparison to other reference conductivity measuring systems (also available as an accredited calibration service). [NOTE—Conductivity measurements are not perfectly linear with concentration.]

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The measured cell constant of the conductivity sensor must be within 5% of the nominal value indicated by the sensor certificate, unless otherwise prescribed.

Modern conductivity sensors normally do not change their cell constant over their lifetime. If a change of the cell constant is detected during calibration, a cleaning of the sensor is appropriate according to the manufacturer's recommendations. Following that, the calibration procedure should be repeated. Sometimes "memory effects" appear, particularly when changing from high to low concentrations if the sensor is not well flushed.

CALIBRATION OF TEMPERATURE

In addition to verifying the sensor's cell constant, the embedded temperature device (or external temperature device) should be appropriately calibrated for the application to apply the temperature compensation algorithm accurately. The temperature accuracy that is required depends on the criticality of the temperature to the application. An accuracy of ±1° typically suffices.

CALIBRATION OF MEASUREMENT ELECTRONICS

The measurement circuit of the system is fundamentally an AC resistance measuring device. Appropriate verification and/or calibration of the measuring circuit is required for measurement systems with signal transfer via analog cable. This is accomplished by disconnecting the measuring circuit from the sensor's electrodes, attaching traceable resistors of known value with the cable of the measurement system to the measuring circuit, and verifying that the measured resistance agrees with the resistor value to an acceptable level. A typical acceptance criterion for the resistance accuracy is <2% of the reading at resistances >100 Ω, and increasing to 5% at lower resistances. However, the application criticality should ultimately determine the desired accuracy.

For conductivity systems that cannot have the resistance-measuring circuit disconnected from the electrodes (e.g., measurement circuit and electrodes in one mutual housing), it may be difficult to directly adjust or verify the circuit accuracy, depending on the sensor design. An alternative method of verifying the measurement system integrity is a system calibration according to the procedures for the cell constant determination for each measuring circuit that is intended to be used.

If verification/calibration of the sensor's cell constant, temperature device, and measuring circuit are done at the same service interval, the measuring circuit should be verified first, the temperature device next, and the cell constant last. Because all of these parameters are typically very stable due to modern electronics and stable sensor construction, frequent calibration (such as daily) is not usually required. Comparison to qualified reference systems is also a proper means of calibration. Calibration is performed at appropriate intervals as defined in the quality management system.

TEMPERATURE COMPENSATION

Because the conductivity of a fluid is temperature dependent, temperature compensation of the conductivity measurement is necessary unless otherwise prescribed (e.g., purified water, water for injection). An appropriate temperature compensation algorithm will ensure that changes in the conductivity measurement can be ascribed to concentration changes and not temperature changes. Conductivity measurements are normally referenced to 25°. A common form of linear temperature compensation uses *Equation 4:*

$$\kappa_{25} = \frac{\kappa_T}{[1 + \alpha(T - 25)]}$$

κ_{25} = conductivity compensated to 25°
κ_T = conductivity at T
α = temperature coefficient of the conductivity
T = measured temperature

A temperature coefficient of 2.1% per 1° is commonly used for many salt solutions. Most salt-based solutions have linear compensation factors ranging from 1.9% to 2.2% per 1°. Depending on the fluid samples, other forms of temperature compensation may be appropriate. Non-linear temperature compensation data for a variety of solutions is widely available (e.g., as described in ISO 7888 *Water quality—Determination of electrical conductivity*). In cases of very low conductivity (<10 µS/cm), such as purified pharmaceutical waters, two compensations need to be made. One is for the intrinsic conductivity of water, and the other is for the other ionic species in water. These compensations are normally combined and embedded in the microprocessor-controlled conductivity measurement systems. This is not supplied in all conductivity measurement technologies.

CONDUCTIVITY MEASUREMENT OF FLUIDS

For off-line batch measurements, rinse the cleaned sensor with the fluid to be measured. Then immerse the sensor in the fluid to be measured, and record the temperature and the temperature-compensated conductivity as required. Be sure that the position of the sensor in the container does not affect the conductivity measurement, because the container walls can affect the measurement for some electrode designs.

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For continuous on-line or at-line measurements, install the cleaned sensor into the pipe, tank, or other containment vessel, and flush, if necessary. Make sure proper installation procedures are applied to prevent bubbles or particles from collecting between the electrodes. Be sure that the position of the sensor in the pipe or tank does not affect the conductivity measurement, because the nearby surfaces can affect the measurement for some electrode designs.

Record the temperature and the temperature-compensated conductivity as required.

For all batch or continuous measurements, ensure that the wetted components of the sensor are compatible with the fluid and the temperature to be measured. ▲ (USP 1-Aug-2019)

⟨645⟩ WATER CONDUCTIVITY

INTRODUCTION

Electrical conductivity in water is a measure of the ion-facilitated electron flow through it. Water molecules dissociate into ions as a function of pH and temperature and result in a very predictable conductivity. Some gases, most notably carbon dioxide, readily dissolve in water and interact to form ions, which predictably affect conductivity also. For the purpose of this discussion, these ions and their resulting conductivity can be considered intrinsic to the water.

Water conductivity is also affected by the presence of extraneous ions. The extraneous ions used in modeling the conductivity specifications described below are the chloride and ammonia ions. The conductivity of the ubiquitous chloride ion (at the theoretical endpoint concentration of 0.47 ppm when chloride was a required attribute test in *USP 22* and earlier revisions) and the ammonium ion (at the limit of 0.3 ppm) represents a major portion of the allowed water ionic impurity level. A balancing quantity of anions (such as chloride, to counter the ammonium ion) and cations (such as sodium, to counter the chloride ion) is included in this allowed impurity level to maintain electroneutrality. Extraneous ions such as these may have a significant effect on the water's chemical purity and suitability for use in pharmaceutical applications.

The procedure in the section *Bulk Water* is specified for measuring the conductivity of waters such as *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, and the condensate of *Pure Steam*. The procedure in the section *Sterile Water* is specified for measuring the conductivity of waters such as *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*.

The procedures below shall be performed using instrumentation that has been calibrated, has conductivity sensor cell constants that have been accurately determined, and has a temperature compensation function that has been disabled for *Bulk Water* Stage 1 testing. For both online and offline measurements, the suitability of instrumentation for quality control testing is also dependent on the sampling location(s) in the water system. The selected sampling instrument location(s) must reflect the quality of the water used.

INSTRUMENT SPECIFICATIONS AND OPERATING PARAMETERS

Water conductivity must be measured accurately with calibrated instrumentation. An electrical conductivity measurement consists of the determination of the conductance, G (or its inverse, resistance, R), of the fluid between and around the electrodes. The conductance ($1/R$) is directly affected by the geometrical properties of the electrodes; i.e., the conductance is inversely proportional to the distance (d) between the electrodes and proportional to the area (A) of the electrodes. This geometrical ratio (d/A) is known as the cell constant, Θ. Thus the measured conductance is normalized for the cell constant to determine the conductivity, κ, according to the following equation:

$$\text{conductivity, } \kappa \text{ (S/cm)} = \Theta \text{ (cm}^{-1}\text{)}/R \text{ (}\Omega\text{)}$$

It is the cell constant and the resistance measurement that must be verified and adjusted, if necessary.

Cell Constant

The cell constant must be known within ±2%. The cell constant can be verified directly by using a solution of known or traceable conductivity, or indirectly by comparing the instrument reading taken with the conductivity sensor in question to readings from a conductivity sensor of known or traceable cell constant. If necessary, adjust the cell constant following the manufacturer's instrument protocol. The frequency of verification/calibration is a function of the sensor design.

Resistance Measurement

Calibration (or verification) of the resistance measurement is accomplished by replacing the conductivity sensor electrodes with precision resistors having standards traceable to NIST or equivalent national authorities in other countries (accurate to ±0.1% of the stated value) to give a predicted instrument conductivity response. The accuracy of the resistance measurement is acceptable if the measured conductivity with the traceable resistor is within ±0.1 µS/cm of the calculated value according to the equation above. For example, the traceable resistor is 50 kΩ, and the cell constant, Θ, is 0.10 cm^{-1}. The calculated value is 2.0×10^{-6} S/cm or 2.0 µS/cm. The measured value should be 2.0 ± 0.1 µS/cm. The instrument must have a minimum resolution of 0.1 µS/cm on the lowest range.

The target conductivity value(s) should be based on the type of water to be analyzed, and it should be equal to or less than the water conductivity limit for that type of water. Multiple measuring circuits may be embedded in the meter or the sensor,

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and each circuit may require separate verification or calibration before use. The frequency of recalibration is a function of instrument system design.

System Verification

The cell constant of the user's sensor can be determined with the user's resistance measurement system, or the cell constant can be determined with an independent resistance measurement system. If the cell constant is determined with an independent resistance measurement system, it is recommended that the user verify that the sensor has been properly connected to the resistance measurement system to ensure proper performance. Verification can be made by comparing the conductivity (or resistivity) values displayed by the measuring equipment with those of an external calibrated conductivity-measuring device. The two non–temperature-compensated conductivity (or resistivity) values should be equivalent to or within ±5% of each other, or should have a difference that is acceptable on the basis of product water criticality and/or the water conductivity ranges in which the measurements are taken. The two conductivity sensors should be positioned close enough together to measure the same water sample at the same temperature and water quality.

Temperature Compensation and Temperature Measurements

Because temperature has a substantial effect on conductivity readings of specimens at high and low temperatures, many instruments automatically correct the actual reading to display the value that theoretically would be observed at the nominal temperature of 25°. This is typically done using a temperature sensor embedded in the conductivity sensor and a software algorithm embedded in the instrument. This temperature compensation algorithm may not be accurate for the various water types and impurities. For this reason, conductivity values used in the *Stage 1* test for *Bulk Water* are non–temperature-compensated measurements. Other conductivity tests that are specified for measurement at 25° can use either temperature-compensated or non–temperature-compensated measurements.

A temperature measurement is required for the *Stage 1* test or for the other tests at 25°. It may be made using the temperature sensor embedded in the conductivity cell sensor. An external temperature sensor positioned near the conductivity sensor is also acceptable. Accuracy of the temperature measurement must be ±2°.

BULK WATER

The procedure and test limits in this section are intended for *Purified Water*, *Water for Injection*, *Water for Hemodialysis*, the condensate of *Pure Steam*, and any other monographs that specify this section.

This is a three-stage test method to accommodate online or offline testing. Online conductivity testing provides real-time measurements and opportunities for real-time process control, decision, and intervention. Precautions should be taken while collecting water samples for offline conductivity measurements. The sample may be affected by the sampling method, the sampling container, and environmental factors such as ambient carbon dioxide concentration and organic vapors. This procedure can be started at *Stage 2* if offline testing is preferred.

Procedure

STAGE 1

Stage 1 is intended for online measurement or may be performed offline in a suitable container.

1. Determine the temperature of the water and the conductivity of the water with a non–temperature-compensated conductivity reading.
2. Using *Table 1*, find the temperature value that is NMT the measured temperature, i.e., the next lower temperature. The corresponding conductivity value on this table is the limit.

[NOTE—Do not interpolate.]

3. If the measured conductivity is NMT the table value determined in step 2, the water meets the requirements of the test for conductivity. If the conductivity is higher than the table value, proceed with *Stage 2*.

Table 1. Stage 1—Temperature and Conductivity Requirements (for non–temperature-compensated conductivity measurements only)

Temperature	Conductivity Requirement (µS/cm)
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5

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Table 1. Stage 1—Temperature and Conductivity Requirements (for non–temperature-compensated conductivity measurements only) *(continued)*

Temperature	Conductivity Requirement (µS/cm)
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

STAGE 2

4. Transfer a sufficient amount of water to a suitable container, and stir the test specimen. Adjust the temperature, if necessary, and, while maintaining it at 25 ± 1°, begin vigorously agitating the test specimen while periodically observing the conductivity. When the change in conductivity (due to uptake of atmospheric carbon dioxide) is less than a net of 0.1 µS/cm per 5 min, note the conductivity.

[NOTE—Conductivity measurements at this stage may be temperature-compensated to 25° or non–temperature-compensated.]

5. If the conductivity is not greater than 2.1 µS/cm, the water meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 µS/cm, proceed with *Stage 3*.

STAGE 3

6. Perform this test within approximately 5 min of the conductivity determination in step 5, while maintaining the sample temperature at 25 ± 1°. Add a saturated potassium chloride solution to the same water sample (0.3 mL per 100 mL of the test specimen), and determine the pH to the nearest 0.1 pH unit, as directed in *pH* ⟨791⟩.

7. Referring to *Table 2*, determine the conductivity limit at the measured pH value. If the measured conductivity in step 4 is NMT the table value determined in step 6, the water meets the requirements of the test for conductivity. If either the measured conductivity is greater than this value or the pH is outside the range of 5.0–7.0, the water does not meet the requirements of the test for conductivity.

Table 2. Stage 3—pH and Conductivity Requirements (for atmosphere- and temperature-equilibrated samples only)

pH	Conductivity Requirement (µS/cm)
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3

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Table 2. Stage 3—pH and Conductivity Requirements (for atmosphere- and temperature-equilibrated samples only) *(continued)*

pH	Conductivity Requirement (µS/cm)
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

STERILE WATER

The procedure and test limits are intended for *Sterile Purified Water*, *Sterile Water for Injection*, *Sterile Water for Inhalation*, and *Sterile Water for Irrigation*, and any other monographs that specify this section. The sterile waters are derived from *Purified Water* or *Water for Injection*, and therefore have been determined to be compliant with the *Bulk Water* requirements before being stored in the container. The specification provided represents the maximum allowable conductivity value, taking into consideration the limitation of the measurement method and reasonable container leaching. Such specification and the sampling volume choices should be defined and validated on the basis of the intended purpose of the water.

Procedure

Obtain a sample that suitably reflects the quality of water used. Before opening, vigorously agitate the package to homogenize the water sample. Several packages may be required to collect sufficient water for analysis.

Transfer a sufficient amount of water to a suitable container, and stir the test specimen. Adjust the temperature, if necessary, and, while maintaining it at 25 ± 1°, begin vigorously agitating the test specimen while periodically observing the conductivity. When the change in conductivity (due to uptake of ambient carbon dioxide) is less than a net of 0.1 µS/cm per 5 min, note the conductivity.

For containers with a nominal volume of 10 mL or less, if the conductivity is NMT 25 µS/cm, the water meets the requirements. For containers with a nominal volume greater than 10 mL, if the conductivity is NMT 5 µS/cm, the water meets the requirements.

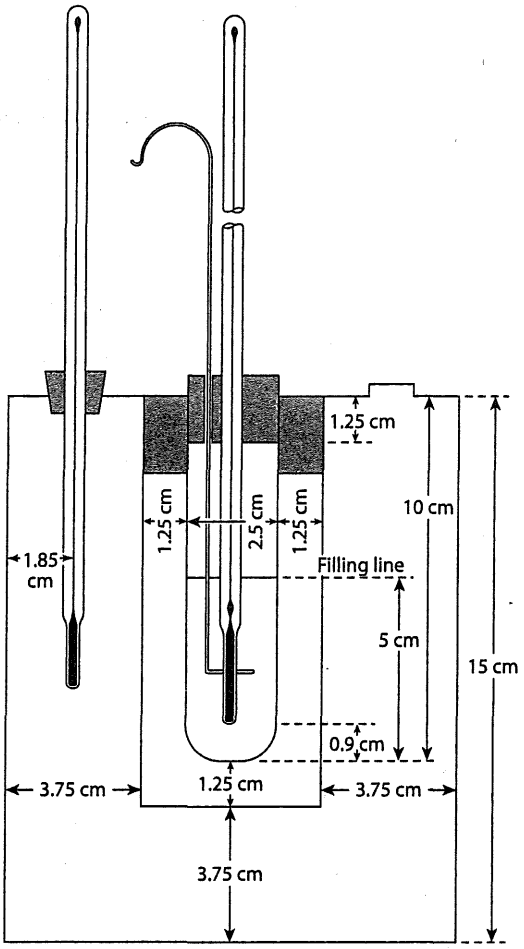
⟨651⟩ CONGEALING TEMPERATURE

The temperature at which a substance passes from the liquid to the solid state upon cooling is a useful index to purity if heat is liberated when the solidification takes place, provided that any impurities present dissolve in the liquid only, and not in the solid. Pure substances have a well-defined freezing point, but mixtures generally freeze over a range of temperatures. For many mixtures, the congealing temperature, as determined by strict adherence to the following empirical methods, is a useful index of purity. The method for determining congealing temperatures set forth here is applicable to substances that melt between −20° and 150°, the range of the thermometer used in the bath. The congealing temperature is the maximum point (or lacking a maximum, the point of inflection) in the temperature-time curve.

APPARATUS

Assemble an apparatus similar to that *illustrated,*

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Congealing Temperature Apparatus

in which the container for the substance is a 25- × 100-mm test tube. This is provided with a suitable, short-range thermometer suspended in the center, and a wire stirrer, about 30 cm long, bent at its lower end into a horizontal loop around the thermometer. Use a thermometer having a range not exceeding 30°, graduated in 0.1° divisions, and calibrated for, but not used at, 76-mm immersion. A suitable series of thermometers, covering a range from –20° to +150°, is available as the ASTM E1 series 89C through 96C. Other temperature-measuring devices may be used if they are validated for this procedure (see *General Notices, 6.80.30. Temperature Reading Devices*). Dimensions should be within ±20% of those given in the illustration.

The specimen container is supported, by means of a cork, in a suitable water-tight cylinder about 50 mm in internal diameter and 11 cm in length. The cylinder, in turn, is supported in a suitable bath sufficient to provide not less than a 37-mm layer surrounding the sides and bottom of the cylinder. The outside bath is provided with a suitable thermometer.

PROCEDURE

Melt the substance, if a solid, at a temperature not exceeding 20° above its expected congealing point, and pour it into the test tube to a height of 50 to 57 mm. Assemble the apparatus with the bulb of the test tube thermometer immersed halfway between the top and bottom of the specimen in the test tube. Fill the bath to about 12 mm from the top of the tube with suitable fluid at a temperature 4° to 5° below the expected congealing point.

In case the substance is a liquid at room temperature, carry out the determination using a bath temperature about 15° below the expected congealing point.

When the test specimen has cooled to about 5° above its expected congealing point, adjust the bath to a temperature 7° to 8° below the expected congealing point. Stir the specimen continuously during the remainder of the test by moving the loop up and down between the top and bottom of the specimen, at a regular rate of 20 complete cycles per minute.

Congelation frequently may be induced by rubbing the inner walls of the test tube with the thermometer, or by introducing a small fragment of the previously congealed substance. Pronounced supercooling may cause deviation from the normal pattern of temperature changes. If the latter occurs, repeat the test, introducing small particles of the material under test in solid form at 1° intervals as the temperature approaches the expected congealing point.

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Record the reading of the test tube thermometer every 30 seconds. Continue stirring only so long as the temperature is gradually falling, stopping when the temperature becomes constant or starts to rise slightly. Continue recording the temperature in the test tube every 30 seconds for at least 3 minutes after the temperature again begins to fall after remaining constant.

The average of not less than four consecutive readings that lie within a range of 0.2° constitutes the congealing temperature. These readings lie about a point of inflection or a maximum, in the temperature-time curve, that occurs after the temperature becomes constant or starts to rise and before it again begins to fall. The average to the nearest 0.1° is the congealing temperature.

⟨659⟩ PACKAGING AND STORAGE REQUIREMENTS

(A portion of the *Associated Components* section of this chapter will become official on May 1, 2019, and a portion of the *Packaging* section of this chapter will become official on May 1, 2020, as indicated. Early adoption of the requirements in this chapter and *Plastic Materials of Construction* ⟨661.1⟩ and *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩ are permitted by USP.)

INTRODUCTION

The purpose of this chapter is to provide packaging definitions, auxiliary packaging information, and storage condition definitions relevant to the storage and distribution of active ingredients, excipients, and medical products, such as pharmaceuticals, devices, combination products (e.g., drug-eluting stents), and dietary supplements.

Change to read:

PACKAGING

Packaging materials must not interact physically or chemically with a packaged article in a manner that causes its safety, identity, strength, quality, or purity to fail to conform to established requirements. ▲Any plastic material used to construct a *Packaging system* must meet the applicable requirements of *Plastic Materials of Construction* ⟨661.1⟩.▲ (Official 1-May-2020) All *Packaging systems* must meet the applicable requirements specified in *Containers—Glass* ⟨660⟩, *Plastic Packaging Systems and Their Materials of Construction* ⟨661⟩, ▲*Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩,▲ (Official 1-May-2020) and *Auxiliary Packaging Components* ⟨670⟩. All elastomeric closures must meet the applicable requirements in *Elastomeric Closures for Injections* ⟨381⟩.

Every monograph in *USP–NF* must have packaging and storage requirements. For the packaging portion of the statement, the choice of containers is provided in this chapter. For active pharmaceutical ingredients (APIs), the choice would be a tight, well-closed, or, where needed, light-resistant container. For excipients, given their typical presentation as large-volume commodity items (*Packaging systems* ranging from drums to tank cars), a well-closed container is an appropriate default requirement. Articles must be protected from moisture, freezing, and excessive heat (see *General Definitions*) when no specific directions or limitations are provided.

The compendial requirements for the use of specified containers apply also to articles packaged by *Dispensers*, *Repackagers*, or other individuals, unless otherwise indicated in the individual drug product monograph.

POISON PREVENTION PACKAGING ACT

This act, which is administered by the United States Consumer Product Safety Commission (CPSC), requires special packaging for most human oral prescription drugs, oral controlled drugs, certain non-oral prescription drugs, certain dietary supplements, and many over-the-counter (OTC) drug preparations, to protect the public from personal injury or illness from misuse of these preparations (16 CFR §1700.14).

The primary packaging of substances regulated under the Poison Prevention Packaging Act (PPPA) must comply with the special packaging standards (16 CFR §1700.15). These apply to all packaging types, including reclosable, non-reclosable, and unit-dose types.

Special packaging is not required for drugs dispensed within a hospital setting for inpatient administration. Also, special packaging does not need to be used by manufacturers and packagers of bulk-packaged prescription drugs that will be repackaged by the pharmacist. PPPA-regulated prescription drugs may be dispensed in non-*Child-resistant packaging* upon the request of the purchaser or when directed in a legitimate prescription (15 USC §1473).

Manufacturers or packagers of PPPA-regulated OTC preparations are allowed to package one size in non-*Child-resistant packaging* as long as popular-size, special packages are also supplied. The non-*Child-resistant packaging* requires special labeling (16 CFR §1700.5).

TEMPERATURE AND STORAGE

Specific directions are stated in some monographs with respect to storage conditions (e.g., the temperature or humidity) at which an article must be stored and shipped. Such directions apply except where the label on the article has different storage conditions that are based on stability studies. Where no specific directions or limitations are provided in the article's labeling, articles must be protected from moisture, freezing, and excessive heat, and, where necessary, from light during shipping and distribution. Drug substances are exempt from this standard.

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GENERAL DEFINITIONS

Packaging Definitions

Packaging system (also referred to as a Container–closure system): The sum of *Packaging components* and materials that together contain and protect the article. This includes *Primary packaging components* as well as *Secondary packaging components* when such components are required to provide additional protection.

Container: A receptacle that holds an intermediate compound, API, excipient, or dosage form, and is in direct contact with the article (e.g., ampules, vials, bottles, syringes, and pen injectors).

Closure: A material that seals an otherwise open space of a *Container* and provides protection for the contents. It also provides access to the contents of the *Container* (e.g., screw caps and stoppers).

Packaging component: Any single part of the *Package* or *Container–closure system,* including: the *Container* (e.g., ampules, syringes, vials, and bottles); *Closures* (e.g., screw caps and stoppers); ferrules and overseals; *Closure* liners (e.g., tube cartridge liners); inner seals; administration ports; overwraps; administration accessories; labels; cardboard boxes; and shrink wrap.

Primary packaging component: A *Packaging component* that is in direct contact with or may come into direct contact with the article.

Secondary packaging component: A *Packaging component* that is in direct contact with a *Primary packaging component* and may provide additional protection for the article.

Tertiary packaging component: A *Packaging component* that is in direct contact with a *Secondary packaging component* and may provide additional protection for the article during transportation and/or storage.

Ancillary component: A component or entity that may come into contact with a *Tertiary packaging component* during the distribution, storage, and/or transportation of the packaged article (e.g., pallets, skids, and shrink wrap).

Associated component: A *Packaging component* that is typically intended to deliver the drug article to the patient but is not stored in contact with the article for its entire shelf life (e.g., spoons, *Dosing cups,* and dosing syringes).

Materials of construction: The materials (e.g., glass, plastic, elastomers, and metal) of which a *Packaging component* consists.

Small-volume injection (Small-volume parenteral): An injectable dosage form that is packaged in *Containers* labeled as containing 100 mL or less.

Large-volume injection (Large-volume parenteral): An injectable dosage form that is packaged in *Containers* labeled as containing more than 100 mL.

Child-resistant packaging: A *Packaging system* designed or constructed to meet CPSC standards pertaining to opening by children (16 CFR §1700.20 et seq. and 16 CFR §1700.15).

Senior-friendly packaging: A *Packaging system* designed or constructed to meet CPSC standards pertaining to opening by senior adults (16 CFR §1700.15 and 16 CFR §1700.20).

Restricted delivery system: A *Packaging system* designed or constructed to restrict (control) the amount of the drug product that may be delivered in order to limit unintended access by children and other similarly vulnerable populations. *Restricted delivery systems* should meet and may exceed CPSC standards for special packaging [*Child-resistant* and *Senior-friendly packaging* (16 CFR §1700.15 et seq.)]. For oral medicinal liquids, surface and flow characteristics vary. It is the responsibility of the manufacturer to ensure that all components of the *Restricted delivery system* provide the intended safety protection. One component of the *Restricted delivery system* is the flow restrictor, which is a *Packaging component* that restricts the flow of liquid. The flow restrictor may be used as part of a *Restricted delivery system* or as an adaptor to facilitate use of a measuring device for oral medicinal liquids. A flow restrictor should not compromise CPSC standards for special packaging [*Child-resistant* and *Senior-friendly packaging* (16 CFR §1700.15 et seq.)].

Tamper-evident packaging: A *Packaging system* that may not be accessed without obvious destruction of the seal or some portion of the *Packaging system. Tamper-evident packaging* must be used for sterile drug products intended for ophthalmic or otic use, except where extemporaneously compounded for immediate dispensing on prescription. Drug products intended for sale without prescription are also required to comply with the *Tamper-evident packaging* and labeling requirements of the Food and Drug Administration (FDA) where applicable (21 CFR §221.132). Preferably, the immediate *Container* and/or the outer *Container* or protective packaging used by a manufacturer or distributor for all dosage forms that are not specifically exempt is designed to show evidence of any tampering with the contents.

Reclosable packaging: A package that after it has been initially opened is capable of being reclosed with a similar degree of security and is capable of being used a sufficient number of times to dispense the total contents without loss of security. *Reclosable packaging* may incorporate child-resistance capabilities.

Non-reclosable packaging: A package or part of a package that cannot be closed again after all or part of the contents have been removed. Examples of *Non-reclosable packaging* are blisters, sachets, strips, and other *Single-unit containers. Non-reclosable packaging* may include cold-formed foil blisters, foil strip packs, and PVC/Aclar® combining multilayer materials that are thermo-formed or cold-formed foil blisters. *Non-reclosable packaging* may be child resistant depending on the intended use and place of use. Household non-reclosables are subject to the PPPA as defined in 16 CFR §1700.14.

Hermetic container: A *Container–closure system* that is impervious to air or any other gas under the ordinary or customary conditions of handling, shipment, storage, and distribution.

Tight container: A *Container–closure system* that protects the contents from contamination by extraneous liquids, solids, or vapors; from loss of the article; and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and distribution, and is capable of tight reclosure. Where a tight container is specified, it may be replaced by a hermetic container for a single dose of an article. [NOTE—Where packaging and storage in a tight container or well-closed container is specified in the individual monograph, the container used for an article when dispensed on prescription meets the requirements in *Containers—Performance Testing* ⟨671⟩.]

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Well-closed container: A *Container–closure system* that protects the contents from contamination by extraneous solids and from loss of the article under the ordinary or customary conditions of handling, shipment, storage, and distribution. See ⟨671⟩.

Light-resistant container: A *Container–closure system* that protects the contents from the effects of light by virtue of the specific properties of the material of which it is composed, including any coating applied to it. A clear and colorless or a translucent container may be made light-resistant by means of an opaque covering or by use of secondary packaging, in which case the label of the container bears a statement that the opaque covering or secondary packaging is needed until the articles are to be used or administered. Where it is directed to "protect from light" in an individual monograph, preservation in a light-resistant container is intended. See *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩, *Spectral Transmission Requirements for Light-Resistant Containers.*

Equivalent container–closure system: A *Container–closure system* that is as protective as or more protective than the original manufacturer's *Packaging system* in terms of moisture-vapor transmission rate, oxygen transmission, light transmission, and compatibility. System equivalency extends to any special protective materials, such as those for seals or desiccants associated with the original *Packaging system.*

Table 1. Packaging Systems Definitions: Injection versus Noninjection

Injection	Noninjection
Multiple-dose	Multiple-unit
Single-dose	Single-unit
—	Unit-dose
—	Unit-of-use
Pharmacy bulk package	—
Imaging bulk package	—

Injection Packaging Systems

Multiple-dose container (also referred to as Multi-dose): A *Container–closure system* that holds a sterile medication for parenteral administration (injection or infusion) that has met antimicrobial effectiveness testing requirements, or is excluded from such testing requirements by FDA regulation. A *Multiple-dose container* is intended to contain more than one dose of a drug product. When space permits, a *Multiple-dose container* is labeled as such. *Multiple-dose containers* are generally expected to contain 30 mL or less of medications. The beyond-use date for an opened or entered (e.g., needle-punctured) *Multiple-dose container* is 28 days unless otherwise specified by the manufacturer on the label. An example of a *Multiple-dose container* is a vial.

Single-dose container: A *Container–closure system* that holds a sterile medication for parenteral administration (injection or infusion) that is not required to meet the antimicrobial effectiveness testing requirements. A *Single-dose container* is designed for use with a single patient as a single injection/infusion.[1] When space permits, a *Single-dose container* is labeled as such and should include on the label appropriate discard statements. Examples of *Single-dose containers* are vials, ampules, and prefilled syringes.

Pharmacy bulk package: A *Container–closure system* of a sterile preparation for parenteral use that contains many single doses. The contents are intended for use in a pharmacy admixture program and are restricted to the preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile syringes. The *Closure* must be penetrated only one time after constitution, if necessary, with a suitable sterile transfer device or dispensing set that allows measured dispensing of the contents. The *Pharmacy bulk package* is to be used only in a suitable work area such as a laminar flow hood (or an equivalent clean-air compounding area). Designation as a *Pharmacy bulk package* is limited to injection, for injection, or injectable emulsion dosage forms as defined in *Nomenclature* ⟨1121⟩, *General Nomenclature Forms.*

Pharmacy bulk packages, although containing more than one single dose, are exempt from the *Multiple-dose container* volume limit of 30 mL and the requirement that they contain a substance or suitable mixture of substances to prevent the growth of microorganisms. See *Labeling* ⟨7⟩ for labeling requirements.

Imaging bulk package: A container of a sterile preparation for parenteral use that contains many single doses of a contrast agent (medical imaging drug product) for use with a medical imaging device. The contents are restricted to use in direct conjunction with a device with features to mitigate the risk of cross-contamination (i.e., an automated contrast injection system or contrast management system approved or cleared for use with an *Imaging bulk package*). The sterility assurance of the *Imaging bulk package* contents in part is dependent upon the automated contrast injection system or the contrast management system.

The *Imaging bulk package* is to be used only in a room designated for radiological procedures that involve intravascular administration of a contrast agent. Using aseptic technique, the *Imaging bulk package* closure must be penetrated only one time with a suitable sterile component of the automated contrast injection system or contrast management system. If the integrity of the *Imaging bulk package* and the delivery system cannot be assured through direct continuous supervision, the *Imaging bulk package* and all associated disposables for the automated contrast injection system or contrast management system should be discarded.

Designation as an *Imaging bulk package* is limited to injection, for injection, or injectable emulsion dosage forms as defined in *Nomenclature* ⟨1121⟩, *General Nomenclature Forms. Imaging bulk packages,* although containing more than one single dose, are exempt from the multiple-dose container volume limit of 30 mL. The contents of the *Imaging bulk package* must have demonstrated the ability to limit the growth of microorganisms over the labeled period of use.

[1] Exceptions may be considered only under conditions described in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩.

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Where a container is offered as an *Imaging bulk package*, the label must (1) state prominently "Imaging Bulk Package" and, in juxtaposition with this statement, include the following use statement: "For use only with an automated contrast injection system or contrast management system approved or cleared for use with this contrast agent in this Imaging Bulk Package"; (2) bear a statement limiting the time frame in which the container may be used once it has been entered, provided it is held under the labeled storage conditions; and (3) bear the statement, "See drug and device labeling for information on devices indicated for use with this Imaging Bulk Package and techniques to help assure safe use".

Noninjection Packaging Systems

Multiple-unit container: A *Container–closure system* that permits withdrawal of successive portions of a noninjection article without changing the safety, strength, quality, or purity of the remaining portion (e.g., bottle of capsules, tablets, and oral or topical liquids).

Single-unit container: A *Container–closure system* that holds a quantity of a noninjection article intended for administration as a single dose or a single finished device intended for use promptly after the *Packaging system* is opened.

Unit-dose container: A single-unit *Container–closure system* for an article intended for administration by other than the parenteral route as a single dose.

Unit-of-use container: A *Container–closure system* that contains a specific quantity of an article that is intended to be dispensed as such without further modification except for the addition of appropriate labeling (see ⟨7⟩). It is not permitted to repackage *Unit-of-use containers* for sale.

Miscellaneous

Repackaging: The act of removing a drug product from the original manufacturer's *Packaging system* and placing it into another *Packaging system*, usually one of smaller size.

Repackager: A firm that repackages drug products or medical devices for distribution (e.g., for resale to distributors, hospitals, or pharmacies). For drug products, this applies to a function that is beyond the regular practice of a pharmacy. The distribution is not patient-specific, in that there are no prescriptions. Repackagers and relabelers of medical devices are also required to register and list and meet the provisions described in 21 CFR §807.

Contract packager/contract repackager: A firm that is contracted by another organization, such as a manufacturer, to package bulk into a marketed *Container* of a drug product. A *Contract packager* does not take ownership from the manufacturer and generally receives the assigned expiration date from the manufacturer.

Dispenser: A licensed or registered practitioner who is legally responsible for providing the patient with a preparation that is in compliance with a prescription or a medication order and contains a specific patient label. In addition, dispensers may prepare limited quantities in anticipation of a prescription or medication order from a physician. *Dispensers* are governed by the board of pharmacy of the individual state. The terms "dispenser" and "pharmacy" are used interchangeably.

Beyond-use date: See ⟨7⟩.

Expiration date: See ⟨7⟩.

Black closure system or black bands: The use of a *Black closure system* on a vial (e.g., a black cap overseal and a black ferrule to hold the elastomeric closure) or the use of a *Black band* or series of bands above the constriction on an ampule is prohibited, except for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products, Potassium Chloride for Injection Concentrate*.

INJECTION PACKAGING

Packaging for sterile products intended for injection must be validated as meeting the containment and protection requirements that are essential for maintaining the article's quality. Refer to *Package Integrity Evaluation—Sterile Products* ⟨1207⟩, *Package Integrity Testing in the Product Life Cycle—Test Method Selection and Validation* ⟨1207.1⟩, *Package Integrity Leak Test Technologies* ⟨1207.2⟩, and *Package Seal Quality Test Technologies* ⟨1207.3⟩ for further information regarding sterile product *Container* closure integrity testing and validation. *Closures* for *Multiple-dose containers* permit the withdrawal of the contents without removal or destruction of the *Closure*. The *Closure* permits penetration by a needle and, upon withdrawal of the needle, closes at once, protecting the contents against contamination. Refer to ⟨381⟩ for *Closure* reseal tests that are useful for screening multiple-dose *Closures* for their reseal properties. Additional testing may be needed to ensure that the specific *Closure* selected for a product package is able to prevent loss of product contents and microbial contamination under anticipated conditions of multiple entry and use. Piggyback *Packaging systems* are usually intravenous infusion *Container–closure systems* that are used to administer a second infusion through a connector of some type or an injection port on the administration set of the first fluid, thereby avoiding the need for another injection site on the patient's body. Piggyback *Packaging systems* also are known as secondary infusion containers.

The volume of injection in a *Single-dose container* provides the amount specified for one-time parenteral administration, and in no case is more than sufficient to permit the withdrawal and administration of 1 L. Preparations intended for intraspinal, intracisternal, or peridural administration are packaged in *Single-dose containers* only. Unless otherwise specified in the individual monograph, a *Multiple-dose container* contains a volume of injection sufficient to permit the withdrawal of NMT 30 mL.

The following injections are exempt from the 1-L restriction of the foregoing requirements relating to packaging:

- Injections packaged for extravascular use as irrigation solutions or peritoneal dialysis solutions.
- Injections packaged for intravascular use as parenteral nutrition or as replacement or substitution fluid to be administered continuously during hemofiltration.

Injections packaged for intravascular use that may be used for intermittent, continuous, or bolus replacement fluid administration during hemodialysis or other procedures, unless excepted above, must conform to the 1-L restriction. Injections labeled for veterinary use are exempt from the packaging and storage requirements concerning the limitation to single-dose *Packaging systems* and the limitation on the volume of *Multiple-dose containers*.

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Packaging for Constitution

Containers, including the *Closures*, for dry solids intended for injection must not interact physically or chemically with the preparation in any manner that alters the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use. A *Packaging system* for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained. Where the Assay in a monograph provides a procedure for the Sample solution, in which the total withdrawable contents are to be withdrawn from a *Single-dose container* with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle NLT 2.5 cm (1 in) in length. Care must be taken to expel any air bubbles, and the contents are then discharged into a *Container* for dilution and assay.

MEDICAL GAS PACKAGING

Gas cylinder: A metallic *Packaging system* constructed of steel or aluminum and designed to hold medical gases under pressure; these gases may include: Carbon Dioxide USP, Helium USP, Medical Air USP, nitric oxide, Nitrous Oxide USP, Nitrogen NF, and Oxygen USP. As a safety measure, for carbon dioxide, helium, medical air, nitrous oxide, and oxygen, the Pin-Index Safety System of matched fittings is recommended for cylinders of Size E or smaller.

Change to read:

ASSOCIATED COMPONENTS

Many *Associated Components* are graduated for measurement and dose administration. *Associated Components* can be packaged with the drug product or sold and purchased separately. It is the responsibility of the manufacturer to ensure that the appropriate measurement and dosing component is provided or that a general purpose component, such as those described in this section, is specified for delivering the appropriate amount/dose with the intended accuracy. Liquid preparations have unique surface and flow characteristics. Consequently, the volume delivered from a measurement/dosing component may vary for each preparation.

The graduated *Associated Components* described in this section are for general use and should be composed of safe materials. Graduated markings should be legible, indelible, and on an extraoral surface that does not contact the product.

▲The associated volume markings must be in metric units only and limited to a single measurement scale that corresponds with the dosing instructions on the OTC or prescription container label (see *Prescription Container Labeling* ⟨17⟩). Under expected conditions of use, the volume error incurred in measuring liquids for individual dose administration by means of such graduated components should be NMT 10% of the indicated amount of the liquid preparation with which the graduated component will be used.▲ (Official 1-May-2019)

Dosing cup: A measuring device consisting of a small cup that may be packaged with oral liquid articles.

Dosing spoon: A measuring device consisting of a bowl and handle that may be packaged with oral liquid articles. The handle may be a graduated tube.

Medicine dropper: A measuring device consisting of a transparent or translucent barrel or tube that is generally fitted with a collapsible bulb. It may be packaged with oral liquid articles.

Oral syringe: A measuring device consisting of a plunger and barrel made of transparent or translucent plastic material and a seal on the end. It may be packaged with oral liquid articles. The syringe should deliver a measured amount of a liquid drug product.

TEMPERATURE AND STORAGE DEFINITIONS

Freezer: A place in which the temperature is controlled between –25° and –10° (–13° and 14° F). It is noted that, in some instances, articles may have a recommended storage condition below –20° (–4° F). In such cases, the temperature of the storage location should be controlled to ±10°.

Refrigerator: A cold place in which the temperature is controlled between 2° and 8° (36° and 46° F).

Cold: Any temperature not exceeding 8° (46° F).

Cool: Any temperature between 8° and 15° (46° and 59° F). [NOTE—An article for which storage in a cool place is directed may, alternatively, be stored and shipped as refrigerated, unless otherwise specified by the individual monograph.]

Room temperature (also referred to as Ambient temperature): The temperature prevailing in a working environment.

Controlled room temperature: The temperature maintained thermostatically that encompasses the usual and customary working environment of 20°–25° (68°–77° F). The following conditions also apply.

Mean kinetic temperature not to exceed 25°. Excursions between 15° and 30° (59° and 86° F) that are experienced in pharmacies, hospitals, and warehouses, and during shipping are allowed. Provided the mean kinetic temperature does not

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exceed 25°, transient spikes up to 40° are permitted as long as they do not exceed 24 h. Spikes above 40° may be permitted only if the manufacturer so instructs.

Articles may be labeled for storage at "controlled room temperature" or at "20°–25°", or other wording based on the same mean kinetic temperature [see also *Good Storage and Distribution Practices for Drug Products* ⟨1079⟩, *Quality Management System, Environmental Management System, Mean Kinetic Temperature (MKT) Calculation*].

An article for which storage at *Controlled room temperature* is directed may, alternatively, be stored and shipped in a cool place or refrigerated, unless otherwise specified in the individual monograph or on the label.

Warm: Any temperature between 30° and 40° (86° and 104° F).

Excessive heat: Any temperature above 40° (104° F).

Dry place: A place that does not exceed 40% average relative humidity at 20° (68° F) or the equivalent water vapor pressure at other temperatures. The determination may be made by direct measurement at the place. Determination is based on NLT 12 equally spaced measurements that encompass either a season, a year, or, where recorded data demonstrate, the storage period of the article. There may be values of up to 45% relative humidity provided that the average value does not exceed 40% relative humidity. Storage in a *Container* validated to protect the article from moisture vapor, including storage in bulk, is considered a *Dry place*.

Protect from freezing: The *Container* label will bear an appropriate instruction to protect the article from freezing in cases where freezing exposes an article to loss of strength or potency or to destructive alteration of its characteristics. These risks are present in addition to the risk that the *Container* may break if exposed to freezing temperatures.

Protect from light: Where light subjects an article to loss of strength or potency or to destructive alteration of its characteristics, the *Container* label bears an appropriate instruction to protect the article from light. The article must be packaged in a light-resistant *Container*.

⟨660⟩ CONTAINERS—GLASS

DESCRIPTION

Glass containers for pharmaceutical use are intended to come into direct contact with pharmaceutical products. Glass used for pharmaceutical containers is either borosilicate (neutral) glass or soda-lime-silica glass. Borosilicate glass contains significant amounts of boric oxide, aluminum oxide, and alkali and/or alkaline earth oxides in the glass network. Borosilicate glass has a high hydrolytic resistance and a high thermal shock resistance due to the chemical composition of the glass itself; it is classified as Type I glass. Soda-lime-silica glass is a silica glass containing alkaline metal oxides, mainly sodium oxide, and alkaline earth oxides, mainly calcium oxide, in the glass network. Soda-lime-silica glass has a moderate hydrolytic resistance due to the chemical composition of the glass itself; it is classified as Type III glass. Suitable treatment of the inner surface of Type III soda-lime-silica glass containers will raise the hydrolytic resistance from a moderate to a high level, changing the classification of the glass to Type II.

The following recommendations can be made as to the suitability of the glass type for containers for pharmaceutical products, based on the tests for hydrolytic resistance. Type I glass containers are suitable for most products for parenteral and nonparenteral uses. Type II glass containers are suitable for most acidic and neutral aqueous products for parenteral and nonparenteral uses. Type II glass containers may be used for alkaline parenteral products where stability data demonstrate their suitability. Type III glass containers usually are not used for parenteral products or for powders for parenteral use, except where suitable stability test data indicate that Type III glass is satisfactory.

The inner surface of glass containers may be treated to improve hydrolytic resistance. The outer surface of glass containers may be treated to reduce friction or for protection against abrasion or breakage. The outer surface treatment is such that it does not contaminate the inner surface of the container.

Information on chemical composition of glass types, the formation of glass containers, and factors that influence inner surface durability of glass containers is provided in *Evaluation of the Inner Surface Durability of Glass Containers* ⟨1660⟩. This chapter also contains recommended approaches to evaluate the potential of a drug product to cause the formation of glass particles and delamination.

Glass may be colored to provide protection from light by the addition of small amounts of metal oxides and is tested as described in *Spectral Transmission for Colored Glass Containers*. A clear and colorless container that is made light resistant by means of an opaque enclosure (see *Packaging and Storage Requirements* ⟨659⟩, *Light-Resistant*) is exempt from the requirements for spectral transmission.

SPECIFIC TESTS

The *Glass Grains Test* combined with the *Surface Glass Test* for hydrolytic resistance determines the glass type. The hydrolytic resistance is determined by the quantity of alkali released from the glass under the conditions specified. This quantity of alkali is extremely small in the case of the more resistant glasses, thus calling for particular attention to all details of the tests and the use of apparatus of high quality and precision. Conducting these tests in conjunction with a glass standard reference material (SRM) on a routine basis will help to ensure the accuracy of the method. Reference materials are available for both borosilicate glass (SRM 623) and soda-lime-silica glass (SRM 622) from the National Institute of Standards and Technology. The tests should be conducted in an area relatively free from fumes and excessive dust. Test selection is shown in *Table 1* and *Table 2*.

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Table 1. Determination of Glass Types

Container Type	Test	Reason
I, II, III	*Glass Grains Test*	Distinguishes Type I borosilicate glass from Type II and III soda-lime-silica glass

The inner surface of glass containers is the contact surface for pharmaceutical preparations, and the quality of this surface is determined by the *Surface Glass Test* for hydrolytic resistance. The *Surface Etching Test* may be used to determine whether high hydrolytic resistance is due to chemical composition or to surface treatement. Alternatively, the comparison of data from the *Glass Grains Test* and the *Surface Glass Test* may be used in *Table 2*.

Table 2. Determination of Inner Surface Hydrolytic Resistance

Container Type	Test	Reason
I, II, III	*Surface Glass Test*	Determines hydrolytic resistance of inner surface; distinguishes between *Type I* and *Type II* containers with high hydrolytic resistance and *Type III* containers with moderate hydrolytic resistance
I, II	*Surface Etching Test* or comparison of *Glass Grains Test* and *Surface Glass Test* data	Where it is necessary, determines whether high hydrolytic resistance is due to inner surface treatment or to the chemical composition of the glass containers

Glass containers must comply with their respective specifications for identity and surface hydrolytic resistance to be classified as Type I, II, or III glass. Type I or Type II containers for aqueous parenteral products are tested for extractable arsenic.

Hydrolytic Resistance

APPARATUS

Autoclave: For these tests, use an autoclave capable of maintaining a temperature of 121 ± 1°, equipped with a thermometer, or a calibrated thermocouple device, allowing a temperature measurement independent of the autoclave system; a suitable recorder; a pressure gauge; a vent cock; and a tray of sufficient capacity to accommodate the number of containers needed to carry out the test above the water level. Clean the autoclave and other apparatus thoroughly with Purified Water before use.
Mortar and pestle: Use a hardened-steel mortar and pestle, made according to the specifications in *Figure 1*.

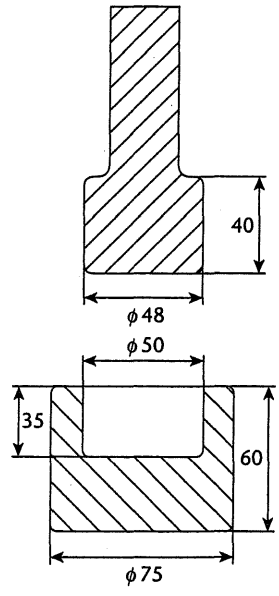


Figure 1. Mortar and pestle for pulverizing glass.

Other apparatus: Also required are a set of three square-mesh stainless steel sieves mounted on frames consisting of US Sieve Nos. 25, 40, and 50 (see *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩, *Table 1. Sizes of Standard Sieve Series in Range of Interest*); a mechanical sieve-shaker or a sieving machine that may be used to sieve the grains; a tempered, magnetic steel hammer; a permanent magnet; weighing bottles; stoppers; metal foil (e.g., aluminum, stainless steel); a hot air oven, capable of maintaining 140 ± 5°; a balance, capable of weighing up to 500 g with an accuracy of 0.005 g; a desiccator; and an ultrasonic bath.

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REAGENTS

Carbon dioxide-free water: This is Purified Water that has been boiled vigorously for 5 min or more and allowed to cool while protected from absorption of carbon dioxide from the atmosphere, or Purified Water that has a resistivity of not less than 18 Mohm-cm.

Methyl red solution: Dissolve 50 mg of methyl red in 1.86 mL of 0.1 M sodium hydroxide and 50 mL of ethanol (96%), and dilute with Purified Water to 100 mL. To test for sensitivity, add 100 mL of carbon dioxide-free water and 0.05 mL of 0.02 M hydrochloric acid to 0.1 mL of the methyl red solution. The resulting solution should be red. NMT 0.1 mL of 0.02 M sodium hydroxide is required to change the color to yellow. A color change from red to yellow corresponds to a change in pH from pH 4.4 (red) to pH 6.0 (yellow).

Glass Grains Test

The *Glass Grains Test* may be performed either on the canes used for the manufacture of tubing glass containers or on the containers.

SAMPLE PREPARATION

Rinse the containers to be tested with Purified Water, and dry in the oven. Wrap at least three of the glass articles in clean paper, and crush to produce two samples of about 100 g each in pieces NMT 30 mm across. Place in the mortar 30–40 g of the pieces between 10 and 30 mm across taken from one of the samples, insert the pestle, and strike it heavily with the hammer once only. Alternatively, transfer samples into a ball mill-breaker, add the balls, and crush the glass. Transfer the contents of the mortar or ball mill to the coarsest sieve (No. 25) of the set. Repeat the operation until all fragments have been transferred to the sieve. Shake the set of sieves for a short time by hand, and remove the glass that remains on sieves No. 25 and No. 40. Submit these portions to further fracture, repeating the operation until about 10 g of glass remains on sieve No. 25. Reject this portion and the portion that passes through sieve No. 50. Reassemble the set of sieves, and shake for 5 min. Transfer to a weighing bottle the glass grains that passed through sieve No. 40 and are retained on sieve No. 50. Repeat the crushing and sieving procedure with the second glass sample until two samples of grains are obtained, each of which weighs more than 10 g.

Spread each sample on a piece of clean glazed paper, and remove any iron particles by passing the magnet over them. Transfer each sample into a beaker for cleaning. Add 30 mL of acetone to the grains in each beaker, and scour the grains, using suitable means such as a rubber-tipped or plastic-coated glass rod. After scouring the grains, allow to settle, and decant as much acetone as possible. Add another 30 mL of acetone, swirl, decant, and add a new portion of acetone. Fill the bath of the ultrasonic vessel with water at room temperature, then place the beaker in the rack, and immerse it until the level of the acetone is at the level of the water; apply the ultrasound for 1 min. Swirl the beaker, allow to settle, and decant the acetone as completely as possible; then repeat the ultrasonic cleaning operation. If a fine turbidity persists, repeat the ultrasonic cleaning and acetone washing until the solution remains clear. Swirl, and decant the acetone. Dry the grains, first by putting the beaker on a warm plate, then by heating at 140° for 20 min in a drying oven. Transfer the dried grains from each beaker into separate weighing bottles, insert the stoppers, and cool in a desiccator.

METHOD

Filling and heating: Weigh 10.00 g of the cleaned and dried grains into two separate conical flasks. Pipet 50 mL of carbon dioxide-free Purified Water into each of the conical flasks (test solutions). Pipet 50 mL of carbon dioxide-free Purified Water into a third conical flask that will serve as a blank. Distribute the grains evenly over the flat bases of the flasks by shaking gently. Close the flasks with neutral glass dishes or aluminum foil rinsed with Purified Water or with inverted beakers so that the inner surfaces of the beakers fit snugly down onto the top rims of the flasks. Place all three flasks in the autoclave containing the water at ambient temperature, and ensure that they are held above the level of the water in the vessel. Carry out the following operations:

1. Insert the end of a calibrated thermometric device in a filled container through a hole of approximately the diameter of the thermocouple and connect it to an external measuring device. If the container is too small to insert a thermocouple, apply a thermocouple in a suitable, similar container. Alternatively, use the internal thermometer of the autoclave.
2. Close the autoclave door or lid securely but leave the vent-cock open.
3. Start automatic recording of the temperature versus time, and heat the autoclave at a regular rate such that steam issues vigorously from the vent-cock after 20–30 min, and maintain a vigorous evolution of steam for a further 10 min. For autoclaves using a steam generator, it is not necessary to maintain the temperature for 10 min at 100°.
4. Close the vent-cock, and raise the temperature from 100° to 121° at a rate of 1°/min within 20–22 min.
5. Maintain the temperature at 121 ± 1° for 30 ± 1 min from the time when the holding temperature is reached.
6. Cool down to 100° at a rate of 0.5°/min, venting to prevent formation of a vacuum, within 40–44 min.
7. Do not open the autoclave until it has cooled to 95°.
8. Remove the hot samples from the autoclave using appropriate safety precautions, and cool the samples cautiously down to room temperature within 30 min, avoiding thermal shock.

Titration: To each of the three flasks add 0.05 mL of *Methyl red solution*. Titrate the blank solution immediately with 0.02 M hydrochloric acid, then titrate the test solutions until the color matches that obtained with the blank solution. Subtract the titration volume for the blank solution from that for the test solutions. Calculate the mean value of the results in mL of 0.02 M hydrochloric acid per g of the sample. Repeat the test if the highest and lowest observed values differ by more than the permissible range given in *Table 3*.

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Table 3. Permissible Range for Values Obtained

Mean of the Values Obtained for the Consumption of Hydrochloric Acid Solution per g of Glass Grains (mL/g)	Permissible Range of the Values Obtained
NMT 0.10	25% of the mean
0.10–0.20	20% of the mean
NLT 0.20	10% of the mean

[NOTE—Where necessary to obtain a sharp endpoint, decant the clear solution into a separate 250-mL flask. Rinse the grains by swirling with three 15-mL portions of carbon dioxide-free water, and add the washings to the main solution. Add 0.05 mL of the *Methyl red solution*. Titrate, and calculate as before. In this case also add 45 mL of carbon dioxide-free Purified Water and 0.05 mL of *Methyl red solution* to the blank solution.]

LIMITS

The volume does not exceed the values indicated in *Table 4*.

Table 4. Test Limits for Glass Grains Test

Filling Volume (mL)	Maximum Volume of 0.02 M HCl per g of Test Glass (mL)	
	Type I	Types II and III
All	0.1	0.85

Surface Glass Test

DETERMINATION OF THE FILLING VOLUME

The filling volume is the volume of Purified Water to be added to the container for the purpose of the test. For vials, bottles, cartridges, and syringes, the filling volume is 90% of the brimful capacity. For ampuls, it is the volume up to the height of the shoulder.

Vials and bottles: Select six dry vials or bottles from the sample lot, or three if their capacity exceeds 100 mL, and remove any dirt or debris. Weigh the empty containers with an accuracy of 0.1 g. Place the containers on a horizontal surface, and fill them with Purified Water to about the rim edge, avoiding overflow and the introduction of air bubbles. Adjust the liquid levels to the brimful line. Weigh the filled containers to obtain the mass of the water expressed to two decimal places, for containers having a nominal volume less than or equal to 30 mL, and expressed to one decimal place, for containers having a nominal volume greater than 30 mL. Calculate the mean value of the brimful capacity in mL, and multiply it by 0.9. This volume, expressed to one decimal place, is the filling volume for the particular container lot.

Cartridges and syringes: Select six dry syringes or cartridges, and seal the small opening (mouth of cartridges; Luer cone or staked needle of syringes), using an inert material. Determine the mean brimful capacity and filling volume according to *Vials and Bottles*.

Ampuls: Place at least six dry ampuls on a flat, horizontal surface, and fill them with Purified Water from a buret until the water reaches point A, where the body of the ampul starts to decrease to the shoulder of the ampul (see *Figure 2*). Read the capacities, expressed to two decimal places, and calculate the mean value. This volume, expressed to one decimal place, is the filling volume for the particular ampul lot. The filling volume may also be determined by weighing.

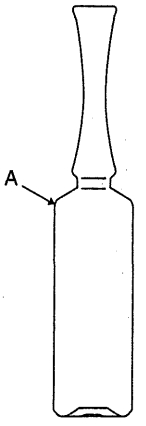


Figure 2. Filling volumes of ampuls up to point A.

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TEST

The determination is carried out on unused containers. The volumes of the test solution necessary for the final determination are shown in *Table 5*.

Table 5. Volume of Test Solution and Number of Titrations

Filling Volume (mL)	Volume of Test Liquid for One Titration (mL)	Number of Titrations
NMT 3	25.0	1
3–30	50.0	2
30–100	100.0	2
NLT 100	100.0	3

METHOD

Cleaning: Remove any debris or dust. Shortly before the test, rinse each container carefully at least twice with Purified Water, refilled, and allow to stand. Immediately before testing, empty the containers; rinse once with Purified Water, then with carbon dioxide-free water; and allow to drain. Complete the cleaning procedure from the first rinsing within 20–30 min. Closed ampules may be warmed in a water bath or in an air oven at about 40° for approximately 2 min before opening to avoid container pressure when opening. Do not rinse before testing.

Filling and heating: The containers are filled with carbon dioxide-free water up to the filling volume. Containers in the form of cartridges or prefillable syringes are closed in a suitable manner with material that does not interfere with the test. Each container, including ampuls, shall be loosely capped with an inert material such as a dish of neutral glass or aluminum foil previously rinsed with Purified Water. Place the containers on the tray of the autoclave. Place the tray in an autoclave containing a quantity of water such that the tray remains clear of the water. Close the autoclave, and carry out autoclaving procedure steps 1–8 as described in the *Glass Grains Test*, except that the temperature is maintained at 121 ± 1° for 60 ± 1 min. If a water bath is used for cooling samples, take care that the water does not make contact with the loose foil caps to avoid contamination of the extraction solution. The extraction solutions are analyzed by titration according to the method described below.

Titration: Carry out the titration within 1 h of the removal of the containers from the autoclave. Combine the liquids obtained from the containers, and mix. Introduce the prescribed volume (see *Table 5*) into a conical flask. Transfer the same volume of carbon dioxide-free water, to be used as a blank, into a second similar flask. Add to each flask 0.05 mL of *Methyl red solution* for each 25 mL of liquid. Titrate the blank with 0.01 M hydrochloric acid. Titrate the test solution with the same acid until the color of the resulting solution is the same as that obtained for the blank. Subtract the value found for the blank titration from that found for the test solution, and express the results in mL of 0.01 M hydrochloric acid per 100 mL of test solution. Express titration values of less than 1.0 mL to two decimal places; express titration values of greater than or equal to 1.0 mL to one decimal place.

LIMITS

The results, or the average of the results if more than one titration is performed, are not greater than the values stated in *Table 6*.

Table 6. Limit Values for the Surface Glass Test

Filling Volume (mL)	Maximum Volume of 0.01 M HCl per 100 mL of Test Solution (mL)	
	Types I and II	Type III
NMT 1	2.0	20.0
1–2	1.8	17.6
2–3	1.6	16.1
3–5	1.3	13.2
5–10	1.0	10.2
10–20	0.80	8.1
20–50	0.60	6.1
50–100	0.50	4.8
100–200	0.40	3.8
200–500	0.30	2.9
NLT–500	0.20	2.2

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Surface Etching Test

The *Surface Etching Test* is used in addition to the *Surface Glass Test* when it is necessary to determine whether a container has been surface treated and/or to distinguish between Type I and Type II glass containers. Alternatively, the *Glass Grains Test* and *Surface Glass Test* may be used. The *Surface Etching Test* may be carried out either on unused samples or on samples used in the *Surface Glass Test*.

METHOD

Vials and bottles: The volumes of test solution required are shown in *Table 5*. Rinse the containers twice with Purified Water, fill to the brimful point with a mixture of one volume of hydrofluoric acid and nine volumes of hydrochloric acid, and allow to stand for 10 min. Empty the containers, and rinse carefully five times with Purified Water. Immediately before the test, rinse once again with Purified Water. Submit these containers to the same autoclaving and determination procedure as described in the *Surface Glass Test*. If the results are considerably higher than those obtained from the original surfaces (by a factor of about 5–10), the samples have been surface treated.

[**CAUTION**—Hydrofluoric acid is extremely aggressive. Even small quantities can cause life threatening injuries.]

Ampuls, cartridges, and syringes: Apply the test method as described in *Vials and Bottles*. If the ampuls, cartridges, and syringes are not surface treated, the values obtained are slightly lower than those obtained in the previous tests.

[NOTE—Ampuls, cartridges, and syringes made from Type I glass tubing are not normally subjected to internal surface treatment.]

DISTINCTION BETWEEN TYPE I AND TYPE II GLASS CONTAINERS

The results obtained from the *Surface Etching Test* are compared to those obtained from the *Surface Glass Test*. For Type I glass containers, the values obtained are close to those found in the *Surface Glass Test*. For Type II glass containers, the values obtained greatly exceed those found in the *Surface Glass Test*; and they are similar to, but not greater than, those obtained for Type III glass containers of the same filling volume.

IMPURITIES

Arsenic ⟨211⟩

Use as the *Test Preparation* 35 mL of the water from one Type I or Type II glass container, or, in the case of smaller containers, 35 mL of the combined contents of several Type I or Type II glass containers, prepared as directed in the *Surface Glass Test*. The limit does not exceed 0.1 µg/g.

FUNCTIONALITY

Spectral Transmission for Colored Glass Containers

APPARATUS

A UV-Vis spectrophotometer, equipped with either a photodiode detector or a photomultiplier tube coupled with an integrating sphere

SAMPLE PREPARATION

Break the glass container or cut it with a circular saw fitted with a wet abrasive wheel, such as a carborundum or a bonded diamond wheel. Select sections representative of the wall thickness, and trim them as suitable for mounting in a spectrophotometer. After cutting, wash and dry each specimen, taking care to avoid scratching the surfaces. If the specimen is too small to cover the opening in the specimen holder, mask the uncovered portion of the opening with opaque paper or tape, provided that the length of the specimen is greater than that of the slit. Before placing in the holder, wash, dry, and wipe the specimen with lens tissue. Mount the specimen with the aid of wax, or by other convenient means, taking care to avoid leaving fingerprints or other marks.

METHOD

Place the specimen in the spectrophotometer with its cylindrical axis parallel to the slit and in such a way that the light beam is perpendicular to the surface of the section and the losses due to reflection are at a minimum. Measure the transmission of the specimen with reference to air in the spectral region of 290–450 nm, continuously or at intervals of 20 nm.

LIMITS

The observed spectral transmission for colored glass containers for products for nonparenteral use does not exceed 10% at any wavelength in the range of 290–450 nm, irrespective of the type and capacity of the glass container. The observed spectral transmission in colored glass containers for parenteral products does not exceed the limits given in *Table 7*.

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Table 7. Limits of Spectral Transmission for Colored Glass Containers for Parenteral Products

Nominal Volume (mL)	Maximum Percentage of Spectral Transmission at Any Wavelength between 290 nm and 450 nm	
	Flame-Sealed Containers	Containers with Closures
NMT 1	50	25
1–2	45	20
2–5	40	15
5–10	35	13
10–20	30	12
NLT 20	25	10

⟨661⟩ PLASTIC PACKAGING SYSTEMS AND THEIR MATERIALS OF CONSTRUCTION

(If the option of early adoption of *Plastic Materials of Construction* ⟨661.1⟩ and *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩ is not used prior to May 1, 2020, the requirements under this chapter will apply.)

INTRODUCTION

It is the purpose of this chapter to provide standards for plastic materials and components used to package medical articles (pharmaceuticals, biologics, dietary supplements, and devices). Definitions that apply to this chapter are provided in *Packaging and Storage Requirements* ⟨659⟩. Standards and tests for the functional properties of containers and their components are provided in *Containers—Performance Testing* ⟨671⟩.

In addition to the standards provided herein, the ingredients added to the polymers, and those used in the fabrication of the containers, must conform to the requirements in the applicable sections of the *Code of Federal Regulations*, Title 21, *Indirect Food Additives*, or have been evaluated by the FDA and determined to be acceptable substances for the listed use.

Plastic articles are identified and characterized by IR spectroscopy and differential scanning calorimetry. Standards are provided in this chapter for the identification and characterization of the different types of plastic, and the test procedures are provided at the end of the chapter. The degree of testing is based on whether or not the container has direct contact with the drug product, and the risk is based on the route of administration.

Plastics are composed of a mixture of homologous polymers, having a range of molecular weights. Plastics may contain other substances such as residues from the polymerization process, plasticizers, stabilizers, antioxidants, pigments, and lubricants. These materials meet the requirements for food contact as provided in the *Code of Federal Regulations*, Title 21. Factors such as plastic composition, processing and cleaning procedures, surface treatment, contacting media, inks, adhesives, absorption and permeability of preservatives, and conditions of storage may also affect the suitability of a plastic for a specific use. Extraction tests are designed to characterize the extracted components and identify possible migrants. The degree or extent of testing for extractables of the component is dependent on the intended use and the degree of risk to adversely impact the efficacy of the compendial article (drug, biologic, dietary supplement, or device). Resin-specific extraction tests are provided in this chapter for polyethylene, polypropylene, polyethylene terephthalate, and polyethylene terephthalate G. Test all other plastics as directed for *Test Methods, Physicochemical Tests*. Conduct the *Buffering Capacity* test only when the containers are intended to hold a liquid product.

Plastic components used for products of high risk, such as those intended for inhalation, parenteral preparation, and ophthalmics, are tested using *Test Methods, Biological Tests*.

Plastic containers intended for packaging products prepared for parenteral use meet the requirements for *Test Methods, Biological Tests* and *Physicochemical Tests*. Standards are also provided for polyethylene containers used to package dry oral dosage forms that are not meant for constitution into solution.

POLYETHYLENE CONTAINERS

Scope

The standards and tests provided in this section characterize containers and components, produced from either low-density polyethylene or high-density polyethylene of either homopolymer or copolymer resins that are interchangeably suitable for packaging dry oral dosage forms not meant for constitution into solution. All polyethylene components are subject to testing by IR spectroscopy and differential scanning calorimetry. Where stability studies have been performed to establish the expiration date of a particular dosage form in the appropriate polyethylene container, then any other polyethylene container meeting these requirements may be similarly used to package such a dosage form, provided that the appropriate stability programs are

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expanded to include the alternative container, in order to ensure that the identity, strength, quality, and purity of the dosage form are maintained throughout the expiration period.

Background

High-density and low-density polyethylene are long-chain polymers synthesized under controlled conditions of heat and pressure, with the aid of catalysts from not less than 85.0% ethylene and not less than 95.0% total olefins. Other olefin ingredients that are most frequently used are butene, hexene, and propylene. High-density polyethylene and low-density polyethylene both have an IR absorption spectrum that is distinctive for polyethylene, and each possesses characteristic thermal properties. High-density polyethylene has a density between 0.941 and 0.965 g per cm^3. Low-density polyethylene has a density between 0.850 and 0.940 g per cm^3. Other properties that may affect the suitability of polyethylene include modulus of elasticity, melt index, environmental stress crack resistance, and degree of crystallinity after molding.

High-Density Polyethylene

INFRARED SPECTROSCOPY—Proceed as directed for *Test Methods, Multiple Internal Reflectance*. The corrected spectrum of the specimen exhibits major absorption bands only at the same wavelengths as the spectrum of USP High-Density Polyethylene RS.

DIFFERENTIAL SCANNING CALORIMETRY—Proceed as directed for *Test Methods, Thermal Analysis*. The thermogram of the specimen is similar to the thermogram of USP High-Density Polyethylene RS, similarly determined, and the temperature of the endotherm (*melt*) in the thermogram of the specimen does not differ from that of the USP Reference Standard by more than 6.0°.

HEAVY METALS AND NONVOLATILE RESIDUE—Prepare extracts of specimens for these tests as directed for *Test Methods, Physicochemical Tests*, except that for each 20.0 mL of *Extracting Medium* the portion shall be 60 cm^2, regardless of thickness.

HEAVY METALS—Containers meet the requirements for *Test Methods, Physicochemical Tests, Heavy Metals*.

NONVOLATILE RESIDUE—Proceed as directed for *Test Methods, Physicochemical Tests, Nonvolatile Residue*, except that the *Blank* shall be the same solvent used in each of the following test conditions: the difference between the amounts obtained from the *Sample Preparation* and the *Blank* does not exceed 12.0 mg when water maintained at a temperature of 70° is used as the *Extracting Medium*; does not exceed 75.0 mg when alcohol maintained at a temperature of 70° is used as the *Extracting Medium*; and does not exceed 100.0 mg when hexanes maintained at a temperature of 50° is used as the *Extracting Medium*.

COMPONENTS USED IN CONTACT WITH ORAL LIQUIDS—Proceed as directed for *Test Methods, Physicochemical Tests, Buffering Capacity*.

Low-Density Polyethylene

INFRARED SPECTROSCOPY—Proceed as directed for *Test Methods, Multiple Internal Reflectance*. The corrected spectrum of the specimen exhibits major absorption bands only at the same wavelengths as the spectrum of USP Low-Density Polyethylene RS.

DIFFERENTIAL SCANNING CALORIMETRY—Proceed as directed for *Test Methods, Thermal Analysis*. The thermogram of the specimen is similar to the thermogram of USP Low-Density Polyethylene RS, similarly determined, and the temperature of the endotherm (*melt*) in the thermogram of the specimen does not differ from that of the USP Reference Standard by more than 8.0°.

HEAVY METALS AND NONVOLATILE RESIDUE—Prepare extracts of specimens for these tests as directed for *Test Methods, Physicochemical Tests, Testing Parameters, Sample Preparation*, except that for each 20.0 mL of *Extracting Medium* the portion shall be 60 cm^2, regardless of thickness.

HEAVY METALS—Containers meet the requirements for *Test Methods, Physicochemical Tests, Heavy Metals*.

NONVOLATILE RESIDUE—Proceed as directed for *Test Methods, Physicochemical Tests, Nonvolatile Residue*, except that the *Blank* shall be the same solvent used in each of the following test conditions: the difference between the amounts obtained from the *Sample Preparation* and the *Blank* does not exceed 12.0 mg when water maintained at a temperature of 70° is used as the *Extracting Medium*; does not exceed 75.0 mg when alcohol maintained at a temperature of 70° is used as the *Extracting Medium*; and does not exceed 350.0 mg when hexanes maintained at a temperature of 50° is used as the *Extracting Medium*.

COMPONENTS USED IN CONTACT WITH ORAL LIQUIDS—Proceed as directed for *Test Methods, Physicochemical Tests, Buffering Capacity*.

POLYPROPYLENE CONTAINERS

Scope

The standards and tests provided in this section characterize polypropylene containers, produced from either homopolymers or copolymers, that are interchangeably suitable for packaging dry solid and liquid oral dosage forms. Where suitable stability studies have been performed to establish the expiration date of a particular dosage form in the appropriate polypropylene container, then any other polypropylene container meeting these requirements may be similarly used to package such a dosage form, provided that the appropriate stability programs are expanded to include the alternative container, in order to ensure that the identity, strength, quality, and purity of the dosage form are maintained throughout the expiration period.

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Background

Propylene polymers are long-chain polymers synthesized from propylene or propylene and other olefins under controlled conditions of heat and pressure, with the aid of catalysts. Examples of other olefins most commonly used include ethylene and butene. The propylene polymers, the ingredients used to manufacture the propylene polymers, and the ingredients used in the fabrication of the containers conform to the applicable sections of the *Code of Federal Regulations,* Title 21.

Factors such as plastic composition, processing and cleaning procedures, contacting media, inks, adhesives, absorption, adsorption and permeability of preservatives, and conditions of storage may also affect the suitability of a plastic for a specific use. The suitability of a specific polypropylene must be established by appropriate testing.

Polypropylene has a distinctive IR spectrum and possesses characteristic thermal properties. It has a density between 0.880 and 0.913 g per cm^3. The permeation properties of molded polypropylene containers may be altered when reground polymer is incorporated, depending on the proportion of reground material in the final product. Other properties that may affect the suitability of polypropylene used in containers for packaging drugs are the following: oxygen and moisture permeability, modulus of elasticity, melt flow index, environmental stress crack resistance, and degree of crystallinity after molding. The requirements in this section are to be met when dry solid and liquid oral dosage forms are to be packaged in a container defined by this section.

INFRARED SPECTROSCOPY—Proceed as directed for *Test Methods, Multiple Internal Reflectance.* The corrected spectrum of the specimen exhibits major absorption bands only at the same wavelengths as the spectrum of the respective USP Homopolymer Polypropylene RS or copolymer polypropylene standard, similarly determined.

DIFFERENTIAL SCANNING CALORIMETRY—Proceed as directed for *Test Methods, Thermal Analysis.* The temperature of the endotherm (*melt*) in the thermogram does not differ from that of the USP Reference Standard for homopolymers by more than 6.0°. The temperature of the endotherm obtained from the thermogram of the copolymer polypropylene specimen does not differ from that of the copolymer polypropylene standard by more than 12.0°.

HEAVY METALS AND NONVOLATILE RESIDUE—Prepare extracts of specimens for these tests as directed for *Test Methods, Physicochemical Tests, Sample Preparation,* except that for each 20 mL of *Extracting Medium* the portion shall be 60 cm^2, regardless of thickness.

HEAVY METALS—Containers meet the requirements for *Test Methods, Physicochemical Tests, Heavy Metals.*

NONVOLATILE RESIDUE—Proceed as directed for *Test Methods, Physicochemical Tests, Nonvolatile Residue,* except that the *Blank* shall be the same solvent used in each of the following test conditions: the difference between the amounts obtained from the *Sample Preparation* and the *Blank* does not exceed 10.0 mg when water maintained at a temperature of 70° is used as the *Extracting Medium;* does not exceed 60.0 mg when alcohol maintained at a temperature of 70° is used as the *Extracting Medium;* and does not exceed 225.0 mg when hexanes maintained at a temperature of 50° is used as the *Extracting Medium.* Containers meet these requirements for *Nonvolatile Residue* for all of the above extracting media.

[NOTE—Hexanes and alcohol are flammable. When evaporating these solvents, use a current of air with the water bath; when drying the residue, use an explosion-proof oven.]

COMPONENTS USED IN CONTACT WITH ORAL LIQUIDS—Proceed as directed for *Test Methods, Physicochemical Tests, Buffering Capacity.*

POLYETHYLENE TEREPHTHALATE BOTTLES AND POLYETHYLENE TEREPHTHALATE G CONTAINERS

Scope

The standards and tests provided in this section characterize polyethylene terephthalate (PET) and polyethylene terephthalate G (PETG) bottles that are interchangeably suitable for packaging liquid oral dosage forms. Where stability studies have been performed to establish the expiration date of a particular liquid oral dosage form in a bottle meeting the requirements set forth herein for either PET or PETG bottles, any other PET or PETG bottle meeting these requirements may be similarly used to package such a dosage form, provided that the appropriate stability programs are expanded to include the alternative bottle in order to ensure that the identity, strength, quality, and purity of the dosage form are maintained throughout the expiration period. The suitability of a specific PET or PETG bottle for use in the dispensing of a particular pharmaceutical liquid oral dosage form must be established by appropriate testing.

Background

PET resins are long-chain crystalline polymers prepared by the condensation of ethylene glycol with dimethyl terephthalate or terephthalic acid. PET copolymer resins are prepared in a similar way, except that they may also contain a small amount of either isophthalic acid (not more than 3 mole percent) or 1,4-cyclohexanedimethanol (not more than 5 mole percent). Polymerization is conducted under controlled conditions of heat and vacuum, with the aid of catalysts and stabilizers.

PET copolymer resins have physical and spectral properties similar to PET and for practical purposes are treated as PET. The tests and specifications provided in this section to characterize PET resins and bottles apply also to PET copolymer resins and to bottles fabricated from them.

PET and PET copolymer resins generally exhibit a large degree of order in their molecular structure. As a result, they exhibit characteristic composition-dependent thermal behavior, including a glass transition temperature of about 76° and a melting temperature of about 250°. These resins have a distinctive IR absorption spectrum that allows them to be distinguished from other plastic materials (e.g., polycarbonate, polystyrene, polyethylene, and PETG resins). PET and PET copolymer resins have a

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density between 1.3 and 1.4 g per cm^3 and a minimum intrinsic viscosity of 0.7 dL per g, which corresponds to a number average molecular weight of about 23,000 Da.

PETG resins are high molecular weight polymers prepared by the condensation of ethylene glycol with dimethyl terephthalate or terephthalic acid and 15 to 34 mole percent of 1,4-cyclohexanedimethanol. PETG resins are clear, amorphous polymers, having a glass transition temperature of about 81° and no crystalline melting point, as determined by differential scanning calorimetry. PETG resins have a distinctive IR absorption spectrum that allows them to be distinguished from other plastic materials, including PET. PETG resins have a density of approximately 1.27 g per cm^3 and a minimum intrinsic viscosity of 0.65 dL per g, which corresponds to a number average molecular weight of about 16,000 Da.

PET and PETG resins, and other ingredients used in the fabrication of these bottles, conform to the requirements in the applicable sections of the *Code of Federal Regulations*, Title 21, regarding use in contact with food and alcoholic beverages. PET and PETG resins do not contain any plasticizers, processing aids, or antioxidants. Colorants, if used in the manufacture of PET and PETG bottles, do not migrate into the contained liquid.

INFRARED SPECTROSCOPY—Proceed as directed for *Test Methods, Multiple Internal Reflectance*. The corrected spectrum of the specimen exhibits major absorption bands only at the same wavelengths as the spectrum of USP Polyethylene Terephthalate RS, or USP Polyethylene Terephthalate G RS, similarly determined.

DIFFERENTIAL SCANNING CALORIMETRY—Proceed as directed for *Test Methods, Thermal Analysis*. For polyethylene terephthalate, the thermogram of the specimen is similar to the thermogram of USP Polyethylene Terephthalate RS, similarly determined: the melting point (T_m) of the specimen does not differ from that of the USP Reference Standard by more than 9.0°, and the glass transition temperature (T_g) of the specimen does not differ from that of the USP Reference Standard by more than 4.0°. For polyethylene terephthalate G, the thermogram of the specimen is similar to the thermogram of USP Polyethylene Terephthalate G RS, similarly determined: the glass transition temperature (T_g) of the specimen does not differ from that of the USP Reference Standard by more than 6.0°.

COLORANT EXTRACTION—Select three test bottles. Cut a relatively flat portion from the side wall of one bottle, and trim it as necessary to fit the sample holder of the spectrophotometer. Obtain the visible spectrum of the side wall by scanning the portion of the visible spectrum from 350 to 700 nm. Determine, to the nearest 2 nm, the wavelength of maximum absorbance. Fill the remaining two test bottles, using 50% alcohol for PET bottles and 25% alcohol for PETG bottles. Fit the bottles with impervious seals, such as aluminum foil, and apply closures. Fill a glass bottle having the same capacity as that of the test bottles with the corresponding solvent, fit the bottle with an impervious seal, such as aluminum foil, and apply a closure. Incubate the test bottles and the glass bottle at 49° for 10 days. Remove the bottles, and allow them to equilibrate to room temperature. Concomitantly determine the absorbances of the test solutions in 5-cm cells at the wavelength of maximum absorbance (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩), using the corresponding solvent from the glass bottle as the blank. The absorbance values so obtained are less than 0.01 for both test solutions.

HEAVY METALS, TOTAL TEREPHTHALOYL MOIETIES, AND ETHYLENE GLYCOL—

EXTRACTING MEDIA—

Purified Water—See monograph.

50 Percent Alcohol—Dilute 125 mL of alcohol with water to 238 mL, and mix.

25 Percent Alcohol—Dilute 125 mL of *50 Percent Alcohol* with water to 250 mL, and mix.

n-Heptane

GENERAL PROCEDURE— [NOTE—Use an *Extracting Medium* of *50 Percent Alcohol* for PET bottles and *25 Percent Alcohol* for PETG bottles.] For each *Extracting Medium,* fill a sufficient number of test bottles to 90% of their nominal capacity to obtain not less than 30 mL. Fill a corresponding number of glass bottles with *Purified Water*, a corresponding number of glass bottles with *50 Percent Alcohol* or *25 Percent Alcohol*, and a corresponding number of glass bottles with *n-Heptane* for use as *Extracting Media* blanks. Fit the bottles with impervious seals, such as aluminum foil, and apply closures. Incubate the test bottles and the glass bottles at 49° for 10 days. Remove the test bottles with the *Extracting Media* samples and the glass bottles with the *Extracting Media* blanks, and store them at room temperature. Do not transfer the *Extracting Media* samples to alternative storage vessels.

HEAVY METALS—Pipet 20 mL of the *Purified Water* extract of the test bottles, filtered if necessary, into one of two matched 50-mL color-comparison tubes, and retain the remaining *Purified Water* extract in the test bottles for use in the test for *Ethylene Glycol*. Adjust the extract with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH paper as an external indicator. Dilute with water to about 35 mL, and mix.

Into the second color-comparison tube, pipet 2 mL of freshly prepared (on day of use) *Standard Lead Solution* (see *Test Methods, Physicochemical Tests, Heavy Metals*), and add 20 mL of *Purified Water*. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH paper as an external indicator. Dilute with water to about 35 mL, and mix.

To each tube add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of *pH 3.5 Acetate Buffer* (see *Test Methods, Physicochemical Tests, Heavy Metals*), dilute with water to 50 mL, and mix: any color produced within 10 minutes in the tube containing the *Purified Water* extract of the test bottles does not exceed that in the tube containing the *Standard Lead Solution*, both tubes being viewed downward over a white surface (1 ppm in extract).

TOTAL TEREPHTHALOYL MOIETIES—Determine the absorbance of the *50 Percent Alcohol* or *25 Percent Alcohol* extract in a 1-cm cell at the wavelength of maximum absorbance at about 244 nm (see ⟨857⟩), using as the blank the corresponding *Extracting Medium* blank: the absorbance of the extract does not exceed 0.150, corresponding to not more than 1 ppm of total terephthaloyl moieties.

Determine the absorbance of the *n-Heptane* extract in a 1-cm cell at the wavelength of maximum absorbance at about 240 nm (see ⟨857⟩), using as the blank the *n-Heptane Extracting Medium*: the absorbance of the extract does not exceed 0.150, corresponding to not more than 1 ppm of total terephthaloyl moieties.

ETHYLENE GLYCOL—

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PERIODIC ACID SOLUTION—Dissolve 125 mg of periodic acid in 10 mL of water.

DILUTE SULFURIC ACID—To 50 mL of water add slowly and with constant stirring 50 mL of sulfuric acid, and allow to cool to room temperature.

SODIUM BISULFITE SOLUTION—Dissolve 0.1 g of sodium bisulfite in 10 mL of water. Use this solution within 7 days.

DISODIUM CHROMOTROPATE SOLUTION—Dissolve 100 mg of disodium chromotropate in 100 mL of sulfuric acid. Protect this solution from light, and use within 7 days.

STANDARD SOLUTION—Dissolve an accurately weighed quantity of ethylene glycol in water, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 1 µg per mL.

TEST SOLUTION—Use the *Purified Water* extract.

PROCEDURE—Transfer 1.0 mL of the *Standard Solution* to a 10-mL volumetric flask. Transfer 1.0 mL of the *Test Solution* to a second 10-mL volumetric flask. Transfer 1.0 mL of the *Purified Water Extracting Medium* to a third 10-mL volumetric flask. To each of the three flasks, add 100 µL of *Periodic Acid Solution,* swirl to mix, and allow to stand for 60 minutes. Add 1.0 mL of *Sodium Bisulfite Solution* to each flask, and mix. Add 100 µL of *Disodium Chromotropate Solution* to each flask, and mix.

[NOTE—All solutions should be analyzed within 1 hour after addition of the *Disodium Chromotropate Solution.*] Cautiously add 6 mL of sulfuric acid to each flask, mix, and allow the solutions to cool to room temperature. [**CAUTION**—Dilution of sulfuric acid produces substantial heat and can cause the solution to boil. Perform this addition carefully. Sulfur dioxide gas will be evolved. Use of a fume hood is recommended.] Dilute each solution with *Dilute Sulfuric Acid* to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard Solution* and the *Test Solution* in 1-cm cells at the wavelength of maximum absorbance at about 575 nm (see ⟨857⟩), using as the blank the solution from the *Purified Water Extracting Medium*: the absorbance of the solution from the *Test Solution* does not exceed that of the solution from the *Standard Solution,* corresponding to not more than 1 ppm of ethylene glycol.

TEST METHODS

Multiple Internal Reflectance

APPARATUS—Use an IR spectrophotometer capable of correcting for the blank spectrum and equipped with a multiple internal reflectance accessory and a KRS-5 internal reflection plate.[1] A KRS-5 crystal 2-mm thick having an angle of incidence of 45° provides a sufficient number of reflections.

SPECIMEN PREPARATION—Cut two flat sections representative of the average wall thickness of the container, and trim them as necessary to obtain segments that are convenient for mounting in the multiple internal reflectance accessory. Taking care to avoid scratching the surfaces, wipe the specimens with dry paper or, if necessary, clean them with a soft cloth dampened with methanol, and permit them to dry. Securely mount the specimens on both sides of the KRS-5 internal reflection plate, ensuring adequate surface contact. Prior to mounting the specimens on the plate, they may be compressed to thin uniform films by exposing them to temperatures of about 177° under high pressures (15,000 psi or more).

GENERAL PROCEDURE—Place the mounted specimen sections within the multiple internal reflectance accessory, and place the assembly in the specimen beam of the IR spectrophotometer. Adjust the specimen position and mirrors within the accessory to permit maximum light transmission of the unattenuated reference beam. (For a double-beam instrument, upon completing the adjustments in the accessory, attenuate the reference beam to permit full-scale deflection during the scanning of the specimen.) Determine the IR spectrum from 3500 to 600 cm^{-1} for polyethylene and polypropylene and from 4000 to 400 cm^{-1} for PET and PETG.

Thermal Analysis

GENERAL PROCEDURE—Cut a section weighing about 12 mg, and place it in the test specimen pan. [NOTE—Intimate contact between the pan and the thermocouple is essential for reproducible results.] Determine the thermogram under nitrogen, using the heating and cooling conditions as specified for the resin type and using equipment capable of performing the determinations as specified under *Thermal Analysis* ⟨891⟩.

FOR POLYETHYLENE—Determine the thermogram under nitrogen at temperatures between 40° and 200° at a heating rate between 2° and 10° per minute followed by cooling at a rate between 2° and 10° per minute to 40°.

FOR POLYPROPYLENE—Determine the thermogram under nitrogen at temperatures ranging from ambient to 30° above the melting point. Maintain the temperature for 10 minutes, then cool to 50° below the peak crystallization temperature at a rate of 10° to 20° per minute.

FOR POLYETHYLENE TEREPHTHALATE—Heat the specimen from room temperature to 280° at a heating rate of about 20° per minute. Hold the specimen at 280° for 1 minute. Quickly cool the specimen to room temperature, and reheat it to 280° at a heating rate of about 5° per minute.

FOR POLYETHYLENE TEREPHTHALATE G—Heat the specimen from room temperature to 120° at a heating rate of about 20° per minute. Hold the specimen at 120° for 1 minute. Quickly cool the specimen to room temperature, and reheat it to 120° at a heating rate of about 10° per minute.

[1] The multiple internal reflectance accessory and KRS-5 plate are available from several sources, including Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, CA 92634, and from Perkin Elmer Corp., Main Ave., Norwalk, CT 06856.

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Biological Tests

The in vitro biological tests are performed according to the procedures set forth under *Biological Reactivity Test, In Vitro* ⟨87⟩. Components that meet the requirements of the in vitro tests are not required to undergo further testing. No plastic class designation is assigned to these materials. Materials that do not meet the requirements of the in vitro tests are not suitable for containers for drug products.

If a plastic class designation is needed for plastics and other polymers that meet the requirements under ⟨87⟩, perform the appropriate in vivo test specified for *Biological Reactivity Test, In Vivo* ⟨88⟩, *Classification of Plastics*.

Physicochemical Tests

The following tests, designed to determine physical and chemical properties of plastics and their extracts, are based on the extraction of the plastic material, and it is essential that the designated amount of the plastic be used. Also, the specified surface area must be available for extraction at the designated temperature.

Testing Parameters—

Extracting Medium—Unless otherwise directed in a specific test below, use *Purified Water* (see monograph) as the *Extracting Medium,* maintained at a temperature of 70° during the extraction of the *Sample Preparation*.

Blank—Use *Purified Water* where a blank is specified in the tests that follow.

Apparatus—Use a water bath and the *Extraction Containers* as described in *Biological Reactivity Tests, In Vivo* ⟨88⟩, *Classification of Plastics, Apparatus*. Proceed as directed in the first paragraph of *Classification of Plastics, Preparation of Apparatus*. [NOTE—The containers and equipment need not be sterile.]

Sample Preparation—From a homogeneous plastic specimen, use a portion, for each 20.0 mL of *Extracting Medium,* equivalent to 120 cm^2 total surface area (both sides combined), and subdivide into strips approximately 3 mm in width and as near to 5 cm in length as is practical. Transfer the subdivided sample to a glass-stoppered, 250-mL graduated cylinder of Type I glass, and add about 150 mL of *Purified Water*. Agitate for about 30 seconds, drain off and discard the liquid, and repeat with a second washing.

Sample Preparation Extract—Transfer the prepared *Sample Preparation* to a suitable extraction flask, and add the required amount of *Extracting Medium.* Extract by heating in a water bath at the temperature specified for the *Extracting Medium* for 24 hours. Cool, but not below 20°. Pipet 20 mL of the prepared extract into a suitable container. [NOTE—Use this portion in the test for *Buffering Capacity*.] Immediately decant the remaining extract into a suitably cleansed container, and seal.

Nonvolatile Residue—Transfer, in suitable portions, 50.0 mL of the *Sample Preparation Extract* to a suitable, tared crucible (preferably a fused-silica crucible that has been acid-cleaned), and evaporate the volatile matter on a steam bath. Similarly evaporate 50.0 mL of the *Blank* in a second crucible. [NOTE—If an oily residue is expected, inspect the crucible repeatedly during the evaporation and drying period, and reduce the amount of heat if the oil tends to creep along the walls of the crucible.] Dry at 105° for 1 hour: the difference between the amounts obtained from the *Sample Preparation Extract* and the *Blank* does not exceed 15 mg.

Residue on Ignition ⟨281⟩— [NOTE—It is not necessary to perform this test when the *Nonvolatile Residue* test result does not exceed 5 mg.] Proceed with the residues obtained from the *Sample Preparation Extract* and from the *Blank* in the test for *Nonvolatile Residue* above, using, if necessary, additional sulfuric acid but adding the same amount of sulfuric acid to each crucible: the difference between the amounts of residue on ignition obtained from the *Sample Preparation Extract* and the *Blank* does not exceed 5 mg.

Heavy Metals—Pipet 20 mL of the *Sample Preparation Extract,* filtered if necessary, into one of two matched 50-mL color-comparison tubes. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH paper as an external indicator, dilute with water to about 35 mL, and mix.

Into the second color-comparison tube pipet 2 mL of *Standard Lead Solution,* and add 20 mL of the *Blank*. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH paper as an external indicator, dilute with water to about 35 mL, and mix. To each tube add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of *pH 3.5 Acetate Buffer,* dilute with water to 50 mL, and mix: any brown color produced within 10 minutes in the tube containing the *Sample Preparation Extract* does not exceed that in the tube containing the *Standard Lead Solution,* both tubes being viewed downward over a white surface (1 ppm in extract).

Lead Nitrate Stock Solution—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard Lead Solution—On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per gram of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

pH 3.5 Acetate Buffer—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Buffering Capacity—Titrate the previously collected 20-mL portion of the *Sample Preparation Extract* potentiometrically to a pH of 7.0, using either 0.010 N hydrochloric acid or 0.010 N sodium hydroxide, as required. Treat a 20.0-mL portion of the *Blank* similarly: if the same titrant was required for both the *Sample Preparation Extract* and the *Blank,* the difference between the two volumes is not greater than 10.0 mL; and if acid was required for either the *Sample Preparation Extract* or the *Blank* and alkali for the other, the total of the two volumes required is not greater than 10.0 mL.

(The text above is official until April 30, 2020. The text beginning below becomes official on May 1, 2020.)

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Change to read:

▲INTRODUCTION

Systems are used to package therapeutic products (pharmaceuticals, biologics, dietary supplements and devices). Such systems and their associated materials and components of construction are considered and defined in *Packaging and Storage Requirements* ⟨659⟩. Such systems may be constructed from plastic materials and components. The plastics used in packaging systems are composed of homologous polymers with a range of molecular weights and contain additives such as antioxidants, stabilizers, lubricants, plasticizers, colorants, and others. The nature and amount of additives in the plastics used for packaging systems are dictated by the type of polymer, the polymer's use, and the process used to convert the polymer into components, containers, or packaging systems.

Therapeutic products come into direct contact with packaging systems and their plastic materials of construction as the product is manufactured, stored, and administered. Such contact may result in an interaction between the therapeutic products and the packaging systems and its materials or components of construction. These interactions must be such that the suitability for use (including its safety and efficacy) of the therapeutic product and the packaging systems is not adversely affected by the interaction. Although suitability for use includes several quality aspects of the packaged drug product and its performance, the suitability for use aspect addressed in this chapter is patient safety. Obtaining such a necessary and desirable outcome is facilitated by the use of well-characterized plastic materials of construction in components, containers, and packaging systems and by the appropriate testing of packaging systems.

SCOPE

Establishing the suitability of plastic packaging systems for therapeutic products involves multiple tests and testing procedures, as briefly outlined below:

- Material screening: Characterization of a packaging system's materials of construction to evaluate ingredients as probable extractables and potential leachables. Such a characterization facilitates the identification of materials that are suitable for use in packaging systems.
- Controlled extraction (simulation) study: Worst-case controlled extraction (simulation) study to determine the extent to which extractables may become probable leachables (for additional information, see *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1663⟩).
- Product assessment: Actual-case measurement of confirmed leachables in the therapeutic product in the pharmaceutical packaging/delivery system intended for the commercial market (for additional information, see *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1664⟩).

Additionally, information provided by the vendor(s) of plastic packaging systems and their associated materials or components of construction can facilitate suitability assessments, as such information may be appropriate additions to or surrogates for the results obtained by performing the tests noted previously.

The process of manufacturing a packaged therapeutic product is complex. Considering the packaging system specifically, packaging systems typically consist of components that are individually manufactured from plastic materials of construction. These individual plastic materials of construction are initially generated from reagents that are reacted to produce a base polymer, which is then compounded with various additives to produce a base resin. Individual base resins either are materials of construction themselves or may be combined with additional additives and processing aids to form a plastic material of construction. Testing of these plastic materials of construction to establish that they are well characterized and suitable for use, specifically considering safety, in packaging systems is within the scope of this series of chapters and is addressed in *Plastic Materials of Construction* ⟨661.1⟩.

Individual plastic materials of construction are combined to form components of the packaging system. The packaging system is completed by assembling its various components into its final form. Testing of packaging systems to establish that they are suited for their intended uses, specifically considering safety, is within the scope of this series of chapters and is addressed in *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩.

Assembled packaging systems are filled to contain the therapeutic product by various means and at various points in the packaging system manufacturing process, thereby generating the packaged therapeutic product. Testing of packaged therapeutic products to establish that they are suited for their intended uses is addressed in compendial monographs relevant to the specific therapeutic product and falls outside of the scope of this series of chapters.

For more information on the scope of, applicability of, and other topics related to the ⟨661⟩ suite of general chapters, see *Evaluation of Plastic Packaging Systems and Their Materials of Construction with Respect to Their User Safety Impact* ⟨1661⟩.▲ (CN 1-May-2020)

(Postponed until May 1, 2020.)

⟨661.1⟩ PLASTIC MATERIALS OF CONSTRUCTION

(This chapter will become official on May 1, 2020. Early adoption of the requirements in this chapter and its companion chapter *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩ **are permitted by USP. When early adoption is not used,** *Plastic Packaging Systems and Their Materials of Construction* ⟨661⟩ **will apply and must be met wherever this chapter or** ⟨661.2⟩ **is referenced in the** ***USP–NF.*****)**

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Introduction

The use of well-characterized materials to construct packaging systems is a primary means of ensuring that the packaging system is suited for its intended use. Materials are characterized so that their properties and characteristics can be matched to the performance requirements of the packaging system, thus facilitating the intentional selection of appropriate materials. For the purposes of this chapter, a plastic material of construction is deemed to be well characterized for its intended use if the following characteristics have been adequately established: its identity, biocompatibility (biological reactivity), general physicochemical properties, and composition (i.e., additives and extractable metals likely to be present).

Scope

The purpose of this chapter is to provide test methods and specifications for plastic materials of construction used in packaging systems. Individual plastic materials of construction are deemed to be well characterized and appropriate for use if they meet the requirements in this chapter or are used in a packaging system that meets the requirements in *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩. The testing and qualification of plastic packaging systems and components for pharmaceutical use are covered in ⟨661.2⟩.

This chapter contains tests, methods, and specifications for the following materials: cyclic olefins; polyamide 6; polycarbonate; polyethylene; polyethylene terephthalate; polyethylene terephthalate G; poly(ethylene-vinyl acetate); polypropylene; polyvinyl chloride; and polyvinyl chloride, plasticized.

Plastic packaging systems could be constructed from materials that are not specifically addressed in this chapter; such materials of construction are termed "unaddressed materials". For an unaddressed material to be deemed compliant with this chapter, it must be characterized in ways that are comparable to those used for the materials specified in this chapter. Specifically, the unaddressed material of construction must be identified by appropriate methodology and tested for biocompatibility, physicochemical properties, additives, and relevant extracted metals.

Specifications must be established for unaddressed materials, and such specifications should be consistent with the specifications for materials addressed in this chapter. For example, unaddressed materials whose aqueous extracts are tested for their total organic carbon (TOC) levels must have a specification for TOC that is consistent with the TOC specification for materials addressed in this chapter.

Alternatively, individual plastic materials of construction are deemed to be well characterized and appropriate for use if they are used in a packaging system that meets the requirements in ⟨661.2⟩. However, such materials are appropriate for use only in the packaging system that meets the requirements of ⟨661.2⟩. The appropriateness for use of such materials in other packaging systems must be established for the other packaging systems via proper testing.

Table 1 provides guidance on the appropriate application of the chemical tests for oral and topical dosage forms, which include oral tablets, oral hard and soft gelatin capsules, oral powders, solutions and suspensions, topical powders, and aqueous-based topical solutions and suspensions. *Table 2* provides guidance on the appropriate application of the chemical tests and biological reactivity tests for all other dosage forms. [NOTE—For aqueous-based oral drug products that contain cosolvents (or if, for any reason, it may be expected to extract greater amounts of substances from plastic packaging components than water), additional extractables information may be needed to address safety issues. If additional information is required, perform *Extractable metals* and *Plastic additives* tests as directed in *Table 2*.]

Table 1. Guidelines for Application of Tests for Oral and Topical Dosage Forms

Biological Reactivity Tests	Chemical Tests
Not required	• *Identification, Physicochemical tests,* and *Extractable metals* • Provide appropriate reference to the Indirect Food Additive regulations in 21 CFR 174–186, specifically those addressing the purity criteria and limitations pertaining to use • Materials that do not meet these requirements are not suitable for packaging for these dosage forms unless the materials are established to be suitable by other means that have been approved by an appropriate regulatory authority

Table 2. Guidelines for Application of Tests for All Other Dosage Forms

Biological Reactivity Tests	Chemical Tests
• *Biological Reactivity Tests, In Vitro* ⟨87⟩ • *Biological Reactivity Tests, In Vivo* ⟨88⟩, *Classification of Plastics* • Materials that do not meet the requirements of the in vivo or in vitro tests are not suitable for containers for these dosage forms	• *Identification, Physicochemical tests, Extractable metals,* and *Plastic Additives* • Materials that do not meet these requirements are not suitable for containers for these dosage forms unless the materials are established to be suitable by other means that have been approved by an appropriate regulatory authority

Change to read:

Specifications

Specifications are provided for absorbance and TOC. If the specification for absorbance or TOC is exceeded, then the material can still be deemed to be compliant with this chapter if the chemicals responsible for the test results can be established (identity and concentration) and the chemicals are characterized to establish that the probable risk posed by all the chemicals, considered individually, is within acceptable parameters.

• CYCLIC OLEFINS

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Cyclic Olefin Polymer Reference Standard (RS) or USP Cyclic Olefin Copolymer RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical

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variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: Given the amorphous nature of these polymers and their compositional variety, material-to-material variations in the melting peak temperature can be anticipated. [NOTE—The identification of the cyclic olefins needs compliance with only one test procedure to be established.]

Physicochemical tests

[NOTE—For all extraction solutions, see *Table 3*.]

Absorbance (Solution S1): Maximum absorbance is 0.2.

Acidity or alkalinity: NMT 1.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 1.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Aluminum: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Arsenic, cadmium, lead, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.

Titanium: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Zinc: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Test results for additional relevant extractable metals are similarly reported.

Plastic additives, phenolic antioxidants, nonphenolic antioxidants, copolymer of dimethyl succinate and (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)ethanol, amides, and stearates: The test results from these analyses are reported.

• **POLYAMIDE 6**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Polyamide 6 RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polyamide 6 RS, and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 8.0°. Note that the results of the differential scanning calorimetry (DSC) analysis are strongly dependent on the amount of plasticizer in the test article. [NOTE—The identification of polyamide 6 needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): NMT 0.25

Acidity or alkalinity: NMT 1.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 4.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Free base functions: The difference between the titration volumes, extract versus extraction blank, is NMT 0.4 mL.

Extractable metals

Arsenic, lead, cadmium, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g. Additional acceptance criteria for certain metals are provided as follows.

Test results for additional relevant extractable metals are similarly reported.

Related substances

Caprolactam: NMT 1%

• **POLYCARBONATE**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197A.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Polycarbonate RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polycarbonate RS, and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 8.0°. Note that the results of the DSC analysis are strongly dependent on the amount of plasticizer in the test article. [NOTE—The identification of polycarbonate needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): NMT 0.20

Acidity or alkalinity: NMT 1.5 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

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Extractable metals

Arsenic, lead, cadmium, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g. Additional acceptance criteria for certain metals are provided as follows.

Test results for additional relevant extractable metals are similarly reported.

Related substances

Residual solvents

Methylene chloride: NMT 200 µg/g

Toluene: NMT 200 µg/g

Sum of toluene and ethylbenzene: NMT 200 µg/g

Chlorobenzene: NMT 500 µg/g

Bisphenol A: NMT 100 µg/g

• **POLYETHYLENE**

Identification

Low-density polyethylene

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Low-Density Polyethylene RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Low-Density Polyethylene RS, and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 8.0°. [NOTE—The identification of low-density polyethylene needs compliance with only one test procedure to be established.]

High-density polyethylene

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP High-Density Polyethylene RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP High-Density Polyethylene RS, and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 6.0°. [NOTE—The identification of high-density polyethylene needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): Maximum absorbance is 0.2.

Acidity or alkalinity: NMT 1.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 1.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Aluminum: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Arsenic, cadmium, lead, mercury, cobalt, and nickel: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.

Chromium: *Solution S3* contains NMT 0.02 mg/L (ppm), corresponding to 0.05 µg/g.

Titanium: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Vanadium: *Solution S3* contains NMT 0.04 mg/L (ppm), corresponding to 0.1 µg/g.

Zinc: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Zirconium: *Solution S3* contains NMT 0.04 mg/L (ppm), corresponding to 0.1 µg/g.

Test results for additional relevant extractable metals are similarly reported.

Plastic additives, phenolic antioxidants, nonphenolic antioxidants, amides, and stearates: The test results from these analyses are reported.

• **POLYETHYLENE TEREPHTHALATE AND POLYETHYLENE TEREPHTHALATE G**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Polyethylene Terephthalate RS or USP Polyethylene Terephthalate G RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry

Polyethylene terephthalate: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polyethylene Terephthalate RS and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 4.0°. [NOTE—The identification of polyethylene terephthalate needs compliance with only one test procedure to be established.]

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Polyethylene terephthalate G: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polyethylene Terephthalate G RS. The melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 6.0°. [NOTE—The identification of polyethylene terephthalate G need be established by using one test procedure.]

Physicochemical tests

Absorbance: Maximum absorbance is 0.2 for *Solution S1* and 0.05 for *Solution S5*. In addition, for colored polyethylene terephthalate, maximum absorbance between 400 and 800 nm is 0.05 for *Solution S1*.

Acidity or alkalinity: NMT 0.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 0.5 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Aluminum: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Antimony: *Solution S4* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Arsenic, cadmium, lead, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.

Barium: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Germanium: *Solution S4* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Manganese: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Titanium: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Zinc: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

Test results for additional relevant extractable metals are similarly reported.

• **POLY(ETHYLENE-VINYL ACETATE)**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Poly(ethylene-vinyl acetate) RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Poly(ethylene-vinyl acetate) RS, and the melting point temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 6.0°. [NOTE—The identification of poly(ethylene-vinyl acetate) needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): Maximum absorbance is 0.2.

Acidity or alkalinity: NMT 1.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

All metals: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.

Test results for additional relevant extractable metals are similarly reported.

Phenolic antioxidants, amides, and stearates: The test results from these analyses are reported.

Related substances

Content of vinyl acetate

For containers: NMT 25% by weight

For tubing: NMT 30% by weight

• **POLYPROPYLENE**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of the USP Homopolymer Polypropylene RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The melting peak temperature in the thermal analysis curve does not differ from that of USP Homopolymer Polypropylene RS by more than 12.0°. [NOTE—The identification of polypropylene needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): Maximum absorbance is 0.2.

Acidity or alkalinity: NMT 1.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 1.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Aluminum: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.

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Arsenic, cadmium, lead, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.
Chromium: *Solution S3* contains NMT 0.02 mg/L (ppm), corresponding to 0.05 µg/g.
Titanium: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.
Zinc: *Solution S3* contains NMT 0.4 mg/L (ppm), corresponding to 1 µg/g.
Test results for additional relevant extractable metals are similarly reported.
Plastic additives, phenolic antioxidants, nonphenolic antioxidants, amides, and stearates: The test results from these analyses are reported.

• **POLYVINYL CHLORIDE**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197F.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Polyvinyl Chloride RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.
Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polyvinyl Chloride RS, and the melting peak temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS by more than 8.0°. Note that the results of the DSC analysis are strongly dependent on the amount of plasticizer in the test article. [NOTE—The identification of polyvinyl chloride needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): NMT 0.25 for containers for non-injectable aqueous solutions. NMT 0.30 for containers for dry dosage forms for oral administration.
Absorbance (Solution S6): NMT 0.2 for tin-stabilized materials used as containers for non-injectable aqueous solutions. NMT 0.4 for other materials used as containers for non-injectable aqueous solutions. NMT 1.0 for materials that do not contain 1-phenyleicosane-1,3-dione used as containers for dry dosage forms for oral administration. NMT 0.4 for materials containing 1-phenyleicosane-1,3-dione used as containers for dry dosage forms for oral administration.
Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Aluminum, arsenic, lead, cadmium, mercury, cobalt, nickel, vanadium, and zinc: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.
Barium: *Solution S3* contains NMT 0.10 mg/L (ppm), corresponding to 2 µg/g.
Cadmium: *Solution S3* contains NMT 0.03 mg/L (ppm), corresponding to 0.6 µg/g.
Zinc: *Solution S3* contains NMT 5 mg/L (ppm), corresponding to 100 µg/g.
Test results for additional relevant extractable metals are similarly reported.

Plastic additives

Epoxidized soya oil of which the oxiran oxygen content is 6%–8% and the iodine value is NMT 6: For tin-stabilized materials, NMT 2%. For non-tin-stabilized materials, NMT 3%.
Calcium, magnesium, or zinc salts for aliphatic fatty acids with more than seven carbon atoms: NMT 1.5% of one salt or NMT 1.5% of a mixture of salts
Lubricants: For individual lubricants: waxes, NMT 4%; liquid paraffin, NMT 1.5%; hydrogenated oils or esters of aliphatic fatty acids, NMT 2%. Total lubricants: NMT 4%.
Macrogol esters: NMT 1.5%
Sorbitol: NMT 1.5%
2,4-Dinonylphenyl phosphite or di(4-nonylphenyl) phosphite or tris(nonylphenyl) phosphite: NMT 1%
Calcium carbonate: For materials used for containers for dry dosage forms for oral administration, NMT 1%
Silica: For materials used for containers for dry dosage forms for oral administration, NMT 1%
Vinyl chloride: NMT 1 ppm. Note that vinyl chloride is not an additive but is monitored as a residual monomer.
Tin: Tin-stabilized materials, NMT 0.25 weight %. Non-tin-stabilized materials, NMT 25 µg/g (ppm).
Colorants: May contain a colorant or pigment or may be opacified by titanium dioxide

Stabilizers

They may contain one of the following groups of stabilizers (where isooctyl is, for example, 2-ethylhexyl).
Tin as di(isooctyl) 2,2′-[(dioctylstannylene)bis(thio)]-diacetate containing about 27% of tri(isooctyl)2,2′,2″-[(monooctylstannylidyne)tris(thio) triacetate: NMT 0.25%
Tin as a mixture containing NMT 76% of di(isooctyl) 2,2′-[(dioctylstannylene)bis(thio)]-diacetate and NMT 85% of tri(isooctyl)2,2′,2″-[(monooctylstannylidyne)tris(thio) triacetate: NMT 0.25%
1-Phenyleicosane-1,3-dione (benzoylstearoylmethane): NMT 1%

Related substances

Content of chlorine: NLT 80% by weight, expressed as polyvinyl chloride

• **POLYVINYL CHLORIDE, PLASTICIZED**

Identification

▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*: 197A.▲ (CN 1-May-2020) Determine the infrared spectrum from 3800 cm^{-1} to 650 cm^{-1} (2.6–15 µm). The specimen exhibits an absorption spectrum that is substantially equivalent to that of USP Polyvinyl Chloride, Plasticized RS. Substantial, as opposed to exact, equivalence allows for minor spectral differences arising from the natural compositional and/or physical variation among polymers of this class. Substantial

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equivalence is achieved when all differences between the sample and RS spectra can be explained in the context of such natural compositional and/or physical variations.

Differential scanning calorimetry: The thermal analysis curve of the specimen is similar to the thermal analysis curve of USP Polyvinyl Chloride, Plasticized RS, and the glass transition temperature obtained from the thermal analysis curve of the specimen does not differ from that of the RS. The nature of these polymers and compositional variety, material-to-material variations in the melting peak temperature can be anticipated. Note that the results of the DSC analysis are strongly dependent on the amount of plasticizer in the test article. [NOTE—The identification of polyvinyl chloride, plasticized needs compliance with only one test procedure to be established.]

Physicochemical tests

Absorbance (Solution S1): NMT 0.25

Acidity or alkalinity: NMT 1.5 mL of 0.01 N sodium hydroxide is required to change the color of the indicator to blue. NMT 1.0 mL of 0.01 N hydrochloric acid is required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The difference between the sample and blank TOC concentrations is NMT 5 mg/L.

Extractable metals

Arsenic, cadmium, lead, mercury, cobalt, nickel, and vanadium: Report the measured value in *Solution S3* at values above 0.01 mg/L (ppm), corresponding to 0.025 µg/g. If the measured values are below these values, report the result as less than 0.01 mg/L (ppm), corresponding to less than 0.025 µg/g.

Barium: *Solution S3* contains NMT 0.25 mg/L (ppm), corresponding to 5 µg/g.

Calcium: *Solution S3* contains NMT 35 mg/L (ppm), corresponding to 0.07 weight %.

Tin: *Solution S3* contains NMT 1 mg/L (ppm), corresponding to 20 µg/g.

Zinc: *Solution S3* contains NMT 100 mg/L (ppm), corresponding to 0.2 weight %.

Test results for additional relevant extractable metals are similarly reported.

Plastic additives

Di(2-ethylhexyl)phthalate: Residue is NMT 40 mg.

***N′ N″*-Diacylethylenediamines:** Residue is NMT 20 mg.

Epoxidized soya oil: The difference between the masses of both residues is NMT 10 mg.

Epoxidized linseed oil: The difference between the masses of both residues is NMT 10 mg.

Related substances

Vinyl chloride: NMT 1 ppm. Note that vinyl chloride is not an additive but is monitored as a residual monomer.

Test Methods

• IDENTIFICATION

The identification testing described in this chapter is required for all materials of construction used in packaging systems. The identification test should be accomplished by using the procedures specified in this chapter (infrared spectrophotometry or thermal analysis). If neither of these procedures are applicable for a particular material, then an alternative procedure can be used. The alternate procedure must establish the identity on the basis of obtaining substantially equivalent results for the test article and its appropriate USP RS.

Specifications must be established for materials that are not specified in this chapter, and such specifications should be consistent with the specifications established for materials that are specified in this chapter. For example, a DSC specification for a material that is not currently listed in this chapter should be consistent, in language and in rigor, with a DSC specification for a material that is listed in this chapter (e.g., melting peak temperature agreement between sample and reference material).

• INFRARED SPECTROPHOTOMETRY

Apparatus: Use an infrared spectrophotometer capable of correcting for the blank spectrum and able to measure in transmission mode or equipped with an internal reflectance accessory and an appropriate internal reflectance plate.

Sample preparation

Transmission mode: Prepare a specimen of appropriate thickness (polyethylene about 250 µm; polypropylene about 100 µm) without visible defects (cracks or holes). The specimens can be compressed to form a thin, uniform film by exposure to elevated temperatures and pressures (2000 psi or more). The temperatures at which the thin films are generated represent a trade-off between producing a melt (which dictates the lowest temperature necessary) and degrading the sample (which dictates the highest temperature allowed). Ultimately, the temperatures that are used are appropriate if the film produced is conducive to the infrared analysis.

Internal reflectance mode: Prepare a flat section and trim it as necessary to obtain a segment that is convenient for mounting in the internal reflectance accessory. Taking care to avoid scratching the surfaces, wipe the specimen with dry paper or, if necessary, a soft cloth dampened with methanol, and permit the surfaces to dry. Then securely mount the specimen on the internal reflection plate, ensuring adequate surface contact.

Procedure, all materials unless otherwise noted: Place the mounted specimen sections in the sample compartment of the infrared spectrophotometer or the internal reflectance accessory, and place the assembly in the specimen beam of the infrared spectrophotometer. For internal reflectance, adjust the specimen position and mirrors within the accessory to permit maximum light transmission of the unattenuated reference beam. (For a double-beam instrument, attenuate the reference beam after completing the adjustment in the accessory to permit full-scale deflection during the scanning of the specimen.)

Polycarbonate: Prepare a hot-pressed film. Otherwise, dissolve 0.5 g of test material in 10 mL methylene chloride by boiling under a reflux condenser for 15 min. Place a few drops of the resulting solution on a sodium chloride slide and evaporate the solvent in an oven at 80°.

Polyvinyl chloride: Dissolve residue A from *Solution S6* in 5 mL of tetrahydrofuran. Apply a few drops of this solution to a sodium chloride plate and evaporate to dryness in an oven at 100°–105°.

Thermal analysis

Refer to *Thermal Analysis* ⟨891⟩.

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Sample preparation: Place an appropriately sized sample in the test specimen pan. [NOTE—Intimate contact between the pan and the thermocouple is essential for obtaining reproducible results.] Determine the thermal analysis curve under nitrogen, using heating/cooling conditions specified for the polymer type and using equipment capable of performing the determinations as described in ⟨891⟩. Thermal analysis curves are obtained for the test materials and their associated USP RS.

Procedure

Cyclic olefins: Given the amorphous nature of these polymers and their compositional variety, material-to-material variations in the melting peak temperature can be anticipated. Thus, it is neither recommended nor required that DSC be performed.

Polyamide 6: Heat the specimen from room temperature to 500° at a heating rate of about 20°/min. Quickly cool the specimen to room temperature.

Polycarbonate: Heat the specimen from –20° to 300° at a heating rate of about 10°/min. Quickly cool the specimen to room temperature.

Polyethylene: Determine the thermal analysis curve under nitrogen at temperatures between 40° and 200° at a heating rate between 2° and 10°/min, followed by cooling at a rate between 2° and 10°/min, to 40°.

Poly(ethylene-vinyl acetate): Heat the specimen from –50° to 120° at a heating rate of about 10°/min. Quickly cool the specimen to room temperature.

Polyethylene terephthalate: Heat the specimen from room temperature to 280° at a heating rate of about 20°/min. Hold the specimen at 280° for 1 min. Quickly cool the specimen to room temperature and reheat it to 280° at a heating rate of 5°/min.

Polyethylene terephthalate G: Heat the specimen from room temperature to 120° at a heating rate of about 20°/min. Hold the specimen at 120° for 1 min. Quickly cool the specimen to room temperature and reheat it to 120° at a heating rate of 10°/min.

Polypropylene: Determine the thermal analysis curve under nitrogen at temperatures ranging from ambient to 30° above the melting point. Maintain the temperature for 10 min, then cool to 50° below the peak crystallization temperature at a rate of 10°–20°/min.

Polyvinyl chloride: Heat the specimen from –20° to 120° at a heating rate of about 10°/min. Quickly cool the specimen to room temperature.

Polyvinyl chloride, plasticized: Heat the specimen from –20° to 120° at a heating rate of about 10°/min. Quickly cool the specimen to room temperature.

Other materials: Specimens must be heated and cooled in a manner that is appropriate for the test material and that facilitates the generation of a useable thermal analysis curve.

• **EXTRACTIONS**

Physicochemical testing of the plastic material requires that it be extracted or dissolved. Different tests are facilitated by various extraction methods. *Table 3* describes the extracts that are generated and the tests that are performed on those extracts. Subsequent discussions address methods for producing the extracts. Note that these extracts may be used for tests other than the physicochemical tests.

Table 3. Extractions Performed for Various Chemical Tests

		Tests Performed on Plastics Using the Specified Extracting Solution						
Extraction	**Extracting Solution**	**Cyclic Olefin, Polyethylene, and Polypropylene**	**Polyamide 6**	**Polycarbonate**	**Polyethylene Terephthalate and Polyethylene Terephthalate G**	**Poly(ethylene-vinyl acetate)**	**Polyvinyl Chloride**	**Polyvinyl Chloride, Plasticized**
Solution S1	Water	Absorbance Acidity/alkalinity TOC	Absorbance Acidity/alkalinity TOC	Absorbance[a] Acidity/alkalinity TOC	Absorbance Acidity/alkalinity TOC	Absorbance Acidity/alkalinity TOC	Absorbance Acidity/alkalinity TOC	Absorbance, Acidity/alkalinity TOC
Solution S2	Toluene	Phenolic antioxidants, nonphenolic antioxidants, amides, and stearates[a]	N/A	N/A	N/A	Phenolic antioxidants, amides, and stearic acid[a]	N/A	N/A
Solution S3	Acid	Extractable metals: Al, As, Cd, Co, Cr,[b] Hg, Ni, Pb, Ti, V, Zn, and Zr[c]	Extractable metals: Al, As, Ba, Cd, Co, Hg, Mn, Ni, Pb, Ti, V, and Zn	Extractable metals: As, Ba, Ca, Cd, Co, Hg, Ni, Pb, Sn, V, and Zn	Extractable metals: Al, As, Ba, Cd, Co, Hg, Mn, Ni, Pb, Ti V, and Zn	Extractable metals: Al, As, Cd, Co, Hg, Ni, Pb, V, and Zn	Extractable metals: Al, As, Ba,[d] Cd, Co, Hg, Ni, Pb, Ti, V, and Zn	Extractable metals: As, Ba, Ca, Cd, Co, Hg, Ni, Pb, Sn, V, and Zn
Solution S4	Alkali	N/A	Extractable metals: Sb and Ge	N/A	Extractable metals: Sb and Ge	N/A	N/A	N/A
Solution S5	Alcohol	N/A	N/A	N/A	Absorbance	N/A	N/A	N/A
Solution S6	Tetrahydrofuran	N/A	N/A	N/A	N/A	N/A	Absorbance	N/A

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Table 3. Extractions Performed for Various Chemical Tests *(continued)*

		Tests Performed on Plastics Using the Specified Extracting Solution						
Extraction	Extracting Solution	Cyclic Olefin, Polyethylene, and Polypropylene	Polyamide 6	Polycarbonate	Polyethylene Terephthalate and Polyethylene Terephthalate G	Poly(ethylene-vinyl acetate)	Polyvinyl Chloride	Polyvinyl Chloride, Plasticized
Solution S7	Phenol	N/A	Free base function	N/A	N/A	N/A	N/A	N/A

[a] Although this extract is suitable for use with these specific ingredient methods, such an extract could be useful for other tests designed to establish a material's composition.
[b] For polyethylene only.
[c] Not applicable for cyclic olefins and polypropylene.
[d] For material used for containers for non-injectable aqueous solutions.

Water extraction, Solution S1

Polyethylene, cyclic olefins, polypropylene, polycarbonate, and poly(ethylene-vinyl acetate): Place 25 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 500 mL of *Purified Water*, and boil under reflux conditions for 5 h. Allow to cool, and filter the extracting solution through a sintered-glass filter. Collect the filtrate in a 500-mL volumetric flask and dilute with *Purified Water* to volume; the diluted solution is designated *Solution S1*. Use *Solution S1* within 4 h of preparation.

Polyethylene terephthalate and polyethylene terephthalate G: Place 10 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 200 mL of *Purified Water*, and heat at 50° for 5 h. Allow to cool, decant the solution into a 200-mL volumetric flask, and dilute with *Purified Water* to volume; the diluted sample is designated *Solution S1*. Use *Solution S1* within 4 h of preparation.

Polyvinyl chloride and polyvinyl chloride, plasticized: Place 25 g of the test material into a borosilicate glass flask. Add 500 mL of *Purified Water*, cover the flask's neck with aluminum foil or a borosilicate beaker, and heat in an autoclave at 121 ± 2° for 20 min. Allow the solution to cool and the solids to settle, decant the solution into a 500-mL volumetric flask, and dilute with *Purified Water* to volume; the diluted solution is designated *Solution S1*.

Polyamide 6: Place 25.0 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 500 mL of *Purified Water* and boil under a reflux condenser for 5 h. Allow the solution to cool to ambient temperature, decant and filter the solution through a sintered glass filter; the filtered solution is designated *Solution S1*.

Toluene extraction, Solution S2

Polyethylene, cyclic olefins, polypropylene, and poly(ethylene-vinyl acetate): Place 2.0 g of the test material in a 250-mL borosilicate glass flask with a ground-glass neck. Add 80 mL of toluene and boil under a reflux condenser for 1.5 h, stirring constantly. Allow to cool to 60° and add, with continued stirring, 120 mL of methanol. Pass the resulting solution through a sintered-glass filter. Rinse the flask and the filter with 25 mL of a mixture of 40 mL of toluene and 60 mL of methanol, add the rinsings to the filtrate, and dilute to 250 mL with the same mixture of solvents to produce *Solution S2*. Prepare a blank solution.

Acid extraction, Solution S3

Polyethylene, cyclic olefins, polypropylene, poly(ethylene-vinyl acetate), polycarbonate, and polyamide 6: Place 100 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 250 mL of 0.1 N hydrochloric acid and boil under a reflux condenser for 1 h with constant stirring. Allow to cool, decant the solution into a 250-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume; the diluted solution is designated *Solution S3*.

Polyethylene terephthalate and polyethylene terephthalate G: Place 20 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 50 mL of 0.1 N hydrochloric acid, and heat at 50° for 5 h. Allow to cool, decant the solution into a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume; the diluted solution is designated *Solution S3*. Use *Solution S3* within 4 h of preparation.

Polyvinyl chloride and polyvinyl chloride, plasticized: Place 5 g in a borosilicate glass flask with a ground-glass neck. Add 100 mL of 0.1 N hydrochloric acid, and boil under a reflux condenser for 1 h with constant stirring. Allow to cool and the solids to settle, decant the solution into a 100-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume; the diluted solution is designated *Solution S3*.

Alkali extraction, Solution S4

Polyethylene terephthalate and polyethylene terephthalate G: Place 20 g of test material in a borosilicate glass flask with a ground-glass neck. Add 50 mL of 0.01 N sodium hydroxide and heat at 50° for 5 h. Allow to cool and the solids to settle, decant the solution into a 50-mL volumetric flask, and dilute with 0.01 N sodium hydroxide to volume; the diluted solution is designated *Solution S4*. Use *Solution S4* within 4 h of preparation.

Alcohol extraction, Solution S5

Polyethylene terephthalate and polyethylene terephthalate G: Place 10 g of the test material in a borosilicate glass flask with a ground-glass neck. Add 100 mL of alcohol, absolute, and heat at 50° for 5 h. Allow to cool and the solids to settle, then decant the solution, producing *Solution S5*. Use *Solution S5* within 4 h of preparation.

Tetrahydrofuran extraction, Solution S6

Polyvinyl chloride: Dissolve 5.0 g of the test material in 80 mL of tetrahydrofuran and dilute to a volume of 100 mL with the same solvent. Filter if necessary; the solution may remain opaque. Slowly and dropwise add 70 mL of ethanol to 20 mL of this solution. Cool the mixture in ice for 1 h. Filter or centrifuge the mixture, collecting residue A. Wash residue A with ethanol. Collect the washings and add them to the solution remaining after filtration or centrifugation. Transfer the solution to a 100-mL volumetric flask and dilute to volume with ethanol. This process produces *Solution S6*. Prepare a blank solution.

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Phenol extraction, Solution S7

Polyamide 6: Dissolve 1.0 g of the test material in 50 mL of phenol (procured as a viscous liquid) by heating at 50° for 4 h with constant stirring. This process produces *Solution S7*. Prepare a blank solution.

• **PHYSICOCHEMICAL TESTS**

Absorbance

Refer to *Ultraviolet-Visible Spectroscopy* ⟨857⟩.

Polyethylene, cyclic olefins, polypropylene, poly(ethylene-vinyl acetate), polycarbonate, and polyamide 6: Determine the spectrum between 220 and 340 nm in *Solution S1*.

Polyethylene terephthalate and polyethylene terephthalate G: Determine the spectrum between 220 and 340 nm in *Solution S1*. For colored polyethylene terephthalate, determine the spectrum between 400 and 800 nm in *Solution S1*. For colored and noncolored polyethylene terephthalate, determine the spectrum between 400 and 800 nm in *Solution S5*.

Polyvinyl chloride, plasticized: Evaporate 100 mL of *Solution S1* to dryness. Dissolve the resulting residue in 5 mL of hexane to produce the hexane sample. Pass the hexane sample, if necessary, through a filter previously rinsed with hexane. Determine the spectrum between 250 and 310 nm in the hexane sample.

Polyvinyl chloride, Solution S1: Evaporate 100 mL of *Solution S1* to dryness. Dissolve the residue in 5 mL of hexane. If necessary, pass through a filter that has been previously rinsed with hexane. Determine the spectrum between 250 and 330 nm in the dissolved residue.

Polyvinyl chloride, Solution S6: If the polyvinyl chloride contains 1-phenyleicosane-1,3-dione and is used as a container for dry dosage forms for oral administration, dilute *Solution S6* (1 in 10) with ethanol prior to measurement. In all other situations, analyze *Solution S6* with no further preparation. Determine the spectrum between 250 and 330 nm in the dissolved residue.

Acidity or alkalinity

BRP indicator solution: 1.0 mg/mL of bromothymol blue, 0.2 mg/mL of methyl red, and 0.2 mg/mL of phenolphthalein in alcohol. Filter the resulting solution.

Methyl orange solution: Dissolve 100 mg of methyl orange in 80 mL of *Purified Water*, and dilute with alcohol to 100 mL. Test for sensitivity: Add 0.1 mL of *Methyl orange solution* to 100 mL of carbon dioxide-free *Purified Water*. NMT 0.1 mL of 1 N hydrochloric acid is required to change the color from yellow to red.

Polyethylene, cyclic olefins, polypropylene, poly(ethylene-vinyl acetate), polycarbonate, and polyamide 6: To 100 mL of *Solution S1* add 0.15 mL of *BRP indicator solution*. Determine the titration volume of 0.01 N sodium hydroxide required to change the color of the indicator to blue. To a separate, 100-mL portion of *Solution S1* add 0.2 mL of *Methyl orange solution*. Determine the titration volume of 0.01 N hydrochloric acid required to reach the beginning of the color change of the indicator from yellow to orange.

Polyethylene terephthalate and polyethylene terephthalate G: To 50 mL of *Solution S1* add 0.15 mL of *BRP indicator solution*. Determine the titration volume of 0.01 N sodium hydroxide required to change the color of the indicator to blue. To a separate 50 mL of *Solution S1* add 0.2 mL of *Methyl orange solution*. Determine the titration volume of 0.01 N hydrochloric acid required to reach the beginning of the color change of the indicator from yellow to orange.

Polyvinyl chloride, plasticized: To 100 mL of *Solution S1* add 0.15 mL of *BRP indicator solution*. Determine the titration volume of 0.01 N sodium hydroxide required to change the color of the indicator to blue. To 100 mL of *Solution S1* add 0.2 mL of *Methyl orange solution*. Determine the titration volume of 0.01 N hydrochloric acid required to reach the beginning of the color change of the indicator from yellow to orange.

Total organic carbon: The TOC content of *Solution S1* is measured according to the general methodologies outlined in *Total Organic Carbon* ⟨643⟩. However, although ⟨643⟩ is designed for the testing of high-purity water with low TOC values, material extracts may have TOC values that are higher than those of *Purified Water* because of extracted organic substances. Thus, the method used to perform the TOC analyses should have a limit of detection of 0.2 mg/L (ppm) and should have a demonstrated linear dynamic range from 0.2 to 20 mg/L (which encompasses the TOC limit). A linear range with a higher upper concentration can be used if linearity is established. If sample extracts exceed this upper linear range, they must be diluted appropriately for analysis.

• **FREE BASE FUNCTIONS**

Titrant (perchloric acid in phenol): Dissolve approximately 0.72 g (target 0.710–0.7250 g) of perchloric acid in 50 mL of phenol (procured as a viscous liquid).

Polyamide 6: Potentiometrically titrate 50 mL of *Solution S7* with *Titrant*, determining the point of equivalence. Similarly titrate 50 mL of phenol (procured as a viscous liquid) as a blank. The difference in the amount of titrant used is the amount of titrant used for the blank.

• **EXTRACTABLE METALS**

The extractable metals testing described in this chapter is required for all plastic materials of construction used in packaging systems. Specifically, all materials must be tested for those extractable metals listed in *Table 3*.

Procedure for extraction: Plastic materials used in packaging systems for medical articles do not dissolve under the conditions of use. Rather, substances derived from packaging systems accumulate in the packaged articles by the process of leaching (extraction). Thus, the appropriate and relevant sample-preparation process for assessing metals in a packaging system's materials of construction is extraction, as opposed to complete digestion, of the plastic material. *Solution S3* (acidic extraction) and *Solution S4* (alkaline extraction) prepared for *Physicochemical Tests* (*Table 2*) are the material extracts that are tested for extractable metals.

Procedure for extract analysis: Instrumentation and methods are those specified in *Elemental Impurities—Procedures* ⟨233⟩ and include an inductively coupled plasma–atomic emission spectrometer and an inductively coupled plasma–mass spectrometer (see *Plasma Spectrochemistry* ⟨730⟩), as directed.

• **PLASTIC ADDITIVES**

Polyethylene, cyclic olefins, and polypropylene: These tests should be carried out in whole or in part as required due to the stated composition of the material.

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Phenolic antioxidants

Solvent mixture: Acetonitrile and tetrahydrofuran (50:50, v/v)

Sample solution S8: Evaporate 50 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the resulting residue with 5.0 mL of the *Solvent mixture* to produce *Sample solution S8*. Prepare a blank solution from the blank solution corresponding to *Solution S2*.

Sample solution S9: Evaporate 50 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the residue with 5.0 mL of methylene chloride to produce *Sample solution S9*. Prepare a blank solution from the blank solution corresponding to *Solution S2*.

Reference solutions: Of the following reference solutions, prepare only those that are necessary for the analysis of the phenolic antioxidants stated in the composition of the substance to be examined.

Reference solution A: 0.1 mg/mL of USP Butylated Hydroxytoluene RS and 0.24 mg/mL of USP Plastic Additive 1 RS prepared in the *Solvent mixture*

Reference solution B: 0.24 mg/mL of USP Plastic Additive 2 RS and 0.24 mg/mL of USP Plastic Additive 3 RS prepared in the *Solvent mixture*

Reference solution C: 0.24 mg/mL of USP Plastic Additive 4 RS and 0.24 mg/mL of USP Plastic Additive 5 RS prepared in methylene chloride

Reference solution D: 0.1 mg/mL of USP Butylated Hydroxytoluene RS prepared in the *Solvent mixture*

Reference solution E: 0.24 mg/mL of USP Plastic Additive 1 RS prepared in the *Solvent mixture*

Reference solution F: 0.24 mg/mL of USP Plastic Additive 6 RS prepared in the *Solvent mixture*

Reference solution G: 0.24 mg/mL of USP Plastic Additive 2 RS prepared in the *Solvent mixture*

Reference solution H: 0.24 mg/mL of USP Plastic Additive 3 RS prepared in the *Solvent mixture*

Reference solution I: 0.24 mg/mL of USP Plastic Additive 4 RS prepared in the methylene chloride

Reference solution J: 0.24 mg/mL of USP Plastic Additive 5 RS prepared in the methylene chloride

Test A: If the substance to be examined contains additive butylated hydroxytoluene and/or additive ethylene bis[3,3-bis[3-(1,1-dimethylethyl)-4-hydroxyphenyl]butanoate]

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Liquid Chromatography*.)

Mobile phase: Acetonitrile and *Purified Water* (70:30, v/v)

Detector: UV 280 nm

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

Flow rate: 2 mL/min

Injection volume: 20 µL of *Sample solution S8*, corresponding blank solution, *Reference solution A*, and *Reference solution D*, *Reference solution E*, or both

Run time: 30 min

System suitability

Resolution: Minimum 5.0 between the additive butylated hydroxytoluene and USP Plastic Additive 1 RS (ethylene bis[3,3-bis[3-(1,1-dimethylethyl)-4-hydroxyphenyl]butanoate]) peaks, *Reference solution A*

Sample solution S8 shows only peaks caused by antioxidants stated in the composition and minor peaks that also correspond to the blank solution.

Analysis: The peak areas of *Sample solution S8* are less than the corresponding peak areas of *Reference solution D* or *Reference solution E*.

Test B: If the substance to be examined contains one or more of the following antioxidants: pentaerythrityl tetrakis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate] (USP Plastic Additive 2 RS); 2,2′,2″,6,6′,6″-hexa-*tert*-butyl-4,4′,4″-[(2,4,6-trimethyl-1,3,5-benzene-triyl)trismethylene]triphenol (USP Plastic Additive 3 RS); octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (USP Plastic Additive 4 RS); tris(2,4-di-*tert*-butylphenyl) phosphite (USP Plastic Additive 5 RS); 1,3,5-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-*s*-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (USP Plastic Additive 6 RS)

Chromatographic system: Carry out the test as described in *Test A* with the following modifications.

Mobile phase: Acetonitrile, tetrahydrofuran, and *Purified Water* (60:30:10, v/v/v)

Detector: UV 280 nm

Flow rate: 1.5 mL/min

Injection volume: 20 µL of *Sample solution S8*, corresponding blank solution, *Reference solution B*, and any *Reference solutions* of the antioxidants listed above that are stated in the composition

System suitability

Resolution: Minimum 2.0 between USP Plastic Additive 2 RS (pentaerythrityl tetrakis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate]) and USP Plastic Additive 3 RS (2,2′,2″,6,6′,6″-hexa-*tert*-butyl-4,4′,4″-[(2,4,6-trimethyl-1,3,5-benzene-triyl)trismethylene]triphenol peaks), *Reference solution B*

Sample solution S8 shows only peaks caused by antioxidants stated in the composition and minor peaks that also correspond to the blank solution.

Analysis: The peak areas of *Sample solution S8* are less than the corresponding areas of the *Reference solutions* of the antioxidants that are listed above and that are stated in the composition.

Test C: If the substance to be examined contains USP Plastic Additive 4 RS (octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate) and/or USP Plastic Additive 5 RS (tris(2,4-di-*tert*-butylphenyl) phosphite)

Chromatographic system: Carry out the test as described in *Test A* with the following modifications.

Mobile phase: Methanol, 2-propanol, and *Purified Water* (50:45:5, v/v/v)

Detector: UV 280 nm

Flow rate: 1.5 mL/min

Injection volume: 20 µL of *Sample solution S9*, corresponding blank solution, *Reference solution C*, and either *Reference solution I* or *Reference solution J*

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System suitability
Resolution: Minimum 2.0 between USP Plastic Additive 4 RS (octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate) and USP Plastic Additive 5 RS (tris(2,4-di-*tert*-butylphenyl) phosphite) peaks, *Reference solution C*
Sample solution S8 shows only peaks due to antioxidants stated in the composition and minor peaks that also correspond to the blank solution.
Analysis: The peak areas of *Sample solution S9* are less than the corresponding peak areas of *Reference solution I* or *Reference solution J*.

Nonphenolic antioxidants
Methylene chloride, acidified: To 100 mL of methylene chloride add 10 mL of hydrochloric acid, shake, allow to stand, and separate the two layers. Use the lower layer.
Iodine in ethanol detection solution: Dissolve 10 g of iodine in 100 mL of alcohol absolute. Store protected from light.
Sample solution S10: Evaporate 100 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the resulting residue with 2 mL of *Methylene chloride, acidified*.
Reference solution M: 6.0 mg/mL of USP Plastic Additive 8 RS prepared in methylene chloride. Dilute 2 mL of the solution with *Methylene chloride, acidified* to 10 mL.
Reference solution N: 6.0 mg/mL of USP Plastic Additive 9 RS prepared in methylene chloride. Dilute 2 mL of the solution with *Methylene chloride, acidified* to 10 mL.
Reference solution O: 6.0 mg/mL of USP Plastic Additive 10 RS prepared in methylene chloride. Dilute 2 mL of the solution with *Methylene chloride, acidified* to 10 mL.
Reference solution P: 6.0 mg/mL of USP Plastic Additive 10 RS, and 6.0 mg/mL of USP Plastic Additive 9 RS prepared in methylene chloride. Dilute 2 mL of the solution with *Methylene chloride, acidified* to 10 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Plate: TLC silica gel GF_{254}
Mobile phase A: Hexane
Mobile phase B: Methylene chloride
Application: 20 µL of *Sample solution S10* and the reference solutions corresponding to all of the phenolic and nonphenolic antioxidants expected to be present in the test material
Development A: Over a path of 18 cm with *Mobile phase A*; dry in air
Development B: Over a path of 17 cm with *Mobile phase B*; dry in air
Detector: UV 254 nm; spray with *Iodine in ethanol detection solution* and examine after 10–15 min
System suitability
Resolution: The chromatogram shows two clearly separated spots, *Reference solution P*.
Analysis: Any spots in the chromatogram of *Sample solution S10* are not more intense than the spots in the same positions in the chromatograms of the *Reference solutions*.

Copolymer of dimethyl succinate and (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)ethanol (cyclic olefins only)
Solvent mixture: Hexane and anhydrous ethanol (89:11, v/v)
Sample solution S11: Evaporate 25 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the residue with 10 mL of toluene and 10 mL of a 10-g/L solution of tetrabutylammonium hydroxide in a mixture of 35 volumes of toluene and 65 volumes of anhydrous ethanol. Boil under a reflux condenser for 3 h. Allow to cool, and filter if necessary, to produce *Sample solution S11*.
Reference solution Q: 0.6 mg/mL of USP Plastic Additive 11 RS prepared in toluene. Add 1 mL of this solution to 25 mL of the blank solution corresponding to *Solution S2*, and evaporate to dryness under vacuum at 45°. Prepare a blank solution from the blank solution corresponding to *Solution S2*. Dissolve the residue with 10 mL of toluene and 10 mL of a 10-g/L solution of tetrabutylammonium hydroxide in a mixture of 35 volumes of toluene and 65 volumes of anhydrous ethanol. Boil under a reflux condenser for 3 h. Allow to cool, and filter if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Liquid Chromatography*.)
Mobile phase: Hexane and anhydrous ethanol (89:11, v/v)
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 2 mL/min
Injection volume: 20 µL of *Sample solution S11*, the corresponding blank solution, and *Reference solution Q*
System suitability
Resolution: Minimum of 7 between the peaks of the diol component and the diluents of *Reference solution Q*
Analysis: The peak area of the diol component in *Sample solution S11* is less than the corresponding peak areas of *Reference solution Q*.

Amides and stearates
Sample solution: Use *Sample solution S10* described in *Nonphenolic antioxidants*.
Reference solution R: 2.0 mg/mL of USP Stearic Acid RS prepared in methylene chloride
Reference solution S: 2.0 mg/mL of USP Plastic Additive 12 RS prepared in methylene chloride
Reference solution T: 2.0 mg/mL of USP Plastic Additive 13 RS prepared in methylene chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Plate: TLC silica gel GF_{254}
Test A
Mobile phase: 2,2,4-trimethylpentane and anhydrous ethanol (75:25, v/v)
Application: 10 µL of *Sample solution S10* and *Reference solution R*
Development: Over a path of 10 cm with *Mobile phase*; dry in air

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Detector: Spray with a 2-g/L solution of 2,6-dichlorophenol-indophenol sodium in dehydrated alcohol and heat in an oven at 120° for a few minutes to intensify the spots.
Analysis: Any spot corresponding to additive stearic acid in *Sample solution S10* is identical in position (R_F about 0.5) but is not more intense than the spot in the same position in *Reference solution R*.

Test B
Mobile phase A: Hexane
Mobile phase B: Methylene chloride and methanol (95:5, v/v)
Application: 10 µL of *Sample solution S10*, *Reference solution S*, and *Reference solution T*
Development A: Over a path of 13 cm with *Mobile phase A*; dry in air
Development B: Over a path of 10 cm with *Mobile phase B*; dry in air
Detector: Spray with a 40-g/L solution of phosphomolybdic acid in alcohol, dehydrated, and heat in an oven at 120° until spots appear.
Analysis: Any spots corresponding to additives oleamide or erucamide in *Sample solution S10* are identical in position (R_F about 0.2) but are not more intense than the corresponding spots in *Reference solution S* and *Reference solution T*.

• **POLY(ETHYLENE-VINYL ACETATE)**
These tests should be carried out in whole or in part as required due to the stated composition of the material.

Phenolic antioxidants
Solvent mixture: Acetonitrile and tetrahydrofuran (50:50, v/v)
Sample solution S12: Evaporate 50 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the resulting residue with 5.0 mL of the *Solvent mixture* to produce *Sample solution S12*. Prepare a blank solution from the blank solution corresponding to *Solution S2*.
Sample solution S13: Evaporate 50 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the residue with 5.0 mL of methylene chloride to produce *Sample solution S13*. Prepare a blank solution from the blank solution corresponding to *Solution S2*.
Reference solutions
Of the following reference solutions, prepare only those that are necessary for the analysis of the phenolic antioxidants stated in the composition of the substance to be examined.
Reference solution K: 0.1 mg/mL of USP Butylated Hydroxytoluene RS, 0.16 mg/mL of USP Plastic Additive 2 RS, 0.16 mg/mL of USP Plastic Additive 3 RS, and 0.16 mg/mL of USP Plastic Additive 4 RS prepared in the *Solvent mixture*
Reference solution L: 0.16 mg/mL of USP Plastic Additive 4 RS and 0.16 mg/mL of USP Plastic Additive 5 RS prepared in methylene chloride

Test A
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Liquid Chromatography*.)
Mobile phase: Tetrahydrofuran, acetonitrile, and *Purified Water* (30:60:10, v/v)
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL of *Sample solution S12*, corresponding blank solution, and *Reference solution K*
Run time: 30 min
System suitability
Resolution: Minimum 2.0 between USP Plastic Additive 2 RS and USP Plastic Additive 3 RS, *Reference solution K*
Column efficiency: Minimum 2500 theoretical plates, calculated for USP Butylated Hydroxytoluene RS, *Reference solution K*
Analysis: *Sample solution S12* shows only peaks caused by antioxidants in *Reference solution K* and minor peaks that also correspond to the blank solution. The peak areas of *Sample solution S12* are less than the corresponding peak areas of *Reference solution K*.

Test B: If the chromatogram obtained via *Test A* for *Sample solution S12* shows a peak with the same retention time as the last antioxidant eluted from *Reference solution K*
Chromatographic system: Carry out the test as described in *Test A* with the following modifications.
Mobile phase: 2-propanol, methanol, and *Purified Water* (45:50:5, v/v/v)
Detector: UV 280 nm
Flow rate: 1.5 mL/min
Injection volume: 20 µL of *Sample solution S13*, corresponding blank solution, *Reference solution L*
System suitability
Resolution: Minimum of 2.0 between USP Plastic Additive 4 RS and USP Plastic Additive 5 RS, *Reference solution L*
Analysis: *Sample solution S13* shows only peaks caused by antioxidants in *Reference solution L* and minor peaks that also correspond to the blank solution. The peak areas of *Sample solution S13* are less than the corresponding peak areas of *Reference solution L*.

Amides and stearic acid
Sample solution S14: Evaporate 100 mL of *Solution S2* to dryness under vacuum at 45°. Dissolve the resulting residue with 2 mL of acidified methylene chloride to produce *Sample solution S14*.
Reference solution R: 2.0 mg/mL of USP Stearic Acid RS prepared in methylene chloride
Reference solution S: 0.8 mg/mL of USP Plastic Additive 12 RS prepared in methylene chloride
Reference solution T: 0.8 mg/mL of USP Plastic Additive 13 RS prepared in methylene chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Plate: TLC silica gel GF_{254}

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Test A

Mobile phase: Anhydrous ethanol and trimethylpentane (25:75, v/v)
Application: 10 µL of *Sample solution S14* and *Reference solution R*
Development: Over a path of 10 cm with *Mobile phase*; dry in air
Detector: Spray with a 2-g/L solution of 2,6-dichlorophenol-indophenol sodium in dehydrated alcohol and heat in an oven at 120° for a few minutes to intensify the spots.
Analysis: Any spot corresponding to additive stearic acid in *Sample solution S14* is identical in position and is not more intense than the spot in the same position in *Reference solution R*.

Test B

Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Plate: TLC silica gel GF_{254}
Mobile phase A: Hexane
Mobile phase B: Methylene chloride and methanol (95:5, v/v)
Application: 10 µL of *Sample solution S14*, *Reference solution S*, and *Reference solution T*
Development A: Over a path of 13 cm with *Mobile phase A*; dry in air
Development B: Over a path of 10 cm with *Mobile phase B*; dry in air
Detector: Spray with a 40-g/L solution of phosphomolybdic acid in alcohol, dehydrated, and heat in an oven at 120° until spots appear.
Analysis: Any spots corresponding to additives oleamide or erucamide in *Sample solution S14* are identical in position but are not more intense than the corresponding spots in *Reference solution S* and *Reference solution T*.

Polyvinyl chloride, plasticized: Additives are di(2-ethylhexyl) phthalate, *N'N"*-diacylethylenediamines, epoxidized soya oil, and epoxidized linseed oil. Vinyl chloride monomer (VCM) is also monitored, although it is a residual monomer and not an additive.

Solution A1: Add 2.0 g of the test material to 200 mL of peroxide-free ether and heat under a reflux condenser for 8 h. Separate the resulting residue B and extraction solution A by filtration. Evaporate extraction solution A to dryness under reduced pressure in a water bath at 30°, producing residue C. Dissolve residue C in 10 mL of toluene to produce *Solution A1*.
Precipitate B2: Dissolve residue B in 60 mL of ethylene chloride heating on a water bath under a reflux condenser, producing solution D. Filter the resulting solution D. Add the filtered solution D dropwise and with vigorous shaking to 600 mL of heptanes heated almost to boiling. Separate by hot filtration the coagulum B1 and the organic solution E. Allow solution E to cool; separate the precipitate B2 that forms upon cooling, and pass through a tared sintered-glass filter (pore size of 16–40 µm).
Reference solutions U, V, W: 10.0-mg/mL solutions of USP Plastic Additive 14 RS, USP Plastic Additive 15 RS, and USP Plastic Additive 16 RS, respectively, in toluene
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Plate: TLC silica gel GF_{254} (1-mm thick)
Method: Apply 0.5 mL of *Solution A1* to the plate as a 30-mm × 3-mm band. Apply 5 µL each of *Reference solutions U, V,* and *W* to the plate. Develop the plate over a path of 15 cm using toluene. Dry the plate carefully.
Additive di(2-ethylhexyl) phthalate: UV 254 nm; locate the zone corresponding to additive di(2-ethylhexyl) phthalate, USP Plastic Additive 14 RS (R_F about 0.4). Remove the area of silica gel corresponding to this zone, mix with 40 mL of ethyl ether, and shake for 1 min. Filter, rinse filter with two quantities each of 10 mL of ethyl ether, add the rinsings to the filtrate, and evaporate to dryness. The residue weighs NMT 40 mg.
Additives epoxidized soya oil and epoxidized linseed oil: Expose the plate to iodine vapor for 5 min. Examine the chromatogram, and locate the band corresponding to additives epoxidized soya oil, USP Plastic Additive 15 RS, and epoxidized linseed oil, USP Plastic Additive 16 RS ($R_F = 0$). Remove the area of silica gel corresponding to this band. Similarly, remove a corresponding area of silica gel as a blank reference. Separately mix both samples with separate 40-mL portions of methanol, shaking for 15 min. Filter, rinse the filter with two quantities of 10 mL of methanol, add the rinsings to the filtrate, and evaporate to dryness. The difference between the masses of both residues is NMT 10 mg.
Additive *N,N'*-diacylethylenediamines: Wash precipitate B2 with alcohol, absolute. Dry to constant mass over diphosphorus pentoxide, and weigh the filter. The precipitate weighs NMT 20 mg.

Polyvinyl chloride

Additives and stabilizers: The supplier of the material must be able to provide sufficient compositional information to establish whether the material meets the specifications for additives and stabilizers.

Vinyl chloride

Internal standard solution: Using a microsyringe, inject 10 µL of ethyl ether into 20.0 mL of *N,N*-dimethylacetamide, immersing the tip of the needle in the solvent. Immediately before use, dilute the solution with *N,N*-dimethylacetamide to 1000 times its volume.
Sample solution: Place 1.0 g of the test material in a 50-mL vial, and add 10.0 mL of the *Internal standard solution*. Close the vial, and secure with a stopper. Shake, avoiding contact between the stopper and the liquid. Place the vial in a water bath at 60 ± 1° for 2 h.
Vinyl chloride primary solution: [NOTE—Prepare under a ventilated hood.] Place 50.0 mL of *N,N*-dimethylacetamide in a 50-mL vial, stopper the vial, secure the stopper, and weigh to the nearest 0.1 mg. Fill a 50-mL polyethylene or polypropylene syringe with gaseous vinyl chloride, allow the gas to remain in contact with the syringe for about 3 min, empty the syringe, and fill again with 50 mL of gaseous vinyl chloride. Fit a hypodermic needle to the syringe, and reduce the volume of gas in the syringe from 50 to 25 mL. Inject the remaining 25 mL of vinyl chloride slowly into the vial, shaking gently and avoiding contact between the liquid and the needle. Weigh the vial again; the increase in mass

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is about 60 mg (1 µL of the solution obtained contains about 1.2 µg of vinyl chloride). Allow to stand for 2 h. Store the primary solution in a refrigerator.

Vinyl chloride standard solution: To one volume of the *Vinyl chloride primary solution* add three volumes of *N,N*-dimethylacetamide.

Reference solutions: Place 10.0 mL of the *Internal standard solution* in each of six 50-mL vials. Close the vials, and secure the stoppers. Inject 1, 2, 3, 5, and 10 µL, respectively, of the *Vinyl chloride standard solution* into five of the vials. The six solutions thus obtained contain, respectively, 0, 0.3, 0.6, 0.9, 1.5, and 3 µg of vinyl chloride. Shake, avoiding contact between the stopper and the liquid. Place the vials in a water bath at 60 ± 1° for 2 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography.*)

Column: Stainless steel 3-m × 3-mm packed with silanized diatomaceous earth for gas chromatography impregnated with 5% m/m of dimethylstearylamide and 5% m/m of polyethylene glycol 400

Gas carrier: Nitrogen for chromatography

Flow rate: 30 mL/min

Temperatures

Injection port: 100°

Column: 45°

Detector: 150°

Analysis

Sample: Inject 1 mL of the head space of each vial containing the *Sample solution* and the *Reference solutions*. Calculate the amount of vinyl chloride in the *Sample solution* by comparing the test result of the *Sample solution* with the test results of the *Reference solutions*. Calculate the amount of vinyl chloride in the test material by dividing the amount of vinyl chloride in the *Sample solution* by 1.0 g, producing a result in µg/g or ppm.

• **TIN IN TIN-STABILIZED MATERIALS**

Reference solution U: 0.81 mg/mL of USP Plastic Additive 18 RS prepared in tetrahydrofuran is diluted from 20 to 100 mL with ethanol.

Sample solution: Add 0.1 mL of *Solution S6* to a test tube. Add 0.05 mL of 1 M hydrochloric acid, 0.5 mL of potassium iodide solution, and 5 mL of ethanol to the test tube. Mix thoroughly and wait for 5 min. Add 9 mL of water and 0.1 mL of a 5-g/L solution of sodium sulfite and mix thoroughly. Add 1.5 mL of dithizone solution freshly diluted 100-fold with methylene chloride, shake for 15 s and allow to stand for 2 min.

Standard solution: Take 0.1 mL of *Reference solution U* through the same procedure as the 0.1 mL of *Solution S6*.

Analysis: Compare the violet color in the lower layer of the *Sample solution* to the violet color in the lower layer of the *Standard solution*. The color in the *Sample solution* should not be as intense as the color in the *Standard solution*.

• **TIN IN NON-TIN-STABILIZED MATERIALS**

Standard solution: Take 0.05 mL of *Reference solution U* through the same procedure as the 0.1 mL of *Solution S6*.

Sample solution: Add 5 mL of *Solution S6* to a test tube. Add 0.05 mL of 1 M hydrochloric acid, 0.5 mL of potassium iodide solution, and 5 mL of ethanol to the test tube. Mix thoroughly and wait for 5 min. Add 9 mL of water and 0.1 mL of a 5-g/L solution of sodium sulfite and mix thoroughly. If the solution is not colorless, add the sodium sulfite in 0.05-mL fractions. Add 1.5 mL of dithizone solution freshly diluted 100-fold with methylene chloride, shake for 15 s and allow to stand for 2 min.

Analysis: Compare the violet color in the lower layer of the *Sample solution* to the violet color in the lower layer of the *Standard solution*. The color in the *Sample solution* should not be as intense as the color in the *Standard solution*.

• **RELATED SUBSTANCES**

Polyamide 6

Caprolactam

Sample solution: Weigh approximately 1.0 g of the test material and place it in a 10-mL volumetric flask, dissolve by adding anhydrous formic acid. Dilute with anhydrous formic acid to volume.

Caprolactam primary solution: Place 125 mg of USP Caprolactam RS in a 50-mL volumetric flask, dissolve by adding anhydrous formic acid. Dilute with anhydrous formic acid to volume. The caprolactam concentration of this primary solution is approximately 2500 mg/L.

Reference solutions: Pipet 0, 2, 4, 6, 8, and 10 mL of the *Caprolactam primary solution* into six 20-mL volumetric flasks. Dilute with anhydrous formic acid to volume. The six reference solutions thus obtained (*Reference solution blank* and *Reference solution WS1* through *WS5*) contain, respectively, 0, 250, 500, 750, 1000, and 1250 mg/L of caprolactam.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography.*)

Column: 30-m × 0.25-mm; 0.25-µm phase G25

Gas carrier: Helium for chromatography

Flow rate: 1 mL/min

Temperatures

Injection port: 250°

Column: Hold at 160° for 2 min, ramped to 210° at 5°/min, and hold at 210° for 10 min

Detector: FID 250°

Injection volume: 1 µL

Injection type: Split ratio, 3:1

Analysis

Conditioning: Inject the *Reference solution blank* three times into the chromatographic system.

System suitability: Inject *Reference solution WS4* five times into the chromatographic system. The % relative standard deviation of the peak areas obtained for these injections must be NMT 5%. The symmetry factor for the caprolactam peak obtained for the third injection must be between 0.8 and 1.3.

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Rinsing: Inject *Reference solution blank* once.
Calibration, front of bracket: Inject each of the five *Reference solutions* once. Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their caprolactam concentrations. The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.99.
Rinsing: Inject the *Reference solution blank* once.
Sample: Inject *Sample solution* once. Inject NMT six *Sample solutions.*
Rinsing: Inject *Reference solution blank* once.
Calibration, back of bracket: Inject each of the five *Reference solutions* once.
Calculations: Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their caprolactam concentrations (both front and back of bracket). The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.99. Calculate the amount of caprolactam in the *Sample solution* by putting the peak area obtained for the *Sample solution* into the calibration curve. Calculate the amount of caprolactam in the test material by multiplying this result by a factor of 10 and dividing the product by the weight of the test material in grams, producing a result in weight %.

• **POLYCARBONATE**

Residual solvents

Sample solution: Weigh approximately 1.0 g of the test material and place it in a 20-mL headspace vial. Add 10 mL of *N,N′*-dimethylformamide, cap the vial closed, and sonicate for 4 h. Cool to room temperature. Prepare a sample blank in a similar fashion.
Residual solvents primary solution: Accurately weigh 500 mg each of dichloromethane, toluene, and ethylbenzene and 1250 mg of chlorobenzene into a 50-mL volumetric flask; dissolve and adjust with *N,N′*-dimethylformamide to volume.
Residual solvents stock solution: Transfer 5 mL of the *Residual solvents primary solution* into a 100-mL volumetric flask; adjust with *N,N′*-dimethylformamide to volume. This solution has theoretical concentrations of 500 mg/L for dichloromethane, toluene, and ethylbenzene and 1250 mg/L for chlorobenzene.
Reference solutions: Pipet 0, 2, 3, 4, 5, and 6 mL of the *Residual solvents stock solution* into individual 100-mL volumetric flasks, dilute with *N-N′*-dimethylformamide to volume and mix well. The six reference solutions thus obtained (*Reference solution blank* and *Reference solution WS1* through *WS5*) contain, respectively, 0, 10, 15, 20, 25, and 30 mg/L of dichloromethane, toluene, and ethylbenzene and 0, 25, 37.5, 50, 62.5, and 75 mg/L of chlorobenzene. Transfer 10 mL of the individual reference solutions to 20-mL headspace vials and cap the vials closed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography.*)
Headspace autosampler
Temperatures
Thermostating: 115°
Needle: 110°
Transfer: 120°
Times
Thermostating: 60 min
Pressurization: 0.5 min
Injection: 0.1 min
Withdrawal: 0.2 min
Carrier gas pressure: 20 psi
Column: Stainless steel, 30-m × 0.32-mm, containing stationary phase (0.5 µm) coated with 100% bonded and cross-linked polyethylene glycol, phase G39
Temperatures
Injection port: 140°
Column: Start at 50°, hold for 20 min. Heat to 165° at 6°/min, hold for 20 min.
Detector: FID 250°
Gas carrier: Helium for chromatography
Flow rate: Adequate to provide a constant pressure of 10 psi
Injection volume: 1 µL, split
Analysis
Conditioning: Inject the *Reference solution blank* two times into the chromatographic system.
System suitability: Inject *Reference solution WS3* five times into the chromatographic system. Note that one injection is done from each autosampler vial. The % relative standard deviation of the peak areas obtained for each analyte for these injections must be NMT 5%.
Rinsing: Inject the *Reference solution blank* once.
Calibration, front of bracket: Inject each of the five *Reference solutions* once. Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their analyte concentrations for each analyte. The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.99.
Rinsing: Inject the *Reference solution blank* once.
Sample: Inject *Sample solution* once, including the *Sample blank.* Inject NMT six *Sample solutions.*
Rinsing: Inject *Reference solution blank* once.
Calibration, back of bracket: Inject each of the five *Reference solutions* once.
Calculations: Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their analyte concentrations (using the front and back of the bracket). The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.99. Calculate the amount of each analyte in the *Sample solution* by putting the peak area obtained for the *Sample solution* into the calibration curve.

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Calculate the amount of each in the test material by multiplying this result by a factor of 10 and dividing the product by the weight of the test material in g, producing a result in µg/g.

$$\text{Analyte (µg/g)} = [\text{analyte in } \textit{Sample solution} \text{ (mg/L)} \times 10]/\text{weight of test material (g)}$$

• **BISPHENOL A**

[NOTE—Bisphenol A is monitored although it is a residual monomer and not an additive.]

Sample solution: Weigh approximately 1.0 g of the test material and place it in a 250-mL round-bottom flask. Add 50 mL of methylene chloride and slightly heat at approximately 35° for 1 h under a reflux condenser to dissolve the test material. Cool the solution to room temperature and slowly add 75 mL of methanol to the room-temperature solution, stirring continuously. Place in a refrigerator for 2 h to cool the resulting solution. Filter the cooled solution through a sintered-glass filter. Wash the round-bottom flask and the filter twice with 15 mL of methanol. Evaporate the filtrate to dryness under vacuum at 45°. Dissolve the residue in 5 mL of methylene chloride. Add 0.5 mL of this solution and 0.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide to a 1.5-mL vial and close the vial immediately. Heat the closed vial at 40° for 2 h and then cool to room temperature. Prepare a sample blank in a similar fashion.

Bisphenol A primary solution: Accurately weigh 20 mg of USP Bisphenol A RS in a 200-mL volumetric flask; dissolve and dilute with methylene chloride to volume. The bisphenol A concentration of this primary solution is approximately 100 mg/L.

Reference solutions: Pipet 0, 5, 10, 20, 30, and 40 mL of the *Bisphenol A primary solution* into six 100-mL volumetric flasks. Dilute with methylene chloride to volume and mix well. The six reference solutions thus obtained (*Reference solution blank* and *Reference solution WS1* through *WS5*) contain, respectively, 0, 5, 10, 20, 30, and 40 mg/L of bisphenol A. Add 0.5 mL each of the *Reference solutions* and 0.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide to separate 1.5-mL vials and close the vials immediately. Heat the closed vials at 40° for 2 h and then cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography*.)

Column: Stainless steel, 25-m × 0.25-mm; stationary phase (0.25 µm) coated with 100% dimethylpolysiloxane, phase G38

Temperatures

Injection port: 300°

Column: 250°

Detector: FID 300°

Gas carrier: Helium for chromatography

Flow rate: Adequate to provide a constant pressure of 13 psi

Injection volume: 2 µL, split

Analysis

Conditioning: Inject the *Reference solution blank* three times into the chromatographic system.

System suitability: Inject *Reference solution WS3* five times into the chromatographic system. The % relative standard deviation of the peak areas obtained for these injections must be NMT 5%.

Rinsing: Inject the *Reference solution blank* twice.

Calibration, front of bracket: Inject each of the five *Reference solutions* once. Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their bisphenol A concentrations. The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.98.

Rinsing: Inject the *Reference solution blank* once.

Sample: Inject *Sample solution* once, including the *Sample blank*. Inject NMT six *Sample solutions*.

Rinsing: Inject the *Reference solution blank* once.

Calibration, back of bracket: Inject each of the five *Reference solutions* once.

Calculations: Construct a linear calibration curve of the peak areas obtained for the *Reference solutions* versus their bisphenol A concentrations (front and back of bracket). The correlation coefficient (*r*) obtained for the best-fit linear regression line must be NLT 0.99. Calculate the amount of bisphenol A in the *Sample solution* by putting the peak area obtained for the *Sample solution* into the calibration curve.

Calculate the amount of bisphenol A in the test material by multiplying this result by a factor of five and dividing the product by the weight of the test material in g, producing a result in µg/g.

$$\text{Bisphenol A (µg/g)} = [\text{bisphenol A in } \textit{Sample solution} \text{ (mg/L)} \times 5]/\text{weight of test material (g)}$$

• **POLY(ETHYLENE-VINYL ACETATE)**

Vinyl acetate

Alcoholic potassium hydroxide: Dissolve 6.6 g of potassium hydroxide in 50 mL of *Purified Water* and dilute with alcohol, dehydrated to 1000 mL.

Sample solution: Place 0.25–1.0 g of the test material into a 300-mL conical flask containing a magnetic stirrer. Prepare an extraction blank starting with an otherwise empty 300-mL conical flask. Add 40 mL of xylene and boil under a reflux condenser with stirring for 4 h. After heating, continue stirring, allowing the solution to cool to the point that precipitation starts. Slowly add 25 mL of alcoholic potassium hydroxide. Boil again under a reflux condenser for 3 h with continued stirring. While stirring, allow the solution to cool, rinse the condenser with 50 mL of water and add 30 mL of 0.05 M sulfuric acid to the flask. Transfer the contents of the flask to a 400-mL beaker, rinsing the flask with the following:

- 2 quantities, 50 mL each, of a 200-g/L solution of anhydrous sodium sulfate
- 3 quantities, 20 mL each, of water

Add the rinsings to the beaker.

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Method: Titrate the excess sulfuric acid in *Sample solution* with 0.1 M sodium hydroxide, determining the endpoint potentiometrically. Carry out a titration of the extraction blank.

Calculation: Determine the amount of titrant (mL) required by subtracting the titrant volume used for the extraction blank (mL) from the titrant volume used for the extract (mL). Determine the amount of vinyl acetate by multiplying the volume of titrant required by the quantity (8.609 mg/mL). The content of vinyl acetate is calculated as:

$$\text{Content of vinyl acetate (weight \%)} = [\text{amount of vinyl acetate (mg)}/\text{weight of material extracted (g)}]/10$$

• **POLYVINYL CHLORIDE**

Chlorine content

Preparation: Prepare the sample using *Oxygen Flask Combustion* ⟨471⟩. Perform the combustion with 50.0 mg of the test material. Absorb the combustion products with 20 mL of 1 M sodium hydroxide.

Analysis: Add 2.5 mL of nitric acid, 10 mL of 0.1 M silver nitrate solution, 5 mL of ferric ammonium sulfate solution, and 1 mL of dibutyl phthalate to the *Preparation* solution. Titrate with 0.005 M ammonium thiocyanate solution until a reddish-yellow color is obtained. Carry out a blank titration.

Calculation: Calculate the titration volume by subtracting the volume of titrant used in the blank from the volume of titrant used in the *Preparation*. Each milliliter of titrant volume is equal to 6.25 mg of polyvinyl chloride. The chlorine content, in weight %, is calculated as follows:

$$\text{Chlorine content (weight \%)} = \{[\text{titrant volume (in mL)} \times 6.25 \text{ mg/mL}]/\text{weight of sample (mg)}\} \times 100\%$$

• **POLYVINYL CHLORIDE, PLASTICIZED**

Vinyl chloride

Internal standard solution: Using a microsyringe, inject 10 µL of ethyl ether into 20.0 mL of *N,N*-dimethylacetamide, immersing the tip of the needle in the solvent. Immediately before use, dilute the solution with *N,N*-dimethylacetamide to 1000 times its volume.

Sample solution: Place 1.0 g of the test material in a 50-mL vial, and add 10.0 mL of the *Internal standard solution*. Close the vial, and secure with a stopper. Shake, avoiding contact between the stopper and the liquid. Place the vial in a water bath at 60 ± 1° for 2 h.

Vinyl chloride primary solution: [NOTE—Prepare under a ventilated hood.] Place 50.0 mL of *N,N*-dimethylacetamide in a 50-mL vial, stopper the vial, secure the stopper, and weigh to the nearest 0.1 mg. Fill a 50-mL polyethylene or polypropylene syringe with gaseous vinyl chloride, allow the gas to remain in contact with the syringe for about 3 min, empty the syringe, and fill again with 50 mL of gaseous vinyl chloride. Fit a hypodermic needle to the syringe, and reduce the volume of gas in the syringe from 50 to 25 mL. Inject the remaining 25 mL of vinyl chloride slowly into the vial, shaking gently and avoiding contact between the liquid and the needle. Weigh the vial again; the increase in mass is about 60 mg (1 µL of the solution obtained contains about 1.2 µg of vinyl chloride). Allow to stand for 2 h. Store the primary solution in a refrigerator.

Vinyl chloride standard solution: To one volume of the *Vinyl chloride primary solution* add three volumes of *N,N*-dimethylacetamide.

Reference solutions: Place 10.0 mL of the *Internal standard solution* in each of six 50-mL vials. Close the vials, and secure the stoppers. Inject 1, 2, 3, 5, and 10 µL, respectively, of the *Vinyl chloride standard solution* into five of the vials. The six solutions thus obtained contain, respectively, 0, 0.3, 0.6, 0.9, 1.5, and 3 µg of vinyl chloride. Shake, avoiding contact between the stopper and the liquid. Place the vials in a water bath at 60 ± 1° for 2 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography*.)

Column: Stainless steel 3-m × 3-mm packed with silanized diatomaceous earth for gas chromatography impregnated with 5% m/m of dimethylstearylamide and 5% m/m of polyethylene glycol 400

Temperatures

Injection port: 100°

Column: 45°

Detector: FID 150°

Gas carrier: Nitrogen for chromatography

Flow rate: 30 mL/min

Analysis: Inject 1 mL of the head space of each vial containing the *Sample solution* and the *Reference solutions*. Calculate the amount of vinyl chloride in the *Sample solution* by comparing the test result of the *Sample solution* with the test results of the *Reference solutions*. Calculate the amount of vinyl chloride in the test material by dividing the amount of vinyl chloride in the *Sample solution* by 1.0 g, producing a result in µg/g or ppm.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

Polymer standards

USP Cyclic Olefin Copolymer RS
USP Cyclic Olefin Polymer RS
USP Polyamide 6 RS
USP Polycarbonate RS
USP High-Density Polyethylene RS
USP Homopolymer Polypropylene RS
USP Low-Density Polyethylene RS
USP Polyethylene Terephthalate RS
USP Polyethylene Terephthalate G RS
USP Poly(ethylene-vinyl acetate) RS

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USP Polyvinyl Chloride RS
USP Polyvinyl Chloride, Plasticized RS

Plastic additive standards

USP Plastic Additive 1 RS

Ethylene bis[3,3-bis[3-(1,1-dimethylethyl)-4-hydroxyphenyl]butanoate].
[CAS-32509-66-3]
USP Plastic Additive 2 RS

Pentaerythrityl tetrakis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate].
[CAS-6683-19-8]
USP Plastic Additive 3 RS

2,2′,2″,6,6′,6″-Hexa-*tert*-butyl-4,4′,4″-[(2,4,6-trimethyl-1,3,5-benzenetriyl)trismethylene]triphenol.
[CAS-1709-70-2]
USP Plastic Additive 4 RS

Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate.
[CAS-2082-79-3]
USP Plastic Additive 5 RS

Tris(2,4-di-*tert*-butylphenyl) phosphite.
[CAS-31570-04-4]
USP Plastic Additive 6 RS

1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-*s*-triazine-2,4,6(1*H*,3*H*,5*H*)-trione.
[CAS-27676-62-6]
USP Plastic Additive 8 RS

Dioctadecyl disulfide.
[CAS-2500-88-1]
USP Plastic Additive 9 RS

Didodecyl 3,3′-thiodipropionate.
[CAS-123-28-4]
USP Plastic Additive 10 RS

Dioctadecyl 3,3′-thiodipropionate.
[CAS-693-36-7]
USP Plastic Additive 11 RS

Copolymer of dimethyl succinate and (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)ethanol.
[CAS-65447-77-0]
USP Plastic Additive 12 RS

Oleamide.
[CAS-301-02-0]
USP Plastic Additive 13 RS

Erucamide.
[CAS-112-84-5]
USP Plastic Additive 14 RS

Di(2-ethylhexyl) phthalate.
[CAS-117-81-7]
USP Plastic Additive 15 RS

Epoxidized soya oil.
[CAS-8013-07-8]
USP Plastic Additive 16 RS

Epoxidized linseed oil.
[CAS-8016-11-3]
USP Plastic Additive 18 RS

Mixture of Di(isooctyl) 2,2′-[dioctylstannylene)-bis(thio)diacetate and Tri(isooctyl) 2,2′,2″-[monooctylstannylidyne) tris(thio)]triacetate.
[CAS-26401-97-8; CAS-26401-86-5]

Related substances standards

USP Bisphenol A RS
[CAS-80-05-07]
USP Butylated Hydroxytoluene RS
[CAS-128-37-0]
USP Caprolactam RS
[CAS-105-60-2]
USP Stearic Acid RS
[CAS-57-11-4]

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⟨661.2⟩ PLASTIC PACKAGING SYSTEMS FOR PHARMACEUTICAL USE

(This chapter will become official on May 1, 2020. Early adoption of the requirements in this chapter and its companion chapter *Plastic Materials of Construction* ⟨661.1⟩ are permitted by USP. When early adoption is not used, *Plastic Packaging Systems and Their Materials of Construction* ⟨661⟩ will apply and must be met wherever ⟨661.1⟩ or this chapter is referenced in the *USP–NF*.)

INTRODUCTION

A packaging system, as defined in *Packaging and Storage Requirements* ⟨659⟩, contains or is intended to contain a medical article, such as a pharmaceutical drug product. As such, a packaging system provides the means for manufacturing, distributing, and storing these articles and products, and potentially for administering a drug product. A plastic packaging system is composed wholly or of a substantial portion of plastic materials. The term "plastic packaging system" refers to the sum of packaging components that together contain the pharmaceutical product, including closures. This sum of packaging components includes: 1) primary packaging components, which are those that directly contact the pharmaceutical product at some time during the product's manufacturing, distribution, storage, or use; and 2) secondary packaging components, which are those that may interact with the pharmaceutical product during the product's manufacturing, distribution, storage, and use, although the component does not directly contact the pharmaceutical product.

SCOPE

This chapter applies specifically to plastic packaging systems. The testing of materials of construction used in packaging systems is addressed in *Plastic Materials of Construction* ⟨661.1⟩. A product's packaging system is deemed chemically suited for its intended use, with respect to safety, if it meets the requirements in this chapter.

The applicant who secures and owns the regulatory approval of a packaging system or packaged drug product is responsible for establishing that the product's packaging system meets these expectations, and thus is suited for its intended use, by ensuring that the packaging system itself and/or the packaged pharmaceutical product has been appropriately tested and that the test results have been appropriately evaluated. A packaging system is chemically suited for its intended use with respect to safety if:

- The packaging system is constructed from well-characterized materials that have been intentionally chosen for use as established by testing according to ⟨661.1⟩.
- The packaging system's general physicochemical properties have been established.
- The packaging system's biocompatibility (biological reactivity) has been appropriately established.
- The packaging system has been established to be safe by means of the appropriate chemical testing, such as extractables or leachables profiling, and toxicological assessment of the test data. This combination of chemical testing and toxicological assessment is termed "chemical safety assessment".

TEST METHODS

Biological Reactivity

In vitro biological tests are performed on the packaging systems according to the test procedures described in *Biological Reactivity Tests, In Vitro* ⟨87⟩. In vitro testing described in ⟨87⟩ and the in vivo testing described in *Biological Reactivity Tests, In Vivo* ⟨88⟩ are not required for packaging systems used for solid and aqueous-based oral and topical dosage forms. Packaging systems that meet the requirements of the in vitro tests are not required to undergo any further in vivo testing. Packaging systems that do not meet the requirements of the biological reactivity tests (⟨87⟩ and ⟨88⟩, if appropriate) are not suitable as packaging systems for pharmaceutical use. If a plastic class designation (classes I–VI) is needed, analysts should perform the appropriate in vivo tests specified by ⟨88⟩. Information about the appropriate plastic class that should be selected is provided in *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* ⟨1031⟩.

Physicochemical Tests

WATER EXTRACTION

Solution C1: Fill the packaging system to its nominal capacity with *Purified Water* and close it, if possible, using the normal means of closure. Otherwise, close with an inert closure. Heat in an autoclave until 121 ± 2° is reached (typically in 20–30 min), and maintain at this temperature for 30 min. If heating at 121° leads to the deterioration of the container, heat at 100 ± 2° for 2 h or at 70 ± 2° for 24 ± 2 h. Cool the filled packaging system and empty its contents. The emptied contents are *Solution C1*.

If the test is being performed on a component, then the component is placed in an inert extraction vessel and put into contact with an amount of *Purified Water* that is equal to the packaging system's nominal capacity. The extraction vessel is closed and then heated as described above for a packaging system. Cool the extraction vessel and empty its contents. The emptied contents are *Solution C1*.

Prepare a blank by heating *Purified Water* in a borosilicate glass flask closed with an inert closure; heat the flask at the same temperature and for the same length of time as used for the preparation of *Solution C1*.

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Use *Solution C1* and the blank within 4 h of preparation.

Appearance of solution, color

Preparations

STANDARD SOLUTION CS1: Mix 3 mL of cobaltous chloride CS, 3 mL of ferric chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of 10 g/L of hydrochloric acid to produce the standard solution.

REFERENCE SOLUTION RS1: Add 1.0 mL of *Standard solution CS1* to a 100-mL volumetric flask and dilute with 10 g/L of hydrochloric acid to volume.

Procedure: Transfer equal portions of *Reference solution RS1*, *Purified Water*, and *Solution C1* to individual identical, colorless, transparent, neutral, flat-based glass vessels (internal diameter of 15–25 mm). Compare the colors in diffuse daylight, viewing vertically against a white background.

Solution C1 is colorless if it has the appearance of *Purified Water* and is not more intensely colored than *Reference solution RS1*.

Appearance of solution, clarity (visual method)

Preparations

HYDRAZINE SULFATE SOLUTION: Dissolve 1.0 g of hydrazine in *Purified Water* and dilute with *Purified Water* to 100 mL. Allow to stand for 4–6 h.

HEXAMETHYLENETETRAMINE SOLUTION: Using a 100-mL stoppered flask, dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of *Purified Water*.

PRIMARY OPALESCENT SUSPENSION: Add 25 mL of the *Hydrazine sulfate solution* to the volumetric flask containing the *Hexamethylenetetramine solution*. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided that it does not adhere to the glass and it is well mixed prior to use.

STANDARD OF OPALESCENCE: Dilute 15 mL of the *Primary opalescent suspension* with *Purified Water* to 1000 mL. This solution can be stored for 24 h.

REFERENCE SUSPENSION: Add 5 mL of the *Standard of opalescence* and 95 mL of *Purified Water* and mix well.

Procedure: Transfer equal portions of *Reference suspension*, *Purified Water*, and *Solution C1* to individual identical, colorless, transparent, neutral, flat-based glass vessels (internal diameter of 15–25 mm). Compare the solutions in diffuse daylight 5 min after preparation, viewing vertically against a black background.

Solution C1 is clear if its clarity is the same as *Purified Water* and its opalescence is not more pronounced than that of the *Reference suspension*.

Absorbance: Determine the spectrum of *Solution C1* between 230 and 360 nm, using the *Solution C1* blank as the compensation liquid.

Acidity or alkalinity: Conduct the test for *Acidity or alkalinity* only when packaging systems are intended to hold a liquid product or a product that is dissolved in its container before use.

To 20 mL of *Solution C1* obtained either as a portion of the fill solution or by combining the fill solution from several containers, add 0.1 mL of *phenolphthalein TS*; note the solution's color. Add 0.4 mL of 0.01 N *sodium hydroxide*; note the solution's color. Add 0.8 mL of 0.01 N *hydrochloric acid* and 0.1 mL of methyl red TS 2; note the solution's color.

Methyl red TS 2: Test for sensitivity: Add 0.1 mL of methyl red solution to 100 mL of carbon dioxide-free *Purified Water* and 0.05 mL of 0.02 N hydrochloric acid. NMT 0.1 mL of 0.02 N sodium hydroxide is required to change the color from red to yellow.

TOTAL ORGANIC CARBON

Refer to *Total Organic Carbon* ⟨643⟩.

The total organic carbon (TOC) content of *Solution C1* is measured according to ⟨643⟩. However, ⟨643⟩ is designed for testing high-purity water that has low TOC values. Because of extracted organic substances, material extracts may have TOC values that are much higher than those of *Purified Water*. Thus, the TOC analyses performed have a limit of detection of 0.2 mg/L (ppm) and have a demonstrated linear dynamic range of 0.2–20 mg/L (which encompasses the TOC limit). A linear range with a higher upper concentration can be used if linearity is established. If sample extracts exceed this upper linear range, then they should be diluted appropriately for analysis.

TOTAL TEREPHTHALOYL MOIETIES IN POLYETHYLENE TEREPHTHALATE AND POLYETHYLENE TEREPHTHALATE G PACKAGING SYSTEMS

Preparations

Polyethylene terephthalate extracting media: 50% alcohol (dilute 125 mL of *alcohol* with *Purified Water* to 238 mL, and mix), *n-heptane*, and *Purified Water*. For each extracting medium, fill a sufficient number of test packaging systems to 90% of their nominal capacity to obtain NLT 30 mL. Fill a corresponding number of glass bottles with each extracting medium for use as blanks. Fit the bottles with impervious seals such as aluminum foil and apply closures. Incubate the test packaging systems and the glass bottles at 49° for 10 days. Remove the test systems and glass bottles, and store at room temperature. Do not transfer the extracting medium samples to alternative storage vessels.

Polyethylene terephthalate G extracting media: 25% alcohol (dilute 125 mL of 50% alcohol with *Purified Water* to 250 mL, and mix), *n-heptane*, and *Purified Water*. Proceed as directed in *Polyethylene terephthalate extracting media*.

Procedure: Determine the absorbance of the 50% alcohol or 25% alcohol extracts in a 1-cm cell at the wavelength of maximum absorbance at about 244 nm (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). For the blank, use the corresponding extracting medium blank.

Determine the absorbance of the *n*-heptane extract in a 1-cm cell at the wavelength of maximum absorbance at about 240 nm (see ⟨857⟩). For the blank, use the *n*-heptane extracting medium.

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ETHYLENE GLYCOL IN POLYETHYLENE TEREPHTHALATE AND POLYETHYLENE TEREPHTHALATE G PACKAGING SYSTEMS

Preparations

Periodic acid solution: Dissolve 125 mg of *periodic acid* in 10 mL of *Purified Water.*

Dilute sulfuric acid: To 50 mL of *Purified Water* slowly add, with constant stirring, 50 mL of *sulfuric acid,* and allow to cool to room temperature.

Sodium bisulfite solution: Dissolve 0.1 g of sodium bisulfite in 10 mL of *Purified Water.* Use this solution within 7 days.

Disodium chromotropate solution: Dissolve 100 mg of *disodium chromotropate* in 100 mL of *sulfuric acid.* Protect this solution from light and use within 7 days.

Standard solution: Dissolve an accurately weighed quantity of *ethylene glycol* in *Purified Water,* and dilute quantitatively and stepwise if necessary to obtain a solution having a known concentration of about 1 µg/mL.

Sample solution: Use the *Purified Water* extract from *Total Terephthaloyl Moieties in Polyethylene Terephthalate and Polyethylene Terephthalate G Packaging Systems.*

Procedure: Transfer 1.0 mL of the *Standard solution* to a 10-mL volumetric flask. Transfer 1.0 mL of the *Sample solution* to a second 10-mL volumetric flask. Transfer 1.0 mL of the *Purified Water* extracting medium to a third 10-mL volumetric flask to serve as the method blank. To each of the three flasks, add 100 µL of *Periodic acid solution,* swirl to mix, and allow to stand for 60 min. Add 1.0 mL of *Sodium bisulfite solution* to each flask, and mix. Add 100 µL of *Disodium chromotropate solution* to each flask, and mix. [NOTE—All solutions should be analyzed within 1 h after addition of the *Disodium chromotropate solution.*] Cautiously add 6 mL of *sulfuric acid* to each flask, mix, and allow the solutions to cool to room temperature.

[**CAUTION**—Dilution of sulfuric acid produces substantial heat and can cause the solution to boil. Perform this addition carefully. Sulfur dioxide gas will be evolved. Use of a fume hood is recommended.]

Dilute each solution with *Dilute sulfuric acid* to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard solution* and the *Sample solution* in 1-cm cells at the wavelength of maximum absorbance at about 575 nm (see ⟨857⟩), using the solution from the *Purified Water* extracting medium as the method blank.

Chemical Safety Assessment

The safety of the packaging system must be established on the basis of relevant and appropriate chemical testing of 1) the packaging system, 2) its materials of construction, 3) its components of construction, as appropriate, or 4) the packaged drug product. Appropriate chemical testing of materials of construction is specified in ⟨661.1⟩ and may include the demonstration of conformance with the appropriate sections of 21 CFR Indirect Food Additives regulations. With regard to the testing of the packaging system (and/or its components of construction as appropriate) and the packaged drug product, an appropriate and rigorous chemical safety assessment would include extractables testing of the packaging system and leachables testing of the packaged drug product. It is expected that the design of the extractables and leachables study would be based on sound and justifiable scientific principles, and that the studies themselves would be consistent with 1) the nature of both the packaging system and packaged drug product, 2) the clinical use of the packaged drug product, and 3) the perceived safety risk associated with the packaging system and dosage form. Although no dosage form is excluded from this testing requirement, it is anticipated that the nature and degree of testing would be dosage form-dependent and consistent with a risk-based approach. For example, the testing of packaging systems for low-risk dosage forms, such as solid and aqueous-based oral and topicals, should be consistent with the low risk associated with these dosage forms. In view of the considerable diversity of packaging systems, dosage forms, and packaged drug products, it is not possible to provide specific test conditions for performing extractables and leachables studies. Nevertheless, general essential principles and demonstrated best-practices recommendations for extractable and leachable studies can be found in *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1663⟩ and *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1664⟩, respectively. These chapters may serve as helpful resources for designing and justifying rigorous and appropriate studies.

Alternative testing strategies for chemical safety assessment may be appropriate in justified circumstances, subject to agreement by an appropriate regulatory authority.

SPECIFICATIONS

Biological Reactivity

Test results are consistent with the relevant chapters (⟨87⟩ or ⟨88⟩) when applicable.

Physicochemical Tests

Appearance of solution: *Solution C1* is clear and colorless.

Absorbance: NMT 0.20

Acidity or alkalinity: The solution is colorless after the addition of *phenolphthalein* solution, pink after the addition of 0.01 N *sodium hydroxide,* and orange-red or red after the addition of 0.01 N *hydrochloric acid* and 0.1 mL of methyl red solution.

Total organic content: The difference in TOC concentrations between *Solution C1* and a suitable blank is NMT 8 mg/L.

Ethylene glycol in polyethylene terephthalate and polyethylene terephthalate G packaging systems: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution,* corresponding to NMT 1 ppm of *ethylene glycol.*

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Total terephthaloyl moieties in polyethylene terephthalate and polyethylene terephthalate G packaging systems: The absorbance of the 50% alcohol, 25% alcohol, and *n*-heptane extracts does not exceed 0.150, corresponding to NMT 1 ppm of total terephthaloyl moieties.

If the specification for absorbance or TOC is exceeded, then the packaging system can still be deemed to be acceptable if the chemicals responsible for the test results can be established (identity and concentration) and the chemicals are safety assessed to establish that the probable safety risk posed by all the chemicals, considered individually, is within acceptable parameters.

Chemical Safety Assessment

The data and information obtained in the *Chemical Safety Assessment* must be interpreted in the context of establishing the patient safety risk associated with the use of the packaging system and the administration of the packaged drug product. Most typically, such an interpretation of the chemical data involves the toxicological safety assessment of extractables and leachables data, supported, as appropriate, by other relevant testing. In this circumstance, the toxicological safety assessment should be performed for each individual relevant member of the packaging system's extractables profile (or each relevant member of the contained product's leachables profile, as appropriate). The assessment should demonstrate that the user safety risk associated with each individual relevant leachable (or extractable as a worst-case leachable) is acceptable and that the probable safety risk posed by all leachables (or extractables as worst-case leachables), considered individually, is within acceptable parameters. The term "relevant extractable or leachable" refers to those extractables that are present in a packaging system and those leachables that are present in a packaged drug product at levels sufficiently high that they have been deemed to have a potential safety impact, based, for example, on a comparison of the levels of extractables or leachables with a recognized and well-established safety alert threshold. Establishing and justifying the acceptable parameters used to assess the safety impact is the responsibility of the applicant who secures and owns the regulatory approval of a packaging system or packaged drug product; such acceptable parameters must be based on and derived from the sound application of established principles of toxicological safety assessment.

For leachables that are also elemental impurities, note that limits for elemental impurities in marketed pharmaceutical drug products (but not specifically packaging systems) can be found in *Elemental Impurities—Limits* ⟨232⟩.

Alternative chemical safety assessment specifications may be appropriate in justified circumstances, subject to agreement by an appropriate regulatory authority.

FUNCTIONALITY

Spectral Transmission Requirements for Light-Resistant Containers

APPARATUS[1]

Use a UV-visible spectrophotometer of suitable sensitivity and accuracy, adapted for measuring the amount of light transmitted by plastic materials used for pharmaceutical containers. In addition, the spectrophotometer is capable of measuring and recording light transmitted in diffused as well as parallel rays.

METHOD

Select sections to represent the average wall thickness. Cut circular sections from two or more areas of the container, and trim them as necessary to give segments of a size convenient for mounting in the spectrophotometer. After cutting, wash and dry each specimen, taking care to avoid scratching the surfaces. If the specimen is too small to cover the opening in the specimen holder, mask the uncovered portion of the opening with opaque paper or masking tape, provided that the length of the specimen is greater than that of the slit in the spectrophotometer. Immediately before mounting in the specimen holder, wipe the specimen with lens tissue. Mount the specimen with the aid of a tacky wax, or by other convenient means, taking care to avoid leaving fingerprints or other marks on the surfaces through which light must pass. Place the section in the spectrophotometer with its cylindrical axis parallel to the plane of the slit and approximately centered with respect to the slit. When properly placed, the light beam is normal to the surface of the section, and reflection losses are at a minimum.

Continuously measure the transmittance of the section with reference to air in the spectral region of interest with a recording instrument or at intervals of about 20 nm with a manual instrument, in the region of 290–450 nm.

SPECIFICATIONS

The observed spectral transmission does not exceed the limits given in *Table 1* for containers intended for parenteral use. The observed spectral transmission for plastic containers for products intended for oral or topical administration does not exceed 10% at any wavelength in the range of 290–450 nm.

Table 1. Limits for Plastic Containers

Nominal Size (mL)	Maximum Percentage of Spectral Transmission at Any Wavelength between 290 and 450 nm
1	25

[1] For further details regarding the apparatus and procedures, reference may be made to the latest edition of ASTM Standard D1003 *Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics*, from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959.

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Table 1. Limits for Plastic Containers *(continued)*

Nominal Size (mL)	Maximum Percentage of Spectral Transmission at Any Wavelength between 290 and 450 nm
2	20
5	15
10	13
20	12
50	10

[NOTE—Any container of a size intermediate to those listed in *Table 1* exhibits a spectral transmission NMT that of the next larger size container listed in *Table 1*. For containers larger than 50 mL, the limits for 50 mL apply.]

⟨670⟩ AUXILIARY PACKAGING COMPONENTS

Auxiliary packaging components are articles that are used to support or enhance container–closure systems. These articles include, but are not limited to, pharmaceutical coil and desiccants for containers. The components covered in this chapter must meet the applicable requirements provided and the additional applicable requirements provided in other specified chapters.

PHARMACEUTICAL COIL

Pharmaceutical coil is used as a filling material in multiple-unit containers for solid oral dosage forms to prevent breakage of tablets or capsules during shipment. The filling material should be discarded once the bottle is opened.

- SOLUTIONS

Iodinated zinc chloride solution: Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of *Purified Water*. Add 0.5 g of iodine, and shake for 15 min. Filter if necessary. Protect from light.

Zinc chloride–formic acid solution: Dissolve 20 g of zinc chloride in 80 g of an 850 g/L solution of anhydrous formic acid.

1% DuPont Fiber Identification Stain No. 4[1] solution: Dissolve 3.8 g of powdered stain in 378.5 mL of deionized water.

- COTTON PHARMACEUTICAL COIL

Purified cotton is the hair of the seed of cultivated varieties of *Gossypium hirsutum* Linné, or of other species of *Gossypium* (Fam. Malvaceae). It is deprived of fatty matter and bleached, and does not contain more than traces of leaf residue, pericarp, seed coat, or other impurities. Cotton pharmaceutical coil is used in bottles of solid oral dosage forms to prevent breakage.

Identification

A. When examined under a microscope, each fiber is seen to consist of a single cell, up to about 4 cm long and 40 µm wide, in the form of a flattened tube with thick and rounded walls that are often twisted.

B. When treated with *Iodinated zinc chloride solution*, the fibers become violet.

C. To 0.1 g of fibers add 10 mL of *Zinc chloride–formic acid solution*, heat to 40°, and allow to stand for 2 h, shaking occasionally: the fibers do not dissolve.

D. Weigh about 5 g of fibers, wet with water, and squeeze out the excess. Add fibers to 100 mL of a boiling solution of a *1% DuPont Fiber Identification Stain No. 4 solution*, and gently boil for at least 1 min. Remove the fibers, rinse well in cold water, and squeeze out the excess moisture: the fibers become green.

Acidity or alkalinity: Immerse about 10 g of fibers in 100 mL of recently boiled and cooled *Purified Water*, and allow to macerate for 2 h. Decant 25-mL portions of the water, with the aid of a glass rod, into each of two dishes. To one portion add 3 drops of phenolphthalein TS, and to the other portion add 1 drop of methyl orange TS. Neither portion appears pink when viewed against a white background.

Fluorescence: Examine a layer about 5 mm in thickness under UV light at 365 nm. It displays only a slight brownish-violet fluorescence and a few yellow particles. It shows no intense blue fluorescence, apart from that which may be shown by a few isolated fibers.

Residual hydrogen peroxide concentration: Place 1 g of fibers in a beaker containing 30 mL of *Purified Water*, and stir for 3 min with a stirring rod. Pour contents into another clean container (do not squeeze sample), or alternatively, remove the fibers from the solution with clean tweezers. Remove a peroxide analytical test strip[2] from its container, and immerse the test end into the sample liquid for 2 s. Shake to remove the excess liquid, immediately insert the test strip into a suitable reflectometry instrument, and record the reading in mg/kg (ppm), and calculate residual hydrogen peroxide concentration in ppm.

For an alternate method, place 20 g in a beaker, add 400 mL of *Purified Water*, stir, add 20 mL of 20% sulfuric acid, and stir the contents. Titrate with 0.100 N potassium permanganate solution to a faint pink color that remains for 30 s. Record the amount of titer, and calculate the concentration in ppm.

NMT 50 ppm is found using either method.

Loss on Drying ⟨731⟩

Analysis: Dry 5 g of fibers in an oven at 105° to constant weight.

Acceptance criteria: NMT 8.0%

[1] DuPont Fiber Identification Stain No. 4 is available from Pylam Products Co., 2175 East Cedar Street, Tempe, AZ 85281; www.pylamdyes.com.

[2] A suitable analysis system consisting of Reflectoquant® peroxide test strips and a RQflex® reflectometry instrument may be obtained from EMD Chemicals Inc., 480 S. Democrat Road, Gibbstown, NJ 08027; www.emdchemicals.com.

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Residue on Ignition ⟨281⟩
Analysis: Place 5 g of fibers in a porcelain or platinum dish, and moisten with 2 N sulfuric acid. Gently heat the cotton until it is charred, then ignite more strongly until the carbon is completely consumed.
Acceptance criteria: NMT 0.20%
Water-soluble substances: Place 10 g of fibers in a beaker containing 1000 mL of *Purified Water*, and boil gently for 30 min, adding water as required to maintain the volume. Pour the water through a funnel into another vessel, and press out the excess water from the cotton with a glass rod. Wash the cotton in the funnel with two 250-mL portions of boiling water, pressing the cotton after each washing. Filter the combined extract and washings, and wash the filter thoroughly with hot water. Evaporate the combined extract and washings to a small volume, transfer to a tared porcelain or platinum dish, evaporate to dryness, and dry the residue at 105° to constant weight. The residue weighs NMT 0.35%.
Fatty matter: Pack 10 g of fibers in a Soxhlet extractor provided with a tared receiver, and extract with ethyl ether for 4 h at a rate such that the ether siphons over NLT 4 times per h. The ethyl ether solution in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at 105° for 1 h. The weight of the residue does not exceed 0.7%.
Dyes: Pack about 10 g of fibers in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL. When observed downward through a column 20 cm in depth, the percolate may show a yellowish color, but not a blue or a green tint.
Other foreign matter: Pinches contain no oil stains or metallic particles by visual inspection.

• **RAYON PHARMACEUTICAL COIL**
Rayon pharmaceutical coil is a fibrous form of bleached, regenerated cellulose, to be used as a filler in bottles of solid oral dosage forms to prevent breakage. It consists exclusively of rayon fibers except for a few isolated foreign fibers that may be present. [NOTE—Rayon pharmaceutical coil has been found to be a potential source of dissolution problems for gelatin capsules or gelatin-coated tablets resulting from gelatin cross-linking.]
Identification
A. When treated with *Iodinated zinc chloride solution*, the fibers become violet.
B. Add 10 mL of *Zinc chloride–formic acid solution* to 0.1 g of fibers, heat to 40°, and allow to stand for 2 h, shaking occasionally: the fibers dissolve completely, except for mat rayon fibers where titanium particles remain.
C. Weigh about 5 g of fibers, wet with water, and squeeze out the excess. Add fibers to 100 mL of a boiling solution of a *1% DuPont Fiber Identification Stain No. 4 solution*, and gently boil for at least 1 min. Remove the fibers, rinse well in cold water, and squeeze out the excess moisture: the fibers become blue–green.
Acidity or alkalinity, Fluorescence, Fatty matter, Dyes, and **Other foreign matter:** Proceed as directed under *Cotton Pharmaceutical Coil*, except use rayon pharmaceutical coil. Sample weight for fatty matter is 5 g and weight of residue does not exceed 0.5%.
Loss on Drying ⟨731⟩
Analysis: Dry 5 g of fibers in an oven at 105° to constant weight.
Acceptance criteria: NMT 11.0%
Residue on Ignition ⟨281⟩**:** NMT 1.50%, determined on a 5-g test specimen
Acid-insoluble ash: To the residue obtained in the test for *Residue on Ignition*, add 25 mL of 3 N hydrochloric acid, and boil for 5 min. Collect the insoluble matter on a tared filtering crucible, wash with hot water, ignite, and weigh: the residue weighs NMT 1.25%.
Water-soluble substances: Proceed as directed under *Cotton Pharmaceutical Coil*, except to use rayon pharmaceutical coil. The residue weighs NMT 1.0%.

• **POLYESTER PHARMACEUTICAL COIL**
Polyester pharmaceutical coil is a white odorless material, to be used as a filler in bottles of solid oral dosage forms to prevent breakage.
Identification
A. Proceed as directed under *Infrared spectroscopy* in the *Test Methods* section. Determine the IR spectrum from 4000 to 650 cm^{-1} (2.5 to 15 µm). The spectrum obtained from the specimen exhibits major absorption bands only at the same wavelengths as the spectrum of USP Polyethylene Terephthalate RS.
B. Weigh about 5 g of fibers, wet with water, and squeeze out excess. Add fibers to 100 mL of a boiling solution of a *1% DuPont Fiber Identification Stain No. 4 solution*, and gently boil for at least 1 min. Remove the fibers, rinse well in cold water, and squeeze out the excess moisture: the fibers become pale orange.
Acidity or alkalinity: Proceed as directed under *Cotton Pharmaceutical Coil*, except to use polyester pharmaceutical coil.
Loss on Drying ⟨731⟩
Analysis: Dry 5 g of fibers in an oven at 105° to constant weight.
Acceptance criteria: NMT 1.0%
Residue on Ignition ⟨281⟩**:** NMT 0.5%, determined on a 5-g test specimen
Finish on fibers: The finish on fibers used for processing should comply with FDA food contact regulations.

• **TEST METHODS**
Infrared spectroscopy[3]
Apparatus: FTIR or a double-beam spectrophotometer capable of scanning from 4000 to 650 cm^{-1} (2.5–15 µm).

Change to read:

Specimen preparation: The ATR ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197A*▲ (CN 1-May-2020) technique can be used as alternative methods where the Reference Standard spectra is similarly obtained.

[3] Additional information on fiber identification methods may be found in "Standard Test Methods for Identification of Fibers in Textiles". Current version of ASTM Method D276, published by ASTM International, 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, PA 19428-2959; www.astm.org.

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Method 1 (potassium bromide disc): Use scissors to cut polyester fibers (1–3 mg) into short lengths (less than 1 mm long), mix with 200 mg of powdered potassium bromide, and grind in a ball mill for 1–2 min. Transfer to potassium bromide-disc die, and form a disc.

Method 2 (melt film): Produce film by pressing polyester fibers between TFE-fluorocarbon sheets and place between heated plates.

DESICCANTS

Desiccants are used to remove moisture from air in containers in order to protect drug products, particularly solid oral dosage forms. They are supplied in a number of different packaging materials including cotton, Kraft paper, rayon and polyester cloth bags, perforated plastic, polymer films, polymer housings or Tyvek®. The most common types of commercial desiccants are bentonite, calcium chloride, calcium oxide, molecular sieves, and silica gel. Other desiccants are subject to appropriate testing to ensure suitability for the intended application.

Where desiccants are incorporated directly into the wall or cap of packaging containers or bound by a carrier material, use unincorporated desiccant in the test methods. For desiccants that are loaded with a predetermined moisture content, perform the testing before the moisture has been loaded or after the desiccant has been regenerated.

• **BENTONITE**

Bentonite clay (also referred to as montmorillonite clay) is a native, colloidal, hydrated aluminum silicate.

Appearance: Grayish-white powder or pellets with a yellowish or pinkish tint

Identification—X-Ray Diffraction ⟨941⟩: For *Sample preparation A*, the largest peak corresponds to a *d* value between 15.0 and 17.2 Å. The major peak in the region between 1.48 and 1.54 Å from the pattern of *Sample preparation B* is between 1.492 and 1.504 Å.

Identification—precipitation: Formation of a gelatinous white precipitate

Inorganic impurities

Arsenic: NMT 10 mg/kg

Lead: NMT 15 mg/kg

Specific tests

pH ⟨791⟩: 4.5–10.5. Disperse 4.0 g in 200 mL of water, mix vigorously to facilitate wetting.

Loss on Drying ⟨731⟩: Dry a 5–10 g sample at 110° to a constant weight: it loses NMT 3.0%.

[NOTE—Conduct assay immediately after opening the original container.]

Moisture adsorption capacity

NLT 13% at 40% ± 5% relative humidity (RH) and 25 ± 2°

NLT 23% at 80% ± 5% RH and 25 ± 2°

Test methods

Identification—X-ray diffraction

Sample preparation A: Add 2 g in small portions to 100 mL of water with intense agitation. Allow to stand for 12 h to ensure complete hydration. Place 2 mL of the mixture so obtained on a suitable glass slide, and allow to air-dry at room temperature to produce an oriented film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that ethylene glycol saturates the desiccator chamber. Allow the slide to stand for 12 h.

Sample preparation B: Prepare a random powder specimen of the sample.

Analysis: *Sample preparation A* and *Sample preparation B*. Record the X-ray diffraction pattern of the samples, and determine the *d* values.

Identification—precipitation

Sample: 5 g

Analysis: Add 1 g of potassium nitrate and 3 g of anhydrous sodium carbonate to the *Sample* contained in a metal crucible, heat until the mixture has melted, and allow it to cool. Add 20 mL of boiling water to the residue, mix, filter, and wash the residue with 50 mL of water. Add 1 mL of hydrochloric acid and 5 mL of water to the residue, and filter. Add 1 mL of 10 N sodium hydroxide to the filtrate, filter, and add 3 mL of 2 M ammonium chloride.

Arsenic

Sample solution: Transfer 8.0 g of dried sample into a 250-mL beaker containing 100 mL of dilute hydrochloric acid (1 in 25), mix, and cover with a watch glass. Boil gently, with occasional stirring, for 15 min without allowing excessive foaming. Pass the hot supernatant liquid through a rapid-flow filter paper into a 200-mL volumetric flask, and wash the filter with four 25-mL portions of hot dilute hydrochloric acid (1 in 25), collecting the washings in the volumetric flask. Cool the combined filtrates to room temperature, add dilute hydrochloric acid (1 in 25) to volume and mix.

Arsenic trioxide stock solution: Accurately weigh 132.0 mg of arsenic trioxide, previously dried at 105° for 1 h, and dissolve in 5 mL of sodium hydroxide solution (1 in 5) in a 1000-mL volumetric flask. Neutralize the solution with 2 N sulfuric acid, add an additional 10 mL of 2 N sulfuric acid, and bring to volume with recently boiled and cooled water and mix.

Standard arsenic solution: Dilute the *Arsenic trioxide stock solution* to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument. Keep in an all-glass container, and use within 3 days.

Analysis: Proceed according to *Elemental Impurities—Procedures* ⟨233⟩.

Lead

Sample solution: Transfer 3.75 g of sample into a 250-mL beaker containing 100 mL of dilute hydrochloric acid (1 in 25), stir, and cover with a watch glass. Boil for 15 min, then cool to room temperature, and pass through a rapid-flow filter paper into a 400-mL beaker. Wash the filter with four 25-mL portions of hot water, collecting the washings in the 400-mL beaker. Concentrate the combined extracts by gentle boiling to approximately 20 mL. If a precipitate forms, add 2–3 drops of nitric acid, heat to boiling, and cool to room temperature. Pass the concentrated extracts through a rapid-flow filter paper into a 50-mL volumetric flask. Transfer the remaining contents of the 400-mL beaker through the filter paper and into the flask with water. Dilute with water to volume.

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Lead nitrate stock solution: Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard lead solution: On the day of use, dilute the *Lead nitrate stock solution* to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.

Analysis: Proceed according to ⟨233⟩.

Moisture adsorption capacity

Equipment: Temperature-humidity chambers capable of controlled humidity at 40% ± 5% RH and 80% ± 5% RH at 25 ± 2° or a desiccator containing water-saturated salts that provide %RH at the required level plus an oven capable of maintaining 25 ± 2°.

Method: 5–10 g. Remove the sample from the packaging material. Where absorbents are incorporated directly into the wall or cap of packaging containers, use unincorporated desiccant. Add the sample to the humidity chamber or desiccator and measure the weight gain over time until this reaches equilibrium when two successive consecutive weighings do not differ by more than 3 mg/g of substance taken, the second weighing following an additional 3 ± 1 h of storage at the required temperature and humidity conditions. Calculate the adsorption capacity as percentage weight gained over the initial sample weight. Where two absorbents are packaged in combination, the moisture adsorption capacity specification must be calculated in proportion of the mix. For example, a mixture of 60% molecular sieve and 40% silica gel, the moisture adsorption capacity would be NLT 16.6% (9.0% + 7.6%) when the test condition is 40% ± 5% RH and 25 ± 2° and the moisture adsorption capacities taken at their minimum specification.

Calculation

Molecular sieve: 60% by weight × 15% moisture adsorption capacity = NLT 9.0% moisture adsorption capacity

Silica gel: 40% by weight × 19% moisture adsorption capacity = NLT 7.6% moisture adsorption capacity

• **CALCIUM CHLORIDE, ANHYDROUS**

Identification—calcium: Passes tests

Identification—chloride: Passes test

Assay: NLT 93.0% and NMT 100.5% of calcium chloride ($CaCl_2$)

Inorganic impurities

Arsenic: NMT 3 ppm

Fluoride: NMT 0.004%

Lead: NMT 5 ppm

Magnesium and alkali salts: NMT 25 mg of residue (NMT 5.0%)

Specific tests

pH ⟨791⟩: 4.5–11.0 (1:20 aqueous solution)

Moisture adsorption capacity: NLT 28% at 80% ± 5% RH and 25 ± 2°

Test methods

Identification—calcium

Sample solution—100 mg/mL: Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner. Using 2 drops of methyl red TS as the indicator, neutralize a 1:20 solution of a calcium salt with 6 N ammonia, then add 2.7 N hydrochloric acid, dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate TS. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Identification—chloride

Sample solution—100 mg/mL: Solutions of chlorides yield with silver nitrate TS a white, curdy precipitate that is insoluble in nitric acid but soluble in a slight excess of 6 N ammonia.

Assay

Sample: 1.5 g

Analysis: Transfer the *Sample* into a 250-mL volumetric flask, dissolve it in a mixture of 100 mL of water and 5 mL of 2.7 N hydrochloric acid, dilute with water to volume, and mix. Transfer 50 mL of this solution into a suitable container and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Then, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator. Continue the titration to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 5.55 mg of calcium chloride ($CaCl_2$).

Arsenic

Sample solution: 1 g in 10 mL

Arsenic trioxide stock solution and **Standard arsenic solution:** Proceed as directed under *Bentonite*.

Analysis: Proceed as directed under *Bentonite*.

Fluoride

Sodium fluoride solution—5 µg/mL: Transfer 2.210 g of sodium fluoride, previously dried at 200° for 4 h and accurately weighed, into a 400-mL plastic beaker, add 200 mL of water, and stir until dissolved. Quantitatively transfer this solution into a 1000-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Store this stock solution in a plastic bottle. On the day of use, transfer 5.0 mL of the stock solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Calibration curve: Transfer 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 mL of the *Sodium fluoride solution* into separate 250-mL plastic beakers. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1 M sodium citrate, and 10 mL of 0.2 M disodium EDTA to each beaker and mix. Transfer each solution into separate 100-mL volumetric flasks, dilute with water to volume, and mix. Transfer a 50-mL portion of each solution into separate 125-mL plastic beakers, and measure the potential of each solution with a suitable ion-selective electrode apparatus (such as the Orion Model No. 94–09, with solid-state membrane), using a suitable reference electrode (such as the Orion Model No. 90–01,

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with single junction). Plot the calibration curve on two-cycle semi-logarithmic paper (such as K & E No. 465130) or with the use of a suitable graphing calculator or spreadsheet program, with µg of F per 100 mL solution on the logarithmic scale.

Analysis: Transfer 1.00 g of sample into a 150-mL glass beaker, add 10 mL of water, and, while stirring continuously, slowly add 20 mL of 1 N hydrochloric acid to dissolve the sample. Boil rapidly for 1 min, then transfer into a 250-mL plastic beaker, and cool rapidly in ice water. Add 15 mL of 1 M sodium citrate and 10 mL of 0.2 M disodium EDTA, and mix. Adjust the pH to 5.5 ± 0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide, if necessary. Transfer into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer a 50-mL portion of this solution into a 125-mL plastic beaker, and measure the potential of the solution with the apparatus described under *Calibration curve*. Determine the fluoride content, in µg, of the sample from the *Calibration curve*. Determine the percentage of fluoride in the sample taken:

$$\text{Result} = (C/WS) \times F \times 100$$

C = content of fluoride (µg)
WS = sample weight (g)
F = factor converting µg to g, 0.000001

Lead

Sample solution: 1 g in 20 mL

Lead nitrate stock solution and **Standard lead solution:** Proceed as directed under *Bentonite*.

Analysis: Proceed as directed under *Bentonite*.

Magnesium and alkali salts

Sample: 1 g

Analysis: Dissolve the *Sample* in 50 mL of water, add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS. Then add 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute with water to 100 mL, and let it stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, and transfer 50 mL of the clear filtrate to a platinum dish. Add 0.5 mL of sulfuric acid to the dish and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight.

Moisture adsorption capacity: Proceed as directed under *Bentonite*.

• **CALCIUM OXIDE**

Identification—calcium: Passes tests

Assay: NLT 95.0% and NMT 100.5% of calcium oxide (CaO), on the ignited basis

Inorganic impurities

Acid-insoluble substances: NMT 1%

Arsenic: NMT 3 ppm

Fluoride: NMT 0.015%

Lead: NMT 2 mg/kg

Magnesium and alkali salts: NMT 3.6%

Specific tests

Loss on ignition: NMT 10.0%

Moisture adsorption capacity: NLT 28% at 80% ± 5% RH and 25 ± 2°

Test methods

Identification—calcium

Sample solution: Shake 1 g of sample with 20 mL of water and add glacial acetic acid until the sample is dissolved.

Analysis: Proceed as directed under *Calcium Chloride, Anhydrous*.

Assay

Sample: 1 g of sample ignited to a constant weight (see *Loss on ignition* below)

Analysis: Dissolve the *Sample* in 20 mL of 2.7 N hydrochloric acid. Cool the solution, dilute with water to 500.0 mL, and mix. Pipet 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Then, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator. Continue the titration with disodium EDTA to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 2.804 mg of calcium oxide (CaO).

Arsenic

Sample solution: 1 g in 15 mL of 2.7 N hydrochloric acid

Arsenic trioxide stock solution and **Standard arsenic solution:** Proceed as directed under *Bentonite*.

Analysis: Proceed as directed under *Bentonite*.

Fluoride

Sample: 1.0 g

Analysis: Proceed as directed under *Calcium Chloride, Anhydrous*.

Lead

Sample solution: 1 g in 15 mL of 2.7 N hydrochloric acid

Lead nitrate stock solution and **Standard lead solution:** Proceed as directed under *Bentonite*.

Analysis: Proceed as directed under *Bentonite*.

Acid-insoluble substances

Sample solution: Shake 5 g of sample, and then mix it with 100 mL of water and sufficient hydrochloric acid, added dropwise, to dissolve it.

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Analysis: Boil the *Sample solution*, cool, add hydrochloric acid, if necessary, to make the solution distinctly acid, and pass through a tared glass filter crucible. Wash the residue with water until free of chlorides, dry at 105° for 1 h, cool, and weigh.

Magnesium and alkali salts

Sample: 500 mg

Analysis: Dissolve the *Sample* in 30 mL of water and 15 mL of 2.7 N hydrochloric acid. Heat the solution, boil for 1 min, and rapidly add 40 mL of oxalic acid TS, and stir vigorously. Add 2 drops of methyl red TS, and neutralize the solution with 6 N ammonium hydroxide to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h and allow it to cool. Dilute the mixture with water to 100 mL, mix well, and filter. Add 0.5 mL of sulfuric acid to 50 mL of the filtrate. Then evaporate to dryness and ignite to constant weight in a tared platinum crucible at 800 ± 25°.

Loss on ignition

Sample: 1 g

Analysis: Ignite the *Sample* to constant weight in a tared platinum crucible at 1100 ± 50°.

Moisture adsorption capacity: Proceed as directed under *Bentonite*.

• **MOLECULAR SIEVES**

Molecular sieves are synthetic porous crystalline alkali-metal aluminosilicates in a beaded form with a tightly controlled pore size. The most commonly used molecular sieves for desiccants are described as types 3A, 4A, 5A, and 13X with a pore size range of approximately 3–10 Å.

Identification A: The drop of water becomes turbid.

Identification B: Passes tests

Inorganic impurities

Lead: NMT 5 ppm

Specific tests

pH ⟨791⟩: 6.5–12 (200 mg/mL in carbon dioxide-free water)

Loss on Drying ⟨731⟩: Dry a 5–10 g sample at 575 ± 25° to a constant weight: it loses NMT 4.5%. [NOTE—Conduct assay immediately after opening the original container.]

Moisture adsorption capacity

NLT 15.0% weight at 40% ± 5% RH and 25 ± 2°

NLT 16.5% weight at 80% ± 5% RH and 25 ± 2°

Test methods

Identification A

Sample: 500 mg

Analysis: Mix the *Sample* with 2.5 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 mL of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it into a beaker with the aid of about 50 mL of water. Gradually add hydrochloric acid until no effervescence is observed, add 10 mL more of the acid, and evaporate to dryness on a steam bath. Cool, add 20 mL of water, boil, and pass through ash-free filter paper. An insoluble residue of silica remains. [NOTE—Retain the filtrate for *Identification B*.] Transfer the gelatinous residue to a platinum dish, and cautiously add 5 mL of hydrofluoric acid. [**CAUTION**—Handle hydrofluoric acid in a fume hood with appropriate precautions.] The precipitate dissolves. (If it does not dissolve, repeat the treatment with hydrofluoric acid.) Heat the solution and introduce a glass stirring rod with a drop of water on the tip into the resulting vapors.

Identification B—aluminum

Sample: Use 2 portions of the filtrate obtained in *Identification A*.

Analysis: Solution of a first portion of the filtrate obtained in *Identification A* yields a white, gelatinous precipitate with 6 N ammonia that is insoluble in an excess of this reagent. Solution of a second portion of the filtrate obtained in *Identification A* yields a white precipitate with a 1 N sodium hydroxide that is dissolved in an excess of this reagent.

Lead

Sample solution: Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 N hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute with water to volume, and mix.

Lead nitrate stock solution and **Standard lead solution:** Proceed as directed under *Bentonite*.

Analysis: Proceed as directed under *Bentonite*.

Moisture adsorption capacity: Proceed as directed under *Bentonite*.

• **SILICA GEL**

Silica gel is silicon dioxide ($SiO_2 \cdot H_2O$) that has been manufactured by the addition of sodium silicate solution to a mineral acid to produce a gelatinous precipitate that is washed, then dehydrated to produce colorless silica gel in a bead, granular, or micronized form in a range of mesh sizes.

Appearance: A white or translucent bead or granule

Identification A: A deep yellow color is produced.

Identification B: A green-blue spot develops.

Assay: NLT 94.0% of silicon dioxide (SiO_2) on the ignited basis

Inorganic impurities

Lead: NMT 5 ppm

Soluble ionizable salts (as Na_2SO_4): The conductance produced by the sample is NMT that produced by the control solution (equivalent to NMT 5.0%).

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Specific tests
pH ⟨791⟩: 4–8 in a slurry (1 in 20)
Loss on Drying ⟨731⟩: Dry a 5–10-g sample at 145° for 3 h: it loses NMT 3.0% of its weight.
[NOTE—Conduct assay immediately after opening the original container.]
Moisture adsorption capacity
NLT 19% wt at 40% ± 5% RH and 25 ± 2°
NLT 27% wt at 80% ± 5% RH and 25 ± 2°
Test methods
Identification A
Sample: 5 mg
Analysis: Place into a platinum crucible, mix with 200 mg of anhydrous potassium carbonate, and ignite over a burner at a red heat for about 10 min. Cool, dissolve the melt in 2 mL of freshly distilled water, warming if necessary, and slowly add 2 mL of ammonium molybdate TS.
Identification B—aluminum
Sample: Solution remaining from *Identification A*
Analysis: Place 1 drop of the *Sample* from *Identification A* on a filter paper, and evaporate the solvent. Add 1 drop of a saturated solution of *o*-tolidine in glacial acetic acid, and place the paper over ammonium hydroxide.
[NOTE—Avoid contact with *o*-tolidine when performing this test, and conduct the test in a well-ventilated hood.]
Assay
Sample: 1 g, previously dried
Analysis: Transfer the *Sample* into a tared platinum crucible, ignite at 1000 ± 25° to constant weight, cool in a desiccator, and weigh to obtain the ignited sample weight (*W1*). Moisten the residue with a few drops of alcohol, add 3 drops of sulfuric acid, then add enough hydrofluoric acid to cover the wetted sample.
[**CAUTION**—Handle hydrofluoric acid in a well-ventilated fume hood with appropriate precautions.]
Evaporate to dryness on a hot plate, using medium heat (95°–105°), then add a few mL of hydrofluoric acid enough to cover the *Sample*, swirl the dish carefully to wash down the sides, and again evaporate to dryness taking care that the *Sample* does not spatter as dryness is approached. Heat the crucible to a red heat using a Meker burner, a propane torch, or in a muffle furnace. Ignite the residue at 1000 ± 25° for 30 min, cool in a desiccator, and weigh to obtain the final weight (*W2*). If a residue remains, repeat the *Analysis* beginning with the addition of hydrofluoric acid until a constant weight is obtained. The difference between the ignited sample weight and the final weight (*W1* – *W2*) represents the weight, in g, of silicon dioxide (SiO_2) in the initially ignited sample. Express the result as a percentage of the initially ignited basis.
Lead
Sample solution: Transfer 5.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 N hydrochloric acid, cover with a watch glass, and slowly heat to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No. 3, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute with water to volume, and mix.
Lead nitrate stock solution and **Standard lead solution:** Proceed as directed under *Bentonite*.
Analysis: Proceed as directed under *Bentonite*.
Soluble ionizable salts (as Na_2SO_4)
Sample: 5 g, previously dried
Control solution: 1 mg/mL of anhydrous sodium sulfate, made to 250 mL
Analysis: Stir the *Sample* with 150 mL of water for at least 5 min in a high-speed mixer. Filter with the aid of suction, and wash the mixer and filter with 100 mL of water in divided portions, adding the washings to the filtrate. Dilute the filtrate with water to 250 mL. Determine the conductances of the diluted filtrate and of the *Control solution* with a suitable conductance bridge assembly.
Moisture adsorption capacity: Proceed as directed under *Bentonite*.
Reagents—test solutions
Ammonium molybdate TS: Dissolve 6.5 g of finely powdered molybdic acid in a mixture of 14 mL of water and 14.5 mL of ammonium hydroxide. Cool the solution, and add it slowly, with stirring, to a well-cooled mixture of 32 mL of nitric acid and 40 mL of water. Allow to stand for 48 h, and filter through a fine-porosity, sintered-glass crucible. This solution deteriorates upon standing and is unsuitable for use if, upon the addition of 2 mL of dibasic sodium phosphate TS to 5 mL of the solution, an abundant yellow precipitate does not form at once or after slight warming. Store it in the dark. If a precipitate forms during storage, use only the clear supernatant.
Oxalic acid TS: Dissolve 6.3 g of oxalic acid in water to make 100 mL.

⟨671⟩ CONTAINERS—PERFORMANCE TESTING

INTRODUCTION
MOISTURE VAPOR TRANSMISSION FOR PACKAGING SYSTEMS
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Introduction

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Classification Based on Desiccant Method
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SPECTRAL TRANSMISSION
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INTRODUCTION

It is the purpose of this chapter to provide standards for the functional properties of packaging systems used for solid oral dosage forms (SODFs) and liquid oral dosage forms (LODFs). The tests in this chapter may be applied to other dosage forms with the appropriate justification. The tests that follow are provided to determine the moisture vapor transmission rate (water vapor permeation rate) for plastic packaging systems for manufacturers, packagers, and repackagers (*Table 1*). This chapter also includes a classification system that allows pharmacists and institutional repackagers to select appropriate containers to repackage SODFs and LODFs. Definitions that apply to this classification system are provided in *Packaging and Storage Requirements* ⟨659⟩.

Table 1. Moisture Vapor Transmission Test Methods for Packaging Systems

Section	Moisture Vapor Transmission for Packaging Systems	Classification System for Packaging Systems		
Subsection		Classification Based on Desiccant Method		Classification Based on Water Method
Application	Multiple-unit containers with seal intact or broached state and single-unit or unit-dose containers	Multiple-unit containers with closure applied and seal intact or broached state	Single-unit and unit-dose containers in sealed state	Multiple- and single-unit containers
Users	Manufacturers	Manufacturers Packagers Repackagers Pharmacies	Manufacturers Packagers Repackagers Pharmacies	Manufacturers Packagers Repackagers Pharmacies

MOISTURE VAPOR TRANSMISSION FOR PACKAGING SYSTEMS

Introduction

The purpose of the moisture vapor transmission test method, which is based upon ASTM method D7709,[1] is to obtain reliable and specific moisture vapor transmission rates that can be used to distinguish barrier performance of packaging systems used for regulated articles by manufacturers, packagers, and repackagers. This test method will establish a value that represents the moisture vapor transmission rate of the container–closure system being evaluated. There may be additional packaging systems where the test methods in this section could be applied; however, any deviation should be described. If other methods are used to measure moisture vapor transmission rate, these methods should be described in sufficient detail to justify their use.

Desiccant Method for Packaging Systems for Oral Dosage Forms

This section describes moisture vapor transmission test methods for multiple-unit containers (see *Method 1*), high barrier single-unit and unit-dose containers (see *Method 2*), and low barrier single-unit and unit-dose containers (see *Method 3*) used to package SODFs.

This method includes the following:

- A specific moisture vapor transmission value for a container rather than a classification
- Sufficient sensitivity and precision to allow differentiation among moisture barrier performance for containers
- Conditions used for testing packaging systems that are the same as those used for accelerated stability testing of the primary packaging of regulated articles [typically 40°/75% relative humidity (RH)]

EQUIPMENT

The following equipment should be used to complete the test method:

- A balance for weighing the test specimens that has sufficient capacity to weigh the test specimens throughout the period of the test (see *Weighing on an Analytical Balance* ⟨1251⟩). The balance must have sensitivity adequate to measure small differences in weight from one time point to the next. The weighing uncertainty must be less than 5% of the weight gain from one time point to the next. The weighing uncertainty is typically three times the balance resolution/sensitivity. As an

[1] ASTM D7709. *Standard Test Methods for Measuring Water Vapor Transmission Rate (WVTR) of Pharmaceutical Bottles and Blisters* published by ASTM International, 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, PA 19428-2959.

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example, a balance with a resolution of 0.1 mg is acceptable for packaging systems whose weight gain per time interval is more than or equal to 6 mg [(0.1 × 3)/5%], which is 60 times the balance sensitivity

- A chamber capable of maintaining 40 ± 2° and 75 ± 5% RH. Other chamber conditions can be used as long as the balance sensitivity is adequate to measure small weight differences from one time point to the next

DESICCANT

Method 1: The desiccant is anhydrous calcium chloride in granular form. Other desiccants, such as a molecular sieve or silica gel, may be suitable. If anhydrous calcium chloride is used, pre-dry at 215 ± 5° for 7¼±¼ h to ensure that any hexahydrate present is fully converted to the anhydrate. Cool the desiccant in a desiccator for at least 2 h before use.

[NOTE—It has been shown[2] that anhydrous calcium chloride may contain calcium hexahydrate, which loses water only when the temperature reaches 200°.]

Methods 2 and 3: The desiccant is silica gel molded in a form to fit the size and shape of the blister cavity used. Other desiccants may be suitable, such as a molecular sieve. If silica gel is used, pre-dry in a circulating hot air oven at one of two conditions: 155 ± 5° for 3¼±¼ h or 150 ± 5° for 4¼±¼ h. If a molecular sieve is used, pre-dry according to the manufacturer's specifications. Cool the desiccant in a desiccator for at least 2 h before use.

PROCEDURE

Method 1: Use 15 multiple-unit containers and 15 closures representative of the system to be tested. Prepare the test specimens by filling each multiple-unit container two-thirds with desiccant and then, for screw-type closures, apply the closure using the torque that is within the range of tightness specified in *Table 2*. For other closure types, apply the closure according to the intended method. Ensure that a proper seal has been made with the intended membrane to the land area of the bottle finish. Identify each multiple-unit container with indelible ink. Do not use a label. If there is a need to increase the precision of the method, the user can test the system without the closure as long as an impervious seal remains on the container. If desired, weigh each multiple-unit container at ambient temperature and RH. Record this weight for time 0, but do not use it in the calculation of permeation. Place all containers in the test chamber (40°/75% RH) within 1 h of weighing. Weigh all multiple-unit containers at time intervals of 7 days ± 1 h. Weigh the multiple-unit containers at 7, 14, 21, 28, and 35 days to get steady-state data points. (The time interval from time 0 to day 7 is the period of equilibration.) Prior to weighing at each time interval, equilibrate the containers for about 30 min at the weighing temperature and RH. Limit the time out of the chamber to less than 2 h. Record the weights in an appropriate manner for later computation of the regression line.

Table 2. Torque Applicable to Screw-Type Container

Closure Diameter[a] (mm)	Suggested Tightness Range with Manually Applied Torque[b] (inch lbs.)	Suggested Tightness Range with Manually Applied Torque[b] (Newton-meters)
8	5	0.56
10	6	0.68
13	8	0.90
15	5–9	0.56–1.02
18	7–10	0.79–1.13
20	8–12	0.90–1.36
22	9–14	1.02–1.58
24	10–18	1.13–2.03
28	12–21	1.36–2.37
30	13–23	1.47–2.60
33	15–25	1.69–2.82
38	17–26	1.92–2.94
43	17–27	1.92–3.05
48	19–30	2.15–3.39
53	21–36	2.37–4.07
58	23–40	2.60–4.52
63	25–43	2.82–4.86
66	26–45	2.94–5.08
70	28–50	3.16–5.65
83	32–65	3.62–7.35

[2] Chen Y, Li Y. Determination of water vapor transmission rate (WVTR) of HDPE bottles for pharmaceutical products. *Int J Pharm*. 2008;358(1-2):137–143.

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Table 2. Torque Applicable to Screw-Type Container *(continued)*

Closure Diameter[a] (mm)	Suggested Tightness Range with Manually Applied Torque[b] (inch lbs.)	Suggested Tightness Range with Manually Applied Torque[b] (Newton-meters)
86	40–65	4.52–7.35
89	40–70	4.52–7.91
100	45–70	5.09–7.91
110	45–70	5.09–7.91
120	55–95	6.22–10.74
132	60–95	6.78–10.74

[a] The torque designated for the next larger closure diameter is to be applied in testing containers having a closure diameter intermediate to the diameters listed.
[b] A suitable apparatus is available from SecurePak, http://www.secure-pak.com. MRA model of a spring torque tester with indicators on both the removal and application sides is available in the following ranges: 1) 0–25 inch lbs., read in 1-inch lb. increments; 2) 0–50 inch lbs., read in 2-inch lb. increments; and 3) 0–100 inch lbs., read in 5-inch lb. increments.

Method 2: Use 10 test units for this method. Provide a minimum of 10 blister cavities for each test unit. If the card contains fewer than 10 cavities, bundle the cards to form a single test unit of at least 10 cavities. This is required to provide sufficient weight gain at each time interval. Fill with pre-dried desiccant, and seal the blisters on equipment that is capable of correctly filling and sealing the blister. The desiccant should fill the cavity. The total weight of desiccant must be sufficient to meet the quantity required to avoid partial saturation of the desiccant before completion of the test. Fill the blisters in a low-humidity atmosphere (as low as possible, but not greater than 50% RH). Do not expose the desiccants to room humidity for more than 30 min before sealing. Identify each test specimen with indelible ink; do not use a label..A test unit will be one or more test specimens. Bundle test specimens into test units, and it should be noted that bundling of the blisters during storage can impact air flow and correspondingly increase data variability.

Weigh each test unit at ambient temperature and RH. Record this weight for time 0. Place all test units in the test chamber (40°/75% RH) within 1 h of weighing. Weigh all test units at time intervals of 7 days ± 1 h. Weigh the test units at 7, 14, 21, 28, and 35 days to get five steady-state data points. (The time interval from time 0 to day 7 is the period of equilibration.) Prior to weighing at each time interval, equilibrate the containers for 30 ± 5 min at the controlled weighing temperature and RH. Limit the time out of the chamber to less than 2 h. Record the weights in an appropriate manner for later computation of the regression line.

Ultra-high barrier blisters may not show the full measure of precision and sensitivity this method can provide. For ultra-high barrier blisters, test units should have more than 10 cavities, but NMT 30 cavities. Examples are foil-foil blisters or very small blisters formed from other materials. An alternative approach is to double or triple the length of weighing intervals to achieve at least a 6-mg weight gain per time interval by the test specimen.

Method 3: Prepare the test units as directed in *Method 2*. Place all test units in the test chamber (40°/75% RH) within 1 h of weighing. Weigh the test units at the end of 2 days (48 ± 1 h). At this time, the difference in weight (the weight gain) is divided by the number of blisters and days (2) in each test unit and this is taken as the moisture vapor transmission rate in mg/day/blister. The number of blisters tested depends on the barrier characteristics of the material, the size of the blister, and the sensitivity of the balance used in the test. For this method, the requirement of five consecutive weighings is waived because the desiccant quickly becomes saturated when packed in a low-barrier package and stored at 40°/75% RH. [NOTE—For single-unit and unit-dose low barrier containers, the weight gain after the second day displays a curvilinear profile typical of approaching saturation of the desiccant. To obtain five weighings within 2 days is not viable and is likely to increase variability. *Methods 2* and *3* may require that the blister cards be bundled in multiples to achieve periodic weight gains of sufficient magnitude to use the balance sensitivity; when bundled, these cards or test specimens are called test units. The weight gain in each weighing period must be 20 times the sensitivity of the balance, and the balance sensitivity is three times the balance precision. In other words, the minimal weight gain per time interval should be at least 60 times the balance precision.]

CALCULATIONS

Methods 1 and 2: Perform the regression analysis for each test unit (see *Regression Equation* below). Typically, the initial data point (at day 0) is not included in fitting the regression line. The slope of the regression line is the moisture vapor transmission rate of each test unit. For *Method 1*, the slope is the moisture vapor transmission rate for the corresponding multiple-unit container. For *Method 2*, the moisture vapor transmission rate of each blister cavity is calculated by dividing the slope by the number of cavities in each test unit.

Method 3: Calculate the weight gain in mg/day/blister from day 0 to day 2 using the 10 test units (see *Calculations* below). The moisture vapor transmission rate of each blister is calculated by dividing the weight gain by 2 (for 2 days) and the number of blisters in each test unit.

Regression Equation:

$$W = I + MT$$

Calculations:

$$Slope(M) = \sum_{i=1}^{N}[(W_i - \overline{W})(T_i - \overline{T})] / \sum_{i=1}^{N}(T_i - \overline{T})^2$$

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$$\text{Intercept } (I) = \overline{W} - M\overline{T}$$

W = measured weight
I = regression line intercept (point where regression line intersects the vertical axis)
M = regression line slope
T = time point
N = number of data points (each point consists of a weight and a time)
$\overline{W}$ = overall weight mean
$\overline{T}$ = overall time point mean

$$\sum_{i=1}^{N}[(W_i - \overline{W})(T_i - \overline{T})]$$

equals the sum of cross-products (for example, for each of the N data points, subtract the overall weight mean from the measured weight and the overall time point mean from the time point and multiply the two differences to get a cross-product, then sum all N cross-products).

$$\sum_{i=1}^{N}(T - \overline{T})^2$$

equals the sum of squared deviations (for example, for each of the N data points, subtract the overall time point mean from the time point and square the difference, then use the sum of all N squared differences).

RESULTS

METHOD 1: Report the moisture vapor transmission rate as the average value, in mg/day/container, and the standard deviation of the 15 slopes. Properly describe the container–closure system tested.

METHOD 2: Report the moisture vapor transmission rate as the average value, in mg/day/cavity, and the standard deviation of the 10 test unit slopes. Properly describe the container–closure system tested.

METHOD 3: Report the moisture vapor transmission rate as the average value from day 0 to day 2, in mg/day/blister, and the standard deviation of the 10 test unit slopes. Properly describe the container–closure system tested.

CLASSIFICATION SYSTEM FOR PLASTIC PACKAGING SYSTEMS

Introduction

A classification system is included to provide methods that allow pharmacists and institutional repackagers to select appropriate containers for repackaging. Repackaging is the act of taking a finished drug product from the container in which it was distributed by the original manufacturer and placing it into a different container without further manipulation of the drug. The classification system provided can also be used by the drug product manufacturer for testing packaging systems that have had the primary seal broached.

Classification Based on Desiccant Method

This section describes the methods for assigning the classification for multiple-unit containers (*Method 4*), multiple-unit containers with induction seals (*Method 5*), and single-unit and unit-dose containers (*Method 6*) used by pharmacists and institutional repackagers.

DESICCANT

Methods 4 and 5: Place a quantity of 4- to 8-mesh, anhydrous calcium chloride[3] in a shallow container, taking care to exclude any fine powder, dry at 215 ± 5° for 7¼±¼ h, and cool in a desiccator.

[NOTE—It has been shown[2] that anhydrous calcium chloride may contain calcium hexahydrate, which loses water only when the temperature reaches 200°.]

Method 6: Dry suitable desiccant pellets as described by the desiccant manufacturer. Use pellets weighing approximately 400 mg each and having a diameter of approximately 8 mm.

[NOTE—If necessary because of limited unit-dose container size, pellets weighing less than 400 mg each and having a diameter of less than 8 mm may be used.]

PROCEDURE

Method 4: Select 12 containers of a uniform size and type. Clean the sealing surfaces with a lint-free cloth, and close and open each container 30 times within the range of tightness specified in *Table 2*. Add *Desiccant* to 10 of the containers, designated "test containers", filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63 mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total weight of the container and *Desiccant*; the layer of

[3] Suitable 4- to 8-mesh, anhydrous calcium chloride is available from VWR International.

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Desiccant in such a container must be NLT 5 cm in depth. Close each container immediately after adding *Desiccant*, and apply the torque designated in *Table 2* when closing screw-capped containers. To each of the remaining two containers, designated "controls", add a sufficient number of glass beads to attain a weight approximately equal to that of each of the test containers, and close, applying the torque designated in *Table 2* when closing screw-capped containers. Record the weight of the individual containers to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest milligram if the container volume is 20 mL or more but less than 200 mL, or to the nearest centigram (10 mg) if the container volume is 200 mL or more, and store at 75 ± 3% RH and a temperature of 23 ± 2°. [NOTE—A saturated system of 35 g of sodium chloride with each 100 mL of water placed in the bottom of a desiccator maintains the specified humidity. Other methods may be employed to maintain these conditions.] After 336 ± 1 h (14 days), record the weight of the individual containers in the same manner. Completely fill five empty containers of the same size and type as that of the containers under test with water or a noncompressible, free-flowing solid, such as well-tamped fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, in milliliters.

Method 5: Proceed as directed under *Method 4*, except fit containers with impervious seals obtained by heat-sealing the bottles with an aluminum foil–polyethylene laminate or other suitable seal. If there is a need to increase the precision of the method, the user can test the system without the closure as long as an impervious seal remains on the container. Keeping the cap on causes variation and the absorption of water by the cap liner is not part of the moisture permeation.

Method 6: Use this procedure for packs (e.g., punch-out cards) and blister cards that incorporate one or more separately sealed unit-dose containers or blisters. Seal a sufficient number of packs, such that NLT 4 packs and a total of NLT 10 unit-dose containers or blisters filled with 1 pellet in each unit are tested. Seal a corresponding number of empty packs, each pack containing the same number of unit-dose containers or blisters as used in the test packs, to provide the controls. Store all of the containers at 75 ± 3% RH and at a temperature of 23 ± 2°. [NOTE—A saturated system of 35 g of sodium chloride with each 100 mL of water placed in the bottom of a desiccator maintains the specified humidity. Other methods may be employed to maintain these conditions.] After 24 h, and at each multiple thereof (see *Classification*), remove the packs from the chamber, and allow them to equilibrate for about 45 min. Record the weights of the individual packs, and return them to the chamber. Weigh the control packs as a unit, and divide the total weight by the number of control packs to obtain the average empty pack weight. [NOTE—If any indicating pellets turn pink during the procedure, or if the average pellet weight increase in any pack exceeds 10%, terminate the test, and regard only earlier determinations as valid.] The test and control containers are weighed after every 24 h and after suitable test intervals for the final weighings. W_F and C_F are as follows: 24 h for Class D, 48 h for Class C, 7 days for Class B, and NLT 28 days for Class A.

CALCULATIONS

Methods 4 and 5: Calculate the rate of moisture vapor transmission, in mg/day/L:

$$(1000/14V)[(T_F - T_I) - (C_F - C_I)]$$

V = volume of the container (mL)
T_F = final weight of each test container (mg)
T_I = initial weight of each test container (mg)
C_F = average final weight of the two controls (mg)
C_I = average initial weight of the two controls (mg)

Method 6: Calculate, to two significant figures, the average rate of moisture vapor transmission, in mg/day, for each unit-dose container or blister in each pack taken:

$$[1/(N \times X)][(W_F - W_I) - (C_F - C_I)]$$

N = number of days expired in the test period (beginning after the initial 24-h equilibration period)
X = number of separately sealed units per pack
W_F = final weight of each test pack (mg)
W_I = initial weight of each test pack (mg)
C_F = final weight of the control packs (mg)
C_I = initial weight of the control packs (mg)

RESULTS

Method 4: Packaging systems are "tight" if NMT 1 of the 10 test containers exceeds 100 mg/day/L in moisture vapor transmission, and none exceeds 200 mg/day/L. Packaging systems are well-closed if NMT 1 of the 10 test containers exceeds 2000 mg/day/L in moisture vapor transmission, and none exceeds 3000 mg/day/L.

Method 5: Packaging systems meet the requirements if the moisture vapor transmission exceeds 10 mg/day/L in NMT 1 of the 10 test containers, and none exceeds 25 mg/day/L.

Method 6: Class A: No pack tested exceeds 0.5 mg/day/unit in average blister moisture vapor transmission rate; Class B: No pack tested exceeds 5 mg/day/unit in average blister moisture vapor transmission rate; Class C: No pack tested exceeds 20 mg/day/unit in average blister moisture vapor transmission rate; and Class D: None of the packs tested meet any of the above average blister moisture vapor transmission rate requirements.

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Classification Based on Water Method

The following procedure and classification scheme are provided to evaluate the moisture vapor transmission characteristics of multiple-unit containers. The information gathered should be used to make an informed judgment regarding the suitability of the packaging system for LODFs. [NOTE—Determine the weights of individual container–closure systems (bottle; inner seal, if used; and closure), both as tare weights and fill weights, to the nearest 0.1 mg if the bottle capacity is less than 200 mL, to the nearest milligram if the bottle capacity is 200 mL or more but less than 1000 mL, or to the nearest centigram (10 mg) if the bottle capacity is 1000 mL or more.]

PROCEDURE

Select 12 bottles of a uniform size and type, and clean the sealing surfaces with a lint-free cloth. Fit each bottle with a seal, closure liner (if applicable), and closure. Number each container–closure system, and record the tare weight.

Remove the closures and, using a pipet, fill 10 bottles with water to the fill capacity. Fill two containers with glass beads, to the same weight as the filled test containers. If using screw closures, apply a torque that is within the range specified in *Table 2*, and store the sealed containers at a temperature of 25 ± 2° and 40 ± 2% RH. After 336 ± 1 h (14 days), record the weight of the individual containers, and calculate the water weight loss rate, in %/year, for each bottle taken:

$$[(W_{1i} - W_T) - (W_{14i} - W_T) - (W_{C1} - W_{C14})] \times 365 \times 100/(W_{1i} - W_T) \times 14$$

W_{1i} = initial weight of each individual bottle (i)
W_T = tare weight
W_{14i} = weight of each individual bottle (i) at 14 days
W_{C1} = initial weight of the control container at day 1
W_{C14} = weight of the control container at 14 days

RESULTS

Classification: The packaging system meets the requirements for tight if the percentage of water weight loss does not exceed 2.5%/year in NMT 1 of the 10 test containers and does not exceed 5.0%/year in any of them.

SPECTRAL TRANSMISSION

Apparatus

Use a spectrophotometer of suitable sensitivity and accuracy, adapted for measuring the amount of light transmitted by plastic materials used for pharmaceutical containers. In addition, the spectrophotometer is capable of measuring and recording light transmitted in diffused, as well as parallel, rays.

Procedure

Select sections to represent the average wall thickness. Cut circular sections from two or more areas of the container, and trim them as necessary to give segments of a size convenient for mounting in the spectrophotometer. After cutting, wash and dry each specimen, taking care to avoid scratching the surfaces. If the specimen is too small to cover the opening in the specimen holder, mask the uncovered portion of the opening with opaque paper or masking tape, provided that the length of the specimen is greater than that of the slit in the spectrophotometer. Immediately before mounting in the specimen holder, wipe the specimen with lens tissue. Mount the specimen with the aid of a tacky wax, or by other convenient means, taking care to avoid leaving fingerprints or other marks on the surfaces through which light must pass. Place the section in the spectrophotometer with its cylindrical axis parallel to the plane of the slit and approximately centered with respect to the slit. When properly placed, the light beam is normal to the surface of the section and reflection losses are at a minimum.

Continuously measure the transmittance of the section with reference to air in the spectral region of interest with a recording instrument or at intervals of about 20 nm with a manual instrument, in the region of 290–450 nm.

Limits

The observed spectral transmission does not exceed the limits given in *Table 3* for containers intended for parenteral use. The observed spectral transmission for plastic containers for products intended for oral or topical administration does not exceed 10% at any wavelength in the range of 290–450 nm.

Table 3. Limits for Plastic Classes I to VI

Nominal Size (mL)	Maximum Percentage of Spectral Transmission at Any Wavelength Between 290 and 450 nm
1	50
2	45
5	40

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Table 3. Limits for Plastic Classes I to VI *(continued)*

Nominal Size (mL)	Maximum Percentage of Spectral Transmission at Any Wavelength Between 290 and 450 nm
10	35
20	30
50	15

[NOTE—Any container of a size intermediate to those listed above exhibits a spectral transmission not greater than that of the next larger size container listed in *Table 3*. For containers larger than 50 mL, the limits for 50 mL apply.]

GLOSSARY

Blister: Formed, lidded, and sealed plastic or foil dome that contains the capsule or tablet (usually a single unit or unit dose).

Low-barrier blister: Blisters made from low-barrier materials, formed and sealed so that the moisture vapor transmission rate when tested at 40°/75% RH is greater than 1.0 mg/cavity/day.

High-barrier blister: Blisters made from high-barrier material, formed and sealed so that the moisture vapor transmission rate when tested at 40°/75% RH is less than 1.0 mg/cavity/day.

Ultra-high barrier blister: Blisters made from ultra-high barrier material, formed and sealed so that the moisture vapor transmission rate when tested at 40°/75% RH is less than 0.01 mg/cavity/day.

Blister card: A contiguous group of blisters formed and sealed with lid in place. The number of blisters per card commonly ranges from 1 to 10 but may be more. The blister card may sometimes be referred to as a packaging system.

Cavity: Formed, lidded, and sealed plastic or foil dome (see *Blister*).

Moisture vapor transmission rate: The steady-state moisture vapor transmission (in unit time) through a packaging system, under specific conditions of temperature and humidity. These test methods use gravimetric measurement to determine the rate of weight gain as a result of moisture vapor transmission into the packaging system and subsequent uptake by a desiccant enclosed within the packaging system.

Test specimen (or specimen)**:** For multiple-unit containers, the bottle is the test specimen, and for single-unit or unit-dose containers, the blister card containing multiple blister cavities is the test specimen. For blisters, more than 1 card (or specimen) may be grouped into a test unit for conducting the test.

Test unit: For multiple-unit containers, the bottle is the test unit as well as being the test specimen, and for single-unit or unit-dose containers, the test unit is a group of test specimens (blister cards) processed together for temperature and humidity exposure and for weighing at each time point. The purpose of the test unit for single-unit or unit-dose containers is to gain the advantage of additive weight gain resulting from more blister cavities than are present on a single card. The test unit, when applied to bottles, is used to maintain naming congruence among test methods 1, 2, and 3.

⟨691⟩ COTTON

Preparatory to the determination of absorbency and of fiber length, remove the Cotton from its wrappings, and condition it for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° (70 ± 2°F).

ABSORBENCY TEST

Procedure

Prepare a test basket, weighing not more than 3 g, from copper wire approximately 0.4 mm in diameter (No. 26 B. & S.) in the form of a cylinder approximately 5 cm in diameter and 8 cm deep, with spaces of about 2 cm between the wires. Take portions of purified cotton weighing 1 ± 0.05 g from five different parts of the package by pulling, not cutting, the specimens, place the combined portions in the basket, and weigh. Hold the basket on its side approximately 12 mm above the surface of water at 25 ± 1°, and drop it into the water. Determine, preferably by use of a stop watch, the time in seconds required for complete submersion.

Remove the basket from the water, allow it to drain for 10 seconds in the same horizontal position, then place it immediately in a tared, covered vessel, and weigh, deducting the weight of the test basket and of the purified cotton to find the weight of water absorbed.

FIBER LENGTH

For the determination of the length and of the length distribution of cotton fibers in purified cotton use the following method:

Carry out all operations associated with the determination of fiber length of purified cotton in an atmosphere maintained at 65 ± 2% relative humidity at 21 ± 1.1° (70 ± 2°F).

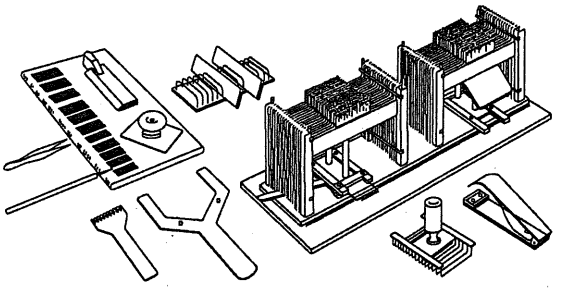
These directions describe the mode of procedure that is well adapted to the sorter* most extensively used in the United States at the present time.

* [NOTE—The method here described is especially adapted to the Suter-Webb Duplex Cotton Fiber sorting apparatus, but with more or less obvious alteration in procedure, may be carried out with two Baer sorters in tandem arrangement, or with a Johannsen or other similar apparatus.]

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Apparatus

The sorter (see *illustration)*



Duplex Cotton Fiber Sorter

consists of two banks of combs rigidly mounted side by side on a common base. Each bank of combs consists of at least 12 individual combs spaced 3.2 mm apart, one behind the other, and mounted in grooves so that as they are approached during the fractionating process and no longer needed, they may be dropped below the working plane. Each individual comb has a single row of accurately aligned and sharply pointed teeth, 12 mm long, consisting of needles 0.38 mm in diameter. The teeth are spaced 62 to 25 mm over an extent of approximately 50 mm.

Accessory equipment consists of fiber-sorter forceps, fiber-depressing grid, fiber-depressing smooth plate, and velvet-covered plates. The sorter forceps consist of two brass pieces approximately 75 mm long, hinged on one end and slightly curved to present a beaked aspect at the gripping end for gripping the protruding fibers close to the surfaces of the combs. Usually, one of the gripping edges has a leather or other fibrous padding. The gripping edge is approximately 19 mm wide.

The fiber-depressing grid consists of a series of brass rods spaced 3.2 mm apart so that they may be placed between the combs to press the fibers down between the teeth. The fiber-depressing smooth plate consists of a polished brass plate approximately 25 × 50 mm, with a knob or handle on the upper surface whereby the plate may be smoothed over the fibers as they are laid on the velvet surface of the array plates. The velvet-covered plates, upon which the fibers may be arrayed, are aluminum sheets approximately 100 mm × 225 mm × 2.4 mm thick, covered on both sides with high-grade velvet, preferably black.

Selection of Cotton

After unrolling the cotton, prepare a representative laboratory test specimen by taking from a package containing from 8 to 16 ounces, 32 pinches (about 75 mg each) well distributed throughout the bulk of the lap, 16 representative pinches being taken from each longitudinal half of the lap. Avoid the cut ends of the lap, and take particular care to secure portions throughout the thickness of the lap. To avoid biased selection of long or short fibers, remove all fibers of the group pinched and do not allow them to slip from between the fingers.

From packages of not more than 4 ounces in weight, take 8 pinches, and from packages weighing more than 4 ounces and not more than 8 ounces, take 16 pinches, all well distributed.

Mix the pinches in pairs promiscuously, and combine each pair by gently drawing and lapping them in the fingers. Then divide each combined pair by splitting longitudinally into two approximately equal parts and utilize one part in the further mixing. (The other part may be discarded or reserved for any further tests or checks.)

Repeat the process described in the preceding paragraph with the successive halves of the bifurcated series until only 1 pinch, the final composite test portion, results. Gently parallel and straighten the fibers of the final composite test portion by drawing and lapping them in the fingers. Take care to retain all of the fibers, including as far as possible those of the neps (specks of entangled fibers) and naps (matted masses of fibers), discarding only motes (immature seed fragments with fibers) and nonfiber foreign material such as stem, leaf, and fragments of seedcoats.

From the final composite portion described in the preceding paragraph, separate longitudinally a test portion of 75 ± 2 mg, accurately weighed. Retain the residue for any check test necessary.

Procedure

With the fiber-depressing grid carefully insert the weighed test portion into one bank of combs of the cotton sorter, so that it extends across the combs at approximately right angles.

With the sorter forceps, grip by the free ends a small portion of the fibers extending through the teeth of the comb nearest to the operator; gently and smoothly draw them forward out of the combs, and transfer them to the tips of the teeth in the second bank of combs, laying them parallel to themselves, straight, and approximately at right angles to the faces of the combs, releasing the gripped ends as near to the face of the front comb as possible. With the depressor grid carefully press the transferred fibers down into the teeth of the combs. Continue the operation until all of the fibers are transferred to the second bank of combs. During this transfer of the fibers, drop the combs of the first bank in succession when and as all of the protruding fibers have been removed.

Turn the machine through 180°, and transfer the cotton fibers back to the *first bank* of combs in the manner described in the preceding paragraph.

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Take great care in evening up the ends of the fibers during both of the above transfers, arranging them as closely as possible to the front surface of the proximal comb. Such evening out of the ends of the protruding fibers may involve drawing out straggling fibers from both the front and rear aspects of the banks of combs, and re-depositing them into and over the main bundle in the combs.

Turn the machine again through 180°. Drop successive combs if necessary to expose the ends of the longest fibers. It may be necessary to re-deposit some straggling fibers. With the forceps withdraw the few most protuberant fibers. In this way continue to withdraw successively the remaining protuberant fibers back to the front face of the proximal comb. Drop this comb and repeat the series of operations in the same manner until all of the fibers have been drawn out. In order not to disturb seriously the portion being tested, and thereby vitiate the length fractionation into length groups, make several pulls (as many as 8 to 10) between each pair of combs.

Lay the pulls on the velvet-covered plates alongside each other, as straight as possible, with the ends as clearly defined as possible, and with the distal ends arranged in a straight line, pressing them down gently and smoothly with the fiber-depressing smooth plate before releasing the pull from the forceps. Employ not fewer than 50 and not more than 100 pulls to fractionate the test portion.

Group together all of the fibers measuring 12.5 mm (about 1/2 inch) or more in length, and weigh the group to the nearest 0.3 mg. In the same manner, group together all fibers 6.25 mm (about 1/4 inch) or less in length, and weigh in the same manner. Finally, group the remaining fibers of intermediate lengths together and weigh. The sum of the three weights does not differ from the initial weight of the test portion by more than 3 mg. Divide the weight of each of the first two groups by the weight of the test portion to obtain the percentage by weight of fiber in the two ranges of length.

⟨695⟩ CRYSTALLINITY

This test is provided to determine compliance with the crystallinity requirement where stated in the individual monograph for a drug substance.

PROCEDURE

A detailed test procedure is described under *Optical Microscopy* ⟨776⟩.

⟨696⟩ CHARACTERIZATION OF CRYSTALLINE SOLIDS BY MICROCALORIMETRY AND SOLUTION CALORIMETRY

For the purpose of this chapter, crystalline material, partially crystalline material, and amorphous material are considered as solids.

INTRODUCTION—THE CONCEPT OF CRYSTALLINITY

The perfectly ordered crystal lattice with every molecule in its expected lattice position is an ideal that is seldom, if ever, achieved. The other extreme is the amorphous state, in which a solid contains the maximum possible density of imperfections (defects of various dimensionalities), such that all long-range order is lost while only the short-range order, imposed by its nearest neighbors, remains. Real crystals lie somewhere between these two extremes. A crystal's position on a scale bounded by these two extremes is termed *crystallinity*.

All real crystals, even in the pure state, possess some lattice imperfections or defects, which increase both the energy (enthalpy under conditions of constant atmospheric pressure) and the disorder (expressed as the entropy) of the crystal lattice. A crystal with a relatively low density of imperfections is said to be highly crystalline and to possess a high crystallinity. By contrast, a particle with a relatively high density of imperfections is said to be partially amorphous and to possess a low crystallinity. In ideal terms, a totally amorphous particle corresponds to zero crystallinity. Amorphous particles may contain somewhat ordered domains that can act as nuclei for crystallization; such so-called amorphous particles are said to possess a low-level, but finite, crystallinity.

The ability to detect and quantify the amount of amorphous material within a highly crystalline substance is of great importance during the development and subsequent manufacture of a pharmaceutical preparation.

In reality, a powder probably contains particles with different degrees of crystallinity, just as it may contain particles with varying sizes and shapes. The lower the crystallinity of a solid, the greater its enthalpy and entropy. The increase in enthalpy is never totally compensated by the increase in entropy; therefore, the Gibbs free energy, which reflects the balance between them, actually increases. Hence, the lower the crystallinity of a material (powder), and consequently the greater its amorphous character, the greater its apparent intrinsic solubility and dissolution rate, but the lower its thermodynamic stability. Because of the great relevance of these properties, crystallinity is also an important property and requires measurement by a suitable method.

In the following chapter, the crystallinity or the content of amorphous parts of a powder are measured by calorimetric methods such as microcalorimetry or solution calorimetry, although other methods could be used (e.g., see general chapter *Characterization of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction (XRPD)* ⟨941⟩).

Many substances are capable of crystallizing in more than one type of crystal lattice, which is known as polymorphism. If water or a solvent is incorporated in the crystal lattice, the crystals are termed hydrates or solvates. Because of the different

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crystal packing, and/or molecular conformation and lattice energy, they usually exhibit different physical properties. For simplicity, calorimetry measurements for degree of crystallinity determination discussed here assume only one solid crystalline form present in the material of interest. The theory and experimental technique can be easily expanded to polymorphic systems with proper consideration of the enthalpy differences among the polymorphs.

METHOD 1—MICROCALORIMETRY (DETERMINATION OF AMORPHOUS CONTENT)

Most chemical, physical, and biological processes are associated with the exchange of heat. Microcalorimetry is a highly sensitive technique to monitor and quantify both exothermic (heat producing) and endothermic (heat absorbing) changes associated with those processes. The technique allows the determination of the rate and extent of chemical reactions, changes of phase, or changes of structure.

Thermal events producing only a fraction of a microwatt can be observed using microcalorimetry. This means that temperature differences less than 10^{-6} K must be detectable. Microcalorimetry typically uses the heat flow (heat leakage) principle, where, in a thermally defined vessel, the heat produced (or absorbed) flows away from (or into) the vessel in an effort to re-establish thermal equilibrium with its surroundings. Exceptional thermal stability with its surrounding has to be achieved either by a heat sink or an electronically regulated surrounding.

Heat energy from an active sample in the reaction vessel is channeled typically through Peltier elements; they act as thermoelectric generators using the Seebeck effect. The heat energy is converted into a voltage signal proportional to the heat flow.

Results are typically presented as a measure of the thermal energy produced per unit of time (Watt) as a function of time.

Apparatus

Microcalorimeters are typically designed as twin systems with a measuring vessel and a reference vessel. Vessels are typically made of glass or stainless steel. For certain applications, specially designed vessels that allow the addition of a gas, a liquid, or a solid material may be used.

Calibration

The microcalorimeter is calibrated for heat flow (energy per time unit) using either calibrated external or internal electrical heat sources or a suitable standard reaction.

Sensitivity

The sensitivity of the microcalorimetric method can be assessed on the basis of an appropriate standard sample analyzed according to the corresponding method in conjunction with the determination of the instrument baseline noise.

Procedure

Weigh an appropriate quantity of the material in a suitable vessel. Close the vessel carefully, to avoid any evaporation of solvents, and place the vessel in the sample holder. If appropriate, allow the vessel to equilibrate at the temperature of the measurement before placing it in the measuring position.

Begin the analysis, and record the heat flow with the time on the abscissa and the heat flow on the ordinate (specify the direction of exothermic or endothermic heat flow).

Detection and Quantification of Amorphous Content in Powders

The amorphous state is metastable with respect to the crystalline state; recrystallization may therefore occur. The measurement of the heat of recrystallization enables the amorphous content to be determined by the area of the recrystallization peak. By relating the output from the microcalorimeter for a sample to that obtained from an amorphous standard, it is possible to quantify the amorphous content of the sample. The range of amorphous content covered by this method depends on the individual substance to be tested. In favorable cases, limits of detection below 1% can be reached.

Recrystallization can be initiated by subjecting the sample to higher relative humidity or an atmosphere containing organic vapor. The sample is typically placed in an ampul that also contains a small test tube containing an aqueous saturated salt solution, an organic solvent, or a solvent mixture.

The heat of recrystallization is typically measured using a fixed sample mass placed in a glass or steel vessel. The test tube containing a saturated salt solution or an organic solvent is chosen large enough to allow a full saturation of the atmosphere above the sample. The mass of the sample and the nature of the vapor atmosphere above the sample is chosen so that recrystallization occurs in such a way that a distinct peak is observed, clearly separated from initial thermal events caused by introduction of the sample.

The conditions under which the transition of the amorphous phase to a thermodynamically more stable crystalline state occurs will have a significant impact on the time of recrystallization. In particular, physical mixtures of purely amorphous and crystalline material will behave differently from a partially crystalline material. These effects should be considered when developing a method.

A typical response for the recrystallization of a mainly amorphous material is shown in *Figure 1*. The first part of the curve represents several concurrent processes taking place simultaneously, such as the absorption of water vapor into the amorphous parts of the powder and by the generation of water vapor from the test tube. After this initial response, there is a large exothermic

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response caused by the recrystallization of the amorphous material. Also included, but not seen, is the expulsion of excess water from the recrystallized parts and its condensation. Thus, the area under this exothermic recrystallization response is proportional to the heat of recrystallization.

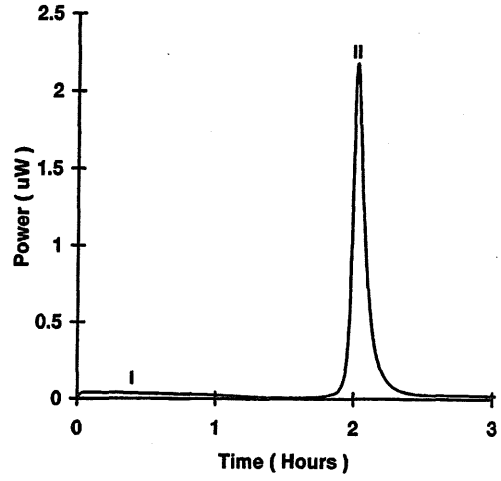


Figure 1. Typical microcalorimetric output of power (in μW) as a function of time (in h): amorphous collapse peak (I) and crystallization peak (II) for mainly amorphous lactose at 25° and 75% relative humidity.

METHOD 2—SOLUTION CALORIMETRY (DETERMINATION OF CRYSTALLINITY)

Solution calorimetry provides a means of determining enthalpy of solution (i.e., heat of solution under constant atmospheric pressure) of a substance. Enthalpy of solution is defined as the enthalpy of the substance dissolved in the solution to a defined concentration minus the enthalpy of the original substance. The solvent for the dissolution process must be such that the mass of solid dissolves within a time frame that matches the response time of the calorimeter, as discussed below. The enthalpy of solution is proportional to the amount of solid being dissolved. This amount may be defined as one mole for molar enthalpy or as one gram for specific enthalpy. If the substance possesses adequate purity (as determined by the degree of accuracy required) and if its molecular mass is known, the molar enthalpy is preferred; otherwise the specific enthalpy must be used. The enthalpy of solution is weakly dependent on both the temperature, which is usually 25.0°, and the final concentration of the dissolved solute.

It is usually preferred to express the crystallinity, P_c, of a substance on a percentage scale. This procedure requires two reference standards, namely a highly crystalline sample assuming 100% crystallinity and having a measured enthalpy of solution of ΔH^s_c, and an amorphous sample assuming 0% crystallinity and having a measured enthalpy of solution of ΔH^s_a. From these values and from the measured enthalpy, ΔH^s_s, of solution of the solid under study, the percentage crystallinity of the solid, P_c, may be calculated as follows:

$$P_c\,(\%) = 100(\Delta H^s_s - \Delta H^s_a)/(\Delta H^s_c - \Delta H^s_a)$$

Clearly, crystallinity expressed on a percentage scale depends on three measured values, and the enthalpies of solution may be replaced by other corresponding physical quantities that depend on crystallinity. The value of the percentage crystallinity of a sample, however, depends not only on the nature and method of preparation of the two reference standards, but also on the choice of the physical quantity that is measured.

The enthalpy of solution is measured either by an isoperibol (constant perimeter, i.e., jacket) solution calorimeter or by an isothermal (constant temperature) solution calorimeter. Typically, at least three measurements are made with each sample. The mean of these values is then calculated. The exact requirements will depend on the equipment capability and degree of accuracy needed.

Isoperibol Solution Calorimetry

In the isoperibol solution calorimeter, the heat change during the solution process causes a corresponding change in temperature of the solvent–solute system (i.e., solution). This temperature change is measured by a temperature sensor, which is wired to an electrical circuit that records an electrical signal corresponding to the temperature change. Typically, this temperature change in an electronic form is measured at precisely defined time intervals to produce temperature–time data that are collected, analyzed by a computer, and then plotted. A blank run without addition of the solid solute to the solvent normally shows no discernible change in the slope of the temperature–time plot.

For isoperibol solution calorimeters, response is fairly rapid, but corrections must be made for any heat losses to or heat gains from the bath. Therefore, isoperibol solution calorimeters are more advantageous than isothermal solution calorimeters when the solution process is relatively fast. For all measurements of enthalpy of solution using isoperibol solution calorimeters, the choice of solvent is critical. The nature and mass of the solvent and the mass of sample allow the total heat change,

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corresponding to total dissolution of the solid, to proceed to completion within five min under vigorous stirring at a constant rotational speed within the range of 400–600 revolutions/min.

The effective heat capacity of the calorimeter cell and its contents is determined for every calorimeter run. This determination is accomplished by electrical heating of the contents of the calorimeter cell. The effective heat capacity is determined according to one of two protocols—either by making one determination after ampul breakage or by making one determination before and a second determination after ampul breakage, and then averaging the two results. The accuracy and reliability of the electrical heating are established by the accuracy and reliability of the aforementioned chemical calibrations.

Isothermal Solution Calorimetry

In the isothermal (constant temperature) solution calorimeter, the heat change during the solution process is compensated for by an equal but opposite energy change, such that the temperature of the solvent–solute system (i.e., solution) remains essentially constant. This equal but opposite energy change is measured and, when its sign is reversed, provides the enthalpy of solution. For isothermal calorimeters, response is relatively slow, but the compensation process eliminates the effects of heat losses to or heat gains from the bath. Therefore, isothermal calorimeters are more advantageous than isoperibol calorimetry when the solution process is relatively slow.

Solution Calorimeter Calibration

To ensure the accuracy of the calorimeter, chemical calibrations must be performed on a regular basis. For an endothermic solution process, the calibration of the calorimeter is checked by measuring the heat absorbed during the dissolution of potassium chloride in distilled water at 298.15 K (25.0°). The established enthalpy change in this endothermic process is 235.5 J/g (17.56 kJ/mol). For an exothermic solution process, the calorimeter is checked by measuring the heat evolved during the dissolution of 5 g/L of tromethamine [tris(hydroxymethyl)aminomethane, THAM] in a 0.1 mol/L aqueous hydrochloric acid solution at 298.15 K (25.0°). The established heat for the aforementioned process is –246.0 J/g (–29.80 kJ/mol).

Sample Handling

The chemical and physical stability of solids may decrease with decreasing crystallinity. In particular, solids of low crystallinity, especially amorphous solids, tend to sorb water vapor from the atmosphere, leading to crystallization and a corresponding gain in crystallinity. For these reasons, anhydrous samples whose crystallinity is to be determined must be stored at zero humidity or below critical humidity levels in sealed chambers containing a desiccant, preferably containing an indicator of effectiveness. If crystallinity–humidity studies are to be carried out, the sample is stored in a sealed chamber containing a saturated salt solution to provide a defined relative humidity.

⟨697⟩ CONTAINER CONTENT FOR INJECTIONS

Each container of an injection contains sufficient excess to allow withdrawal of the labeled quantity of drug (see *Pharmaceutical Dosage Forms* ⟨1151⟩, *Excess Volume in Injections*). Such withdrawal shall be performed according to labeled directions, if provided.

DETERMINATION OF VOLUME OF INJECTION IN CONTAINERS

This section is harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. These pharmacopeias have undertaken not to make any unilateral change to this harmonized section. A portion of the present text (see below) is national *USP* text, and therefore not part of the harmonized text; it is marked with symbols (•,) to specify this fact.

Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to 20°–25° before measuring the volume. •Sterile solid formulations must be constituted according to labeled directions before removing the contents. Contents are then to be measured following the procedures for suspensions, emulsions, or solutions, as appropriate.•

Single-Dose Containers

Select 1 container if the volume of the container is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured and fitted with a 21-gauge needle NLT 2.5 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively, the volume of the contents in mL may be calculated as the mass, in g, divided by the density. For containers with a nominal volume of 2 mL or less, the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement, provided that a separate, dry syringe assembly is used for each container. The contents of

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containers holding 10 mL or more may be determined by means of opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is NLT the nominal volume in the case of containers examined individually or, in the case of containers with a nominal volume of 2 mL or less, is NLT the sum of the nominal volumes of the containers taken collectively.

Multi-Dose Containers

For Injections in multiple-dose containers labeled to yield a specific number of doses of a stated volume, select 1 container, and proceed as directed for single-dose containers, using the same number of separate syringe assemblies as the number of doses specified. The volume is such that each syringe delivers NLT the stated dose.

Injections in Cartridges or Prefilled Syringes

Select 1 container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume, in mL, calculated as the mass, in g, divided by the density.

The volume measured for each of the containers is NLT the nominal volume.

Large-Volume Intravenous Solutions

For intravenous solutions, select 1 container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume is NLT the nominal volume.

⟨698⟩ DELIVERABLE VOLUME

PURPOSE

The following tests are designed to provide assurance that oral liquids will, when transferred from the original container, deliver the volume of dosage form that is declared on the label.

SCOPE

These tests are applicable to products that are dispensed by pouring from the container. The tests apply whether the products are supplied as liquid preparations or liquid preparations that are constituted from solids upon the addition of a designated volume of a specific diluent. They are not required for an article packaged in single-unit containers when the monograph includes the test for *Uniformity of Dosage Units* ⟨905⟩.

DENSITY DETERMINATION

Because of the tendency of oral liquids to entrain air when shaken or transferred, a more accurate method for determining the delivered volume is to first determine the delivered mass, and then, using the density of the material, to convert the mass to delivered volume. In order to do that, a determination of the density of the material is required. The following is one method to determine density:

1. Tare a 100-mL volumetric flask containing 50.0 mL of water.
2. Add approximately 25 g of well-shaken product, and gently swirl the contents to mix.
3. Reweigh the flask.
4. From a buret, add an accurately measured amount of water to bring the flask contents to volume while gently swirling the contents of the flask. Record the volume taken from the buret.
5. Calculate the density of the sample:

$$W/V$$

in which W is the weight, in g, of the material taken; and V is 50.0 mL minus the volume, in mL, of water necessary to adjust the contents of the flask to volume. Other methods to determine the density may be employed depending on the formulation (e.g., substantially nonaqueous formulations).

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TEST PREPARATIONS

For the determination of deliverable volume, select NLT 30 containers, and proceed as follows for the dosage form designated.

Oral Solutions and Oral Suspensions

Shake the contents of 10 containers individually.

Powders That Are Labeled to State the Volume of Oral Liquid That Results When the Powder Is Constituted with the Volume of Diluent Stated in the Labeling

Constitute 10 containers with the volume of diluent stated in the labeling, accurately measured, and shake individually.

PROCEDURE

The deliverable volume can be determined by weight as follows:

1. Discharge the container contents into a suitable tared container (allowing drainage for NMT 5 s for single-dose containers and NMT 10 min for multiple-unit containers).
2. Determine the mass of the contents.
3. Calculate the volume using the density.

Alternatively, the following by volume procedure may be used:

1. Under conditions of use or as instructed in the labeling, carefully discharge the contents of each container into separate dry graduated cylinders of a rated capacity not exceeding two and a half times the volume to be measured, and calibrated "to contain" (see *Volumetric Apparatus* ⟨31⟩). Care must be taken to avoid the formation of air bubbles during the process. In the absence of labeling instructions, support the containers at about a 30° angle to the horizontal, and gently discharge the contents into the graduated cylinder.
2. Allow each container to drain for a period not to exceed 10 min for multiple-unit containers and 5 s for single-unit containers, unless otherwise specified in the monograph.
3. When free from bubbles, measure the volume of each mixture.

ACCEPTANCE CRITERIA

Use the following criteria to determine compliance with this test.

For Multiple-Unit Containers (see *Figure 1*)

The average volume of liquid obtained from the 10 containers is NLT 100%, and the volume of no container is less than 95% of the volume declared in the labeling. If A, the average volume is less than 100% of that declared in the labeling, but the volume of no container is less than 95% of the labeled amount, or if B, the average volume is NLT 100% and the volume of NMT 1 container is less than 95%, but is NLT 90% of the labeled volume, perform the test on 20 additional containers. The average volume of liquid obtained from the 30 containers is NLT 100% of the volume declared in the labeling; and the volume of liquid obtained from NMT 1 of the 30 containers is less than 95%, but NLT 90% of that declared in the labeling.

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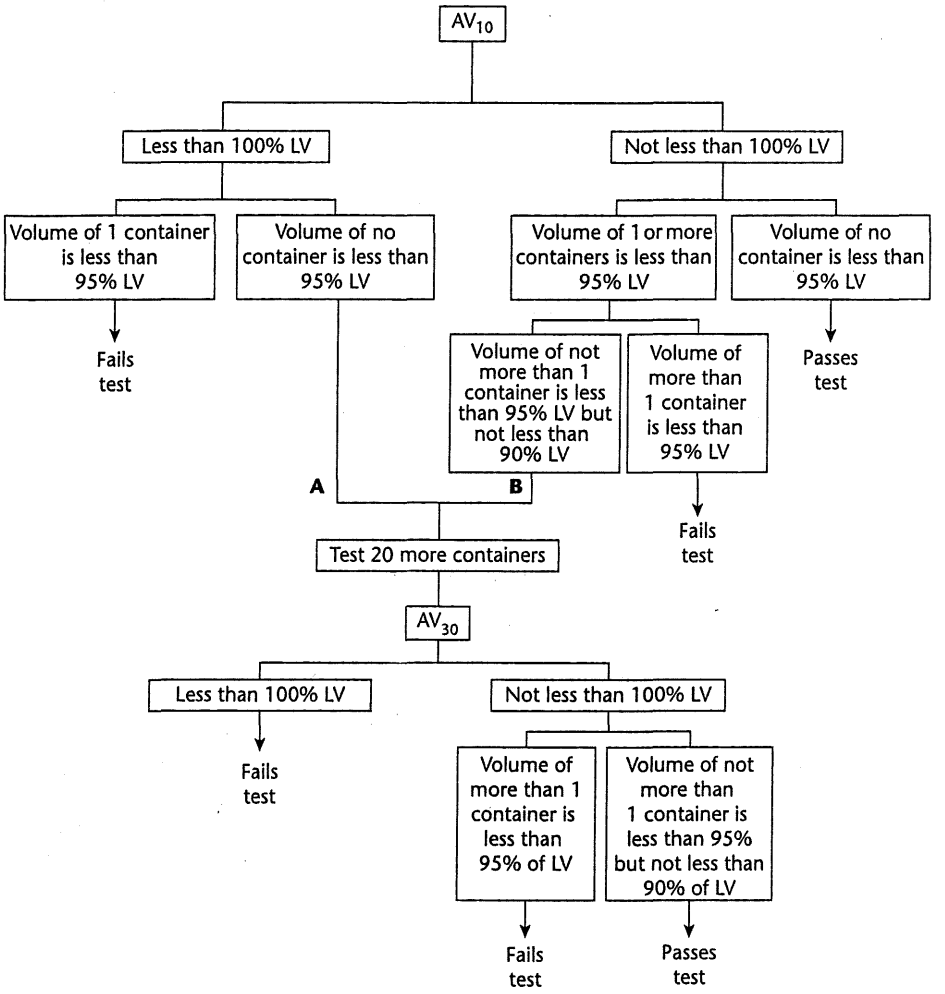


Figure 1. Decision scheme for multiple-unit containers. (AV = Average volume. LV = Labeled volume)

For Single-Unit Containers (see *Figure 2*)

The average volume of liquid obtained from the 10 containers is NLT 100%, and the volume of each of the 10 containers lies within the range of 95%–110% of the volume declared in the labeling. If A, the average volume is less than 100% of that declared in the labeling, but the volume of no container is outside the range of 95%–110%, or if B, the average volume is NLT 100% and the volume of NMT 1 container is outside the range of 95%–110%, but within the range of 90%–115%, perform the test on 20 additional containers. The average volume of liquid obtained from the 30 containers is NLT 100% of the volume declared in the labeling; and the volume obtained from NMT 1 of the 30 containers is outside the range of 95%–110%, but within the range of 90%–115% of the volume declared on the labeling.

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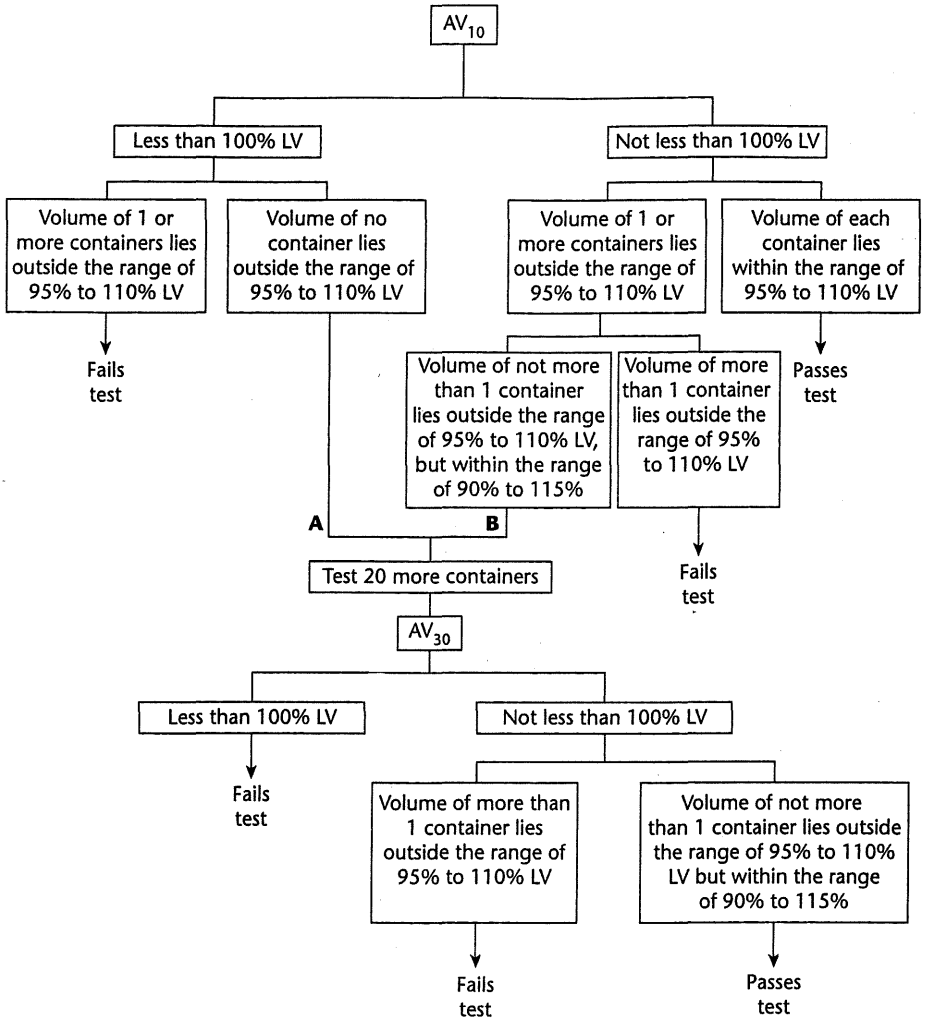


Figure 2. Decision scheme for single-unit containers. (AV = average volume; LV = labeled volume)

⟨699⟩ DENSITY OF SOLIDS

TERMS AND DEFINITIONS

Density refers to the average spatial distribution of mass in a material. The density of solids typically is expressed in g per cm^3, in contrast to fluids, where the density is commonly expressed in g per mL at a stated reference temperature.

The density of a solid particle can assume different values depending on the method used to measure the volume of the particle. It is useful to distinguish among three different possibilities.

The *true density* of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is a property of a particular material, and hence should be independent of the method of determination. The true density of a perfect crystal can be determined from the size and composition of the unit cell.

The *pycnometric density*, as measured by gas pycnometry, is a convenient density measurement for pharmaceutical powders. In a gas pycnometer, the volume occupied by a known mass of powder is determined by measuring the volume of gas displaced by the powder. The quotient of the mass and volume is the pycnometric density. The pycnometric density equals the true density unless the material contains impenetrable voids, or sealed pores, that are inaccessible to the gas used in the pycnometer.

The *granular density* includes contributions to particle volume from open pores smaller than some limiting size. The size limit depends on the method of measurement. A common measurement technique is mercury porosimetry, where the limiting pore size depends upon the maximum intrusion pressure. Because of the additional contribution from pore volume, the granular density will never be greater than the true density. A related concept is the *aerodynamic density*, which is the density of the particle with a volume defined by the aerodynamic envelope of the particle in a flowing stream. Both the closed and open pores

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contribute to this volume, but the open pores fill with the permeating fluid. The aerodynamic density, therefore, depends on the density of the test fluid if the particle is porous.

For brevity, the pycnometric density and the true density are both referred to as density. If needed, these quantities may be distinguished based on the method of measurement.

The density of a material depends on the molecular packing. For gases and liquids, the density will depend only on temperature and pressure. For solids, the density will also vary with the crystal structure and degree of crystallinity. If the solids are amorphous, the density may further depend upon the history of preparation and treatment. Therefore, unlike fluids, the densities of two chemically equivalent solids may be different, and this difference reflects a difference in solid-state structure. The density of constituent particles is an important physical characteristic of pharmaceutical powders.

Beyond these definitions of particle density, the *bulk density* of a powder includes the contribution of interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the packing of powder particles.

GAS PYCNOMETRY FOR THE MEASUREMENT OF DENSITY

Gas pycnometry is a convenient and suitable method for the measurement of the density of powder particles. A simple schematic of one type of gas pycnometer is shown in *Figure 1*.

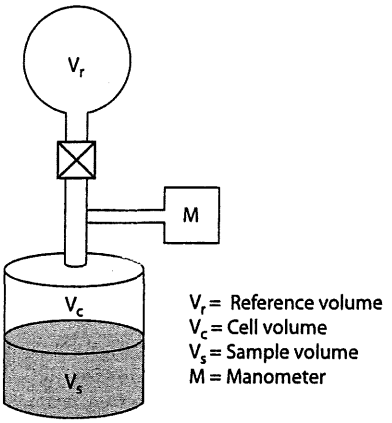


Figure 1. Schematic of gas pycnometer.

The sample, with mass w and volume V_s, is placed inside a sealed test cell with an empty cell volume of V_c. The system reference pressure, P_r, is determined at the manometer while the valve that connects the reference volume with the test cell is open. The valve is closed to separate the reference volume, V_r, from the test cell. The test cell is pressurized with the measurement gas to an initial pressure, P_i. Then the valve is opened to connect the reference volume, V_r, with the test cell, and the pressure drops to the final pressure, P_f. If the measurement gas behaves ideally under the conditions of measurement, the sample volume, V_s, is given by the following expression:

$$V_S = V_C - \frac{V_r}{\left[\frac{P_i - P_r}{P_f - P_r}\right] - 1} \quad (1)$$

The density, ρ, is given by the equation:

$$\rho = \frac{w}{V_s} \quad (2)$$

Details of the instrumental design may differ, but all gas pycnometers rely on the measurement of pressure changes as a reference volume is added to, or deleted from, the test cell.

The measured density is a volume-weighted average of the densities of individual powder particles. The density will be in error if the test gas sorbs onto the powder or if volatile contaminants are evolved from the powder during the measurement. Sorption is prevented by an appropriate choice of test gas. Helium is the common choice. Volatile contaminants in the powder are removed by degassing the powder under a constant purge of helium prior to the measurement. Occasionally, powders may have to be degassed under vacuum. Two consecutive readings should yield sample volumes that are equal within 0.2% if volatile contaminants are not interfering with the measurements. Because volatiles may be evolved during the measurement, the weight of the sample should be taken after the pycnometric measurement of volume.

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Method

Ensure that the reference volume and the calibration volume have been determined for the gas pycnometer by an appropriate calibration procedure. The test gas is helium, unless another gas is specified in the individual monograph. The temperature of the gas pycnometer should be between 15° and 30° and should not vary by more than 2° during the course of the measurement. Load the test cell with the substance under examination that has been prepared according to the individual monograph. Where ⟨699D⟩ is indicated, dry the substance under examination as directed for *Loss on drying* in the monograph unless other drying conditions are specified in the monograph *Density of solids* test. Where ⟨699U⟩ is indicated, the substance under examination is used without drying. Use a quantity of powder recommended in the operating manual for the pycnometer. Seal the test cell in the pycnometer, and purge the pycnometer system with the test gas according to the procedure given in the manufacturer's operating instructions. If the sample must be degassed under vacuum, follow the recommendations in the individual monographs and the instructions in the operating manual for the pycnometer.

The measurement sequence above describes the procedure for the gas pycnometer shown in *Figure 1*. If the pycnometer differs in operation or in construction from the one shown in *Figure 1*, follow the operating procedure given in the manual for the pycnometer.

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume, V_s, agree to within 0.2%. Unload the test cell and measure the final powder weight, w. Calculate the pycnometric density, ρ, of the sample according to *Equation 2*.

⟨701⟩ DISINTEGRATION

Change to read:

This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. The texts of these pharmacopeias are therefore interchangeable, and the methods of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* may be used for demonstration of compliance instead of the present general chapter. These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.

Portions of the present general chapter text that are national *USP* text, and therefore not part of the harmonized text, are marked with symbols (•▲) to specify this fact.

This test is provided to determine whether tablets, capsules, •▲or granules•▲ (USP 1-Aug-2019) disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below. •Compliance with the limits on *Disintegration* stated in the individual monographs is required. •▲ (USP 1-Aug-2019) Determine the type of units under test from the labeling and from observation, and apply the appropriate procedure to 6 or more dosage units.•

For the purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disk, if used, is a soft mass having no palpably firm core.

Change to read:

APPARATUS

The apparatus consists of a basket-rack assembly, a 1000-mL low-form beaker 138–160 mm in height and having an inside diameter of 97–115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid 35°–39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate 29–32 cycles/min through a distance of NLT 53 mm and NMT 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke, the wire mesh remains at least 15 mm below the surface of the fluid and descends to NLT 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-Rack Assembly

The basket-rack assembly consists of 6 open-ended transparent tubes, each ▲75.0–80.0▲ (USP 1-May-2020) mm long and having an inside diameter of 20.7–23 mm and a wall 1.0–2.8 mm thick; the tubes are held in a vertical position by two plates, each 88–92 mm in diameter and 5–8.5 mm in thickness, with 6 holes, each 22–26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8–2.2-mm apertures and with a wire diameter of 0.57–0.66 mm. The parts of the apparatus are assembled and rigidly held by means of 3 bolts passing through the 2 plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis.

The design of the basket-rack assembly may be varied somewhat, provided the specifications for the glass tubes and the screen mesh size are maintained. The basket-rack assembly conforms to the dimensions found in *Figure 1*.

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Disks

The use of disks is permitted only where specified or allowed •in the monograph. If specified in the individual monograph,• each tube is provided with a cylindrical disk ▲9.35–9.65▲ (USP 1-May-2020) mm thick and ▲20.55–20.85▲ (USP 1-May-2020) mm in diameter. The disk is made of a suitable transparent plastic material having a specific gravity of 1.18–1.20. Five parallel ▲1.9–2.1▲ (USP 1-May-2020)-mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are ▲parallel to the cylindrical axis and▲ (USP 1-May-2020) centered ▲5.8–6.2▲ (USP 1-May-2020) mm from the axis on imaginary lines perpendicular to the axis and ▲▲ (USP 1-May-2020) to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of ▲1.5–1.7▲ (USP 1-May-2020) mm, and its bottom edges lie at a depth of 1.5–1.8 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length ▲9.2–9.6▲ (USP 1-May-2020) mm, and its center lies at a depth ▲2.5–2.7▲ (USP 1-May-2020) mm from the cylinder's circumference. All surfaces of the disk are smooth. If the use of disks is specified •in the individual monograph,• add a disk to each tube, and operate the apparatus as directed under *Procedure*. The disks conform to dimensions found in *Figure 1*.[1]

[1] The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimension given in this chapter.

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▲

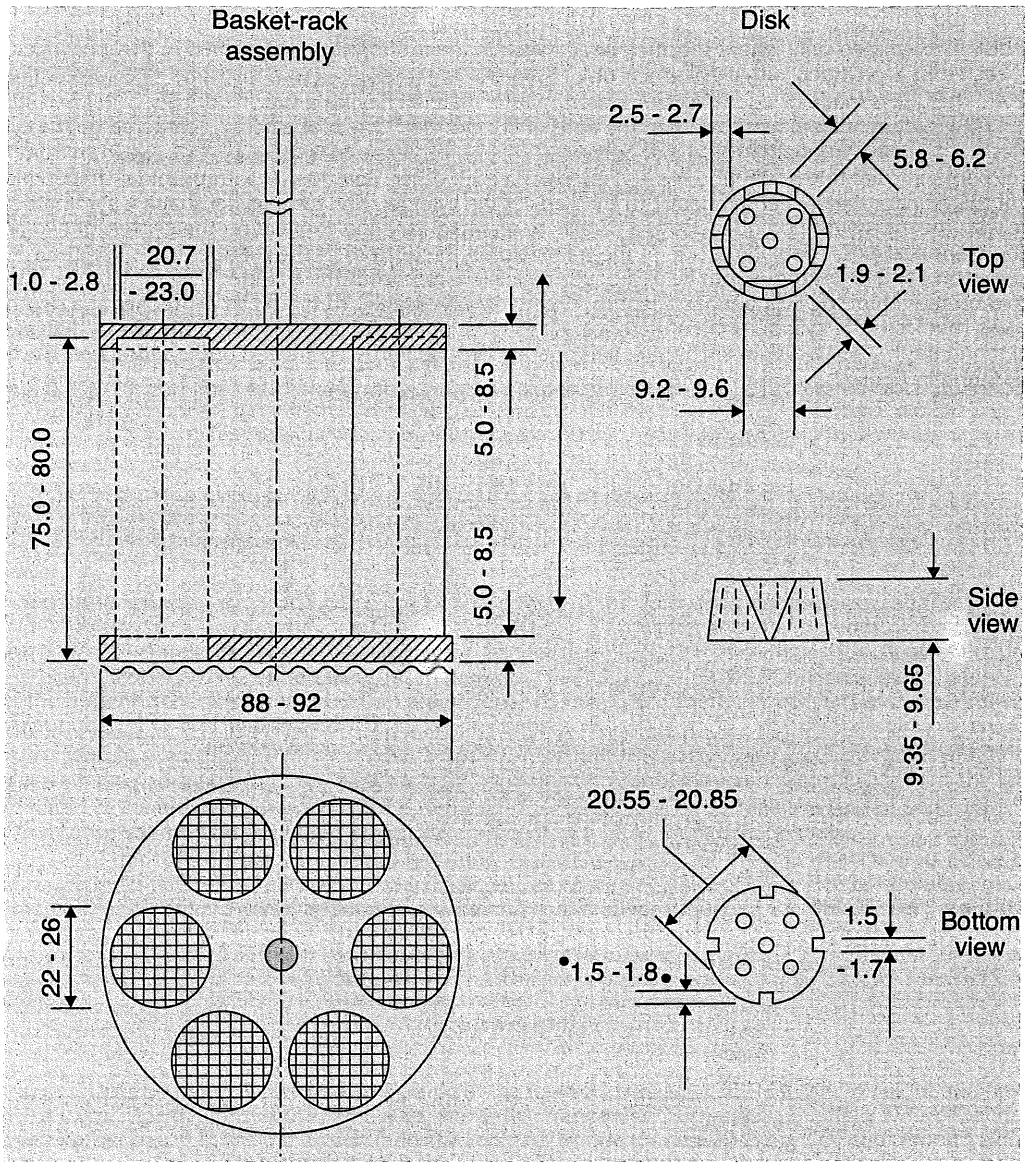


Figure 1. Disintegration apparatus. (All dimensions are expressed in mm.)

▲ (USP 1-May-2020)

Change to read:

PROCEDURE •▲AND CRITERIA

Procedure and Criteria for▲ (USP 1-Aug-2019) Uncoated ▲or Plain-Coated▲ (USP 1-Aug-2019) Tablets

▲PROCEDURE FOR UNCOATED OR PLAIN-COATED TABLETS▲ (USP 1-Aug-2019)◆

Place 1 dosage unit in each of the 6 tubes of the basket▲•-rack assembly◆▲ (USP 1-Aug-2019) and, if prescribed, add a disk. Operate the apparatus, using •water or◆ the specified medium as the immersion fluid, maintained at 37 ± 2°. At the end of the time limit specified •in the monograph,◆ lift the basket▲•-rack assembly◆▲ (USP 1-Aug-2019) from the fluid, and observe the tablets. All of the tablets ▲•should◆▲ (USP 1-Aug-2019) have disintegrated completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets.

General Chapters

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▲◆CRITERIA FOR UNCOATED OR PLAIN-COATED TABLETS

1. If 6 tablets are tested, all 6 of the tablets are disintegrated.
2. If 18 tablets are tested, the requirement is met if not fewer than 16 of the total of 18 tablets are disintegrated.◆▲ (USP 1-Aug-2019)

◆▲Procedure and Criteria for Delayed-Release Tablets and Capsules (tablets or capsules that are formulated with acid-resistant or enteric coatings)

PROCEDURE FOR DELAYED-RELEASE TABLETS AND CAPSULES▲ (USP 1-Aug-2019)

Place 1 ▲dosage unit▲ (USP 1-Aug-2019) in each of the 6 tubes of the basket. ▲If the dosage units are not sugar-coated, proceed to the *Acid stage*. If testing tablets that have a soluble external sugar coating, immerse the basket in water at room temperature for 5 min and then immediately proceed to the *Acid stage*. If specified in the monograph, add 1 disk to each tube.

Acid stage

Immersion fluid: 0.1 M hydrochloric acid, or simulated gastric fluid TS, or as specified in the monograph
Temperature: 37 ± 2°
Time: 1 h

If after 1 h no dosage unit shows evidence of disintegration, cracking, or softening, proceed with the *Buffer stage*.

Buffer stage

Immersion fluid: pH 6.8 phosphate buffer, or simulated intestinal fluid TS, or as specified in the monograph
Temperature: 37 ± 2°
Time: As specified in the individual monograph

CRITERIA FOR DELAYED-RELEASE TABLETS AND CAPSULES

Acid stage: No dosage unit shows evidence of disintegration, cracking, or softening.
Buffer stage: Apply the *Criteria for Uncoated or Plain-Coated Tablets*.

Procedure and Criteria for Buccal Tablets, Sublingual Tablets, Capsules, Tablets for Oral Suspension, Tablets for Oral Solution, Tablets for Topical Solution, Orally Disintegrating Tablets, and Chewable Tablets

Apply the *Procedure and Criteria for Uncoated or Plain-Coated Tablets*.

Procedure and Criteria for Effervescent Tablets for Oral Solution

PROCEDURE FOR EFFERVESCENT TABLETS FOR ORAL SOLUTION

Place 1 tablet in each of 6 beakers containing 200 mL of water. A suitable beaker will have a nominal volume of 250–400 mL.
Time: 5 min or as specified in the individual monograph

CRITERIA FOR EFFERVESCENT TABLETS FOR ORAL SOLUTION

Numerous bubbles of gas are evolved. After 5 min or as specified in the individual monograph, the evolution of gas around each tablet or its fragments has ceased. Each tablet has dissolved or disintegrated and has been dispersed in the water so that no appreciable agglomerates remain.

Procedure and Criteria for Effervescent Granules

PROCEDURE FOR EFFERVESCENT GRANULES

In each of 6 beakers containing 200 mL of water place 1 dose of the effervescent granules. A suitable beaker will have a nominal volume of 250–400 mL.
Time: 5 min or as specified in the individual monograph

CRITERIA FOR EFFERVESCENT GRANULES

Numerous bubbles of gas are evolved. After 5 min or as specified in the individual monograph, the evolution of gas around the granules in each beaker has ceased. The granules have dissolved or disintegrated and have been dispersed in the water.▲ (USP 1-Aug-2019)◆

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⟨705⟩ QUALITY ATTRIBUTES OF TABLETS LABELED AS HAVING A FUNCTIONAL SCORE

PURPOSE

This chapter provides quality attributes for products with approved labeling indicating that the tablets can be split to produce multiple portions that have an accurate fractional dose (labeled as functionally scored). The label claim of the split portions should be a simple fractional part of the claim for the intact tablet based on the number of scores and the size of the split portion (e.g., one-half, one-third, or one-quarter). At the time of splitting, the intact tablets should conform to the monograph specification. With the exception of dose, each split portion from tablets labeled as having a functional score are expected to conform to the quality attributes of the whole tablets. The split portions resulting from subdividing a functionally scored tablet should conform to the tests for *Splitting Tablets with Functional Scoring* and *Dissolution* or *Disintegration* given in this chapter.

SCOPE

This chapter applies to tablets labeled as having a functional score and to the split portions that represent any labeled fraction of the whole functionally scored tablet dose. Tablets should be split as part of the test procedure and the storage conditions for the split portions should be defined in the test procedure. For *Dissolution* or *Disintegration* testing, analysts should use only split portions from tablets determined to be acceptable by the *Splitting Tablets with Functional Scoring* test.

SPLITTING TABLETS WITH FUNCTIONAL SCORING

Test Procedure

1. Take a random sample of 30 intact tablets, and proceed as follows.
2. Accurately weigh each tablet, and record its weight.
3. For each intact tablet, determine the expected weight of the split portions by dividing the whole-tablet weight by the designated number of split portions indicated on the labeling.
4. Split each tablet by hand (without mechanical assistance) into the designed number of split portions, and weigh each split portion.
5. For each tablet, determine the percent of the expected weight in each split portion.

An acceptable tablet breaks into the designed number of segments, and each split portion has NLT 75% and NMT 125% of the expected weight of the split tablet portion. [NOTE—Set aside split tablet portions derived from acceptable tablets for subsequent testing for dissolution or disintegration.]

Acceptance criteria: NLT 28 of the 30 tablets are acceptable.

DISSOLUTION

Use split portions from tablets that are acceptable according to the *Splitting Tablets with Functional Scoring* test.

Immediate-Release Tablets

Dissolution for immediate-release tablets is performed at the S_2 stage (see *Dissolution* ⟨711⟩). Test 12 split tablet portions according to the specified *Medium, Apparatus, Times,* and *Analysis*. The average of the 12 results is NLT Q, and no result is less than $Q - 15\%$.

Extended-Release Tablets

Perform dissolution testing of split tablet portions from extended-release tablets by one of the two alternative procedures. The procedure to be used is specified in the monograph.

Procedure 1 (*Procedure for Extended-Release Dosage Forms, Dissolution* ⟨711⟩): Individually test 12 split tablet portions and 12 intact tablets.

Medium, Apparatus, and **Analysis:** As given in the monograph following the appropriate test number found on the labeling. Dissolution profile test time points are determined as follows. From the appropriate dissolution test in the monograph, use the time points given. At a minimum, use three time points with no more than one time point where the results exceed 85% dissolved.

Calculate the similarity factor (f_2) for the intact-tablet results and the split-tablet portion results:

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$$f_2 = 50 \cdot \log\left\{\left[1+(1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \cdot 100\right\}$$

R_t = cumulative percentage of the labeled drug dissolved at each of the selected n time points of the intact tablets
T_t = cumulative percentage of the labeled drug dissolved at each of the selected n time points of the split tablet portions
Acceptance criteria: The calculated f_2 is NLT 50 (acceptable range: 50–100).
Procedure 2 (*Procedure for Extended-Release Dosage Forms, Dissolution* ⟨711⟩)**:** Use a split-tablet portion as the dosage unit. Individually test 12 dosage units.
Medium, Apparatus, Times, and **Analysis:** As given in the monograph following the appropriate test number found on the labeling.
Acceptance criteria: The percentages of the labeled amount released at the times specified conform to the L_2 level criteria of *Acceptance Table 2* in ⟨711⟩.

DISINTEGRATION

Disintegration testing is necessary only when used as a surrogate for dissolution testing as specified in the monograph. Follow the procedure using split portions from tablets that are acceptable according to the *Splitting Tablets with Functional Scoring* test as the dosage unit (see *Disintegration* ⟨701⟩).

⟨711⟩ DISSOLUTION

General chapter *Dissolution* ⟨711⟩ is being harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. These pharmacopeias have undertaken to not make any unilateral change to this harmonized chapter.

Portions of the present general chapter text that are national *USP* text, and therefore not part of the harmonized text, are marked with symbols (•,▲) to specify this fact.

This test is provided to determine compliance with the dissolution requirements •where stated in the individual monograph,▲ for dosage forms administered orally. In this general chapter, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified. •Of the types of apparatus designs described herein, use the one specified in the individual monograph. Where the label states that an article is enteric coated and a dissolution or disintegration test does not specifically state that it is to be applied to delayed-release articles and is included in the individual monograph, the procedure and interpretation given for *Delayed-Release Dosage Forms* are applied, unless otherwise specified in the individual monograph.

FOR DOSAGE FORMS CONTAINING OR COATED WITH GELATIN

If the dosage form containing gelatin does not meet the criteria in the appropriate *Acceptance Table* (see *Interpretation, Immediate-Release Dosage Forms, Extended-Release Dosage Forms,* or *Delayed-Release Dosage Forms*) because of evidence of the presence of cross-linking, the dissolution procedure should be repeated with the addition of enzymes to the medium, as described below, and the dissolution results should be evaluated starting at the first stage of the appropriate *Acceptance Table*. It is not necessary to continue testing through the last stage (up to 24 units) when criteria are not met during the first stage testing, and evidence of cross-linking is observed.

Gelatin, in the presence of certain compounds and/or in certain storage conditions, including but not restricted to high humidity and temperature, may present cross-linking. A pellicle may form on the external and/or internal surface of the gelatin capsule shell or on the dosage form that prevents the drug from being released during dissolution testing (see more information in *Capsules—Dissolution Testing and Related Quality Attributes* ⟨1094⟩).

[NOTE—All references to a chapter above ⟨1000⟩ are for information purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for this application.]

Dissolution Medium with pH ≤4.0

Enzyme: Pepsin, activity determined by the procedure in *purified pepsin*, in the *Reagent Specifications* section
Amount: A quantity of pepsin that results in an activity of NMT 750,000 Units/L of dissolution medium

Dissolution Medium with pH >4.0 and <6.8

Enzyme: Papain, activity determined by the *Assay* test in the monograph for *Papain*; or bromelain, activity determined by the procedure in *bromelain*, in the *Reagent Specifications* section
Amount: A quantity of papain that results in an activity of NMT 550,000 Units/L of dissolution medium, or a quantity of bromelain that results in an activity of NMT 30 gelatin-digesting units (GDU)/L of dissolution medium

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Dissolution Medium with pH ≥6.8

Enzyme: Pancreatin, protease activity determined by the procedure in *Assay for protease activity* (*Casein digestive power*) in the monograph for *Pancreatin*

Amount: A quantity of pancreatin that results in a protease activity of NMT 2000 Units/L of dissolution medium

Dissolution Medium Containing Surfactant or Other Ingredients Known to Denature the Enzyme

If the dissolution medium contains surfactant or other ingredients that are known to denature the enzyme used, a pretreatment step in the dissolution testing of the dosage form may be applied. This pretreatment step is done using the specified dissolution medium without the surfactant or the ingredient and with the addition of the appropriate amount of enzyme according to the medium pH. The amount of enzyme added is appropriate to the volume of dissolution medium used in the pretreatment. To achieve the specified medium volume for the final dissolution testing, the pretreatment step may be conducted with a smaller volume of medium without the ingredient such that the final volume is obtained when the ingredient is added at the end of the pretreatment step. All of the other conditions of the test (apparatus, rotation, or flow rate) should remain as described in the method or monograph. Typically, the duration of the pretreatment step is NMT 15 min. The required pretreatment time should be evaluated on a case-by-case basis and should be scientifically justified. This time should be included in the total time of the test. As an example, if the total time of the test is 45 min and 15 min are used in the pretreatment step, the test will continue for 30 min after the addition of the ingredient.

APPARATUS

Apparatus 1 (Basket Apparatus)

The assembly consists of the following: a vessel, which may be covered, and made of glass or other inert, transparent material;[1] a motor; a metallic drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device, such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at 37 ± 0.5° during the test and keeps the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating, stirring element. An apparatus that permits observation of the specimen and of the stirring element during the test is preferable. The vessel is cylindrical, with a hemispherical bottom and with one of the following dimensions and capacities: for a nominal capacity of 1 L, the height is 160–210 mm, and its inside diameter is 98–106 mm; for a nominal capacity of 2 L, the height is 280–300 mm, and its inside diameter is 98–106 mm; and for a nominal capacity of 4 L, the height is 280–300 mm, and its inside diameter is 145–155 mm. Its sides are flanged at the top. A fitted cover may be used to retard evaporation.[2] The shaft is positioned so that its axis is NMT 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at the specified rate given in the individual monograph, within ±4%.

Shaft and basket components of the stirring element are fabricated of stainless steel, type 316, or other inert material, to the specifications shown in *Figure 1*. A basket having a gold coating of about 0.0001 inch (2.5 µm) thick may be used. A dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

[1] The materials should not sorb, react, or interfere with the specimen being tested.

[2] If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

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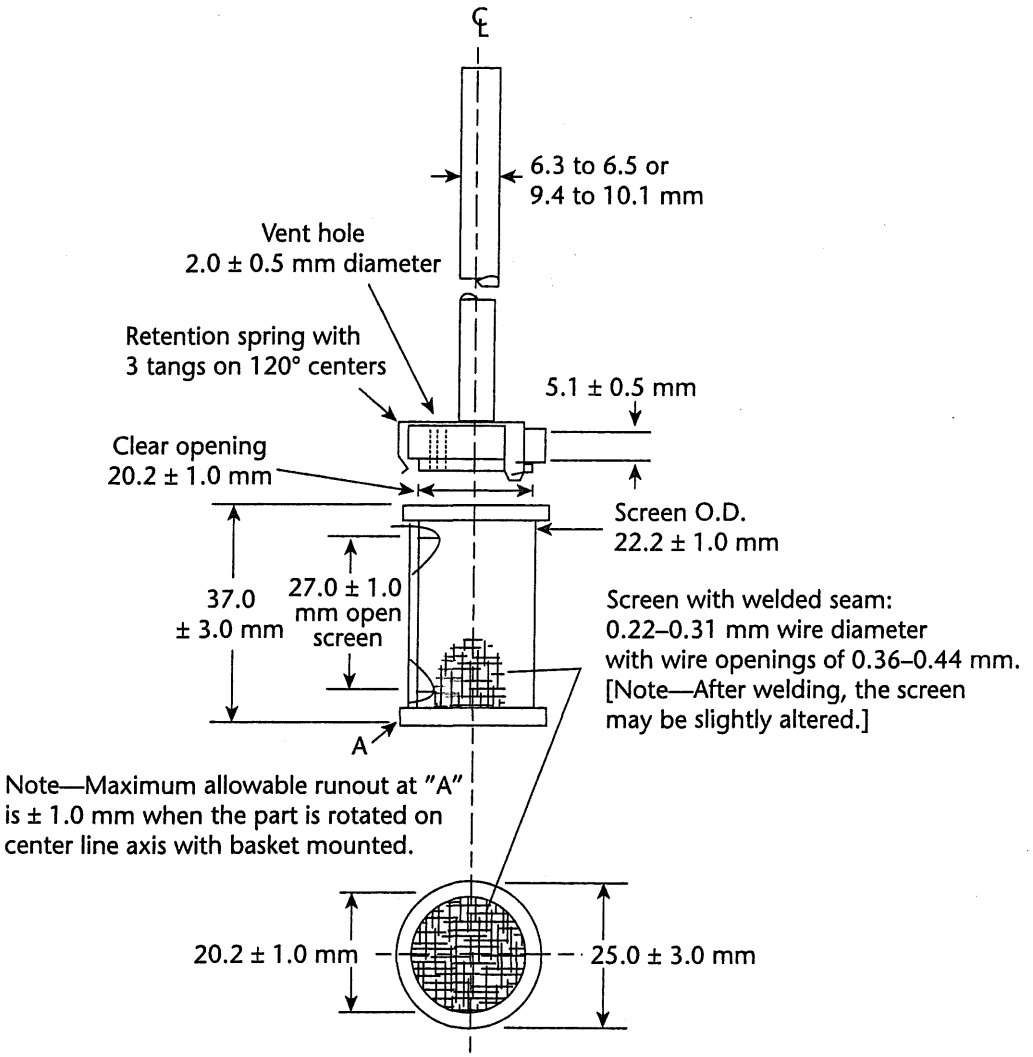


Figure 1. Basket stirring element.

Apparatus 2 (Paddle Apparatus)

Use the assembly from *Apparatus 1*, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is NMT 2 mm from the vertical axis of the vessel at any point and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in *Figure 2*. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft compose a single entity. A suitable two-part, detachable design may be used, provided that the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make both of them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of nonreactive material, such as NMT a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in *Figure 2a*. Other validated sinker devices may be used.

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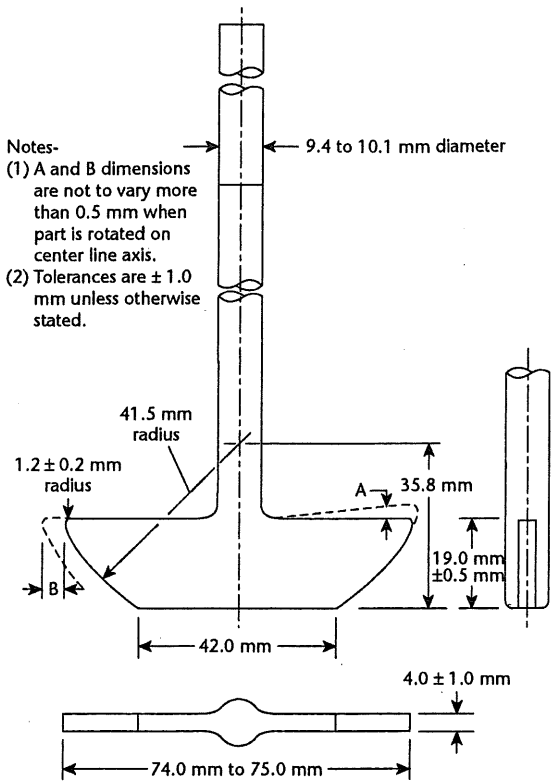


Figure 2. Paddle stirring element.

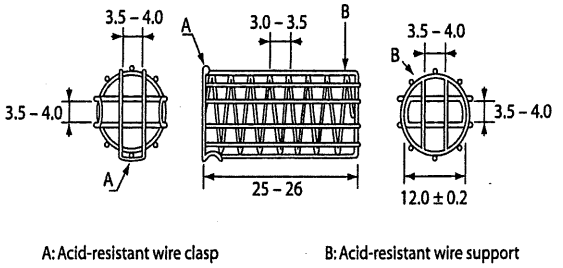


Figure 2a. Alternative sinker. All dimensions are expressed in mm.

Apparatus 3 (Reciprocating Cylinder)[3]

The assembly consists of a set of cylindrical, flat-bottomed glass vessels; a set of glass reciprocating cylinders; inert fittings (stainless steel type 316 or other suitable material), and screens that are made of suitable nonsorbing and nonreactive material and that are designed to fit the tops and bottoms of the reciprocating cylinders; and a motor and drive assembly to reciprocate the cylinders vertically inside the vessels; if desired, index the reciprocating cylinders horizontally to a different row of vessels. The vessels are partially immersed in a suitable water bath of any convenient size that permits holding the temperature at 37 ± 0.5° during the test. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating cylinder. A device is used that allows the reciprocation rate to be selected and maintained at the specified dip rate ▪given in the individual monograph▪, within ±5%. An apparatus that permits observation of the specimens and reciprocating cylinders is preferable. The vessels are provided with evaporation caps that remain in place for the duration of the test. The components conform to the dimensions shown in *Figure 3*, unless otherwise specified ▪in the individual monograph▪.

[3] Not accepted by the *Japanese Pharmacopoeia.*

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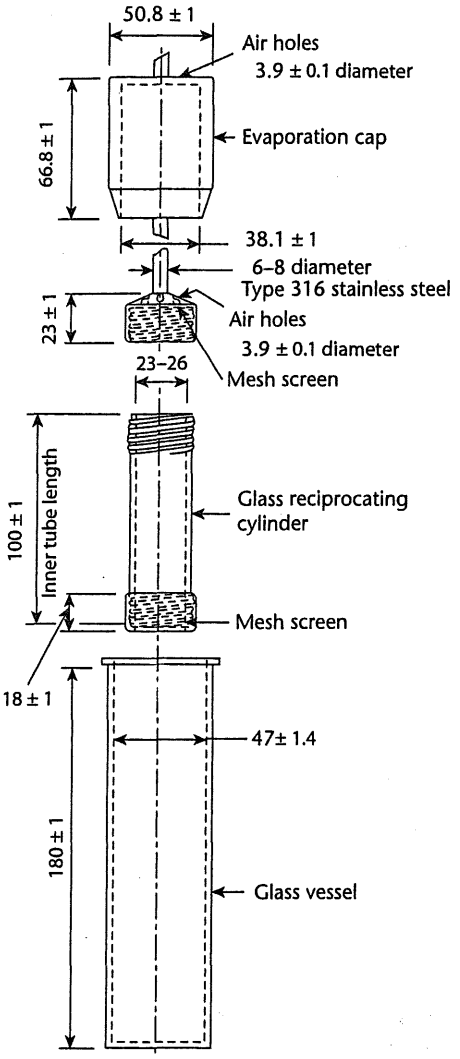


Figure 3. Apparatus 3 (reciprocating cylinder).

Apparatus 4 (Flow-Through Cell)

The assembly consists of a reservoir and a pump for the *Dissolution medium*; a flow-through cell; and a water bath that maintains the *Dissolution medium* at 37 ± 0.5°. Use the specified cell size •as given in the individual monograph,•.

The pump forces the *Dissolution medium* upward through the flow-through cell. The pump has a delivery range between 240 and 960 mL/h, with standard flow rates of 4, 8, and 16 mL/min. It must deliver a constant flow (±5% of the nominal flow rate); the flow profile is sinusoidal with a pulsation of 120 ± 10 pulses/min. A pump without pulsation may also be used. Dissolution test procedures using a flow-through cell must be characterized with respect to rate and any pulsation.

The flow-through cell (see *Figure 4* and *Figure 5*), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph) that prevents escape of undissolved particles from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5 mm, positioned at the apex to protect the fluid entry tube; and a tablet holder (see *Figure 4* and *Figure 5*) is available for positioning of special dosage forms, e.g., inlay tablets. The cell is immersed in a water bath, and the temperature is maintained at 37 ± 0.5°.

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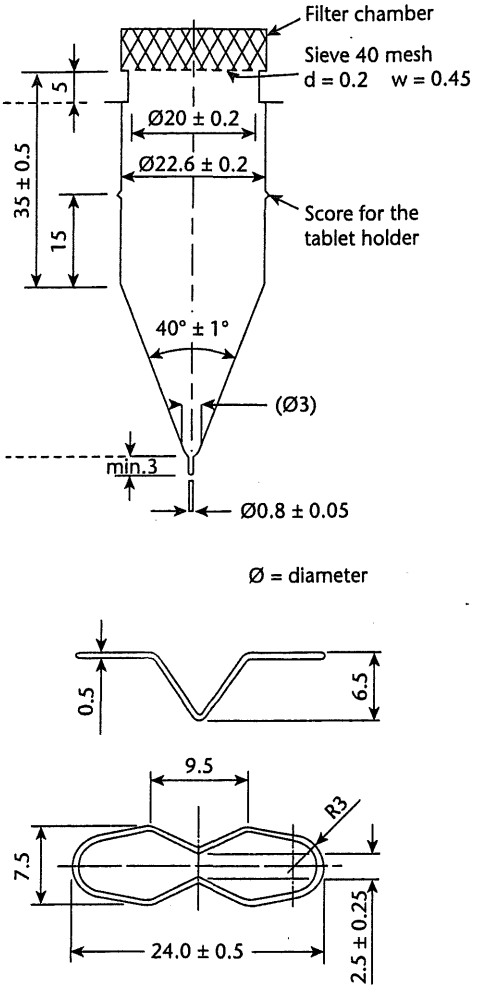


Figure 4. Apparatus 4: large cell for tablets and capsules (top); tablet holder for the large cell (bottom). (All measurements are expressed in mm unless noted otherwise.)

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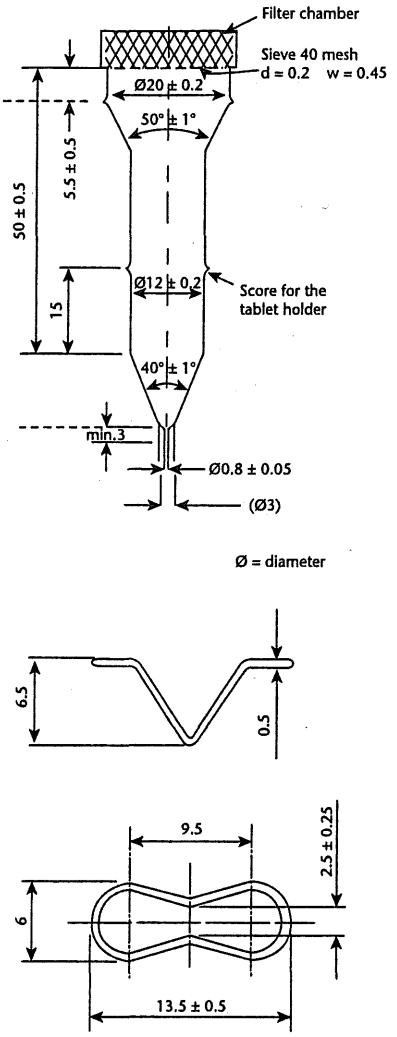


Figure 5. Apparatus 4: small cell for tablets and capsules (top); tablet holder for the small cell (bottom). (All measurements are expressed in mm unless noted otherwise.)

The apparatus uses a clamp mechanism and two O-rings to assemble the cell. The pump is separated from the dissolution unit to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytef, with about a 1.6-mm inner diameter and chemically inert, flanged-end connections.

APPARATUS SUITABILITY

The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and temperature of the *Dissolution medium*, rotation speed (*Apparatus 1* and *Apparatus 2*), dip rate (*Apparatus 3*), and flow rate of medium (*Apparatus 4*).

Determine the acceptable performance of the dissolution test assembly periodically. The suitability for the individual apparatus is demonstrated by the *Performance verification test*.

USP Reference Standards ⟨11⟩: *USP Prednisone Tablets RS*

Performance verification test, Apparatus 1 and Apparatus 2: Test *USP Prednisone Tablets RS* according to the operating conditions specified. The apparatus is suitable if the results obtained are within the acceptable range stated in the technical data sheet specific to the lot used and the apparatus tested.

Performance verification test, Apparatus 3: [To come.]

Performance verification test, Apparatus 4: [To come.]

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PROCEDURE

Apparatus 1 and Apparatus 2

IMMEDIATE-RELEASE DOSAGE FORMS

Place the stated volume of the *Dissolution medium* (±1%) in the vessel of the specified apparatus •given in the individual monograph•, assemble the apparatus, equilibrate the *Dissolution medium* to 37 ± 0.5°, and remove the thermometer. Place 1 dosage unit in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operate the apparatus at the specified rate •given in the individual monograph•. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the *Dissolution medium* and the top of the rotating basket or blade, NLT 1 cm from the vessel wall.

[NOTE—Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh *Dissolution medium* at 37° or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.]

Perform the analysis •as directed in the individual monograph• using a suitable assay method.[4] Repeat the test with additional dosage form units.

If automated equipment is used for sampling or the apparatus is otherwise modified, verification that the modified apparatus will produce results equivalent to those obtained with the standard apparatus described in this general chapter is necessary.

Dissolution medium: A suitable dissolution medium is used. Use the solvent specified •in the individual monograph•. The volume specified refers to measurements made between 20° and 25°. If the *Dissolution medium* is a buffered solution, adjust the solution so that its pH is within 0.05 unit of the specified pH •given in the individual monograph•. [NOTE—Dissolved gases can cause bubbles to form, which may change the results of the test. If dissolved gases influence the dissolution results, dissolved gases should be removed before testing.[5]]

Time: Where a single time specification is given, the test may be concluded in a shorter period if the requirement for the minimum amount dissolved is met. Specimens are to be withdrawn only at the stated times, within a tolerance of ±2%.

•Procedure for a pooled sample for immediate-release dosage forms: Use this procedure where *Procedure for a Pooled Sample* is specified in the individual monograph. Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2* in the *Procedure* section. Combine equal volumes of the filtered solutions of the six or twelve individual specimens withdrawn, and use the pooled sample as the test specimen. Determine the average amount of the active ingredient dissolved in the pooled sample.•

EXTENDED-RELEASE DOSAGE FORMS

Proceed as directed for *Immediate-Release Dosage Forms.*

Dissolution medium: Proceed as directed for *Immediate-Release Dosage Forms.*

Time: The test-time points, generally three, are expressed in hours.

DELAYED-RELEASE DOSAGE FORMS[3]

Use *Method A* or *Method B* and the apparatus specified •in the individual monograph•. All test times stated are to be observed within a tolerance of ±2%, unless otherwise specified.

Method A Procedure •(unless otherwise directedin the individual monograph)•

ACID STAGE

Place 750 mL of 0.1 N hydrochloric acid in the vessel, and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5°. Place 1 dosage unit in the apparatus, cover the vessel, and operate the apparatus at the specified rate •given in the monograph•.

After 2 h of operation in 0.1 N hydrochloric acid, withdraw an aliquot of the fluid, and proceed immediately as directed in the *Buffer Stage.*

Perform an analysis of the aliquot using a suitable assay method. •The procedure is specified in the individual monograph.•

BUFFER STAGE

[NOTE—Complete the operations of adding the buffer and adjusting the pH within 5 min.] With the apparatus operating at the rate specified •in the monograph•, add to the fluid in the vessel 250 mL of 0.20 M tribasic sodium phosphate that has been equilibrated to 37 ± 0.5°. Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05. Continue to operate the apparatus for 45 min, or for the specified time •given in the individual monograph•. At the end of the

[4] Test specimens are filtered immediately upon sampling unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the active ingredient or contain extractable substances that would interfere with the analysis.

[5] One method of deaeration is as follows: Heat the medium, while stirring gently, to about 41°, immediately filter under vacuum using a filter having a porosity of 0.45 µm or less, with vigorous stirring, and continue stirring under vacuum for about 5 min. Other validated deaeration techniques for removal of dissolved gases may be used.

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time period, withdraw an aliquot of the fluid, and perform the analysis using a suitable assay method. •The procedure is specified in the individual monograph. The test may be concluded in a shorter time period than that specified for the *Buffer Stage* if the requirement for the minimum amount dissolved is met at an earlier time.•

Method B Procedure •(unless otherwise directedin the individual monograph)•

ACID STAGE

Place 1000 mL of 0.1 N hydrochloric acid in the vessel, and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5°. Place 1 dosage unit in the apparatus, cover the vessel, and operate the apparatus at the rate specified •in the monograph•. After 2 h of operation in 0.1 N hydrochloric acid, withdraw an aliquot of the fluid, and proceed immediately as directed in the *Buffer Stage*.

Perform an analysis of the aliquot using a suitable assay method. •The procedure is specified in the individual monograph.•

BUFFER STAGE

[NOTE—For this stage of the procedure, use buffer that previously has been equilibrated to a temperature of 37 ± 0.5°.] Drain the acid from the vessel, and add to the vessel 1000 mL of pH 6.8 phosphate buffer, prepared by mixing 0.1 N hydrochloric acid with 0.20 M tribasic sodium phosphate (3:1) and adjusting, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05. [NOTE—This may also be accomplished by removing from the apparatus the vessel containing the acid, then replacing it with another vessel containing the buffer, and transferring the dosage unit to the vessel containing the buffer.]

Continue to operate the apparatus for 45 min, or for the specified time •given in the individual monograph•. At the end of the time period, withdraw an aliquot of the fluid, and perform the analysis using a suitable assay method. •The procedure is specified in the individual monograph. The test may be concluded in a shorter time period than that specified for the *Buffer Stage* if the requirement for minimum amount dissolved is met at an earlier time.•

Apparatus 3 (Reciprocating Cylinder)

IMMEDIATE-RELEASE DOSAGE FORMS[3]

Place the stated volume of the *Dissolution medium* in each vessel of the apparatus, assemble the apparatus, equilibrate the *Dissolution medium* to 37 ± 0.5°, and remove the thermometer. Place 1 dosage form unit in each of the six reciprocating cylinders, taking care to exclude air bubbles from the surface of each dosage unit, and immediately operate the apparatus as specified •in the individual monograph•. During the upward and downward strokes, the reciprocating cylinder moves through a total distance of 9.9–10.1 cm. Within the time interval specified, or at each of the times stated, raise the reciprocating cylinders and withdraw a portion of the solution under test from a zone midway between the surface of the *Dissolution medium* and the bottom of each vessel. Perform the analysis as directed •in the individual monograph•. If necessary, repeat the test with additional dosage-form units.

Dissolution medium: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

Time: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

EXTENDED-RELEASE DOSAGE FORMS

Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 3*.

Dissolution medium: Proceed as directed for *Extended-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

Time: Proceed as directed for *Extended-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

DELAYED-RELEASE DOSAGE FORMS

Proceed as directed for *Delayed-Release Dosage Forms, Method B* in *Apparatus 1 and Apparatus 2*, using one row of vessels for the acid stage media and the following row of vessels for the buffer stage media, and using the volume of medium specified (usually 300 mL).

Time: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

Apparatus 4 (Flow-Through Cell)

IMMEDIATE-RELEASE DOSAGE FORMS

Place the glass beads into the cell specified •in the monograph•. Place 1 dosage unit on top of the beads or, if specified •in the monograph•, on a wire carrier. Assemble the filter head, and fix the parts together by means of a suitable clamping device. Introduce by the pump the *Dissolution medium* warmed to 37 ± 0.5° through the bottom of the cell to obtain the flow rate specified •in the individual monograph• and measured with an accuracy of 5%. Collect the eluate by fractions at each of the times stated. Perform the analysis as directed •in the individual monograph•. Repeat the test with additional dosage form units.

Dissolution medium: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

Time: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

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EXTENDED-RELEASE DOSAGE FORMS

Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 4*.
Dissolution medium: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 4*.
Time: Proceed as directed for *Immediate-Release Dosage Forms* in *Apparatus 4*.

DELAYED-RELEASE DOSAGE FORMS

Proceed as directed for *Delayed-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*, using the specified media.
Time: Proceed as directed for *Delayed-Release Dosage Forms* in *Apparatus 1 and Apparatus 2*.

INTERPRETATION

Immediate-Release Dosage Forms

Unless otherwise specified •in the individual monograph,• the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to *Acceptance Table 1*. Continue testing through the three stages unless the results conform at either S_1 or S_2. The quantity, Q, is the amount of dissolved active ingredient •specified in the individual monograph,• expressed as a percentage of the labeled content of the dosage unit; the 5%, 15%, and 25% values in *Acceptance Table 1* are percentages of the labeled content so that these values and Q are in the same terms.

Acceptance Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is NLT Q + 5%.
S_2	6	Average of 12 units (S_1 + S_2) is $\geq Q$, and no unit is $< Q$ – 15%.
S_3	12	Average of 24 units (S_1 + S_2 + S_3) is $\geq Q$, NMT 2 units are $< Q$ – 15%, and no unit is $< Q$ – 25%.

•Immediate-Release Dosage Forms Pooled Sample

Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient dissolved from the pooled sample conform to the accompanying *Acceptance Table for a Pooled Sample*. Continue testing through the three stages unless the results conform at either S_1 or S_2. The quantity, Q, is the amount of dissolved active ingredient specified in the individual monograph, expressed as a percentage of the labeled content.

Acceptance Table for a Pooled Sample

Stage	Number Tested	Acceptance Criteria
S_1	6	Average amount dissolved is NLT Q + 10%.
S_2	6	Average amount dissolved (S_1 + S_2) is $\geq Q$ + 5%.
S_3	12	Average amount dissolved (S_1 + S_2 + S_3) is $\geq Q$.

•

Extended-Release Dosage Forms

Unless otherwise specified •in the individual monograph,• the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to *Acceptance Table 2*. Continue testing through the three levels unless the results conform at either L_1 or L_2. Limits on the amounts of active ingredient dissolved are expressed in terms of the percentage of labeled content. The limits embrace each value of Q_i, the amount dissolved at each specified fractional dosing interval. Where more than one range is specified •in the individual monograph,• the acceptance criteria apply individually to each range.

Acceptance Table 2

Level	Number Tested	Criteria
L_1	6	No individual value lies outside each of the stated ranges, and no individual value is less than the stated amount at the final test time.
L_2	6	The average value of the 12 units (L_1 + L_2) lies within each of the stated ranges and is NLT the stated amount at the final test time; none is >10% of labeled content outside each of the stated ranges; and none is >10% of the labeled content below the stated amount at the final test time.

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Acceptance Table 2 *(continued)*

Level	Number Tested	Criteria
L_3	12	The average value of the 24 units ($L_1 + L_2 + L_3$) lies within each of the stated ranges and is NLT the stated amount at the final test time; NMT 2 of the 24 units are more than 10% of labeled content outside each of the stated ranges; NMT 2 of the 24 units are >10% of labeled content below the stated amount at the final test time; and none of the units are >20% of labeled content outside each of the stated ranges or >20% of the labeled content below the stated amount at the final test time.

Delayed-Release Dosage Forms[3]

ACID STAGE: Unless otherwise specified •in the individual monograph,• the requirements of this portion of the test are met if the quantities, based on the percentage of the labeled content, of active ingredient dissolved from the units tested conform to *Acceptance Table 3*. Continue testing through all levels unless the results of both the *Acid stage* and *Buffer stage* conform at an earlier level.

Acceptance Table 3

Level	Number Tested	Criteria
A_1	6	No individual value exceeds 10% dissolved.
A_2	6	Average of the 12 units ($A_1 + A_2$) is NMT 10% dissolved, and no individual unit is >25% dissolved.
A_3	12	Average of the 24 units ($A_1 + A_2 + A_3$) is NMT 10% dissolved, and no individual unit is >25% dissolved.

BUFFER STAGE: Unless otherwise specified •in the individual monograph,• the requirements are met if the quantities of active ingredient dissolved from the units tested conform to *Acceptance Table 4*. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of *Q* in *Acceptance Table 4* is 75% dissolved unless otherwise specified •in the individual monograph,•. The quantity, *Q*, •specified in the individual monograph,• is the total amount of active ingredient dissolved in both the *Acid stage* and the *Buffer stage*, expressed as a percentage of the labeled content. The 5%, 15%, and 25% values in *Acceptance Table 4* are percentages of the labeled content so that these values and *Q* are in the same terms.

Acceptance Table 4

Level	Number Tested	Criteria
B_1	6	Each unit is NLT $Q + 5\%$.
B_2	6	Average of 12 units ($B_1 + B_2$) is $\geq Q$, and no unit is $< Q - 15\%$.
B_3	12	Average of 24 units ($B_1 + B_2 + B_3$) is $\geq Q$, NMT 2units are $< Q - 15\%$, and no unit is $< Q - 25\%$.

⟨721⟩ DISTILLING RANGE

To determine the range of temperatures within which an official liquid distills, or the percentage of the material that distills between two specified temperatures, use Method I or Method II as directed in the individual monograph. The *lower limit* of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser, and the *upper limit* is the Dry Point, i.e., the temperature at which the last drop of liquid evaporates from the lowest point in the distillation flask, without regard to any liquid remaining on the side of the flask, or the temperature observed when the proportion specified in the individual monograph has been collected.

[NOTE—Cool all liquids that distill below 80° to between 10° and 15° before measuring the sample to be distilled.]

METHOD I

Apparatus

Use apparatus similar to that specified for *Method II*, except that the distilling flask is of 50- to 60-mL capacity, and the neck of the flask is 10 to 12 cm long and 14 to 16 mm in internal diameter. The perforation in the upper insulating board, if one is used, should be such that when the flask is set into it, the portion of the flask below the upper surface of the insulating material has a capacity of 3 to 4 mL.

Procedure

Proceed as directed for *Method II*, but place in the flask only 25 mL of the liquid to be tested.

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METHOD II

Apparatus

Use an apparatus consisting of the following parts:

DISTILLING FLASK

A round-bottom distilling flask, of heat-resistant glass, of 200-mL capacity, and having a total length of 17 to 19 cm, and an inside neck diameter of 20 to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side-arm 10 to 12 cm long and 5 mm in internal diameter, which forms an angle of 70° to 75° with the lower portion of the neck.

CONDENSER

A straight glass condenser 55 to 60 cm in length with a water jacket about 40 cm in length, or a condenser of other design having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or it may be connected to a bent adapter that serves as a delivery tube.

INSULATING BOARDS

Two pieces of insulating board, 5 to 7 mm thick and 14 to 16 cm square, suitable for confining the heat to the lower part of the flask. Each board has a hole in its center, and the two boards differ only with respect to the diameter of the hole, i.e., the diameters are 4 cm and 10 cm, respectively. In use, the boards are placed one upon the other, and rest on a tripod or other suitable support, with the board having the larger hole on top.

RECEIVER

A 100-mL cylinder graduated in 1-mL subdivisions.

THERMOMETER

In order to avoid the necessity for an emergent stem correction, an accurately standardized, partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°) is recommended. Suitable thermometers are available as the ASTM E-1 series 37C through 41C, and 102C through 107C (see *General Notices, 6.80.30. Temperature Reading Devices*). When placed in position, the stem is located in the center of the neck, and the top of the contraction chamber (or bulb, if 37C or 38C is used) is level with the bottom of the outlet to the side-arm.

HEAT SOURCE

A small Bunsen burner or an electric heater or mantle capable of adjustment comparable to that possible with a Bunsen burner.

Procedure

Assemble the apparatus, and place in the flask 100 mL of the liquid to be tested, taking care not to allow any of the liquid to enter the side-arm. Insert the thermometer, shield the entire burner and flask assembly from external air currents, and apply heat, regulating it so that between 5 and 10 minutes elapse before the first drop of distillate falls from the condenser. Continue the distillation at a rate of 4 to 5 mL of distillate per minute, collecting the distillate in the receiver. Note the temperature when the first drop of distillate falls from the condenser, and again when the last drop of liquid evaporates from the bottom of the flask or when the specified percentage has distilled over. Unless otherwise specified in the individual monograph, apply when necessary the emergent stem correction and report the temperatures adjusting the barometric pressure by the following formula:

$$t = t_0 + [(t_0 10^{-4} + 0.033)(760 - p)]$$

in which t is the corrected boiling temperature, in Celsius scale; t_0 is the measured boiling temperature, in Celsius scale; and p is the barometric pressure at the time of measurement, in mm Hg.

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⟨724⟩ DRUG RELEASE

SCOPE

This test is provided to determine compliance with drug release or dissolution requirements where stated in the individual monographs for transdermal systems (TDS) and other dosage forms. From the types of apparatus described herein, use the one specified in the individual monograph.

GENERAL DRUG RELEASE STANDARDS

Apparatus 5 (Paddle over Disk)

APPARATUS

Use the paddle and vessel assembly as described in *Dissolution* ⟨711⟩, *Apparatus, Apparatus 2*, with the addition of a disk assembly designed to hold the TDS at the bottom of the vessel. This disk assembly is designed to minimize any "dead" volume between the assembly and the bottom of the vessel. The disk assembly holds the TDS flat and is positioned such that the release surface is parallel with the bottom of the paddle blade (see *Figure 1a*, *Figure 1b*, and *Figure 1c*). A distance of 25 ± 2 mm between the paddle blade and the surface of the disk assembly is maintained during the test. Other appropriate devices may be used (see *Figure 2*), provided they do not sorb, react with, or interfere with the specimen being tested. [NOTE—A suitable device is catalog number TRANDE-SC from www.qla-llc.com.]

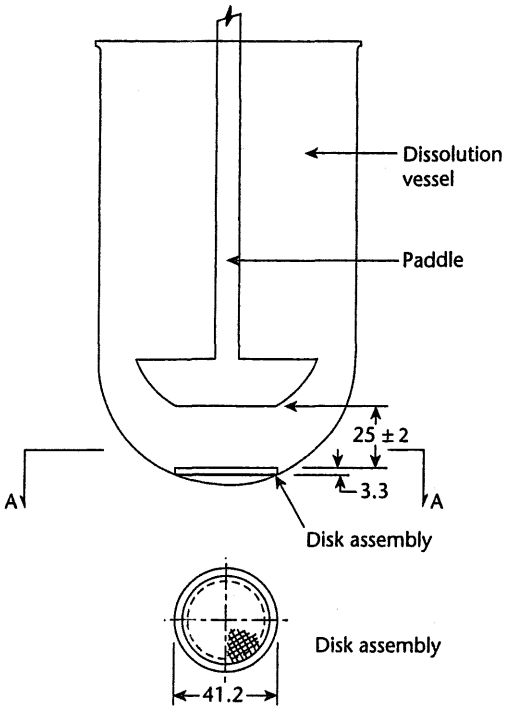


Figure 1a. Paddle over disk. (All measurements are expressed in mm unless noted otherwise.)

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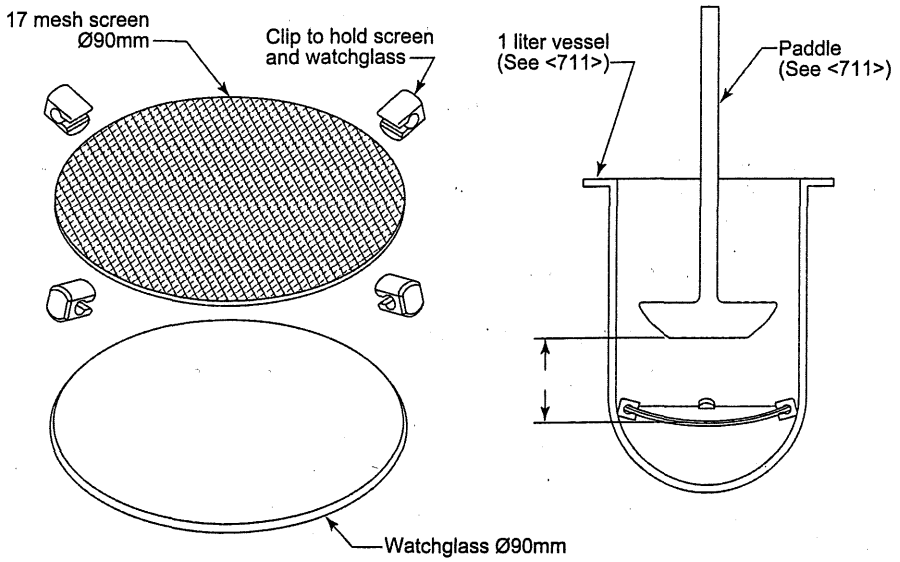


Figure 1b. Paddle over disk (screen and watchglass).

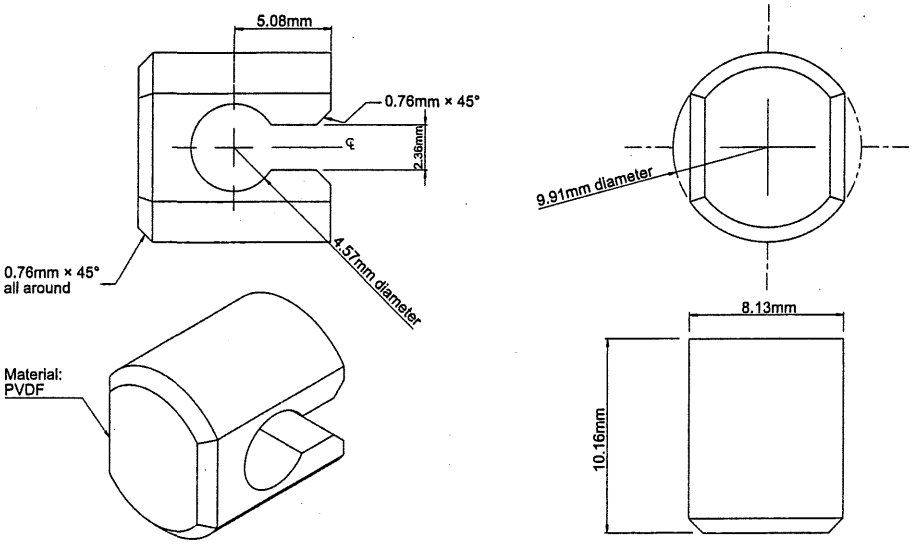


Figure 1c. Clip to hold screen and watchglass.

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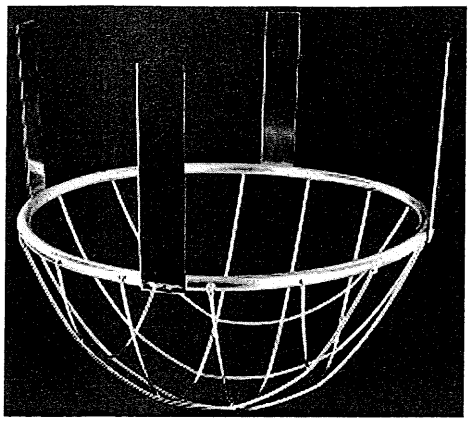


Figure 2. Holding wire screen.

APPARATUS SUITABILITY

Proceed as directed for *Apparatus 2* in Dissolution ⟨711⟩, *Apparatus, Apparatus 4, Apparatus Suitability, Performance verification test, Apparatus 1 and Apparatus 2.*

MEDIUM

Proceed as directed for *Dissolution medium* in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms.*

PROCEDURE

Place the stated volume of *Medium* in the vessel, and equilibrate to 32 ± 0.5°.

Apply the TDS to the disk assembly, assuring that the release surface is as smooth as possible and the TDS is completely and firmly attached to the disk. The TDS, with release surface side up, may be attached to the disk by an appropriate and validated procedure such as use of an adhesive, double-face adhesive tape, screen, or membrane. Care must be taken to avoid the presence of air bubbles between the membrane, if used, and the TDS, or the presence of wrinkles on the surface of the TDS. Carefully remove the protective liner from the TDS without causing damage to the surface of the TDS.

Place the disk assembly flat at the bottom of the vessel with the release surface facing up and parallel to the edge of the paddle blade and surface of the *Medium*. Ensure that the surface of the TDS is devoid of air bubbles. The bottom edge of the paddle is 25 ± 2 mm from the surface of the disk assembly. The vessel may be covered during the test to minimize evaporation.

Immediately operate the apparatus at the rate specified in the monograph.

At each sampling time interval, withdraw a specimen from a zone midway between the surface of the *Medium* and the top of the blade, not less than 1 cm from the vessel wall.

Perform the analysis on each sample aliquot as directed in the individual monograph, correcting for any volume losses, as necessary. Repeat the test with additional TDS as needed.

TIME

The test time points, at least three, are expressed in hours. Specimens are to be withdrawn within a tolerance of ±15 min or ±2% of the stated time, selecting the tolerance that results in the narrowest time interval.

INTERPRETATION

Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* below. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Acceptance Table 1

Level	Number Tested	Criteria
L_1	6	No individual value lies outside the stated range.
L_2	6	The average value of the 12 units ($L_1 + L_2$) lies within the stated range. No individual value is outside the stated range by more than 10% of the average of the stated range.
L_3	12	The average value of the 24 units ($L_1 + L_2 + L_3$) lies within the stated range. Not more than 2 of the 24 units are outside the stated range by more than 10% of the average of the stated range; and none of the units is outside the stated range by more than 20% of the average of the stated range.

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Apparatus 6 (Cylinder)

APPARATUS

Use the vessel assembly as described in *Dissolution* ⟨711⟩, *Apparatus, Apparatus 1*, except replace the basket and shaft with a stainless steel cylinder stirring element with the specifications shown in *Figure 3*. This cylinder stirring element is composed of two parts, one comprising the shaft and the upper cylinder, and the other being the extension cylinder. Since both parts are serially numbered, it is recommended that they be kept paired because the friction fitting is done individually for each cylinder, allowing a very tight fit. The distance between the inside bottom of the vessel and the cylinder is maintained at 25 ± 2 mm during the test.

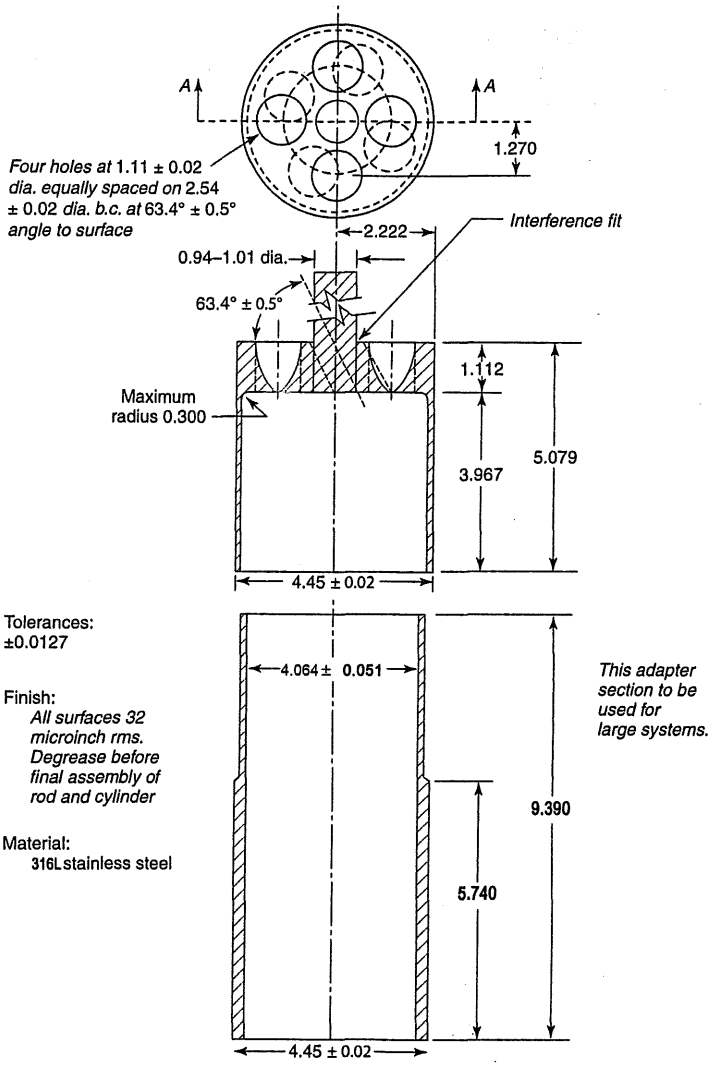


Figure 3. Cylinder stirring element. (All measurements are expressed in cm unless noted otherwise.)

MEDIUM

See *Dissolution medium* in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms.*

PROCEDURE

Place the stated volume of *Medium* in the vessel, and equilibrate to 32 ± 0.5°.

Apply the TDS to the cylinder, assuring that the release surface is as smooth as possible and the TDS is completely and firmly attached to the cylinder. The TDS, with release side facing the *Medium*, may be attached to the cylinder by an appropriate and validated procedure such as use of an adhesive, double-face adhesive tape, membrane, or nylon net. Care must be taken to avoid the presence of air bubbles between the membrane, if used, and the TDS, or the presence of wrinkles on the surface of

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the TDS. Carefully remove the protective liner from the TDS without causing damage to the surface. If additional reinforcement of the TDS to the cylinder is needed, inert metal wire or a polymer ring may be used.

Place the cylinder in the apparatus, and immediately rotate at the rate specified in the individual monograph. The vessel may be covered during the test to minimize evaporation.

At each sampling time interval, withdraw a specimen from a zone midway between the surface of the *Medium* and the top of the rotating cylinder, not less than 1 cm from the vessel wall.

Perform the analysis on each sample aliquot as directed in the individual monograph, correcting for any volume losses, as necessary. Repeat the test with additional TDS, as needed.

TIME

The test time points, at least three, are expressed in hours. Specimens are to be withdrawn within a tolerance of ±15 min or ±2% of the stated time, selecting the tolerance that results in the narrowest time interval.

INTERPRETATION

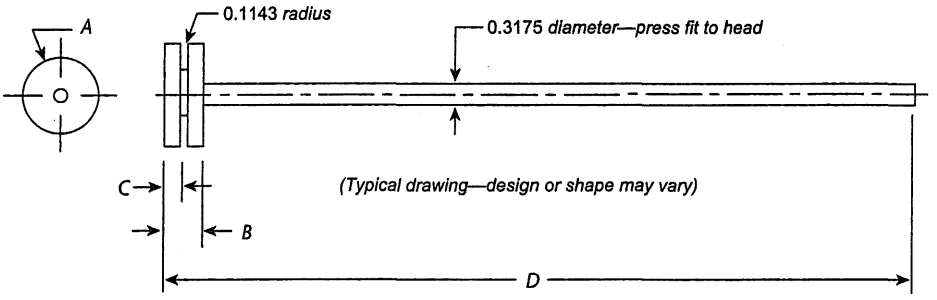
Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* above. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Change to read:

Apparatus 7 (Reciprocating Holder)

APPARATUS

The assembly consists of (1) a set of volumetrically calibrated or tared solution containers made of glass or other suitable inert material that should not sorb, react with, or interfere with the specimen being tested; (2) a motor and drive assembly to reciprocate the system vertically and to index the system horizontally to a different row of vessels, automatically if desired; and (3) a set of suitable sample holders (see *Figure 4*, *Figure 5a*, *Figure 5b*, *Figure 5c*, and *Figure 5d*).



Dimensions are in centimeters.

	HEAD				ROD		O-RING
System[a]	*A (Diameter)*	*B*	*C*	Material[b]	*D*	Material[c]	*(not shown)*
1.6 cm^2	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2–113–V884–75
2.5 cm^2	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2–016–V884–75
5 cm^2	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2–022–V884–75
7 cm^2	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2–124–V884–75
10 cm^2	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2–225–V884–75

[a] Typical system sizes.

[b] SS/VT=Either stainless steel or virgin Teflon.

[c] SS/P=Either stainless steel or Plexiglas.

Figure 4. Reciprocating disk sample holder.

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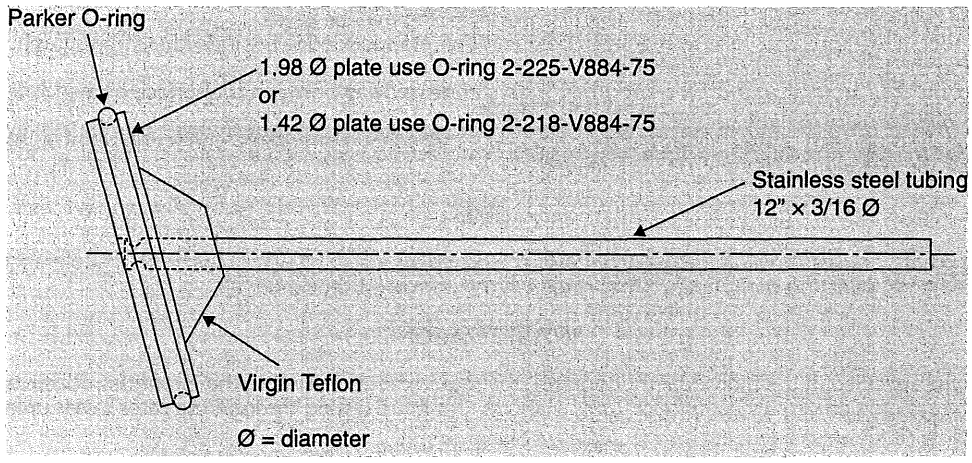


Figure 5a. Transdermal system holder—angled disk.

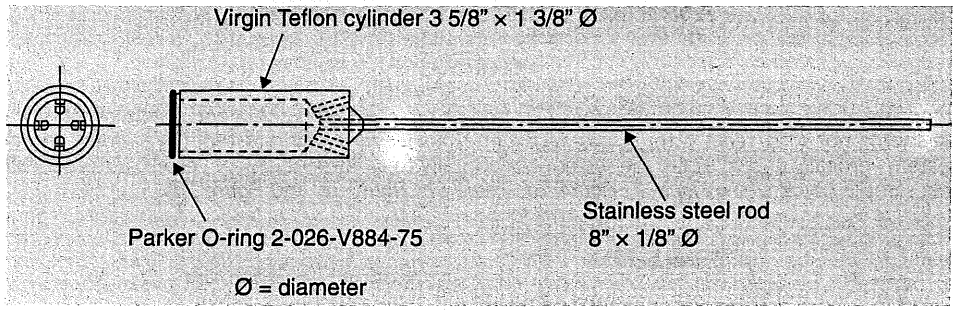


Figure 5b. Transdermal system holder—cylinder.

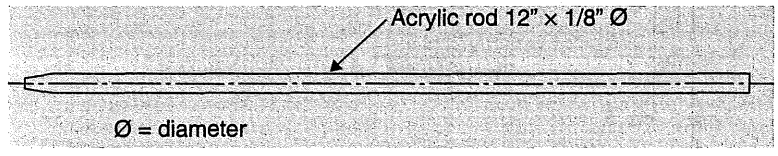


Figure 5c. Oral extended-release tablet holder—rod, pointed for gluing.

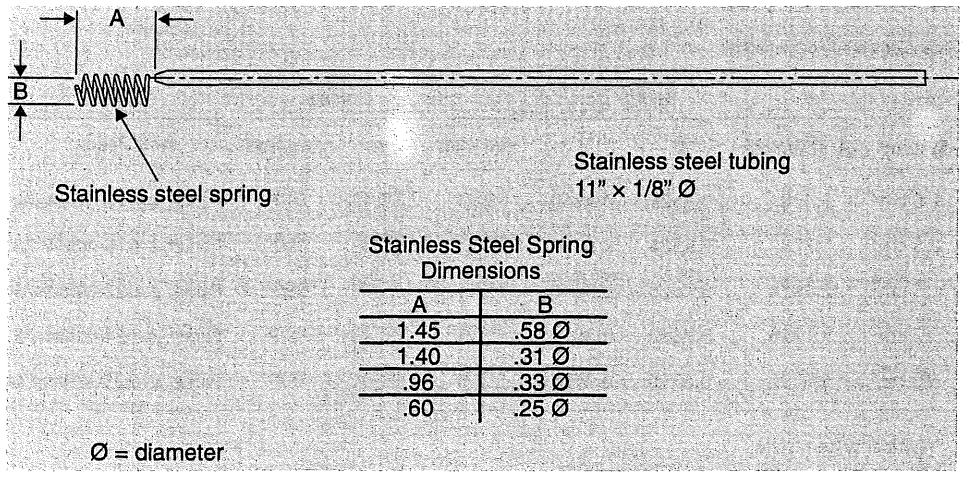


Figure 5d. Oral extended-release tablet holder—spring holder.

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During the test, the solution containers are partially immersed in a suitable water bath of any convenient size that permits maintaining the temperature inside the containers at 32 ± 0.5° for TDS or at 37 ± 0.5° for other dosage forms during the test.

No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating sample holder.

Apparatus that permits observation of the system and holder during the test is preferable.

Use the size container and sample holder as specified in the individual monograph.

MEDIUM

See *Dissolution medium* in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms.*

SAMPLE PREPARATION A (FOR OSMOTIC PUMP TABLETS)

Attach each unit to be tested to a suitable holder by an appropriate and validated procedure such as use of an adhesive to adhere the edge of the tablet to a holder (e.g., *Figure 5c*), the spring holder (*Figure 5d*), a small nylon net bag, or a membrane.

SAMPLE PREPARATION B (FOR TDS)

Apply the TDS to the appropriate holder, assuring that the release surface is as smooth as possible and the TDS is completely and firmly attached to the holder. The TDS, with release side facing the *Medium*, may be attached to the holder by an appropriate and validated procedure such as use of an adhesive, double-face adhesive tape, membrane, or nylon net. Care must be taken to avoid the presence of air bubbles between the membrane, if used, and the TDS, or the presence of wrinkles on the surface of the TDS. Carefully remove the protective liner from the TDS without causing damage to the surface of the TDS. If additional reinforcement of the TDS to the holder is needed, inert metal wire or a polymer ring may be used.

SAMPLE PREPARATION C (FOR OTHER DOSAGE FORMS)

Attach each dosage form to be tested to a suitable holder.

PROCEDURE

Place the stated volume of *Medium* in the solution containers, and equilibrate to the test temperature.

Suspend each prepared sample holder from a vertically reciprocating shaker such that each system is continuously immersed in *Medium* during the entire test.

Reciprocate at a frequency of about 30 cycles/min with an amplitude of about 2 cm, or as specified in the individual monograph, for the specified time.

At each sampling time interval, remove the solution containers from the bath, cool to room temperature, and add sufficient solvent (i.e., water in most cases) to correct for evaporative losses.

Perform the analysis on each sample as directed in the individual monograph. Repeat the test with additional TDS, as needed.

TIME

The test time points, at least three, are expressed in hours. Specimens are to be withdrawn within a tolerance of ±15 min or ±2% of the stated time, selecting the tolerance that results in the narrowest time interval.

INTERPRETATION

Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the TDS conform to *Acceptance Table 1* above, or the appropriate acceptance table in ⟨711⟩ for other dosage forms. Continue testing through the three levels unless the results conform at either L_1 or L_2.

⟨729⟩ GLOBULE SIZE DISTRIBUTION IN LIPID INJECTABLE EMULSIONS

INTRODUCTION

Lipid injectable emulsions for intravenous administration are sterile oil-in-water emulsions of soybean oil, used to provide an ample supply of essential fatty acids, linoleic and linolenic, dispersed with the aid of an emulsifying agent in *Water for Injection*. Alternatively, soybean oil can be mixed with other suitable oils (neutral triglycerides), such as safflower oil, medium-chain triglycerides (MCT) derived from coconut or palm kernel oils, olive oil, or a marine oil, such as menhaden oil. The size of the lipid droplets is critical: because of mechanical filtration, larger-size fat globules (>5 µm) can be trapped in the lungs. The essential size characteristics of a lipid injectable emulsion for intravenous use include the mean diameter of the lipid droplets and the range of the various droplet diameters distributed around the mean diameter, expressed as the standard deviation. In particular, the amounts of fat globules comprising the large-diameter tail of the globule size distribution are especially important with respect to infusion safety. These two regions of the globule size distribution (mean droplet size and large-diameter tail) must be controlled within specified limits.

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The two methods described below are used for determination of the mean lipid droplet diameter and the distribution of large-diameter globule sizes in lipid injectable emulsions. *Method I* and *Method II* must be validated. The methods described below to assess the quality of lipid injectable emulsions are to be performed in two stages.

Change to read:

METHOD I—▲MEASUREMENT OF MEAN DROPLET DIAMETER BY DYNAMIC LIGHT SCATTERING OR CLASSICAL LIGHT SCATTERING▲ (USP 1-AUG-2019)

For the determination of the mean droplet size of lipid injectable emulsions, either of two common light-scattering techniques may be employed: (1) dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), or (2) classical light scattering ▲[laser diffraction (LD)],▲ (USP 1-Aug-2019) based on Mie scattering theory. The DLS, or PCS, technique is based on analyzing the rapid temporal fluctuations in the scattered light intensity that occur due to the random Brownian motion, or diffusion, of any particles, including lipid droplets, suspended in liquid. The intensity is measured at a given angle (usually 90°) by a suitable detector (e.g., photomultiplier tube) able to measure the rapidly fluctuating scattered light intensity produced by the suspended, diffusing droplets. These scattered intensity data are typically used to calculate the intensity autocorrelation function, which is a simple decaying exponential function in time for droplets of uniform size. A distribution of droplet sizes expresses itself by ▲the superposition, or summation, of▲ (USP 1-Aug-2019) exponential functions of different decay times, ▲each representative of a given droplet size.▲ (USP 1-Aug-2019) The autocorrelation function generated by the scattered intensity data obtained from a given emulsion can be "inverted" by means of an appropriate deconvolution algorithm in order to obtain the approximate distribution of intensity-weighted diffusion coefficients. From the latter, the distribution of small-diameter droplets is calculated, using the Stokes–Einstein equation and the rules of classical (Mie) light scattering.

By contrast, classical light scattering based on Mie theory analyzes the spatial ▲(angular),▲ (USP 1-Aug-2019) rather than temporal, variation of the scattered light intensity by measuring the latter as a function of the scattering angle, typically over a large range of detected angles. The temporal fluctuations in the scattering intensity due to Brownian motion are averaged out in time for each angular measurement. This angular variation occurs as a consequence of the mutual interference of individual scattered waves arriving at the detector with different phases from different points within ▲each of the▲ (USP 1-Aug-2019) lipid droplets, ▲summed over all of the▲ (USP 1-Aug-2019) different particles. The extent of the angular variation is significant whenever the droplet diameter is not small compared with the wavelength of the laser light (typically ▲633–▲ (USP 1-Aug-2019)635 nm). Droplets of a given size and refractive index yield a unique curve of scattering intensity versus angle. A distribution of droplet sizes gives rise to a final angular dependence that represents the superposition, or summation, of ▲all of the▲ (USP 1-Aug-2019) individual (different) intensity versus angle curves. The measured angular dependence of the scattering intensity obtained from a given emulsion sample, ▲in principle,▲ (USP 1-Aug-2019) can be inverted by means of an appropriate deconvolution algorithm ▲in combination with▲ (USP 1-Aug-2019) Mie scattering theory in order to obtain the approximate droplet size distribution.

Thus, light scattering, using either dynamic light scattering (i.e., ▲varying▲ (USP 1-Aug-2019) temporal fluctuations due to droplet diffusion) or classical light scattering/Mie theory (i.e., ▲varying,▲ (USP 1-Aug-2019) average intensity versus angle), can provide acceptable results for both the mean diameter and, ▲to a lesser degree, the▲ (USP 1-Aug-2019) standard deviation of the droplet size distribution. For purposes of illustrating the method used in *Method I*, a dynamic light-scattering technique is described. For guidance regarding instruments employing classical Mie-theory light scattering, see *Light Diffraction Measurement of Particle Size* ⟨429⟩.

Apparatus

A suitable DLS/PCS instrument with or without the capability of automatic sample dilution is controlled by validated software and is used to perform the measurement, with the scattering angle typically set at 90°. The intensity-weighted results (mean diameter and standard deviation) are reported, provided it is clearly stated which values are given and that the necessary parameter values required for all requisite calculations are also given.

Water

Pass distilled water through a filter of 0.2-µm pore size, and degas by sonication, or use *Sterile Water for Injection* stored in a glass container.

Standard Preparation

To a pre-established volume of *Water* add an appropriate amount of concentrated suspension, containing National Institute of Standards and Technology (NIST)-traceable polystyrene latex standard particles or other suitable nanospheres. Gently mix the fluids to achieve a homogeneous suspension. The diluted suspension will be slightly turbid in appearance. If the DLS/PCS instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe, with further dilution occurring automatically to optimize the droplet concentration for analysis. Alternatively, the sample would require greater manual dilution with *Water* (typically by at least a factor of 10 over the first dilution), and then this sample would be instilled into a "drop-in" cuvette. The optimum dilution scheme that achieves the proper scattering intensity for the cuvette-based analysis will be determined by the instrument specifications. Thus, the concentration of latex in the final sample must be optimized for the DLS/PCS instrument used. This should be performed separately for three different size standards of approximately 100, 250, and 400 nm (triplicate analyses per size), and the

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corresponding results of intensity-weighted mean diameter and standard deviation should coincide with the expected values within acceptable errors.

Test Preparation

To a pre-established volume of *Water* add an appropriate volume of sample from the lipid injectable emulsion. Gently mix the fluids to achieve a homogeneous suspension. The diluted suspension will be slightly turbid in appearance. If the DLS/PCS instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe. Further dilution of the sample then occurs automatically to optimize the droplet concentration for analysis, ensuring that it is not so high as to cause artifacts due to multiple scattering or interdroplet interactions. Alternatively, the sample would require greater manual dilution with *Water* (typically by at least a factor of 10 over the first dilution), and then this sample would be instilled into a "drop-in" cuvette. The optimum dilution scheme that achieves the proper scattering intensity for the cuvette-based analysis will be determined by the instrument specifications. Thus, the concentration of lipid injectable emulsion in the final sample must be optimized for the DLS/PCS instrument used. ▲Testing should be done in triplicate or, as appropriate, based on method validation data.▲ (USP 1-Aug-2019)

System Suitability

Using the *Standard Preparation,* measure the intensity-weighted mean particle diameter and the corresponding standard deviation. The system is suitable once the sample temperature has reached equilibration and the results have stabilized and triplicate mean droplet diameter measurements are obtained. The coefficient of variation (CV) should not exceed ▲15% (see below)▲ (USP 1-Aug-2019) of the NIST-traceable mean droplet diameter. A larger CV value indicates that the latex microspheres are not suitable as a standard because they either inherently lack uniformity or have become agglomerated to an unacceptable extent. In this case, another standard latex suspension must be selected and tested.

▲[NOTE—The recommended 15% CV limit is increased here because of the differences in the way the mean droplet size is determined by DLS versus classical light scattering.]▲ (USP 1-Aug-2019)

Procedure and Interpretation

If the DLS/PCS instrument is equipped with an automatic dilution system, use a disposable syringe to load the *Standard preparation* or *Test preparation*. If no automatic dilution system is used, transfer the appropriately diluted preparation to a cuvette, and place the cuvette in the ▲temperature-regulated sample holder of the instrument.▲ (USP 1-Aug-2019) Allow the sample to equilibrate to a preset controlled temperature close to ambient (between 20° and 25°, as in the USP definition found in *Packaging and Storage Requirements* ⟨659⟩). Set the instrument scattering angle to 90°, and carry out the measurements. As long as the chi-square (χ^2) goodness-of-fit parameter remains acceptably low (per instrument specifications), the results for the *Test preparation* are acceptable. Excessive values of the χ^2 parameter suggest that the droplet distribution is not normal and may indicate an unstable emulsion. The intensity-weighted mean droplet diameter (MDD) for lipid injectable emulsions must be less than 500 nm or 0.5 µm, irrespective of the concentration of the dispersed lipid phase.

▲Submicron results from the DLS method and classical (Mie) light scattering method may vary due to the different physical principles of the mechanism of testing and the different mathematical procedures used to analyze the underlying data.▲ (USP 1-Aug-2019)

Change to read:

METHOD II—MEASUREMENT OF LARGE GLOBULE CONTENT BY LIGHT OBSCURATION OR EXTINCTION METHOD

For determination of the extent of the large-diameter droplet tail (>5 µm) of lipid injectable emulsions, a light obscuration (LO) or light extinction (LE) method that employs a single-particle (globule) optical sizing (SPOS) technique is used. ▲DLS methodology is effective only for characterizing smaller particle sizes in the nominal size range of about 1–1000 nm; therefore, DLS is complimentary to LE and is particularly effective in sizing droplets smaller than 500 nm. Importantly, DLS is only a sizing method and provides only semi-quantitative information on the relative droplet size distribution. DLS does not count particles or droplets; thus, it is mainly a qualitative method. In contrast, LE both sizes (1.3–400 µm) and counts fat globules providing "reliable" count values at a size threshold of 5 µm.▲ (USP 1-Aug-2019)

During application of the LE/SPOS technique, passage of a droplet through a thin optical sensing zone results in ▲effective▲ (USP 1-Aug-2019) blockage of a portion of the incident light beam, causing a momentary decrease in the light intensity reaching the ▲distant▲ (USP 1-Aug-2019) "extinction" detector. The magnitude of this decrease in the signal is ▲related▲ (USP 1-Aug-2019) to the cross-sectional area of the droplet (assumed smaller than the sensing zone thickness), i.e., to the square of the droplet diameter. ▲The larger the droplet diameter, the larger the height (voltage) of the signal pulse produced by the momentary decrease in the detected light intensity.▲ (USP 1-Aug-2019) During optimization of the LE/SPOS instrument for a given emulsion sample, a series of dilutions should be tested to achieve consistency between samples. The goal is to identify a standard range of dilutions that yield consistent data and are most applicable to the formulation tested. Ideally, when comparing different emulsions, the same approximate number of globules are sized each time, and once a standard is achieved, it should be incorporated into the routine sampling plan for validation testing. As long as the fat globule concentration is below the "coincidence limit" of the sensor (determined by the flow cell and optical design, ▲verified experimentally),▲ (USP 1-Aug-2019) only

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one globule at most will pass through the sensing zone at any given time, allowing it to be counted and accurately sized (with less than 1% ▲particle or globule▲ (USP 1-Aug-2019) coincidence events). Both the coincidence ▲(concentration)▲ (USP 1-Aug-2019) limit and the optimal flow rate must be known for the LE/SPOS sensor used. Furthermore, it is prudent to perform the large-diameter measurements at a reduced emulsion concentration such that the measurable droplet concentration ▲from the▲ (USP 1-Aug-2019) threshold of detection (e.g., >1.8 µm) to an ▲appropriate▲ (USP 1-Aug-2019) upper ▲size▲ (USP 1-Aug-2019) limit (e.g., 50 µm) is only approximately one-third of the nominal coincidence limit for the sensor used. The resulting single pulse heights are converted to droplet diameters using a standard calibration curve previously constructed from NIST-traceable monosized polystyrene microspheres of known diameters. For additional guidance in the use of the light obscuration methodology, see *Particulate Matter in Injections* ⟨788⟩.

Apparatus

A suitable light obscuration instrument with or without the capability of automatic sample dilution and controlled by a personal computer (PC) is used for the measurement. The number- and volume-weighted particle size distribution data are reported, provided that it is clearly stated which values are given and that the necessary parameter values required for all necessary calculations are also given.

Water

Pass distilled water through a filter of 0.2-µm pore size ▲and degas by sonication,▲ (USP 1-Aug-2019) or use *Sterile Water for Injection* stored in a glass container.

Standard Preparation

To a pre-established volume of *Water* add an appropriate amount of concentrated suspension, containing NIST-traceable polystyrene latex standard particles or other suitable microspheres. Gently mix the fluids to achieve a homogeneous suspension. If the light obscuration instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe or Teflon sample line. Further dilution of the sample then occurs automatically to optimize the particle concentration for analysis. Alternatively, the sample would require greater manual dilution with water (typically by at least a factor of 10 over the first dilution). The resulting diluted sample is then instilled in an appropriate, clean container, such as a sterile Type I glass container, before being passed through the sensor. In either case the final particle concentration is caused to lie below the coincidence limit of the sensor. The sizing and counting accuracy of the light obscuration instrument should be obtained using two different size standards of approximately 5 and 10 µm (triplicate analyses per size). For the standards after system calibration, set the instrument threshold of detection at 1.8 µm, extended to an upper limit of 50 µm. The corresponding results for the mean diameter should coincide with the expected values, within 10% of the relative standard deviation and 90%–110% size accuracy. In addition, the number of particle counts obtained per milliliter should also agree within ±10% with the concentration values certified in the documentation provided with each NIST-traceable size standard.

Test Preparation

To a pre-established volume of *Water* add an appropriate volume of sample from the lipid injectable emulsion (triplicate analyses per sample). Gently mix the fluids to achieve a homogeneous suspension. The diluted emulsion will be slightly turbid in appearance. If the light obscuration instrument is equipped with an automatic dilution system, the starting concentrated sample can be analyzed by injection directly into the instrument via a syringe or nonreactive[1] Teflon sample line. Further dilution then occurs automatically to optimize the droplet/globule concentration for analysis. Alternatively, the sample would require greater manual dilution with water (typically by at least a factor of 10 over the first dilution). The resulting diluted sample is then instilled in an appropriate, clean container such as a sterile Type I glass container. In either case the final droplet/globule concentration is caused to lie below the coincidence limit of the sensor.

System Suitability

Perform prior to the test procedure, using the *Standard preparation* of a 5- and 10-µm NIST-traceable particle. Measure in triplicate the number-weighted particle diameter and the counts/mL of the standard. The system is suitable when the triplicate mean number-weighted particle diameter measurements are within 10% of the target value, both in terms of repeatability (CV) and closeness to the certified size on the label of the NIST-traceable standard.

Procedure and Interpretation

If the light obscuration instrument is equipped with an automatic dilution system, use a disposable syringe or Teflon sample line to load the *Standard Preparation* or *Test Preparation*. If no automatic dilution system is used, transfer the sample to an appropriate large-volume, clean container such as a sterile Type I glass vessel containing an appropriate volume of water. Allow the sample and water to mix thoroughly to achieve a homogeneous suspension. Set the instrument threshold of detection at

[1] Polyvinyl chloride (PVC) with diethylhexylphthalate (DEHP) has been shown to induce breakdown of lipid injectable emulsions (Drug Product Problem Reporting System. USP File Access No. 11173, May 15, 1991).

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1.8 µm, extended to an upper ▲size▲ (USP 1-Aug-2019) limit of 50 µm, and vary the concentration and/or data collection times such that there is at least a factor of two in the difference of the total number of globules that measure >5 µm between at least two sample runs. In any case, the number of globules that measure >5 µm should be large enough so that it represents an adequate number of globules that are statistically representative of the large-diameter tail population of the native emulsion. The volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 µm (PFAT5) for a given lipid injectable emulsion, must not exceed 0.05%. ▲Most commonly used instruments provide calculation in software (HIAC Royco, PSS AccuSizer, Klotz). However, the general equation applied is based on the sum of the number of droplets per milliliter of sample per channel multiplied by the volume of a sphere of the geometric mean of each channel, i.e., Σ(#/mL/channel) (πD3/6).▲ (USP 1-Aug-2019)

⟨730⟩ PLASMA SPECTROCHEMISTRY

INTRODUCTION

Plasma-based instrumental techniques, which are useful for pharmaceutical analyses, fall into two major categories: those based on the inductively coupled plasma, and those where a plasma is generated at or near the surface of the sample. The inductively coupled plasma (ICP) is a high-temperature excitation source that desolvates, vaporizes, and atomizes aerosol samples and ionizes the resulting atoms. The excited analyte ions and atoms can then subsequently be detected by observing their emission lines, a method termed inductively coupled plasma–optical emission spectroscopy (ICP–OES; also referred to as inductively coupled plasma–atomic emission spectroscopy), or the excited or ground state ions can be determined by a technique known as inductively coupled plasma–mass spectrometry (ICP–MS). ICP–OES and ICP–MS may be used for either single- or multi-element analysis and used for either sequential or simultaneous analyses with good sensitivity over an extended linear range.

For additional information and discussion of the theory and principles of measurements, see *Plasma Spectrochemistry—Theory and Practice* ⟨1730⟩.

QUALIFICATION OF PLASMA SPECTROPHOTOMETERS

Qualification of the ICP–OES or the ICP–MS can be divided into three elements: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ)—see also the general chapter *Analytical Instrument Qualification* ⟨1058⟩.

Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location, and that the environment, in which the instrument will be used, is adequate.

Operational Qualification

The purpose of OQ is to demonstrate that instrument performance is suitable. In OQ, an instrument's performance is characterized using standards of known spectral properties to verify that the system operates within target specifications. OQ is a check of the key operational parameters performed after installation and after repairs and/or maintenance. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package.

Performance Qualification

PQ determines whether the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement. Depending on typical use, the specifications for PQ may be different from the manufacturer's specifications. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements. A solution used in the initial standard curve of an ICP–OES or ICP–MS instrument must be reassayed as a check standard at appropriate, pre-established intervals throughout the analysis of the sample set.

For single-element ICP–OES analyses, when analytical wavelengths are between 200 and 500 nm, or concentrations are >1 µg/mL, the reassayed standard should agree with its expected value to within ±10%, or as specified in an individual monograph. For multi-element ICP–OES analyses, when analytical wavelengths are <200 nm or >500 nm, or at concentrations of <1 µg/mL, the reassayed standard should agree with its theoretical value to within ±20%, or as specified in an individual monograph. For single-element ICP–MS analyses, when analytical masses that are free of interferences and when concentrations are >1 ng/mL, the reassayed standard should agree with its expected value to within ±10%, or as specified in an individual monograph.

For multi-element ICP–MS analyses, or when concentrations are <1 ng/mL, or for single-element analyses where interferences may be present, the reassayed standard should agree with its expected value to within ±20%, or as specified in an individual monograph.

In cases where an individual monograph provides different guidance regarding the reassayed check standard for ICP–OES or ICP–MS, the requirements of the monograph take precedence. Specific procedures, acceptance criteria, and time intervals for characterizing ICP–OES or ICP–MS performance depend on the instrument and intended application.

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PROCEDURE

Users must evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in ICP–OES and ICP–MS analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents. For some analyses, diligence must be exercised to prevent the adsorption of analytes onto the surface of a vessel; contamination of the sample solutions from metal and ions present in the container also can lead to inaccurate results. For the analysis of a ubiquitous element, it is often necessary to use the purist grade of reagent or solvent available. Check all solutions for elemental contamination before they are used in an analysis.

Standard Solution

Standard solutions are used to standardize the instrument at the time-of-use, and prepare these solutions as directed in the individual monograph. [NOTE—Commercially available single- or multi-element standard solutions, traceable to the National Institute of Standards and Technology or to an equivalent national metrology organization, can be used in the preparation of standard solutions.] Standard solutions, especially those used for ultra-trace analyses, may have limited shelf-life. Stability of standard solutions can vary depending on concentration, analyte of interest, storage container type, and storage conditions. For these reasons, standard solutions with concentrations <10 ppm (w/v) should be retained for NMT 24 h, unless stability is demonstrated experimentally.

The method of standard additions can be used for instrument standardization. This method involves adding a known concentration of the analyte element to the sample at NLT two concentration levels against an unspiked sample preparation. The instrument response is plotted against the concentration of the added analyte element, and a linear regression line is drawn through the data points. The absolute value of the *x*-intercept multiplied by any dilution factor is the concentration of the analyte in the sample, and many instruments will perform the analyte calculation automatically. Once a method has been developed and is in routine use, it is common practice to calibrate with a blank and a single standard. One-point standardizations are suitable for conducting limit tests on production materials and final products, provided that the methodology has been rigorously validated for sufficient specificity, sensitivity, linearity, accuracy, precision, ruggedness, and robustness.

Sample Solution

Although solid samples and slurried samples may be analyzed, it is generally necessary to prepare solutions of samples for analyses as directed in the individual monograph. Samples may be dissolved in any solvent, including organic solvents or acids/bases, that are compatible with the instrument system. Samples that are not soluble in a solvent require digestion. A variety of digestion techniques could be used to dissolve a sample. These include hot-plate and microwave-assisted digestions, including open-vessel and closed-vessel approaches. Note that open-vessel digestion generally is not recommended for the analysis of volatile metals, e.g., selenium and mercury.

Analysis

The instrument must be standardized for quantitation at the time of use, following the procedure as directed in the individual monograph for the instrumental parameters. The response of standard solutions that bracket the target concentration of an analyte in a sample is determined against an appropriate blank. The detector response is plotted as a function of the analyte concentration. When an analysis is performed at or near the detection limit, the analyst cannot always use a bracketing standard. In such a case, a standard prepared at or near (within ±10% of) the detection limit should be run in triplicate, with an acceptance criteria of ±10% of the theoretical value for the standard for single-element standards, and ±20% of the theoretical value for the standard for multi-element standards.

To demonstrate the stability of the system's initial standardization, the analyst must reassay a solution used in the initial standard curve as a check standard at appropriate, pre-established intervals throughout the analysis of the sample set. Unless otherwise indicated in the individual monograph, the reassayed standard should meet the same acceptance criteria as those outlined in the PQ section above. Sample concentrations are calculated versus the working curve generated by plotting the detector response versus the concentration of the analyte in the standard solutions, and most instruments perform this calculation automatically.

VALIDATION AND VERIFICATION

Validation

Validation is required when the ICP–OES or ICP–MS method is intended for use as an alternative to the official procedure for testing an official article. The objective of the ICP–OES or the ICP–MS procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). [NOTE—For definition of different categories, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category of the test, analytical procedure validation for the ICP–OES or ICP–MS requires the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

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General chapter ⟨1225⟩ provides definitions and general guidance on the procedures for analytical validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology.

ACCURACY

For Category I assays or Category II tests, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of elements. It is also an acceptable practice to compare assay results obtained using the ICP–OES or ICP–MS procedure under validation to those of an established analytical procedure. In standard addition methods, accuracy assessments are based on the final intercept concentration, not the recovery calculated from the individual standard additions.
Validation criteria: 95.0%–105.0% mean recovery for the assay, and 70.0%–150.0% mean recovery for the impurity analysis. These criteria apply throughout the intended range.

Precision

REPEATABILITY

The analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration.
Validation criteria: The relative standard deviation is NMT 5.0% for the drug substance assay, NMT 5.0% for the drug product assay, and NMT 20% for the impurity analysis.

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the procedure should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, any combination of these factors totaling three experiments will provide an estimation of intermediate precision. For example, this could be one analyst on each of 3 days, or one analyst on two sets of equipment on two different days for one instrument; three analysts could be on the same piece of equipment.
Validation criteria: The relative standard deviation is NMT 8.0% for the drug substance assay, NMT 8.0% for the drug product assay, and NMT 25.0% for the impurity analysis.

SPECIFICITY

The procedure must be able to unequivocally assess each analyte element in the presence of components that are expected to be present, including any matrix components.
Validation criteria: Demonstrated by meeting the accuracy requirement

QUANTITATION LIMIT

The limit of quantitation (QL) is estimated by calculating the standard deviation of NLT 10 replicate measurements of a blank solution and multiplying by 10. When validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used (see ⟨1225⟩).

A measurement of a test solution prepared from a representative sample matrix spiked at the estimated QL concentration must be performed to confirm accuracy. When validating a procedure using the method of standard additions, the validation criterion applies to the final experimental result, not the spike recovery of the individual standard addition levels.
Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

LINEARITY

A response curve between the analyte concentration and absorbance is prepared from NLT two standard solutions and a blank (for a total of three data points), at concentrations that encompass the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods, such as a least-squares regression.

For experiments that do not yield a linear relationship between analyte concentration and the ICP–OES or ICP–MS response, appropriate statistical methods must be applied to describe the analytical response.
Validation criteria: Correlation coefficient (R), NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests

RANGE

Range is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity that is demonstrated by meeting the precision, accuracy, and linearity requirements.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 70.0%–130.0% of the acceptance criteria.

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ROBUSTNESS

The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. Because certain changes to experimental parameters could result in potential safety issues or damage to equipment, robustness is demonstrated by meeting the intermediate precision requirements set forth above.

Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP–NF*, are not required to validate these procedures if they are provided in a monograph. Instead, users must simply verify their suitability under actual conditions of use.

The objective of ICP–OES or ICP–MS procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, should be executed by the user with suitable accuracy, specificity, and precision using the instruments, analysts, and sample matrices available. According to *Verification of Compendial Procedures* ⟨1226⟩, if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Verification of compendial ICP–OES or ICP–MS methods should, at a minimum, include the execution of the validation parameters for specificity, accuracy, precision, linearity, and limit of quantitation, when appropriate, as indicated in *Validation*.

⟨731⟩ LOSS ON DRYING

The procedure set forth in this chapter determines the amount of volatile matter of any kind that is driven off under the conditions specified. For substances appearing to contain water as the only volatile constituent, the procedure given in the chapter, *Water Determination* ⟨921⟩, is appropriate, and is specified in the individual monograph.

Unless otherwise directed in the individual monograph, conduct the determination on a 1- to 2-g test specimen. Mix the substance to be tested and, if it is in the form of large particles, reduce the particle size to about 2 mm by quickly crushing before weighing out the test specimen. Tare an appropriate glass-stoppered, shallow weighing bottle that has been dried for about 30 minutes under the same conditions to be employed in the determination and cooled to room temperature in a desiccator. Put the test specimen in the bottle, replace the cover, and accurately weigh the bottle and the contents. By gentle, sidewise shaking, distribute the test specimen as evenly as practicable to a depth of about 5 mm generally, and not more than 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the test specimen at the temperature and for the time specified in the monograph.

[NOTE—The temperature specified in the monograph is to be regarded as being within the range of ±2° of the stated figure.] When "dry to constant weight" is specified in a monograph, drying shall be continued until two consecutive weighings do not differ by more than 0.50 mg per g of substance taken, the second weighing following an additional hour of drying. Upon opening the chamber, close the bottle promptly, and allow it to come to room temperature in a desiccator before weighing.

If the substance melts at a lower temperature than that specified for the determination of *Loss on Drying*, maintain the bottle with its contents for 1 to 2 hours at a temperature 5° to 10° below the melting temperature, then dry at the specified temperature.

Where capsules are to be tested, use a portion of the mixed contents of not fewer than 4 capsules.

Where tablets are to be tested, use powder from not fewer than 4 tablets.

Where the individual monograph directs that loss on drying be determined by thermogravimetric analysis, a sensitive electrobalance is to be used.

Where drying in vacuum over a desiccant is directed in the individual monograph, a vacuum desiccator or a vacuum drying pistol, or other suitable vacuum drying apparatus, is to be used.

Where drying in a desiccator is specified, exercise particular care to ensure that the desiccant is kept fully effective by frequent replacement.

Where drying in a capillary-stoppered bottle[1] in vacuum is directed in the individual monograph, use a bottle or tube fitted with a stopper having a 225 ± 25-µm diameter capillary, and maintain the heating chamber at a pressure of 5 mm or less of mercury. At the end of the heating period, admit dry air to the heating chamber, remove the bottle, and with the capillary stopper still in place allow it to cool to room temperature in a desiccator before weighing.

⟨733⟩ LOSS ON IGNITION

This procedure is provided for the purpose of determining the percentage of test material that is volatilized and driven off under the conditions specified. The procedure, as generally applied, is nondestructive to the substance under test; however, the substance may be converted to another form such as an anhydride.

Perform the test on finely powdered material, and break up lumps, if necessary, with the aid of a mortar and pestle before weighing the specimen. Weigh the specimen to be tested without further treatment, unless a preliminary drying at a lower temperature, or other special pretreatment, is specified in the individual monograph. Unless other equipment is designated in the individual monograph, conduct the ignition in a suitable muffle furnace or oven that is capable of maintaining a temperature

[1] Available as an "antibiotic moisture content flask" from Kimble-Kontes, 1022 Spruce St., Vineland, NJ 08362-1502.

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within 25° of that required for the test, and use a suitable crucible, complete with cover, previously ignited for 1 hour at the temperature specified for the test, cooled in a desiccator, and accurately weighed.

Unless otherwise directed in the individual monograph, transfer to the tared crucible an accurately weighed quantity, in g, of the substance to be tested, about equal to that calculated by the formula:

$$10/L$$

in which L is the limit (or the mean value of the limits) for *Loss on ignition*, in percentage. Ignite the loaded uncovered crucible, and cover at the temperature (±25°) and for the period of time designated in the individual monograph. Ignite for successive 1-hour periods where ignition to constant weight is indicated. Upon completion of each ignition, cover the crucible, and allow it to cool in a desiccator to room temperature before weighing.

⟨735⟩ X-RAY FLUORESCENCE SPECTROMETRY

INTRODUCTION

X-ray fluorescence (XRF) spectrometry is an instrumental method based on the measurement of characteristic X-ray photons caused by the excitation of atomic inner-shell electrons by a primary X-ray source. The XRF method can be used for both qualitative and quantitative analysis of liquids, powders, and solid materials. The X-rays produced by an X-ray tube include characteristic lines that correspond to the anode material and a continuum known as Bremsstrahlung radiation. Both types of X-rays can be used to excite atoms and thus induce X-rays. XRF instrumentation can be divided into one of two categories: Wavelength Dispersive X-ray Fluorescence (WDXRF) and Energy Dispersive X-ray Fluorescence (EDXRF). The main factor distinguishing these technologies is the method used to separate the spectrum emitted by the atoms in the sample. The energy of the X-ray photon is characteristic for a given electron transition in an atom and is *qualitative* in nature. The *intensity* of the emitted radiation is indicative of the number of atoms in the sample and constitutes the *quantitative* nature of the method.

QUALIFICATION OF XRF SPECTROMETERS

Installation Qualification

See also *USP* general information chapter *Analytical Instrument Qualification* ⟨1058⟩.

Operational Qualification

The purpose of operational qualification (OQ) is to verify that the system operates within target tolerances using appropriate samples with known spectral properties. OQ is a check of the key operating parameters and should be performed following installation, repairs, or major maintenance that can affect the performance of the instruments. Note that all calibration samples must be handled with cotton or nitrile gloves and must be stored in sealed plastic containers. Alternatively, they may be fixed in the instrument.

The OQ tests and specifications in the following sections are typical examples only (see *Tables 1* and *3*). Other tests and standards can be used to establish tolerances for these purposes. The instrument vendor often makes samples and test parameters available as part of the IQ package.

Table 1. EDXRF OQ Specifications

Test	Procedure	Acceptance Criteria
Peak position	Acquire a spectrum of the Al–Cu energy calibration sample.	The spectrum should have at least 3000 counts at the top of both the Al Kα and the Cu Kα peaks. The energy corresponding to the peaks of Al Kα and Cu Kα should differ less than 0.1% from the tabulated values.
Detector resolution	Calculate the resolution (full width, half maximum) at the same energy and at the same count rate that was used at IQ.	The resolution value should not change more than 10% from the value determined at IQ.
Count rate	Measure the count rate of Al and Cu Kα lines from the energy calibration sample.	<10% change from initial measurements at IQ, for each peak

The Al–Cu energy calibration sample is a disc of Al–Cu alloy (EN AW-AlCu6BiPb; Alloy 2011, ASTM B211) that has been selected for use in EDXRF spectrometers. This alloy is generally available, is resistant to corrosion, and provides adequate intensities for both Al Kα and Cu Kα. These characteristic lines cover the typical energies used for XRF analysis. Also, sufficient information can be obtained from spectra recorded on this material to assess detector resolution. A more complete compositional specification for this calibration sample is given in *Table 2*.

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Table 2. Specification of the Concentration of the Alloying Elements in Al–Cu Alloy (the Remaining Balance Is Aluminum)

Element	Concentration Limits (in % by Weight)
Cu	5–6
Zn	0.3 maximum
Fe	0.7 maximum
Bi	0.2–0.6
Pb	0.2–0.6
Si	0.4 maximum

When the energy range of the analytical lines for which the spectrometer will be used includes energies above 50 keV, a pure W metal (99.5% minimum) should be used instead of the Al–Cu alloy. This metal is stable, is generally available, and provides well-defined characteristic lines at 8.40 keV (L-lines) and 59.3 keV (K-lines).

Table 3. WDXRF OQ Specifications

Test	Procedure	Acceptance Criteria
Peak angle	Perform according to the manufacturer's procedure. Repeat for each crystal.	The angle corresponding to the peak maximum should differ less than 0.10 degree 2θ of the angle measured at IQ.
Detector resolution	Full width half maximum at specified wavelengths and at the same measurement conditions at the time of IQ. Repeat for each detector available.	NMT 20% change
Count rate	Measure count rate from the specified monitor specimen at a specified wavelength at the same measurement conditions at the time of IQ. Repeat for each detector available.	<10% change from initial measurements at IQ

Use Inconel 625 (Special Metals Corporation, New Hartford, NY) as a sample for WDXRF spectrometers. Other designations for this alloy are UNS N06625, DIN 2.4856, ASTM B443, ASME SB-443, and AMS 5599. This is a nickel-based alloy that includes chromium, molybdenum, and niobium as the most important alloying elements. A more complete compositional specification is given in *Table 4*.

Table 4. Elemental Concentrations in Inconel 625

Element	Concentration Limits (in % by Weight)
Ni	58.0 minimum
Cr	20.0–23.0
Fe	5.0 maximum
Mo	8.0–10.0
Nb[a]	3.15–4.15

[a] This could include Ta.

A polished piece of Inconel 625 should never require resurfacing when stored and used appropriately.

The radiation characteristics of nickel can be detected by all detectors that are used in sequential WDXRF, whether they are flow proportional, sealed gas, or scintillation detectors. In combination with Mo K (and L) radiation, the tests regarding peak position and detector response of WDXRF spectrometers can be completed readily. *Table 5* includes the typical wavelengths or energies that are used for XRF analysis.

Table 5. Energies and Wavelengths[a] for Al, Ni, Cu, Mo, and W

	Al	Ni	Cu	Mo	W
$K\alpha_1$ Line transition	$K–L_{2,3}$	$K–L_{2,3}$	$K–L_{2,3}$	$K–L_3$	$K–L_3$
$K\alpha_1$ Energy (eV)	1487	7473	8041	17479	59310
$K\alpha_1$ Wavelength (Å)	8.340	1.659	1.542	0.709	0.209
$L\alpha_1$ Line transition	N/A[b]	N/A	N/A	$L_3–M_5$	$L_3–M_5$
$L\alpha_1$ Energy (eV)	N/A	N/A	N/A	2293.2	8398.2

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Table 5. Energies and Wavelengths[a] for Al, Ni, Cu, Mo, and W *(continued)*

	Al	Ni	Cu	Mo	W
$L\alpha_1$ Wavelength (Å)	N/A	N/A	N/A	5.4066	1.47632

[a] From: Deslattes RD, Kessler EG, Indelicato P, et al. X-ray transition energies: new approach to a comprehensive evaluation. *Rev Mod Phys.* 2003;75(1):35–99. For Al, Ni, and Cu, the $K\alpha_1$ and $K\alpha_2$ energies from *Table V* were averaged with 2:1 weighting. Wavelength conversion uses the *hc/E* value from page 94. Values for Mo and W are taken from *Table VI*.
[b] N/A = not applicable.

Performance Qualification

The purpose of performance qualification (PQ) is to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality measurements.

Depending on typical use, the specifications for PQ may be different from the manufacturer's specifications. Method-specific PQ tests, also known as system suitability tests, may be used in lieu of PQ requirements for validated methods.

Specific procedures, acceptance criteria, and time intervals for characterizing XRF performance depend on the instrument and its intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be obtained from sample spectra using previously validated XRF experiments.

PROCEDURE

General Recommendations

Analysts should check the suitability of all reagents and materials for contamination before using depending on the method used. The analysis of a ubiquitous element often requires the use of the purest grade of reagent or material available. Sample holders and support windows should be appropriate for the analysis and the instrument configuration. Analysts should evaluate the cleaning of equipment used to prepare samples for XRF analysis in order to avoid cross-contamination of samples.

SAMPLES

Liquids: Liquid samples can be introduced directly to the XRF spectrometer provided that the solution consists of a single phase and has sufficiently low volatility. Analysis of liquid samples requires use of a special liquid sample holder and a commercially available support window composed of a suitable polymer film. Alternatively, liquid samples can be transferred onto the surface of a disk and dried before analysis. The experiment typically is conducted using a purge gas. The liquid sample can be spiked directly with solution standards at appropriate concentrations to facilitate accuracy, precision, and specificity tests as required for method validation.

Powders: Prepared powders may be measured directly in a liquid sample holder. Alternatively, they may be pressed into pellets. If the powder has poor self-binding properties, it may require a binder such as a wax or ethyl cellulose.

Powders also can be prepared for XRF analysis by fusing the sample material into a glass using a flux, typically sodium tetraborate, lithium tetraborate, and lithium metaborate. Because the temperatures required to melt the flux and dissolve the sample are relatively high (800°–1300°), this procedure is not suitable for the analysis of volatile elements such as mercury and arsenic.

Powder samples can be mixed with appropriate quantities of a certified reference material to facilitate accuracy, precision, and specificity tests as required for method validation. Alternatively, powder samples can be spiked with appropriate quantities of solution-based standards and then dried, ground if necessary, and thoroughly mixed before analysis. Standard additions can be used in instances when physical or chemical properties of the powder may introduce an analyte response bias.

Standards

Appropriate reference materials that are traceable to the National Institute of Standards and Technology, or equivalent, can be used in the preparation of XRF standards.

Analysis

For the instrumental parameters (if applicable) follow the procedure in the individual monograph. Because of differences in manufacturers' equipment configurations, analysts can use the manufacturer's suggested default conditions. At the time of use, the instrument must be standardized for the intended use. Analysts should use calibration standards to bracket the expected range of typical analyte concentrations. When they perform an analysis at or near the detection limit, analysts cannot always use a bracketing standard, which is an acceptable strategy for limit tests. Analysts should use regression analysis of the standard plot to evaluate the linearity of detector response, and individual monographs may set criteria for the residual error of the regression line.

To demonstrate the stability of the system's initial standardization, at appropriate intervals throughout their tests on the sample set analysts must re-assay the calibration standard used in the initial standard curve as a check standard. The use of an independently prepared standard also is acceptable. Unless otherwise indicated in the individual monograph, the re-assayed standard should agree with its expected value to within ±2% for *Assay* or ±20% for an impurity analysis.

Sample concentrations are calculated versus the working curve generated by plotting the instrument response versus the concentration of the analyte in the standard solutions.

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VALIDATION AND VERIFICATION

Current Good Manufacturing Practice regulations [21 CFR 211.194(a)(2)] indicate that users of analytical methods described in *USP–NF* are not required to validate the accuracy and reliability of these methods but rather must verify their suitability under actual conditions of use. In this context, and according to these regulations, validation is required when an XRF procedure is used to test a nonofficial article and when this procedure is used as an alternative to the official procedure for testing an official article (see *USP–NF General Notices, 6.30 Alternative and Harmonized Methods and Procedures*). On the other hand, verification must be performed the first time an official article is tested using a *USP* procedure (for informational purposes only, refer to *Verification of Compendial Procedures* ⟨1226⟩).

Validation

The objective of an XRF method validation is to demonstrate that the measurement procedure is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). Depending on the category of the test (see *Table 2* in *Validation of Compendial Procedures* ⟨1225⟩), the analytical method validation process for XRF requires the testing of linearity, range, accuracy, specificity, precision, quantitation limit, and robustness.

Performance characteristics that demonstrate the suitability of an XRF method are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in ⟨1225⟩. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the method. The samples for validation should be independent of the calibration set.

ACCURACY

For Category I assays or Category II tests, analysts can determine accuracy by conducting recovery studies using the appropriate matrix spiked with known concentrations of elements. An appropriate certified standard material provided by USP also can be used. It also is an acceptable practice to compare assay results obtained using the XRF method under validation to those from an established analytical method. When analysts use the method of standard additions, accuracy assessments are based on the final intercept concentration, not the recovery calculated from the individual standard additions.
Acceptance Criteria: 98.0%–102.0% recovery for drug substances and drug product assay, 70.0%–150.0% recovery for impurity analysis. These acceptance criteria should be met throughout the validated range.

PRECISION

Repeatability: Analysts should assess the analytical method by measuring the concentrations of six separate standards at 100% of the assay test concentration. Alternatively, they can measure the concentrations of three replicates of three separate samples at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. In this case, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Acceptance Criteria: The relative standard deviation is NMT 1.0% for drug substance assay, NMT 2.0% for drug product assay, and NMT 20.0% for impurity analysis.

Intermediate Precision: Analysts should establish the effect of random events on the method's analytical precision. Typical variables include performing the analysis on different days, using different instrumentation, or having two or more analysts perform the method. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Acceptance Criteria: The relative standard deviation is NMT 1.0% for drug substance assay, NMT 3.0% for drug product assay, and NMT 25.0% for impurity analysis.

SPECIFICITY

The procedure must unequivocally assess each analyte element in the presence of components that may be expected to be present, including any matrix components.
Acceptance Criteria: Demonstrated by meeting the *Accuracy* requirement.

QUANTITATION LIMIT

The limit of quantitation (LOQ) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank and multiplying by 10. Other suitable approaches may be used (see ⟨1225⟩). A measurement of a test sample prepared from a representative sample matrix and spiked so that the concentration is similar to the estimated LOQ concentration must be performed to confirm accuracy.
Acceptance Criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

LINEARITY

Analysts should demonstrate a linear relationship between the analyte concentration and corrected XRF response by preparing no fewer than five standards at concentrations that encompass the anticipated concentration of the test sample. The standard curve then should be evaluated using appropriate statistical methods such as a least squares regression. The correlation coefficient (R), y-intercept, and slope of the regression line must be determined.

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For experiments that do not have a linear relationship between analyte concentration and XRF response, appropriate statistical methods must be applied to describe the analytical response.
Acceptance Criteria: *R* is NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

The range is the interval between the upper and lower concentration (amounts) of analyte in the sample (including the upper and lower concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. Range is demonstrated by meeting the linearity and accuracy requirement.
Acceptance Criteria: For 100.0% centered acceptance criteria: 80.0%–120.0%. For non-centered acceptance criteria: 10% below the lower limit of the specification to 10% above the upper limit of the specification. For content uniformity: 70.0–130.0%. For Category II the range requirements are 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters. For XRF this can include measuring the stability of the analyte under specified storage conditions.
Acceptance Criteria: The measurement of a standard or sample response following a change in experimental parameters should differ from the same standard measured using established parameters by NMT ±2.0% for a drug product assay and NMT ±20.0% for an impurity analysis.

Verification

The objective of an XRF method verification is to demonstrate that the procedure as prescribed in a specific monograph is being executed with suitable accuracy, sensitivity, and precision. According to ⟨1226⟩, if the verification of the compendial procedure according to the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *USP–NF General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Although complete revalidation of a compendial XRF method is not required, verification of compendial XRF methods should at minimum include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated under *Validation* (above).

⟨736⟩ MASS SPECTROMETRY

INTRODUCTION

Mass spectrometry (MS) is an analytical technique based on the measurement of the mass-to-charge ratio of ionic species related to the analyte under investigation. MS can be used to determine the molecular mass and elemental composition of an analyte as well as provide an in-depth structural elucidation of the analyte.

In addition to being recognized as a powerful structure-elucidation tool, MS is also extensively used for quantitative measurements. For additional information, see general information chapter *Applications of Mass Spectrometry* ⟨1736⟩, which provides a detailed discussion of MS.

Currently available MS instrumentation offers a wide range of capabilities for qualitative and quantitative analysis, which results in a wide range of potential MS experimental approaches for a given measurement need. Because of the diversity of approaches, this chapter does not present specific procedures but instead provides experimental and system suitability information for MS procedures.

QUALITATIVE ANALYSIS

MS is a sensitive and highly specific technique for the identification of analytes. Identification or verification of structure (i.e., comparison against an authentic standard) by MS is particularly powerful when used in conjunction with a separation technique such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). Additional degrees of specificity can be obtained by the use of tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS). Also, tighter tolerances on mass accuracy are also afforded using high-resolution mass spectrometry (HRMS).

Experimental Parameters

The following MS experimental parameters should be defined for a qualitative (e.g., identification) procedure.

MASS RESOLUTION

Unit mass resolution is sufficient for most identification tests. When higher resolution is required, the resolution is specified in the procedure, and demonstration of adequate resolution is included in the system suitability tests for the procedure.

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MASS ACCURACY

A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. A mass accuracy or agreement of ±0.05% from a standard should be sufficient for the identification of large molecules (above 2000 m/z) when multiply charged ions are employed for the identification test.

When higher mass accuracy is required, the required mass accuracy is specified in the procedure. A demonstration of the mass accuracy then is included in the system suitability tests for the procedure or as part of the instrument's established performance qualification (PQ) procedure discussed later in this chapter.

MASS RANGE

The mass range to be scanned is also presented in the procedure. The mass range must encompass all ions used as part of the identification confirmation.

System Suitability

The system suitability for the MS procedure should include demonstration of adequate performance for the following experimental attributes.

MASS RESOLUTION

A demonstration of the appropriate resolution is included in the system suitability tests for a procedure. The performance test in the instrument's established PQ, executed daily or prior to the time of use, may suffice. When resolutions greater than unit mass are required for a procedure, the system suitability test includes demonstration of adequate resolution along with acceptance criteria.

MASS ACCURACY

A demonstration of the mass accuracy is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. If higher mass accuracy is required for a given procedure, the appropriate acceptance criteria are specified.

Interpretation Directions

An identification or verification experiment involves comparison of the compound of interest to an authentic standard (e.g., a USP Reference Standard). For this form of identification or verification, the standard and the sample are run under identical conditions and should have results that are within acceptable experimental error for the procedure. Applications of MS for identification purposes in a compendial monograph may incorporate the mass spectral data alone or may combine this spectral identification with the chromatographic retention time if more specificity is dictated by the particular monograph identification needs.

The procedure should also provide specific instructions that define a successful mass spectral match. If the procedure represents the only spectroscopic identification test or if no other identification tests (e.g., peptide mapping or amino acid analysis) provide structural information, the spectrum of the standard should closely resemble that of the sample and a minimum of three structurally relevant ions, preferably one of which is an ion representing the molecular mass of the analyte, should be used for the comparison. In the case where only the ion representing the intact molecule is produced, the accurate mass or MS/MS spectrum of the molecular ion may be used to strengthen the identification. If other structurally relevant identification tests are also conducted, the comparison can be conducted with fewer ions as long as one of the ions represents the molecular mass of the analyte. For example, the MS identification test for a protein may examine only an ion that represents the molecular mass if several other tests are conducted to confirm the protein structure.

QUANTITATIVE ANALYSIS

The sensitivity and specificity of MS also make it a suitable analytical tool for the quantification of analytes. Quantification is particularly powerful when used in conjunction with a separation technique such as GC or HPLC. Further degrees of specificity can be obtained by the use of MS/MS or HRMS.

Experimental Parameters

The following experimental parameters should be defined within a quantitative (e.g., identification) MS procedure.

MASS RESOLUTION

Unit mass resolution is sufficient for most quantitative tests. When higher resolution is required, the resolution is specified in the procedure, and demonstration of the mass accuracy is included in the system suitability tests for the procedure.

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MASS ACCURACY

The mass accuracies listed in the previous qualitative section should be sufficient for most quantitative applications. When higher mass accuracy is required, it is specified in the procedure. A demonstration of the mass accuracy is included in the system suitability tests for the procedure or as part of the instrument's established PQ procedure.

MASS SELECTION

The masses to be monitored (e.g., mass range, individual masses, or MS/MS transitions) are presented in the procedure.

System Suitability

The system suitability for the MS procedure should include demonstration of adequate performance for the following experimental attributes, as appropriate for the procedure.

MASS RESOLUTION

A demonstration of the appropriate resolution is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. When resolutions greater than unit mass are required for a procedure, the system suitability test includes demonstration of adequate resolution along with acceptance criteria.

MASS ACCURACY

A demonstration of the mass accuracy is included in the system suitability tests for the procedure or is part of the instrument's established PQ procedure. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. If higher mass accuracy is required for a given procedure, the appropriate acceptance criteria are specified.

PRECISION

The system suitability includes a demonstration of adequate precision. See *Table 1* for maximum limits for precision. Typically, system suitability limits are set to tighter limits than those employed to ensure adequate precision for the validation experiments. (See also the section on *Validation and Verification of Mass Spectrometry Analytical Procedures*.)

LINEARITY

The system suitability includes a demonstration of adequate linearity. See *Table 1* for appropriate linearity limits. (See also the section on *Validation and Verification of Mass Spectrometry Analytical Procedures*.)

ACCURACY

In certain situations, quality control (or check) samples may also be appropriate for inclusion in the procedure to ensure the quality of the measurement. Typically, these quality control samples are of known analyte concentration and are prepared identically to the test samples. If used, quality control (or check) samples are also prepared as a verification of time-of-application method accuracy. The procedure specifies the number or analysis order of quality control (or check) samples needed. Acceptance criteria for calibration and quality control (or check) sample results should be aligned with the validation requirements as required by the application type (i.e., Category I or II) as outlined in *Table 1*.

QUANTITATION LIMIT

In certain applications (e.g., limits tests), it may be necessary to include demonstration of the ability to detect the analyte at a prescribed level. For these applications, the procedure specifies the limit and success criteria (e.g., signal-to-noise ratio).

QUALIFICATION OF MASS SPECTROMETRY INSTRUMENTS

Qualification of an MS instrument can be divided into three elements: installation qualification (IQ), operational qualification (OQ), and PQ. For additional information, see *Analytical Instrument Qualification* ⟨1058⟩, which may be a helpful, but not mandatory resource.

Installation Qualification

IQ provides evidence that the hardware and software are installed to accommodate safe and effective use of the instrument at the desired location.

Operational Qualification

In OQ, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable for a given application. Because

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so many different approaches are available for measuring MS spectra, OQ using standards with known spectral properties is recommended. Because of the diversity of MS instrumentation, interfaces, and experimental approaches, MS instruments should be qualified against target specifications for the intended application, not simply the specifications supplied by the manufacturer.

Performance Qualification

PQ helps to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality measures. PQ documentation should describe the following:

- the definition of the specific performance criteria and detailed test procedures, including test samples and instrument parameters;
- the elements that will be measured to evaluate the criteria and the predefined specifications;
- the test interval, which may be daily or time-of-use measurements;
- the use of bracketing samples or groups of samples; and
- corrective actions that will be implemented if the spectrometer does not pass the specifications.

Periodic PQ should include a subset of the OQ tests to ensure that the instrument as supplied is performing at a level that produces data that are suitable for their intended use. Depending on typical use, the specifications for PQ may be higher or lower than the manufacturer's installation specifications. Method-specific PQ tests, also known as system suitability tests, may be used in lieu of PQ requirements for validated procedures.

Because of the diversity of MS instrumental configurations and experimental designs, a standard sample or experiment for all PQ assessments may not be available. Thus, method-specific PQ tests or system suitability tests often are needed. The PQ experimental design should be sufficiently robust to ensure proper instrument performance for the intended application, including the specifications associated with the measurement. At minimum, PQ experiments should include the following.

- For qualitative applications, the PQ experiment includes a check of the mass accuracy of the instrumentation. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications.
- For quantitative applications, the PQ experiment includes checks of mass accuracy and precision. A mass accuracy or agreement of ±0.50 mass units for singly charged ions from a known standard should be sufficient for most applications. The success criteria for precision is established via consideration of the instrument and method capability, and provides sufficient controls relative to the specification for the measurement in question.

Characterizing Instrument Performance

Specific procedures, acceptance criteria, and time intervals for characterizing MS spectrometer performance depend on the instrument and its intended applications. Many MS applications use previously validated experiments that relate MS spectra to a chemical property of interest. Analysts typically demonstrate stable instrument performance over extended periods of time. This practice provides some assurance that reliable measurements can be taken from sample spectra using previously validated MS experiments.

VALIDATION AND VERIFICATION OF MASS SPECTROMETRY ANALYTICAL PROCEDURES

Validation is required only when an MS procedure is an alternative to the official procedure for testing an official article.

The objective of validating an MS procedure is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities (Category II), and identification tests (Category IV). [NOTE—For additional information on the different category definitions, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category of the test, analytical procedure validation requires the testing of linearity, range, accuracy, specificity, precision, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

Chapter ⟨1225⟩ provides definitions and general guidance about analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

Measurement Categories for Mass Spectrometry Analytical Procedures

The required validation performance characteristics of an MS analytical procedure, assuming the typical Category I USP specifications of 98.0%–102.0% for drug substances and 95.0%–105.0% for drug products, are listed in *Table 1*. The actual validation performance characteristics would be dependent upon the specifications in place and should provide sufficient evidence that the measurement capability is sufficient for those specifications. A procedure validation protocol must specify the required validation experiments and validation criteria. These criteria are determined according to the intended purpose of the analytical procedure.

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Table 1. Analytical Measurement Requirements

Analytical Performance Characteristics	Category I	Category II Quantitative
Specificity	Ensured by use of a reference standard when possible and demonstrable lack of interference from other components	
Linearity	Correlation coefficient *(R)* NLT 0.995	Correlation coefficient *(R)* NLT 0.99
Range	For 100.0% centered acceptance criteria: 80.0%–120.0%. For noncentered acceptance criteria: 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity: 70.0%–130.0%	50%–120%
Accuracy	98.0%–102.0% (drug substance) 95.0%–105.0% (drug product)	80.0%–120.0%
Repeatability	NMT 1.0% (drug substance) NMT 2.0% (drug product)	NMT 20.0%
Intermediate precision	NMT 1.0% (drug substance) NMT 3.0% (drug product)	NMT 25.0%
Quantitation limit	—	The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.
Robustness	The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters.	

Analytical Procedure Validation

The objective of analytical procedure validation is to demonstrate that the analytical procedure is suitable for its intended purpose by conducting experiments and obtaining results that meet predefined acceptance criteria. MS analytical procedures can include quantitative tests for major component and impurities content, limit tests for the presence of impurities, quantification of a component in a product or formulation, or identification tests.

VALIDATION PARAMETERS

Performance characteristics that demonstrate the suitability of an analytical procedure are similar to those required for any analytical procedure. For additional information on the applicable general principles, see ⟨1225⟩. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the analytical procedure.

The performance characteristics that are required as part of a validation for each of the analytical procedure categories are given in *Table 1*.

Specificity: The purpose of a specificity test is to demonstrate that measurements of the intended analyte signals are free of interference from components and impurities in the test material. Specificity tests can be conducted to compare spectra of components and impurities that are known from synthetic processes, formulations, and test preparations. Specificity is also to be demonstrated for any materials added as part of the procedure (e.g., specificity versus isotope-labeled internal standards).

For an identification MS analytical procedure (Category I and II), validation experiments may include multidimensional MS experiments to validate correct assignments of an ion's structure or origin.

Linearity: A linear relationship is exhibited between the analyte concentration and instrument response. This is demonstrated by measuring analyte responses from NLT five standard solutions at concentrations that encompass the anticipated concentration range of analyte(s) in the test solution. For Category I, standard solutions can be prepared from reference materials in appropriate solvents. For Category II (MS analytical procedures that are used to quantitate impurities), linearity samples are prepared by spiking suitable test samples that contain low amounts of analyte or by spiking matrix samples at concentrations of the expected range. The standard curve then is constructed using appropriate statistical analytical procedures such as a least-squares regression. The correlation coefficient (*R*), *y*-intercept, slope of the regression line, and residual root mean square are then determined. Absolute values determined for these factors are appropriate for the procedure being validated.

Range: The range between the low and high concentrations of analyte is given by the quantitative MS analytical procedure. This typically is based on test article specifications in the *USP* monograph. It is the range within which the analytical procedure can demonstrate an acceptable degree of linearity, accuracy, and precision and can be obtained from an evaluation of that analytical procedure.

Recommended ranges for various MS analytical procedures are as follows.

- For Category I—assay of a drug substance (or a finished product): 80%–120% of the test concentration;
- For Category I—content uniformity: a minimum of 70%–130% of the test concentration;
- For Category II—determination of an impurity: 50%–120% of the acceptance criteria.

Accuracy: The accuracy of a quantitative MS analytical procedure is determined across the required analytical range. Typically, three levels of concentrations are evaluated using triplicate preparations at each level.

Preparation of accuracy samples: For drug substance assays (Category I), accuracy is determined by analyzing a reference standard of known purity. For drug product assays (Category I), a composite sample of reference standard and other components in a pharmaceutical finished product should be used for analytical procedure validation. The assay results are

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compared to the theoretical value of the reference standard to estimate errors or percent recovery. For the quantitation of impurities (Category II), the accuracy of the analytical procedure can be determined by conducting studies with drug substances or products spiked with known concentrations of the analyte under test.

Assay results from the analytical procedure being validated may be compared to those of an established alternative analytical procedure.

Precision:

Repeatability: The analytical procedure is assessed by measuring the concentrations of three replicates of separate standard solutions at three different concentrations that encompass the analytical range. Alternatively, the concentrations of six separate standard solutions at 100% of the test concentration can be measured. The relative standard deviation from the replicate measurements is then evaluated to determine if the solutions meet the acceptance criteria.

Intermediate precision: The effect of random events on the analytical precision of the analytical procedure is to be established. Typical variables include performing the analysis on different days, using different instruments that are suitable as specified in the analytical procedure, or having the analytical procedure performed by two or more analysts.

Quantitation limit: The quantitation limit is validated by measuring six replicates of test samples spiked with analyte at 50% of specification.

From these replicates, analysts are then able to determine accuracy and precision. Examples of specifications for Category II quantitative determinations are that the measured concentration is within 70%–130% of the spike concentration and the relative standard deviation is NMT 15%.

Robustness: The reliability of an analytical measurement is demonstrated with deliberate changes to critical experimental parameters. These can include measuring the stability of the analyte under specified storage, chromatographic, or ionization conditions.

Analytical Procedure Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* do not need to validate procedures that are provided in a monograph. Instead, they must simply verify the suitability of the procedures under actual conditions of use.

The objective of an MS procedure verification is to demonstrate that the procedure as prescribed in a specific monograph can be executed by the user with suitable accuracy, specificity, and precision using the instruments, analysts, and sample matrices available. According to the general information chapter *Verification of Compendial Procedures* ⟨1226⟩, if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures.*

Verification of a compendial MS procedure includes at minimum the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in the *Validation and Verification of Mass Spectrometry Analytical Procedures* section in this chapter.

⟨741⟩ MELTING RANGE OR TEMPERATURE

The terms melting range, melting point, or melting temperature are all used in pharmacopeial contexts. Most substances exhibit a melting transition, spanning the temperatures at which the first detectable change of phase or liquid phase is detected to the temperature at which no solid phase is apparent. The transition may appear instantaneous for a highly pure material, but usually a range is observed from the beginning to the end of the process. Factors influencing this transition include the sample size, the particle size, the efficiency of heat diffusion within the sample, and the heating rate, among other variables, that are controlled by procedure instructions.

For pharmacopeial purposes, the temperatures of the beginning (onset temperature) and end of transition (clear temperature) represent the melting range, except as defined otherwise for *Procedure for Class II* and *Procedure for Class III* below. The terms melting point and melting temperature are considered to be equivalent.

Substances which melt with no decomposition or chemical change are known to melt congruently. In these cases, the melting point is taken to be the end of the melting range, i.e., the temperature at which no solid phase is apparent. In some articles, the melting process is accompanied by simultaneous decomposition, which is visually evidenced as a side event like darkening of the material, charring, bubbling, or other incident. These transitions are known to be non-congruent. The visual impact of this side reaction frequently obscures the end of the melting process, which may be impossible to accurately determine. In those circumstances, only the beginning of the melting can be accurately established, and it is to be reported as the melting point.

Since there may be a thermal lag between the heating medium and the sample within the capillary tube, in order to achieve consistency and repeatability, it is important to perform the melting determination at a heating rate, also referred to as ramp rate, of 1°/min.

The accuracy of the apparatus to be used as described below should be checked at suitable intervals by the use of one or more of the available USP Melting Point Standards, preferably those that melt nearest the melting temperatures of the compounds being tested (see *USP Reference Standards* ⟨11⟩). The USP Melting Point Standards are intended to check the accuracy of the device and may be suitable to calibrate.

Eight procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the *Procedure for Class Ia* for crystalline or amorphous substances and the *Procedure for Class II* for waxy substances.

The procedure known as the mixed-melting point determination, whereby the melting range or temperature of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, e.g., the

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corresponding USP Reference Standard, if available, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture constitutes reliable evidence of chemical identity.

APPARATUS

Apparatus with cameras or other computerized equipment to improve accuracy, sensitivity, or precision may be used provided that the apparatus is properly qualified.

Apparatus I

An example of a suitable melting range *Apparatus I* consists of a glass container for a bath of transparent fluid, a suitable stirring device, an accurate thermometer, and a controlled source of heat. The bath fluid is selected with a view to the temperature required, but light paraffin is used generally and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied by an open flame or electrically. The capillary tube is about 10 cm long and 0.8–1.2 mm in internal diameter with walls 0.2–0.3 mm in thickness.

Apparatus II

An instrument may be used in the *Procedure for Class I*, *Procedure for Class Ia*, and *Procedure for Class Ib*. An example of a suitable melting range *Apparatus II* consists of a block of metal that may be heated at a controlled rate, with its temperature being monitored by a sensor. The block accommodates the capillary tube containing the test substance and permits monitoring of the melting process, typically by means of a beam of light and a detector. The detector signal may be processed by a microcomputer to determine and display the melting point or range, or the detector signal may be plotted to allow visual estimation of the melting point or range. Some approaches broadly used by automated systems employ optical methods such as light absorption or bulk reflection.

PROCEDURES

Procedure for Class I, Apparatus I

Reduce the substance under test to a very fine powder, and, unless otherwise directed, render it anhydrous when it contains water of hydration by drying it at the temperature specified in the monograph, or, when the substance contains no water of hydration, dry it over a suitable desiccant for NLT 16 h (or at the conditions stated in *Loss on Drying* ⟨731⟩, if appropriate).

Charge a capillary glass tube, one end of which is sealed, with a sufficient amount of the dry powder to form a column in the bottom of the tube to a nominal height of 3 mm high when packed down as closely as possible by moderate tapping on a solid surface. Due to the instrument design, alternative sample sizes may be used as instructed by the instrument manufacturer.

Heat the bath until the temperature is about 10° below the expected melting point. Remove the thermometer, and quickly attach the capillary tube to the thermometer by wetting both with a drop of the liquid of the bath or otherwise, and adjust its height so that the material in the capillary is level with the thermometer bulb. Replace the thermometer, and continue the heating, with constant stirring, sufficiently to achieve the ramp rate of about 3°/min. When the temperature is about 3° below the lower limit of the expected melting range, reduce the heating so that the ramp rate of about 1°/min is achieved. Continue heating until melting is complete.

The temperature at which the column of the substance under test is observed to collapse definitely against the side of the tube at any point indicates the beginning of melting, and the temperature at which the test substance becomes liquid throughout corresponds to the end of melting or the melting point. The two temperatures fall within the limits of the melting range. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting (melting point) is within the range specified.

Procedure for Class Ia, Apparatus I

Prepare the test substance and charge the capillary as directed in *Procedure for Class I, Apparatus I*. Heat the bath until the temperature is about 10° below the expected melting point and is rising at a rate of about 1°/min. Insert the capillary as directed in *Procedure for Class I, Apparatus I* when the temperature is about 5° below the lower limit of the expected melting range, and continue heating until melting is complete. Record the melting range as directed in *Procedure for Class I, Apparatus I*.

Procedure for Class Ib, Apparatus I

Place the test substance in a closed container and cool to 10°, or lower, for at least 2 h. Without powdering, charge the cooled material into the capillary tube as directed in *Procedure for Class I, Apparatus I*, then immediately place the charged tube in a vacuum desiccator and dry at a pressure not exceeding 20 mm of mercury for 3 h. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows. Heat the bath until the temperature is about 10° below the expected melting range, then introduce the charged tube, and heat at a rate of rise of about 1°/min until melting is complete. Record the melting range as directed in *Procedure for Class I, Apparatus I*.

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If the particle size of the material is too large for the capillary, precool the test substance as directed above, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class I, Apparatus II

Prepare the substance under test and charge the capillary tube as directed in *Procedure for Class I, Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 10° below the expected melting point. Insert the capillary tube into the heating block, and continue heating at a rate of temperature increase of about 1°/min until melting is complete.

The temperature at which the detector signal first leaves its initial value indicates the beginning of melting, and the temperature at which the detector signal reaches its final value corresponds to the end of melting, or the melting point. The two temperatures fall within the limits of the melting range. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting (melting point) is within the range specified.

Procedure for Class Ia, Apparatus II

Prepare the test substance and charge the capillary as directed in *Procedure for Class I, Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 5° below the expected melting point and is rising at a rate of about 1°/min. Insert the capillary as directed in *Procedure for Class I, Apparatus I*, and continue heating until melting is complete. Record the melting range as directed in *Procedure for Class I, Apparatus I*. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting (melting point) is within the range specified.

Procedure for Class Ib, Apparatus II

Place the test substance in a closed container and cool to 10°, or lower, for at least 2 h. Without previous powdering, charge the cooled material into the capillary tube as directed in *Procedure for Class I, Apparatus I*, then immediately place the charged tube in a vacuum desiccator, and dry at a pressure not exceeding 20 mm of mercury for 3 h. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 10° below the expected melting range, then introduce the charged tube, and heat at a rate of rise of about 1°/min until melting is complete. Record the melting range as directed in *Procedure for Class I, Apparatus I*.

If the particle size of the material is too large for the capillary, precool the test substance as directed above, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class II

Carefully melt the material to be tested at as low a temperature as possible, and draw it into a capillary tube, which is left open at both ends, to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 h, or in contact with ice for at least 2 h. Then attach the tube to the thermometer by suitable means, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level, and heat as directed in *Procedure for Class I, Apparatus I* except, within 5° of the expected melting temperature, to regulate the rate of rise of temperature of about 1.0°/min. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

Procedure for Class III

While stirring, melt a quantity of the test substance slowly until it reaches a temperature of 90°–92°. Remove the source of the heat, and allow the molten substance to cool to a temperature of 8°–10° above the expected melting point. Chill the bulb of a suitable thermometer to 5°, wipe it dry, and while it is still cold dip it into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature NMT 16°.

Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of about 2°/min to 30°, then change to a rate of about 1°/min, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the test substance. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of three determinations is 1° or greater than 1°, make two additional determinations and take the average of the five.

⟨755⟩ MINIMUM FILL

SCOPE

The following tests and specifications apply to articles such as creams, gels, lotions, ointments, pastes, powders, aerosols, and sprays that are packaged in containers. To minimize the impact of entrained air for products labeled by volume, the fill determination is performed by mass from which the volume is calculated by use of the density of the preparation.

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PURPOSE

The test for minimum fill ensures that the amount of material filled into the product conforms to the labeled amount.

PROCEDURE FOR DOSAGE FORMS OTHER THAN AEROSOLS

For Containers Labeled by Weight

Select a sample of 10 filled containers, and remove any labeling that might be altered in weight during the removal of the container contents. Thoroughly cleanse and dry the outside of the containers by a suitable means, and weigh individually. Quantitatively remove the contents from each container, cutting the latter open and washing with a suitable solvent, if necessary, taking care to retain the closure and other parts of each container that were present during the initial weighing. Dry and reweigh each empty container, together with its corresponding parts. Determine the net weight of the contents of the container by difference.

For Containers Labeled by Volume

Proceed as indicated above for products labeled by weight, but convert the mass to volume using the density of the preparation. A suggested approach to determine the density of the materials is as follows:

1. Tare a 100-mL volumetric flask containing 50.0 mL of liquid which is miscible with the formulation.
2. Add approximately 25 mL of a representative sample of the product and gently swirl the contents to mix.
3. Reweigh the flask.
4. From a buret, add an accurately measured amount of the miscible liquid to bring the flask contents to volume while gently swirling the contents of the flask. Record the volume taken from the buret.
5. Calculate the density of the sample:

$$W/V$$

W = weight of the material taken (g)

V = 50.0 mL minus the volume, in mL, of the miscible fluid necessary to adjust the contents of the flask to 100 mL

Other methods of determining the density may be employed depending on the formulation (e.g., substantially nonaqueous formulations). Similarly, if the container contents are less than 25 mL, smaller graduated vessels may be used, with the quantities of miscible liquid adjusted accordingly.

Alternatively, pour the contents of 10 containers into 10 suitable graduated cylinders, and allow to drain completely. Record the volume of the contents of each of the 10 containers.

Acceptance Criteria

This test meets the acceptance criteria either at *Stage 1* or *Stage 2*:

Stage 1:

1. The average net content of the 10 containers is NLT the labeled amount, and the net content of any single container is NLT 90% of the labeled amount where the labeled amount is 60 g or 60 mL or less, or NLT 95% of the labeled amount where the labeled amount is more than 60 g or 60 mL. If these criteria are not met, and the net content of NMT 1 container is less than 90% of the labeled amount where the labeled amount is 60 g or 60 mL or less, or 95% of the labeled amount where the labeled amount is more than 60 g or 60 mL, proceed to *Stage 2*.

Stage 2:

1. Determine the content of 20 additional containers.
2. The average content of the 30 containers is NLT the labeled amount, and the net content of NMT 1 of the 30 containers is less than 90% of the labeled amount where the labeled amount is 60 g or 60 mL or less, or less than 95% of the labeled amount where the labeled amount is more than 60 g or 60 mL.

PROCEDURE FOR AEROSOLS AND SPRAYS

Select a sample of 10 filled containers, and remove any labeling that might be altered in weight during the removal of the container contents. Thoroughly cleanse and dry the outsides of the containers by suitable means, and weigh individually. Remove the contents from each container by employing any safe technique (e.g., chill to reduce the internal pressure, remove the valve, and pour). Remove any residual contents with suitable solvents, then rinse with a few portions of methanol. Retain as a unit the container, the valve, and all associated parts, and heat them at 100° for 5 min. Cool, and again weigh each of the containers together with its corresponding parts. The difference between the original weight and the weight of the empty aerosol container is the net fill weight. Determine the net fill weight for each container tested.

Acceptance Criteria

The requirements are met if the net weight of the contents of each of the 10 containers is NLT the labeled amount.

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⟨761⟩ NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is an analytical method based on the magnetic properties of certain atomic nuclei. As is the case with other types of spectroscopy, absorption or emission of electromagnetic energy at characteristic frequencies provides structural information. NMR differs from other types of spectroscopy because the discrete energy levels between which the transitions take place are present only when the nuclei are placed in a magnetic field.

Although widely recognized as one of the most powerful structure-elucidation tools available, with proper experimental design, it can also be used for accurate qualitative and quantitative measurements. See general information chapter *Applications of Nuclear Magnetic Resonance Spectroscopy* ⟨1761⟩. [NOTE—Above 1000 chapters are for informational purposes only.]

QUALIFICATION OF NMR INSTRUMENTS

Qualification of an NMR instrument can be divided into three elements: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). For further discussion, see general information chapter *Analytical Instrument Qualification* ⟨1058⟩.

Installation Qualification

The IQ requirements provide evidence that the hardware and software are installed to accommodate safe and effective use of the instrument at the desired location.

Operational Qualification

In OQ, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable for a given application. Because so many different approaches are available for measuring NMR spectra, OQ using standards with known spectral properties is recommended. Generally, sealed NMR tubes are available as reference standards for measuring signal-to-noise (S/N) and lineshape.

Performance Qualification

PQ helps to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality (CTQ) measures. PQ documentation should describe the following:

1. The definition of the specific performance criteria and detailed test procedures including test samples and instrument parameters.
2. The elements that will be measured to evaluate the criteria and the predefined specifications.
3. The test interval, which may be time-of-use.
4. The use of bracketing samples or groups of samples.
5. The defined corrective actions that will be implemented if the spectrometer does not pass the specifications.

Periodic PQ should include a subset of the OQ tests to ensure that those aspects of the instrument that are being supplied are performing at a level that produces data that are suitable for its intended use. Depending on typical use, the specifications for PQ may be higher or lower than the manufacturer's installation specifications. Typical CTQs include S/N ratio and resolution tests for all nuclei of interest. Method-specific PQ tests, also known as system suitability tests, may be used in lieu of PQ requirements for validated procedures.

The PQ samples and tests in the following subsections are typical examples only. Other tests and samples may be used to establish specifications for specific purposes. Instrument vendors often provide samples and test parameters that can be used as part of the PQ package.

RESOLUTION AND LINESHAPE MEASUREMENT—^{1}H NMR (see *Figure 1*)

Sample: 1% chloroform in acetone-d_6 (≥ 500 MHz), 3% chloroform in acetone-d_6, degassed and sealed
Spectral width: < 1 KHz
Data acquisition time: NLT 10 s
Tip angle: 90°
Relaxation time: 60 s
Spinning rate: Static or 20 Hz
Pulse sequence: Delay-pulse-acquire with no decoupling
Processing: No line broadening, zero-filling to 128 k

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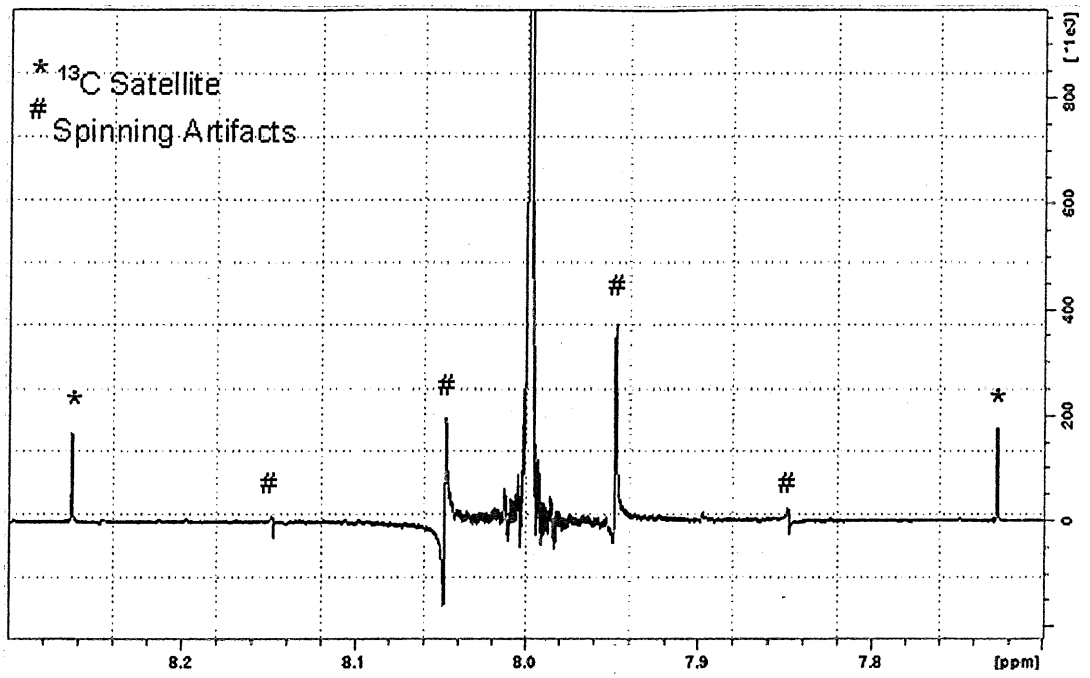


Figure 1. ^{1}H NMR spectrum of chloroform in acetone-d_6 obtained at 400 MHz. The linewidth measured at 0.55% and 0.11% of the ^{13}C satellites was 2.7 and 5.5 Hz, respectively.

Shim the magnet with special attention to the off-axis shims, acquire a single acquisition, phase to pure absorption, and measure the linewidth at 50%, 0.55%, and 0.11% maximum intensity. The linewidth should pass specifications at these positions, and, in addition, the lineshape should be Lorentzian. On modern NMR spectrometers, the lineshape is frequently obtained on a nonspinning sample because the off-axis shims can be set so well that there is essentially no difference between spectra obtained spinning and nonspinning. In addition, two-dimensional spectra should be obtained on a static sample.

S/N MEASUREMENTS—^{1}H NMR (see *Figure 2*)

Sample: 0.1% ethylbenzene in chloroform-d, 1% ethylbenzene in chloroform-d (< 200 MHz) degassed and sealed
Spectral width: 10 ppm
Data acquisition time: 400 ms
Tip angle: 90°
Relaxation delay: 60 s
Spinning rate: 0 or approximately 20 Hz
Pulse sequence: Delay-pulse-acquire with no decoupling
Processing: Exponential with 1-Hz line broadening
Referencing: Tetramethylsilane (TMS) = 0.0 ppm or the center of the quartet = 2.65 ppm

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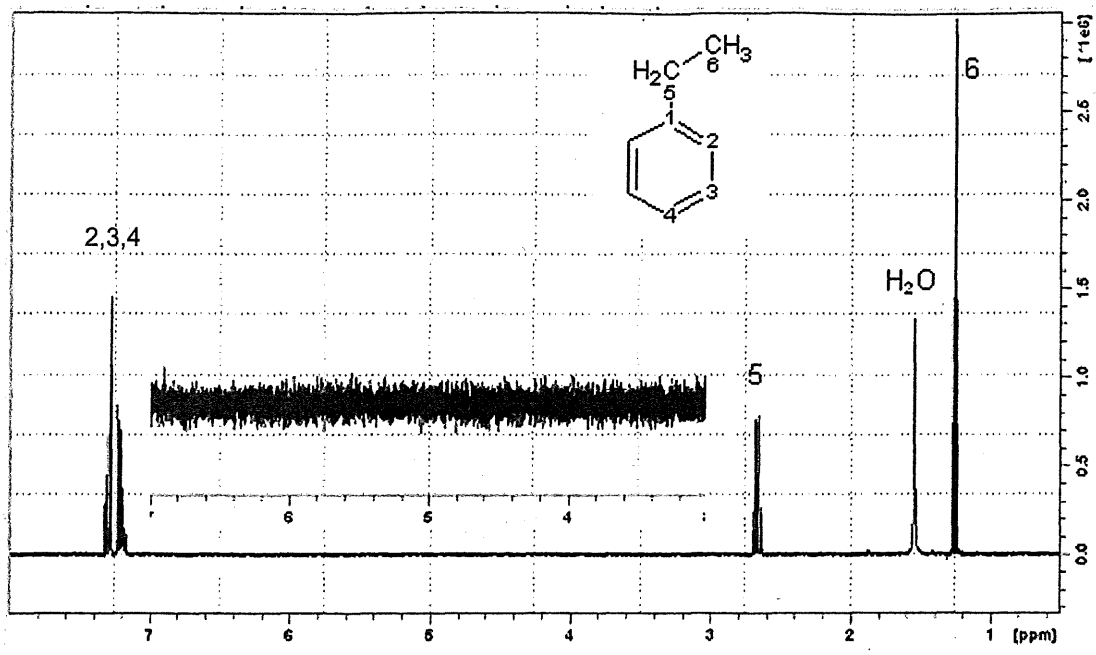


Figure 2. ^{1}H NMR spectrum of 0.1% ethylbenzene obtained at 400 MHz with an S/N ratio of 550:1.

The concentration of ethylbenzene should be chosen to achieve S/N ratio specifications in the range of 20–1000. Concentrations that typically result in measurements outside that range are of limited utility in assessing the performance of the instrument. Nevertheless, established standard solutions are conventionally used. The magnet should be shimmed as well as possible. Ideally, this test should be run immediately after the lineshape test because most of the shims will be nearly maximized. Acquire a single acquisition, phase the spectrum in pure absorption mode, and measure the S/N of the ethylbenzene quartet. This experiment can be run with or without sample spinning. With a spinning sample, the S/N value that is measured should be only about 10% higher than that obtained with a nonspinning sample if the off-axis shims are well adjusted. A higher ratio would indicate that the determination would benefit from further shimming with the off-axis shims.

Most modern spectrometers have software that perform the S/N measurement after the operator has identified the signal and noise regions. Manual calculations can also be made. Measure the amplitude (*A*) from the center of the baseline to the peak of the highest of the central two lines in the quartet. Measure the peak-to-peak noise height (*H*) from the lowest noise peak to the highest noise peak in the 3–5 ppm region. The noise may be vertically multiplied by a factor for accurate measurement of high S/N spectra. Calculate the S/N as follows:

$$S/N = k \times 2.5 \times A/H \quad [1]$$

where *k* is the vertical expansion factor of the noise region used. The factor of 2.5 converts the peak-to-peak S/N to root-mean-squared (rms) noise, which is the standard convention for reporting S/N in NMR spectroscopy. Computerized S/N calculations can be used provided the specifications are set and tested by the same procedure. At the discretion of the spectroscopist, an S/N value lower than that specified by the manufacturer may be used if it is judged to be sufficient for the current application.

S/N MEASUREMENTS ^{13}C NMR (see *Figure 3*)

Sample: 40% *p*-dioxane in benzene-d_6 (v/v) (degassed and sealed)
Spectral width: Approximately 200 ppm
Tip angle: 90°
Relaxation delay: 300 s
Spinning rate: Approximately 20 Hz
Pulse sequence: Delay-pulse-acquire with no decoupling
Processing: Exponential with 3.5-Hz line broadening, zero-filling to 32k
Referencing: TMS = 0.0 ppm or the center of the benzene triplet = 128.4 ppm

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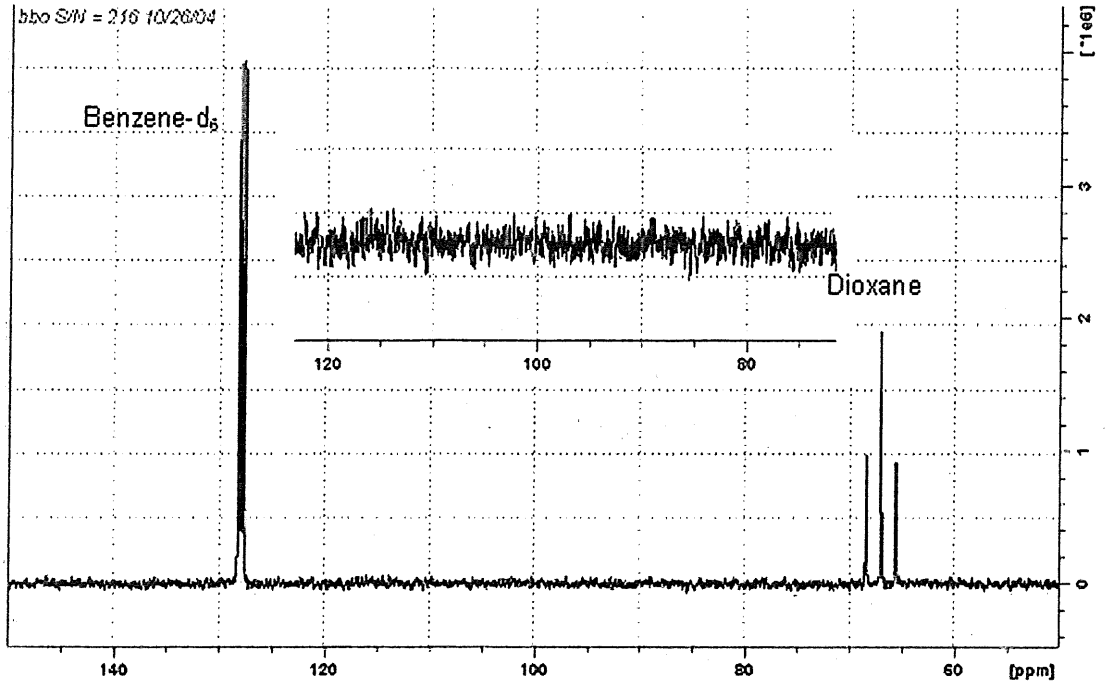


Figure 3. ^{13}C NMR spectrum of the ASTM standard 40% *p*-dioxane in benzene-d_6 (v/v) obtained at 100.6 MHz, with an S/N ratio of 140:1.

With a well-shimmed magnet, acquire a single acquisition following a minimum delay of 300 s, phase the spectrum in pure absorption mode, and measure the height of the benzene triplet at approximately 128.4 ppm from the center of the baseline. The peak-to-peak noise can be measured as above with appropriate vertical expansion of 80–120 ppm. S/N calculations can be made as in *Equation 1* or by computer calculation.

The benzene-d_6 triplet has no nuclear Overhauser enhancement (NOE). Consequently, this test verifies the performance of only the ^{13}C channel.

PERFORMANCE OF BOTH OF THE ^{13}C AND ^{1}H CHANNELS (see *Figure 4*)

Sample: up to 10% ethylbenzene in chloroform-d (degassed and sealed)
Spectral width: 200 ppm
Data acquisition length: 64k points
Tip angle: 90°
Relaxation delay: 300 s
Spinning rate: Approximately 20 Hz
Pulse sequence: Delay-pulse-acquire with composite pulse decoupling set to the center of the ^{1}H spectrum
Processing: Exponential with 0.3-Hz line-broadening
Referencing: TMS = 0.0 ppm or the center of the chloroform-d, triplet = 77.23 ppm

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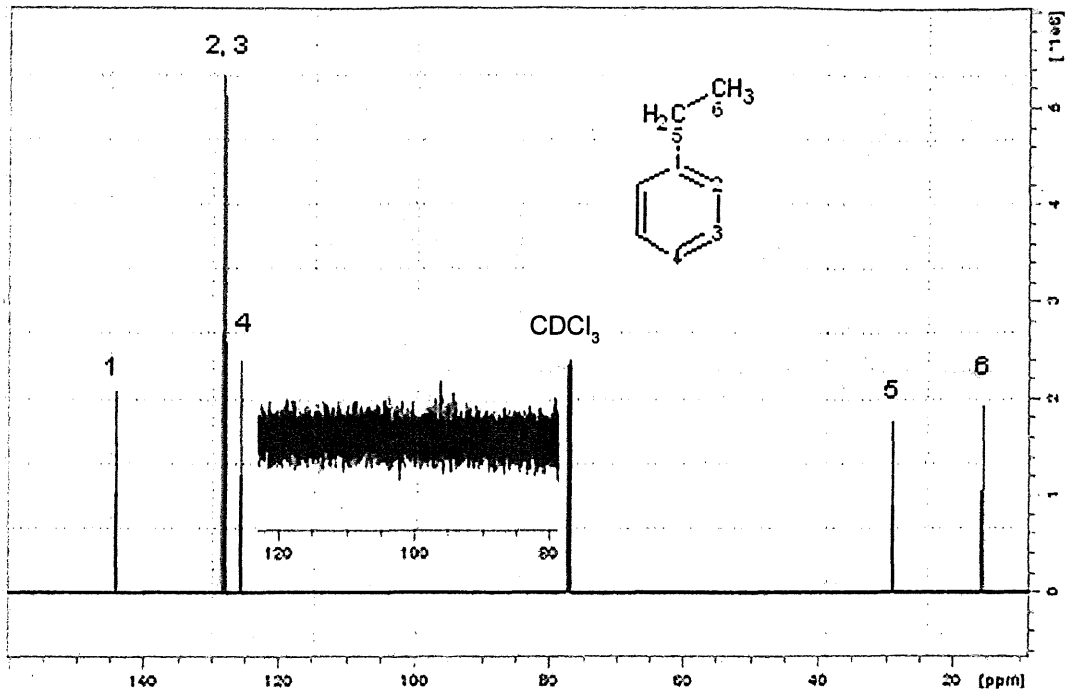


Figure 4. ^{13}C NMR spectrum of 10% ethylbenzene obtained using a cryogenically cooled dual $^{1}H/^{13}C$ probe at 150.9 MHz, with an S/N ratio of 640:1.

The shimming should be sufficient to pass the resolution and lineshape tests described above. The measurement of S/N is done from the peak height of the larger resonance of the two near 128 ppm. The noise is measured as above in the region of 80–120 ppm, with appropriate vertical expansion. S/N is calculated by the computer or as in *Equation 1*.

RELAXOMETRY MEASUREMENTS—LOW FIELD-NMR (LF-NMR)

The PQ should be performed before the collection of experimental data.

Dissolve an accurately weighed quantity of manganese (II) chloride tetrahydrate (MW 197.91) in water, and quantitatively dilute with water to obtain check solutions that have known concentrations of 0.9, 2.7, and 4.5 mM.

Place a portion of each of the solutions into sample holders suitable for the configuration of the specific model of the LF-NMR spectrometer. Warm to 40° for NLT 10 min, and measure the spin-lattice relaxation time (T_1) of water. The average T_1 for replicate measurements must be within 5% of 156, 52, and 32 ms for the 0.9, 2.7, and 4.5 mM solutions, respectively.

Characterizing Instrument Performance

Specific procedures, acceptance criteria, and time intervals for characterizing NMR spectrometer performance depend on the instrument and its intended application. Many NMR applications use previously validated experiments that relate NMR spectra to a physical or chemical property of interest. Stable instrument performance over extended periods of time should be demonstrated. This practice provides some assurance that reliable measurements can be taken from sample spectra using previously validated NMR experiments.

QUALITATIVE AND QUANTITATIVE NMR ANALYSIS

NMR spectroscopy has been used for a wide range of applications such as structure elucidation; thermodynamic, kinetic, and mechanistic studies; and quantitative analysis. Some of these applications are beyond the scope of compendial methods.

All characteristics of the signal—chemical shift, multiplicity, linewidth, coupling constants, relative intensity, and relaxation time—contribute analytical information.

Qualitative Applications

Comparison of a spectrum from the literature or from an authentic standard with that of a test sample may be used to confirm the identity of a compound and to detect the presence of impurities that generate extraneous signals. The NMR spectra of simple structures can be adequately described by the value of the chemical shifts and coupling constants, and by the relative number of nuclei represented by the integral of each signal. (The software of modern instruments have available programs that

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generate simulated spectra using these data.) Experimental details, such as the solvent used, and the chemical shift reference, must also be provided.

For unknown samples, NMR analysis, usually coupled with other analytical techniques, is a powerful tool for structure elucidation. Chemical shifts provide information on the chemical environment of the nuclei. Extensive literature is available with correlation charts and rules for predicting chemical shifts. The multiplicity of the signals provides important structural information. The magnitude of the scalar coupling constant, *J*, between residual protons on substituted aromatic, olefinic, or cycloalkyl structures is used to identify the relative position of the substituents. Routine ^{13}C spectra are obtained under proton decoupling conditions that remove all heteronuclear ^{13}C-^{1}H couplings. As a result of this decoupling, the carbon signals appear as singlets, unless other nuclei that are not decoupled are present (e.g., ^{19}F, ^{31}P).

Chemical exchange is an example of the effect of intermolecular and intramolecular rate processes on NMR spectra. If a proton can experience different environments by virtue of such a process (tautomerism, rotation about a bond, exchange equilibria, ring inversion, etc.), the appearance of the spectrum will be a function of the rate of the process. Slow processes (on an NMR time scale) result in more than one signal from the interconverting species; fast processes average these signals to one line; and intermediate processes produce broad signals, which sometimes cannot be easily found in the spectra.

The software of modern FT-NMR spectrometers allows for sequences of pulses much more complex than the repetitive accumulation of transients described above. Such experiments include homonuclear or heteronuclear *multidimensional analysis*, which determines the correlation of couplings and may simplify the interpretation of otherwise complex spectra.

See chapter ⟨1761⟩ for detailed descriptions of common two-dimensional experiments.

Quantitative Applications

I. General considerations of quantitative NMR: see ⟨1761⟩.

II. The scope of this section is limited to quantitation by one-dimensional NMR. Although any of the NMR active nuclei can be used to obtain quantitative data, the discussion here will be limited to ^{1}H. There are two kinds of quantitation by NMR: relative and absolute.

1. **Relative quantitation** involves measurement of relative amounts of species in a sample based on integration of peaks due to each of the components measured. The integrals are normalized by factor *N*, that is, the integral is divided by the number of equivalent nuclei represented by that peak to give the relative molar concentration of each component.
2. **Absolute quantitation** is the direct measurement of the actual amount of analyte independent of other components contained in that sample. There are two basic methods for absolute quantitation based on the kind of reference standard that is used to calibrate the NMR signal.

A. **Internal reference standard**

I. **Definition:** The reference standard is co-dissolved in the analyte test solution.

II. **Procedure**

Typical NMR solution preparation: An NMR solution is prepared with exact weights of both the analyte and reference standard. The largest source of error in this quantitative NMR method is from weighing, so the use of larger weights is recommended to minimize this error. This quantitative method is based on a comparison of the reference standard and analyte NMR peaks and their respective concentrations. Since the analyte and reference standard are in the same solution, the analyte and reference standard are contained in the same volume, and only their masses are compared. Therefore, the exact volume is not required. Typically, at least three replicates are prepared.

Data acquisition: Data is acquired under quantitative conditions, see ⟨1761⟩. For example, the pulse repetition time should be at least 5 times the longest T_1 when a 90° pulse is used.

Data processing: Process the data, using zero-filling if necessary, such that a sufficient number of points define a peak. For example, experience has shown that at least 16 points gives a good quantitative representation of a peak.

Analysis: Integrate appropriate peaks. For example, avoid using peaks that are overlapped, or due to hydrogens capable of exchanging. The determination of the amount of analyte derives from the basic proportionality between the peak intensity and the concentration of the solute.

$$\frac{[A]_{1H}}{I_A/N_A} = \frac{[RS]_{1H}}{I_{RS}/N_{RS}} \qquad [2]$$

where *I* = integral; *N* = normalization factor; and $[\,]_{^1H} = {}^1H$ relative molar concentration, and the subscripts *A* and *RS* represent the analyte and reference standard, respectively. The mass of the analyte is thus calculated according to the following equation.

$$M_A = \frac{I_A}{I_{RS}} \times \frac{N_{RS}}{N_A} \times \frac{MM_A}{MM_{RS}} \times M_{RS} \times P \qquad [3]$$

where M_A = mass of the analyte, *MM* = molar mass, and *P* = purity of the reference standard.

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III. A common application of absolute quantitation is the determination of the purity of a sample. The weight % purity is given by

$$weight\,\%\,purity = \frac{M_A}{M_S} \times 100\% \qquad [4]$$

where M_S is the total mass of the sample with contributions from the analyte plus any contaminants that may be present in the sample such as water and salts. Combining *Equations 3* and *4*, the weight % purity is given by

$$weight\,\%\,purity = \frac{I_A}{I_{RS}} \times \frac{N_{RS}}{N_A} \times \frac{MM_A}{MM_{RS}} \times \frac{M_{RS}}{M_S} \times P \times 100 \qquad [5]$$

B. **External reference standard**

I. **Definition:** The classical external reference standard method consists of solutions of a reference standard and analyte that are each in separate NMR tubes. One variation of an external reference standard is the standard test solution contained in a coaxial tube and is inserted into an analyte test solution contained in an NMR tube. Another variation is the introduction of a computer-generated signal into the spectrum of a reference standard solution of known concentration to calibrate the signal's response (intensity per ^1H molar concentration, in the case of ^1H NMR), followed by insertion of that calibrated computer-generated signal into the spectrum of an analyte test solution. This section will address the use of an external reference in the classical sense.

II. **Procedure**

NMR solution preparation: NMR solutions of known concentrations of each of the analyte and reference standard are prepared using exact weights and volumes. Again, the use of larger weights is recommended to minimize the weighing error. Typically replicates of the analyte solutions and reference standard solutions are prepared. The analyte and reference must be prepared in the same solvent to minimize probe tuning differences.

Data acquisition and processing: Same as in II.B.1.b, internal reference standard. Apply the same acquisition and processing parameters to the analyte and reference standard spectra.

Analysis: Integrate appropriate peaks in the spectra of the analyte and reference standard. The amount of analyte is calculated according to the following equation:

$$M_A = \frac{I_A}{I_{RS}} \times \frac{N_{RS}}{N_A} \times \frac{V_A}{V_{RS}} \times \frac{MM_A}{MM_{RS}} \times M_{RS} \times P \qquad [6]$$

where V = volume.

Application to weight % purity: Weight % purity values may be similarly calculated as in *Equation 5*.

3. The internal and external reference standard methods each have their own set of advantages and disadvantages.

A. **Chemical interactions:** Preparation of the reference standard and the test material in separate solutions avoids chemical interactions between the test sample and reference standard that may otherwise occur with an internal reference standard.

B. **Spectral overlap:** The use of an external reference standard also avoids potential overlap between peaks of the reference standard and test sample that can occur with an internal standard.

C. **Calibration:** Once an NMR response has been calibrated with external reference standard solutions, this calibration may be applied to any other sample in the same solvent given that i) the instrument has been demonstrated to be stable over the time between when the calibration is done and when data is acquired on the test material, ii) system suitability has been established on the day that the measurement on the test material is made, and iii) absolute integrals are compared. In the case of internal reference standards, the measurement on the reference standard and test sample is made under absolutely identical conditions.

D. **Accuracy and precision:** Multiple external reference standard solutions may be prepared to average the errors in the mass and volume measurements during sample preparation, thereby improving the accuracy of the calibrated NMR response. In the case of internal reference standards, single measurements of the reference standard and analyte are made for each replicate test solution. The combined errors from the mass measurements of the reference standard and test sample as well as instrumental electronic variations determine the standard deviation of the average M_A or weight % purity values.

VALIDATION AND VERIFICATION OF NMR ANALYTICAL PROCEDURES

If an NMR procedure is provided in a monograph, verification of suitability (see ⟨1226⟩) under actual conditions of use is required. Validation is required only when an NMR method is an alternative to the official procedure for testing an official article. The objective of validation of a procedure relying on the NMR method is to demonstrate that the measurement is suitable for its intended purpose, including the following: quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities (Category II), and identification tests (Category IV). [NOTE—For a definition of the different categories, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category

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of the test, analytical procedure validation requires the testing of specificity, linearity, range, accuracy, precision, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

Analytical Procedure Validation

The objective of an analytical procedure validation is to demonstrate that the analytical procedure is suitable for its intended purpose by conducting experiments and obtaining results that meet predefined acceptance criteria. NMR analytical procedures can include the following: quantitative tests for major component and impurities content, limit tests for the presence of impurities, quantification of component in a product or formulation, and/or identification tests.

Performance characteristics that demonstrate the suitability of an analytical procedure are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in chapter ⟨1225⟩. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the analytical procedure.

The performance characteristics that are required as part of a validation for each of the analytical procedure categories is given below.

SPECIFICITY

The purpose of a specificity test is to demonstrate that measurements of the intended analyte signals are free of interference from components and impurities in the test material. Specificity may be applied to all categories and is a requirement for Category IV. Specificity tests can be conducted to compare NMR spectra of other components and impurities that are known from synthetic processes and formulations and test preparations. For an identification NMR analytical procedure (Category IV), validation experiments may include multidimensional NMR experiments to validate correct assignments of chemical shifts and to confirm the structure of the analyte.

Validation criteria: Specificity is ensured by use of a reference standard wherever possible and demonstrable lack of interference from other components.

LINEARITY

A linear relationship is exhibited between the analyte concentration and instrument response; this should be demonstrated by measuring responses of analyte from NLT five standard solutions at concentrations encompassing the anticipated concentration range of analyte(s) of the test solution. For Category I, standard solutions can be prepared from reference materials in an appropriate NMR solvent. For Category II, NMR analytical procedures that are used to quantitate impurities, linearity samples can be prepared by spiking suitable test samples that contain low amounts of analyte or by spiking matrix samples at concentrations of the expected range. The standard curve should then be constructed using appropriate statistical analytical procedures such as a least squares regression. The correlation coefficient (R), y-intercept, and slope of the regression line should be determined. Absolute values determined for these factors should be appropriate for the procedure being validated.

Validation criteria: The correlation coefficient (R) must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

The range between the low and high concentrations of analyte is given by the quantitative NMR analytical procedure. This is normally based on test article specifications in the *USP* monograph. It is the range within which the analytical procedure can demonstrate an acceptable degree of linearity, accuracy, and precision, and may be obtained from an evaluation of that analytical procedure. Recommended ranges for various NMR analytical procedures are given below.

Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, it is 70.0%–130.0%. For Category II quantitative tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ACCURACY

The accuracy of a quantitative NMR analytical procedure should be determined across the required analytical range. Typically, three levels of concentrations are evaluated using triplicate preparations at each level.

For drug substance assays (Category I), accuracy can be determined by analyzing a reference standard of known purity. For drug product (Category I), a composite sample of reference standard and other components in a pharmaceutical finished product should be used for analytical procedure validation. The assay results are compared to the theoretical value of the reference standard to estimate errors or percent recovery. For the quantitation of impurities (Category II), the accuracy of the analytical procedure can be determined by conducting studies with drug substances or products spiked with known concentrations of the analyte under test. It is also acceptable to compare assay results from the analytical procedure being validated to those of an established, alternative analytical procedure.

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Validation criteria: 98.0%–102.0% recovery for drug substances, 95.0%–105.0% recovery for compounded pharmaceutical finished products assay, and 80.0%–120.0% recovery for the quantitative impurity analysis. These criteria should be met throughout the intended range.

PRECISION

Repeatability: The analytical procedure should be assessed by measuring the concentrations of six separate standard solutions at 100% of the test concentration. The relative standard deviation from the replicate measurements should be evaluated to meet acceptance criteria. Alternatively they can measure the concentrations of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for drug substances, NMT 2.0% for compounded pharmaceutical finished products, and NMT 20.0% for the quantitative impurity analysis.

Intermediate precision: The effect of random events on the analytical precision of the analytical procedure should be established. Typical variables include performing the analysis on different days, using different instrumentation that are suitable as specified in the analytical procedure, and/or having the analytical procedure performed by two or more analysts. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.0% for drug substances, NMT 3.0% for compounded pharmaceutical finished products, and NMT 25.0% for quantitative impurity analysis.

QUANTITATION LIMIT (QL)

The QL can be validated by measuring six replicates of test samples spiked with analyte at 50% of specification. From these replicates, accuracy and precision can be determined. Examples of specifications for Category II quantitative determinations are that the measured concentration is within 70.0%–130.0% of the spike concentration and the relative standard deviation is NMT 15%.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated with deliberate changes to critical experimental parameters. This can include measuring the stability of the analyte under specified storage conditions, slightly varied inter-pulse delay, probe temperature, and possible interfering species, to list a few examples. Robustness is required for Category I and Category II, quantitative methods.

Analytical Procedure Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* do not need to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of an NMR procedure verification is to demonstrate that the procedure as prescribed in a specific monograph can be executed by the user with suitable accuracy, specificity, and precision using the instruments, analysts, and sample matrices available. According to general information chapter *Verification of Compendial Procedures* ⟨1226⟩, if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Verification of a compendial NMR procedure should at minimum include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in this section.

GLOSSARY

Internal standard: An internal standard (IS) is a substance added to a sample solution at a known concentration. One should select an IS with at least a single NMR resonance that does not overlap with those of the analyte. The ratio of a specific internal standard peak area and that of an analyte peak area is used to determine the concentration of the analyte. The number of nuclei corresponding to the integrated peaks in the IS and analyte spectra must be known.

NMR reference: An NMR reference, also known as an NMR shift reference, is a substance added to a sample and from which the chemical shift for the δ scale is established. Common examples for proton and carbon NMR analyses are tetramethylsilane (TMS) for use in organic solvents and the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) or sodium-3-trimethylsilylpropionate (TMSP) for use in aqueous media. In both cases, the chemical shift of the methyl peaks is defined as 0.0 ppm.

Reference standard: A reference standard is a substance authenticated by appropriate experimental means to be of a specific chemical structure. In NMR spectroscopy, a reference standard is typically used for the qualitative analysis of a test material. Structure can be confirmed if one directly compares the chemical shifts and multiplicities of the peaks in the NMR spectrum of the test material against the spectrum of the reference standard acquired under comparable experimental conditions.

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⟨771⟩ OPHTHALMIC PRODUCTS—QUALITY TESTS

INTRODUCTION

Ophthalmic products are sterile products that are intended for application to any ocular structure, including any space adjacent to an ocular structure and its immediate surrounding spaces.

The routes of administration of ophthalmic products fall into three general categories: topical, intraocular injections, and extraocular injections. Topical drug products are intended to be administered to an ocular surface component, such as the eyelid, conjunctiva, or cornea, and can produce local or systemic effects. Intraocular and extraocular injections are administered through external boundary tissue. The ophthalmic routes of administration include, but are not limited to: topical, subconjunctival, sub-Tenons, subretinal, subchoroidal, intracorneal, intrascleral, suprachoroidal, intravitreal, intracameral, juxtascleral, and retrobulbar routes (see *Figure 1*). Ophthalmic products are administered to the eye in a wide variety of dosage forms, including but not restricted to: solutions, suspensions, ointments, gels, emulsions, strips, injections, inserts, and implants.

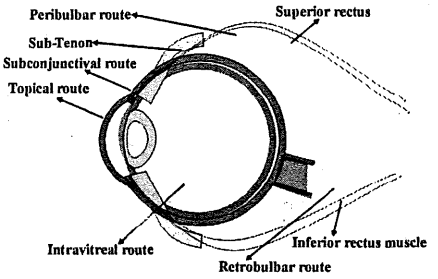


Figure 1. Some of the routes of administration in the eye.

This chapter provides lists of consolidated common product quality test requirements in a concise and coherent fashion. This chapter applies, in part or in its entirety, when referenced in a drug product monograph (see *General Notices, 3.10 Applicability of Standards*). The chapter includes the quality tests for the specific route of administration. The quality tests listed can be used, as appropriate, by manufacturers toward the development of new drug product monographs for submission to the *USP–NF*.

[NOTE—All references to chapters above ⟨1000⟩ are for information purposes only, for use as a helpful resource. These chapters are not mandatory unless explicitly called out for application.]

OPHTHALMIC DOSAGE FORMS

This chapter discusses the specific characteristics of dosage forms that are administered to any structure in the eye. See *Pharmaceutical Dosage Forms* ⟨1151⟩ for additional information pertaining to the description and manufacturing of pharmaceutical dosage forms.

Ophthalmic products have the same or similar requirements as injectables and implants. For more information on the requirements for these dosage forms, see *Injections and Implanted Drug Products* ⟨1⟩.

Depending on the formulation, container–closure system, and sterilization process, degradation and/or morphological changes can occur during the sterilization step of the manufacturing process and should be minimized. Temperatures required for autoclaving can cause irreversible damage to certain dosage forms such as suspensions, semisolids, emulsions, and others, whereas filtration is applicable only to formulations containing particulates with particle sizes <0.2 µm. An alternative is to manufacture the product from sterile ingredients in an aseptic environment.

For multidose products, a suitable antimicrobial preservative is required, and its effectiveness over the shelf life of the product must be verified.

Solutions

[NOTE—Included in this section are those solid products that, when reconstituted according to the label instructions, result in a solution.] The corneal contact time of topical ophthalmic solutions increases with the viscosity of the formulations. Several synthetic polymers, including but not limited to polyvinylalcohol, polyvinylpyrrolidone, polyethylene glycol, polyacrylic acid, and many cellulose derivatives such as hypromellose and hydroxyethylcellulose, are commonly used as viscosity enhancers because of their physiologic compatibility and satisfactory physicochemical properties.

Another approach to increase the corneal contact time involves the use of polymers that provide the liquid formulation with semisolid consistency only when it is placed in the conjunctival or corneal area. In this way, easy instillation of the solution is followed by prolonged permanence as a result of the viscoelastic properties of the formed hydrogel. "Hydrogel", or "water-based gels", are three-dimensional networks of polymer chains containing water within the network. The network can be either physically or chemically cross-linked (a covalent bonding) structures between polymer chains. The water content of

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hydrogels can be adjusted by modulating the composition and conformation of polymers, such as the hydrophilic/hydrophobic balance of polymer chains and pendant groups, and the degree of cross-linking. The in situ gelling phenomenon is caused by a change in the conformation of the polymer(s) that can be triggered by external stimuli such as temperature, pH, ionic content, and lacrimal fluid, upon delivery to the eye. Additionally, some polymers can interact, via noncovalent bonds, with conjunctival mucin and maintain the formulation in contact with corneal tissues.

Solutions can be injected not only by the intravitreal route but also by other routes, such as subconjunctival, sub-Tenons, retrobulbar, suprachoroidal, and subretinal routes.

Suspensions

[NOTE—Included in this section are those solid products that, when reconstituted according to the label instructions, result in a suspension.] Use of aqueous or oily suspensions may be considered for a number of reasons such as the following: 1) for drugs that are poorly water soluble, 2) for drugs that have poor aqueous stability, and 3) in cases where there is a need to increase contact time with the eye and to increase wetting of the eye. The drug particle size is often reduced to levels <10 µm in an attempt to avoid excessive lacrimation.

After topical instillation, particles are expected to be retained in the cul-de-sac, and the drug slowly dissolves or is released from the polymeric structures by diffusion, dissolution, polymer degradation, or ion-exchange.

Suspensions can be injected not only by the intravitreal route but also through other routes, such as subconjunctival, sub-Tenons, retrobulbar, suprachoroidal, and subretinal.

Ointments

Eye ointments are semisolid products usually intended for application to the conjunctiva, cornea, or eyelid. They have longer contact time when compared to many solutions. A suitable ophthalmic ointment base is nonirritating to the eye and permits diffusion of the drug substance throughout the secretions bathing the eye.

The majority of water-free, oleaginous eye ointment bases are composed of white petrolatum and liquid petrolatum (mineral oil), or the eye ointment base is a modification of the petrolatum-based formula. They are designed to melt at body temperature. Other types of lipophilic ointment bases can also be used. An anhydrous vehicle can be advantageous for moisture-sensitive drugs.

Gels

Semisolid gel-type products are an alternative to traditional ointments and are based on the effect of increasing the viscosity to prolong the retention of the drug in the eye. Several types of gelling agents can be used, such as polyacrylic acid derivatives, carbomer, and hypromellose.

Emulsions

Topical ophthalmic emulsions are generally prepared by dissolving or dispersing the drug substance into an oil phase, adding suitable emulsifying and suspending agents, and mixing with water vigorously to form a uniform oil-in-water emulsion. Each phase is typically sterilized before or during charging into the mixing vessel. High-shear homogenation may be used to reduce oil droplet size to submicron size, which may improve the physical stability of the oil micelles so that they do not coalesce. The resulting dosage form should contain small oil droplets, uniformly suspended.

Limited aqueous solubility of the drug substance(s) is the most common rationale for developing an ophthalmic emulsion. The drug substance(s) can be added to the phase in which it is soluble at the beginning of the manufacturing process, or the drug substance can be added after the emulsion is prepared by a suitable dispersion process.

The physical stability of the emulsion can be measured by light-scattering techniques that characterize oil-phase, globule-size distribution. Suitable surfactants may be added to improve emulsion stability.

Strips

Ophthalmic strips can have different ophthalmic uses including, but not limited to, use as a diagnostic tool to visualize defects or aberrations in the corneal epithelium or to measure the amount of tear production. They are made of filter paper, and they can contain compounds such as fluorescein sodium. They are individually packed to preserve sterility until the moment of use.

Injections

Although injections are considered a dosage form for nomenclature purposes, they are not treated as a dosage form in this chapter. Instead, refer to the appropriate physical form, such as solution, suspension, or others, for general information.

Inserts

Ophthalmic inserts and ocular systems are solid dosage forms of appropriate size and shape that are placed in the conjunctival fornix, in the lacrimal punctum (*Figure 2*), or on the cornea. Inserts can usually be removed, if adverse effects develop, and provide extended release of the drug over a certain period of time. Inserts can be classified as erodible (soluble) or nonerodible (insoluble). Drug release from soluble inserts involves two steps: 1) fast release of a portion of the drug as the tear fluid penetrates into the system, and 2) slow release as a gel layer is formed on the surface of the insert. Collagen shields made from porcine

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sclera collagen or bovine corium tissue—and devices obtained by molding, extrusion, or compression (minitablets) of gelling polymers—belong to this category of soluble inserts. Bioerodible polymers (e.g., cross-linked gelatin derivatives and polyesters) can be used to prepare erodible inserts; these matrices act as reservoirs or interact with the drug molecules through labile bonds.

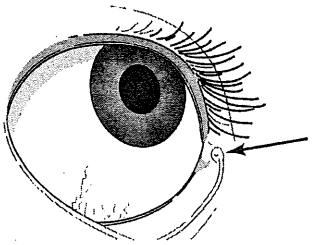


Figure 2. Lacrimal punctum.

Soluble inserts can have the drug incorporated into an erodible matrix, such as hydroxypropyl cellulose, hyaluronic acid, carbomer, or polyacrylic acid. Soluble inserts may be placed in the lower cul-de-sac and generally dissolve within 12–24 h. Erodible polymeric products undergo gradual dissolution while releasing the drug, and the patient does not have to remove the erodible polymeric insert after use.

Insoluble inserts can have a reservoir or matrix structure. Their mechanism of action is based on diffusion of a fluid into the device, dissolving of the drug, and the creation of a saturated solution released to the medium by diffusion out of the insert. These insoluble inserts need to be removed after a certain period of time.

Implants

Implants are injected or implanted into the anterior segment (intracameral, subconjunctival, sub-Tenon, or other parts of the anterior segment) or intravitreal cavities, and the implants can be in different shapes, such as a disk or a thin rod. Implants can be made of nonbiodegradable or biodegradable polymers.

Among the types of nonbiodegradable solid (polymer) implants are reservoir-type implants anchored to the sclera, in which the drug is released across a nonbiodegradable, semipermeable polymer. The reservoir-type implants can be made of a variety of materials, including polyvinyl alcohol–ethylene vinyl acetate. Biodegradable polymers can be used to form injectable solid implants.

Drug–Device Combination Products

An ophthalmic drug–device combination product is constituted, in most cases, of two components. One is a pharmaceutical dosage form containing the drug substance(s), and the other is a device that will activate or facilitate the penetration of the drug substance(s) from the dosage form into a particular region of the eye. Some examples of the devices are those that generate waveforms (heat or light).

This chapter is applicable only to the pharmaceutical dosage form component of the ophthalmic drug–device combination product. The appropriate FDA regulations regarding medical devices should be used for the device component.

DRUG PRODUCT QUALITY

Procedures and acceptance criteria for testing ophthalmic products are divided into two categories: 1) those that assess general quality attributes, e.g., identification, potency, purity (and impurities), sterility, and particulate matter; and 2) those that assess in vitro product performance, i.e., dissolution or drug release of the drug substance from the drug product. Quality tests assess the integrity of the dosage form, whereas the performance tests assess drug release and other attributes that relate to in vivo drug performance. Taken together, quality and performance tests ensure the identity, strength, quality, purity, and efficacy of the drug product. This chapter addresses the quality tests for ophthalmic products. The performance tests (dissolution/drug release) are addressed by *Ophthalmic Products—Performance Tests* ⟨1771⟩.

Universal Tests

In this chapter, the division of the product quality tests into universal and specific tests does not strictly follow the ICH guidance *Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* (available at www.ich.org). Universal tests in this chapter mean the tests that are applicable to all ophthalmic products, regardless of the dosage form type.

DESCRIPTION

A qualitative description of the drug product should be provided. The acceptance criteria should contain the final acceptable appearance, including clarity and color, of the dosage form and packaging. If the color changes during storage, a quantitative procedure may be appropriate. This is not a compendial test but is part of the manufacturer's specification of the drug product.

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IDENTIFICATION

Identification tests are discussed in *General Notices, 5.40 Identification*. Identification tests should establish the identity of the drug or drugs present in the article and should discriminate between compounds of closely related structures that are likely to be present. Identity tests should be specific for the drug substance(s) [e.g., infrared (IR) spectroscopy]. If the test is nonspecific, at least two orthogonal nonspecific tests should be used. Near-infrared (NIR) or Raman spectrophotometric methods could be acceptable for the identification of the drug product. The most widely used identification procedures for drug substance(s) contained in pharmaceutical dosage forms are chromatographic procedures with comparison to the appropriate standards (see *Chromatography* ⟨621⟩ and *Thin-Layer Chromatographic Identification Test* ⟨201⟩). Identification by use of a single chromatographic retention time does not constitute a specific test.

ASSAY

A specific and stability-indicating test should be used to determine the strength (content) of the drug product. In cases where the use of a nonspecific assay test is justified, other supporting analytical procedures should be used to achieve overall specificity. A specific procedure should be used when there is evidence of excipient interference with the nonspecific assay test. Additional information on specific assays may be found in *Antibiotics—Microbial Assays* ⟨81⟩, ⟨621⟩, and *Ion Chromatography* ⟨1065⟩.

IMPURITIES

Process impurities, synthetic byproducts, and other inorganic and organic impurities may be present in the drug substance and excipients used in the manufacture of the drug product. These impurities are controlled by the drug substance and excipient monographs. Organic impurities arising from the degradation of the drug substance in the drug product and those impurities arising during the manufacturing process of the drug product should be monitored. All articles meet the requirements in *Elemental Impurities—Limits* ⟨232⟩ and *Residual Solvents* ⟨467⟩.

pH

See *pH* ⟨791⟩. Normal tears have a pH of about 7.4. The eye can tolerate products over a range of pH values from about 3.0 to about 8.6, depending on the buffering capacity of the formulation. The pH value of the formulation should be the one where the drug product is the most stable. Formulations that target the extremes of the acceptable pH range will have better patient acceptability if the formulations have a low buffering capacity.

OSMOLARITY

See *Osmolality and Osmolarity* ⟨785⟩. Ophthalmic products may be tolerated over a fairly wide range of tonicity (0.5%–5% sodium chloride, equivalent to about 171–1711 mOsm/kg). Hypotonic solutions are better tolerated than hypertonic solutions. Precautions should be taken to ensure that the product maintains its osmolarity during shelf life. Any possible contributions or interferences from the packaging system should be considered.

PARTICULATE AND FOREIGN MATTER

All ophthalmic products should be inspected for package integrity and, to the extent possible, for the presence of observable foreign and particulate matter (visible particulates). These unwanted particles arise from two sources: extrinsic (i.e., foreign matter); and intrinsic, or product-related matter. Extrinsic matter cannot be associated with the product or process. Intrinsic particles are added during assembly of the product or result from a change over time. A third category, inherent matter, describes a physical state or particles that are an expected attribute of the product.

One hundred percent inspection of injectable products in clear packages is required to remove final packages with visible particles. When 100% inspection of the injectable product in the final package is difficult, such as in opaque containers, alternative methods may be used to determine acceptability. *Visible Particulates in Injections* ⟨790⟩ provides further guidance for general inspection methods and a definition of "essentially free of visible particles" for batch compliance.

Two general categories apply for product administration to the tissues in the eye. Intraocular administration includes all ophthalmic products that cross (penetrate) boundary tissue, such as the cornea and sclera. Extraocular administration of ophthalmic products includes all other ocular components and spaces. For subvisible particle content, USP guidance is followed. Products for intraocular use must comply with *Particulate Matter in Ophthalmic Solutions* ⟨789⟩. Products for extraocular use must comply with *Particulate Matter in Injections* ⟨788⟩. Considerations for product evaluation and the background for both subvisible methods are found in *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions* ⟨1788⟩.

STERILITY

Ophthalmic dosage forms must meet the requirements of *Sterility Tests* ⟨71⟩. If the specific ingredients used in the formulation do not lend themselves to routine sterilization techniques, ingredients that meet the sterility requirements described in ⟨71⟩, along with aseptic manufacture, may be used. The immediate container for ophthalmic products shall be sterile at the time of filling and closing. See also *Universal Tests, Container–Closure Integrity.*

ANTIMICROBIAL PRESERVATIVES

Antimicrobial agents must be added to products that are packaged in containers that allow for the withdrawal or administration of multiple doses, unless one of the following conditions prevails: 1) there are different directions in the individual

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monograph; 2) the substance contains a radionuclide with a physical half-life of <24 h; and 3) the drug product, without additional agents, is sufficiently microbicidal to meet the requirements of *Antimicrobial Effectiveness Testing* ⟨51⟩. Substances must meet the requirements of ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩. Acceptance criteria for antimicrobial preservative content in multiple-unit products should be established.

BACTERIAL ENDOTOXINS

All injected ophthalmic drug products shall be prepared in a manner designed to minimize bacterial endotoxins as defined in *Bacterial Endotoxins Test* ⟨85⟩. The limits are NMT 0.5 EU/mL for ophthalmic irrigation products and NMT 2.0 EU/dose/eye for injected or implanted drug products. Typically, this test is not required for topically applied ophthalmic products. This chapter does not address the endotoxin limits for devices that are injected or implanted.

UNIFORMITY OF DOSAGE UNITS

This test is applicable to dosage forms packaged in single-unit containers. It includes both the mass of the dosage form and the content of the drug substance(s) in the dosage form. The test can be performed by either content uniformity or weight variation (see *Uniformity of Dosage Units* ⟨905⟩).

CONTAINER CONTENTS

Container contents of ophthalmic products should be determined (see *Minimum Fill* ⟨755⟩).

LEACHABLES AND EXTRACTABLES

The packaging system should not interact physically or chemically with the product in any manner to alter the strength, quality, or purity of the drug product. The packaging system should meet the requirements in *Elastomeric Closures for Injections* ⟨381⟩, *Containers—Glass* ⟨660⟩, *Plastic Materials of Construction* ⟨661.1⟩, and *Plastic Packaging Systems for Pharmaceutical Use* ⟨661.2⟩. Further information regarding packaging systems testing can be found in *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1663⟩ and *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems* ⟨1664⟩. The assessment of possible leachables and/or extractables and the establishment of any acceptance criteria for these compounds should take into account the risk assessment of the product, its indication, and its packaging system. This assessment is done in a case-by-case approach during product development and re-evaluated when any changes are made to the product including, but not restricted to, changes in manufacturing process, formulation, and packaging material.

Furthermore, it should be noted that a risk assessment of extractable/leachable impact on topical or intraocular route of administration is a challenging undertaking. Toxicological or safety assessments of primary or secondary packaging component extractables and leachables are not typically available for ophthalmic routes of administration. A risk assessment may include evaluation of toxicology and safety from other routes of administration and an assessment of the Total Daily Intake of the extractable/leachable being evaluated. The preponderance of such assessments leads to an estimate of extractable/leachable risk via ocular delivery to the patient.

CONTAINER–CLOSURE INTEGRITY

The packaging system should be closed or sealed in such a manner as to prevent contamination or loss of contents and should provide evidence of being tamper proof. Validation of container integrity must demonstrate no penetration of microbial contamination or of chemical or physical impurities (see *Package Integrity Evaluation—Sterile Products* ⟨1207⟩).

Specific Tests

VISCOSITY

An increase in viscosity increases the residence time in the eye. However, drug diffusion out of the formulation into the eye may be inhibited due to high product viscosity. Ophthalmic ointments are designed to be of very high viscosity to prolong the residence time in the eye. The inclusion of viscosity evaluation in the specification of the product should be based on the type of dosage form and whether changes in product viscosity will affect its performance. This is not a compendial test but is part of the manufacturer's specification of the drug product. See *Viscosity—Capillary Methods* ⟨911⟩, *Viscosity—Rotational Methods* ⟨912⟩, and *Viscosity—Rolling Ball Method* ⟨913⟩.

ANTIOXIDANT CONTENT

If antioxidants are present in the drug product, tests of their content should be established, unless oxidative degradation can be detected by another test method such as impurity testing. Acceptance criteria for antioxidant content should be established. They should be based on the levels of antioxidant necessary to maintain the product's stability at all stages throughout its proposed usage and shelf life.

RESUSPENDABILITY/REDISPERSIBILITY

Consideration must be given to establishing good physical stability of a suspension. If the particles settle and eventually produce a cake at the bottom of the container, they must redisperse readily at the time of use to achieve dosage uniformity.

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PARTICLE SIZE AND PARTICLE SIZE DISTRIBUTION

The potential for any changes in the particle size of ophthalmic suspensions and emulsions needs to be evaluated through stability testing (see *Light Diffraction Measurement of Particle Size* ⟨429⟩).

DROP SIZE

For ophthalmic drug products dispensed as drops, drop sizes may typically range from 20 to 70 µL. Drop size can be controlled by weight or by volume, and it is typically evaluated during product development. Drop size can be determined by any appropriated validated procedure.

ADDED SUBSTANCES

Suitable substances may be added to ophthalmic products to increase stability, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with therapeutic efficacy or with responses to the specified assays and tests. Typically, they should be evaluated during product development. The use of ingredients solely to impart a color, odor, or flavor is prohibited.

⟨776⟩ OPTICAL MICROSCOPY

Optical microscopy for particle characterization can generally be applied to particles 1 µm and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

APPARATUS

Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives, are preferable with apochromats, and are required for appropriate color rendition in photomicrography. Condensers corrected at least for spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the conditions of use and is affected by the actual aperture of the condenser diaphragm and by the presence of immersion oils.

ADJUSTMENT

The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

ILLUMINATION

A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

VISUAL CHARACTERIZATION

The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made. Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

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PHOTOGRAPHIC CHARACTERIZATION

If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photographic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

PREPARATION OF THE MOUNT

The mounting medium will vary according to the physical properties of the test specimen. Sufficient but not excessive contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting medium must include a consideration of the analyte solubility.

CRYSTALLINITY CHARACTERIZATION

The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

LIMIT TEST OF PARTICLE SIZE BY MICROSCOPY

Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10 µg of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.

PARTICLE SIZE CHARACTERIZATION

The measurement of particle size varies in complexity depending on the shape of the particle, and the number of particles characterized must be sufficient to ensure an acceptable level of uncertainty in the measured parameters. Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276. For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see *Figure 1*):

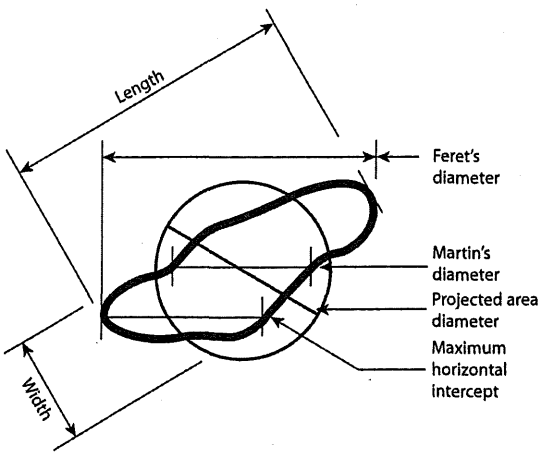


Figure 1. Commonly used measurements of particle size.

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Feret's Diameter—The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.
Martin's Diameter—The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.
Projected Area Diameter—The diameter of a circle that has the same projected area as the particle.
Length—The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.
Width—The longest dimension of the particle measured at right angles to the length.

PARTICLE SHAPE CHARACTERIZATION

For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the powder should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see *Figure 2*):

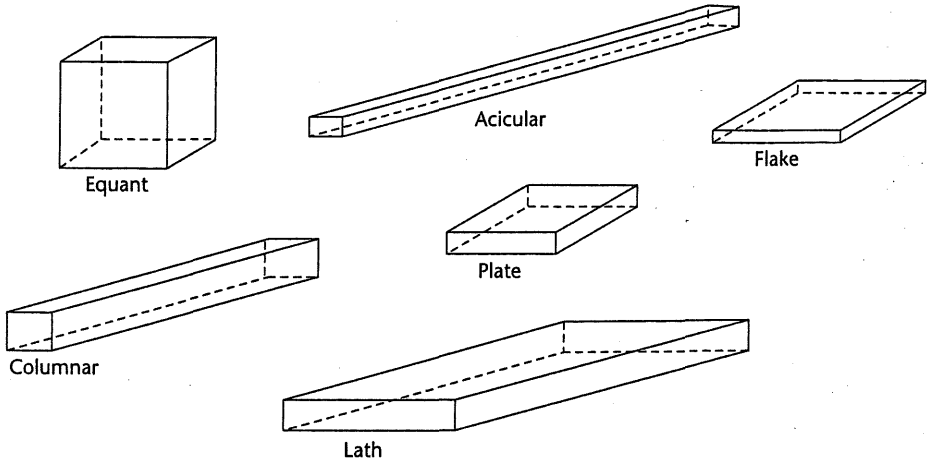


Figure 2. Commonly used descriptions of particle shape.

Acicular—Slender, needle-like particle of similar width and thickness.
Columnar—Long, thin particle with a width and thickness that are greater than those of an acicular particle.
Flake—Thin, flat particle of similar length and width.
Plate—Flat particles of similar length and width but with greater thickness than flakes.
Lath—Long, thin, and blade-like particle.
Equant—Particles of similar length, width, and thickness; both cubical and spherical particles are included.

GENERAL OBSERVATIONS

A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated. This degree of association may be described by the following terms:

Lamellar—Stacked plates.
Aggregate—Mass of adhered particles.
Agglomerate—Fused or cemented particles.
Conglomerate—Mixture of two or more types of particles.
Spherulite—Radial cluster.
Drusy—Particle covered with tiny particles.

Particle condition may be described by the following terms:

Edges—Angular, rounded, smooth, sharp, fractured.
Optical—Color (using proper color-balancing filters), transparent, translucent, opaque.
Defects—Occlusions, inclusions.

Surface characteristics may be described as:

Cracked—Partial split, break, or fissure.
Smooth—Free of irregularities, roughness, or projections.
Porous—Having openings or passageways.
Rough—Bumpy, uneven, not smooth.
Pitted—Small indentations.

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⟨781⟩ OPTICAL ROTATION

INTRODUCTION

Many pharmaceutical substances are optically active in the sense that they rotate an incident plane of polarized light so that the transmitted light emerges at a measurable angle to the plane of the incident light. This property is characteristic of some crystals and of many pharmaceutical liquids or solutions of solids. Where the property is possessed by a liquid or by a solute in solution, it is generally the result of the presence of one or more asymmetric centers, usually a carbon atom with four different substituents. The number of optical isomers is 2^n, where n is the number of asymmetric centers. Polarimetry, the measurement of optical rotation, of a pharmaceutical article may be the only convenient means for distinguishing optically active isomers from each other and thus is an important criterion of identity and purity.

Substances that may show optical rotatory properties are "chiral". Those that rotate light in a clockwise direction as viewed toward the light source are "dextrorotatory", or "(+) optical isomers", and those that rotate light in a counterclockwise direction are called "levorotatory" or "(–) optical isomers". (The symbols *d*- and *l*-, formerly used to indicate dextrorotatory and levorotatory isomers, are no longer sanctioned owing to confusion with D- and L-, which refer to configuration relative to D-glyceraldehyde. The symbols *R* and *S*, or α and β, are also used to indicate configuration, the arrangement of atoms or groups of atoms in space.)

The physicochemical properties of nonsuperimposable chiral substances rotating plane-polarized light in opposite directions to the same extent, "enantiomers", are identical, except for this property and in their reactions with other chiral substances. Enantiomers often exhibit profound differences in pharmacology and toxicology, owing to the fact that biological receptors and enzymes themselves are chiral. Many articles from natural sources, such as amino acids, proteins, alkaloids, antibiotics, glycosides, and sugars, exist as chiral compounds. Synthesis of such compounds from nonchiral materials usually results in equal amounts of the enantiomers, i.e., "racemates". Racemates have a net null optical rotation, and their physical properties may differ from those of the component enantiomers. Use of stereoselective or stereospecific synthetic methods or separation of racemic mixtures can be used to obtain individual optical isomers.

Measurement of optical rotation is performed using a polarimeter.[1] The general equation used in polarimetry is:

$$[\alpha]_{\lambda}^{t} = \frac{100a}{lc}$$

$[\alpha]$	= specific rotation at wavelength λ
t	= temperature
a	= observed rotation in degrees (°)
l	= path length (dm)
c	= concentration of the analyte (g/100 mL)

Thus, [α] is 100 times the measured value, in degrees (°), for a solution containing 1 g in 100 mL, measured in a cell having a path length of 1.0 dm under defined conditions of incident wavelength of light and temperature. For some Pharmacopeial articles, especially liquids such as essential oils, the optical rotation requirement is expressed in terms of the observed rotation, *a*, measured under conditions defined in the monograph.

Historically, polarimetry was performed using an instrument where the extent of optical rotation is estimated by visual matching of the intensity of split fields. For this reason, the D-line of the sodium lamp at the visible wavelength of 589 nm was most often employed.[2] Specific rotation determined at the D-line is expressed by the symbol:

$$[\alpha]_{D}^{25} \text{ or } [\alpha]_{D}^{20}$$

Much of the data available are expressed in this form. Use of lower wavelengths, such as those available with the mercury lamp lines isolated by means of filters of maximum transmittance at approximately 546, 436, 405, 365, and 325 nm in a photoelectric polarimeter, has been found to provide advantages in sensitivity with a consequent reduction in the concentration of the test compound. In general, the observed optical rotation at 436 nm is about double, and at 365 nm, about 3 times that at 589 nm.[2] Reduction in the concentration of the solute required for measurement may sometimes be accomplished by conversion of the substance under test to one that has a significantly higher optical rotation. Optical rotation is also affected by the solvent used for the measurement, and this is always specified.

It is now common practice to use other light sources, such as xenon or tungsten halogen, with appropriate filters, because these may offer advantages of cost, long life, and broad wavelength emission range, over traditional light sources.

[1] Suitable calibrators are available from the Office of Standard Reference Materials, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899, as current lots of Standard Reference Materials, Dextrose and Sucrose. Alternatively, calibration may be checked using a Polarization Reference Standard, which consists of a plate of quartz mounted in a holder perpendicular to the light path. Acceptable periodic calibrations may be made by a National Metrology Institute (NMI) that is signatory to the International Committee of Weights and Measures (CIPM) Mutual Recognition Arrangement (MRA) or an ISO/IEC 17025 accredited laboratory for calibration where the accreditation body is a signatory to the International Laboratory Accreditation Cooperation (ILAC) MRA.

[2] All references of wavelengths are in vacuum. The sodium D-line is 589.44 in vacuum and 589.3 in air.

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PROCEDURES

Specific Rotation

The reference "*Optical Rotation* ⟨781S⟩, *Procedures, Specific Rotation*" in a monograph signifies that specific rotation is to be calculated from observed optical rotations in the *Test solution* or *Sample solution* obtained as directed therein. Unless otherwise directed, measurements of optical rotation are made in a 1.0-dm tube at 589 nm at 25°.[2] Where a photoelectric polarimeter is used, a single measurement, corrected for the solvent blank, is made. Where a visual polarimeter is employed, the average of no fewer than five determinations, corrected for the reading of the same tube with a solvent blank, is used. Temperature, which applies to the solution or the liquid under test, should be maintained within 0.5° of the stated value. Use the same cell for sample and blank. Maintain the same angular orientation of the cell in each reading. Place the cell so that the light passes through it in the same direction each time. Unless otherwise specified, specific rotation is calculated on the dried basis where *Loss on Drying* is specified in the monograph, or on the anhydrous basis where *Water Determination* is specified.

Optical rotation of solutions should be determined within 30 min of preparation. In the case of substances known to undergo racemization or mutarotation, care should be taken to standardize the time between adding the solute to the solvent and introduction of the solution into the polarimeter tube.

Angular Rotation

The reference "*Optical Rotation* ⟨781A⟩, *Procedures, Angular Rotation*" in a monograph signifies, unless otherwise directed, that the optical rotation of the neat liquid is measured in a 1.0-dm tube at 589 nm at 25°, corrected for the reading of the dry, empty tube.[2]

⟨782⟩ VIBRATIONAL CIRCULAR DICHROISM SPECTROSCOPY

1. INTRODUCTION
2. QUALIFICATION OF VCD SPECTROMETERS
   2.1 Installation Qualification
   2.2 Operational Qualification
   2.3 Performance Qualification
3. PROCEDURE
   3.1 Sample
   3.2 Standard
   3.3 Analysis
4. VALIDATION AND VERIFICATION
   4.1 Validation
   4.2 Precision
   4.3 Verification

1. INTRODUCTION

Vibrational circular dichroism (VCD) is a form of chiroptical spectroscopy for which chiral molecules have non-zero spectra with intensities that are identical for mirror-image pairs (enantiomers) of chiral molecules but with signs that are opposite with respect to zero intensity. For a chiral molecular substance, two important properties are 1) absolute configuration (AC), which indicates which of two mirror-image forms of the molecule is present; and 2) enantiomeric excess (EE, also called enantiomeric purity), the fractional excess of one enantiomer of a chiral molecule over its mirror-image enantiomer. The principal chapter in *USP–NF* that addresses molecular chirality is *Optical Rotation* ⟨781⟩. Optical rotation (OR) and electronic circular dichroism (ECD) are the traditional forms of chiroptical spectroscopy, whereas VCD is a newer form of chiroptical spectroscopy that contains more molecular structure information and recently has been adopted more broadly for use in the pharmaceutical industry.

In this chapter, the required methodology for the use of VCD is described in terms of instrument qualification; sign and intensity calibration; procedures, validation, and verification of VCD measurement; calculation; and statistical analysis as needed for the determination of AC and EE of chiral pharmaceutical products. VCD is the extension of ECD from the region of electronic transitions in the UV and visible regions of the spectrum to vibrational transitions in the infrared (IR) region.

VCD is defined as:

$$\Delta A = A_L - A_R$$

ΔA = difference in the IR absorbance
A_L = sample absorbance for left circularly polarized (LCP) radiation
A_R = sample absorbance for right circularly polarized (RCP) radiation

Unpolarized IR intensity is defined as the average of LCP and RCP intensities:

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$$A = (A_L + A_R)/2$$

A = IR absorbance
A_L = sample absorbance for left circularly polarized (LCP) radiation
A_R = sample absorbance for right circularly polarized (RCP) radiation

The IR intensities of enantiomers are identical, whereas enantiomers have equal and opposite-signed VCD intensities.

For discussion of the theory and principles of VCD measurements, as well as a more detailed explanation of the application of VCD to problems of pharmaceutical interest, see *Vibrational Circular Dichroism Spectroscopy—Theory and Practice* ⟨1782⟩.

2. QUALIFICATION OF VCD SPECTROMETERS

This section on qualification is divided into three subsections: *2.1 Installation Qualification* (IQ), *2.2 Operational Qualification* (OQ), and *2.3 Performance Qualification* (PQ) of a Fourier transform (FT)-VCD instrument. Although a VCD instrument can be based on a scanning dispersive spectrometer, no such VCD spectrometers are commercially available, and hence they will not be considered. Additional details on the qualification of instruments can be found in *Analytical Instrument Qualification* ⟨1058⟩.

2.1 Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location. An FT-VCD instrument is a sensitive, circular-polarization-difference, FT-infrared (FT-IR) spectrometer. Its installation requires a stable bench-top surface, access to dry air or nitrogen for purging of water vapor, and standard electrical outlets. Evidence for IQ involves ensuring that the IR interferometer is scanning, that its photoelastic modulator(s) [PEM(s)] is operating, and that the electronics hardware is interacting with both the FT-IR main bench and the PEM. The final evidence for IQ is that the software display for acquisition of a VCD spectrum is ready for operator use.

2.2 Operational Qualification

In OQ, an instrument's performance is characterized using a qualification standard of known spectral properties to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. OQ is a check of the key operational parameters and should be performed after installation and after repairs and/or maintenance.

The OQ tests described in the following sections are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance criteria provided in this section are applicable for general use, whereas specifications for particular instruments and applications may vary, depending on analytical methods used and desired accuracy of the final result.

CHARACTERIZING INSTRUMENT PERFORMANCE

FT-VCD spectrometers measure simultaneously the VCD spectrum and the parent IR spectrum. The VCD spectrum is obtained from a VCD interferogram arising from the combined high-frequency PEM polarization modulation and the Fourier frequencies, whereas the parent IR spectrum arises from the conventional FT-IR interferogram based only on the instrument's Fourier frequencies. Part of the instrument performance is from the underlying FT-IR operation, and the remaining part of the instrument performance is from those components that are uniquely associated with the FT-VCD operation. The combined FT-IR and FT-VCD criteria are wavenumber accuracy and absorbance linearity, whereas those uniquely associated with the VCD operation are VCD sign and intensity calibration, empty-beam baseline accuracy, sample-associated baseline deviations and artifact levels, baseline stability and spectral reproducibility, and signal-to-noise ratio. As described in ⟨1782⟩, two standard samples are commonly used for specifying VCD performance. For sign and intensity calibration, neat α-pinene is used as a validation standard, whereas for baseline characteristics, a solution of camphor in carbon tetrachloride (CCl_4) is used as the qualification standard. VCD spectra presented in ⟨1782⟩ serve as examples of performance characteristics of typical spectra for VCD sign and intensity calibration, baseline stability, and signal-to-noise ratio.

WAVENUMBER ACCURACY

The wavenumber accuracy of FT-VCD instruments is based on the corresponding wavenumber accuracy of the parent FT-IR operation. This accuracy is determined by fringe counting of the visible laser co-operating with the FT-IR interferometer. The frequency calibration is accurate to within the spectral resolution of the instrument and requires no calibration standard. The accuracy of wavenumber frequency should be checked by measuring the IR spectrum of a 35-µm-thick polystyrene film, as described in the *USP–NF* chapter on mid-IR spectroscopy, *Mid-Infrared Spectroscopy* ⟨854⟩, with additional information in *Mid-Infrared Spectroscopy—Theory and Practice* ⟨1854⟩. Because the FT-VCD and FT-IR spectra are simultaneously measured by the action of the same interferometer, the wavenumber accuracy of the VCD spectrum is the same as that of the FT-IR operation of the instrument.

LINEARITY AND STABILITY

Commercial FT-IR instruments have excellent linearity, provided that the infrared detector remains unsaturated. FT-IR spectra are particularly prone to detector saturation, because the intensity of the entire spectrum is present simultaneously at the detector. Detector saturation can be avoided by the use of a long-wavelength pass filter or appropriate adjustment of the gain

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of the preamplifier to ensure that the maximum of the FT-IR interferogram lies within the range of preamplifier voltage capability. The hallmark of the onset of nonlinearity is distortion of the FT-IR interferogram that can lead to negative FT-IR absorbance values at the endpoint frequency of the spectral coverage, which is clearly incorrect for an absorbing sample. Aperture reduction, insertion of screens, or neutral density filters can be used to avoid detector saturation.

These same considerations apply to the linearity of FT-VCD measurements. The linearity of VCD is more sensitive to the onset of detector saturation than is the parent IR spectrum. VCD spectra are unstable and distorted when the parent FT-IR spectrum has any saturation, and in some cases linearity in the VCD is not restored until the FT-IR intensity is well below saturation. Saturation can be tested by varying the throughput of the instrument by changing the sample path length and making sure that all of the points across the spectrum are changed by the same factor, i.e., there are no relative shape changes in the spectrum. These relative shape changes should be less than the apparent noise level or, at most, 2% (1.00 ± 0.02), depending on the accuracy needed for the VCD measurements.

PHASE CORRECTION, SIGN, AND INTENSITY CALIBRATION

The raw VCD spectrum is measured as the ratio of the FT of the VCD interferogram to that of the FT of the IR interferogram. Both of these interferograms must be phase-corrected before performing the FT. The phase correction for the IR is straightforward, but the phase correction of the VCD interferogram requires transfer of a phase file from a VCD interferogram representing only positive intensities. Most commercial FT-VCD instruments provide such phase files for the measurement of VCD.

Next, the measured raw VCD spectrum must be calibrated such that VCD spectra with the correct signs and intensities are produced. The signs can be determined by measuring the qualification standard of (–)-camphor or (+)-camphor in carbon tetrachloride solution and comparing it to a previously published standard VCD spectrum of this molecule, such as that provided in ⟨1782⟩. Either all of the signs agree, in which case the sign is correctly set, or all of the signs are opposite, in which case the phase of the VCD synchronous detection (lock-in amplifier or numerical processing) must be changed by 180° to reverse the signs of all VCD bands, positive-to-negative and negative-to-positive.

Calibration of VCD intensities is performed by dividing the raw VCD by a calibration curve representing the magnitude of unit VCD intensity for the VCD spectrometer. The shape of the calibration curve follows a first-order Bessel function, which in turn depends on the settings of the PEM generating LCP and RCP radiation. The maximum of the calibration curve corresponds to the maximum wavenumber value set on the PEM controller. The calibration curve for a given PEM setting can be obtained from the crossing points of curves generated by placing a multiple-wave plate followed by a polarizer in place of the sample and measuring the VCD spectrum in the usual manner. Commercial VCD instruments provide such curves, and the correct calibration curve must be used for each wavenumber setting of the PEM. For an example of a calibration curve for the PEM setting at 1200 cm^{-1}, see ⟨1782⟩.

After calibration of the intensities of the VCD instrument, a measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride measured in a 100-µm path-length cell should be approximately 2×10^{-4}. After baseline subtraction, described below and in ⟨1782⟩, this difference should be a positive peak ΔA value of $+1 \times 10^{-4}$ absorbance units at 1240 cm^{-1} and a negative ΔA peak value of -1×10^{-4} at 1040 cm^{-1}.

EMPTY BEAM BASELINE CHARACTERISTICS

To avoid significant levels of baseline deviations from the absorbance features of a sample, the empty beam baseline for a VCD instrument should be as close as possible to the true zero of the measurement. A minimum standard for FT-VCD spectrometers is to have empty beam baselines sufficiently close to zero so that deviations from true zero are <25% of the span of maximum positive-to-negative absorbance intensity of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a 100-µm path-length cell. This magnitude, noted above, is approximately 2×10^{-4} as determined from a positive peak value of $+1 \times 10^{-4}$ at 1240 cm^{-1} and a negative peak value of -1×10^{-4} at 1040 cm^{-1}. The value of 25% of this intensity range is approximately 5×10^{-5}. VCD empty beam baseline deviations (maximum to minimum), over the mid-IR range from 900 to 1800 cm^{-1}, should not exceed $\pm 5 \times 10^{-4}$ and should not deviate more than $+5 \times 10^{-4}$ or -5×10^{-4} from the zero of VCD intensity over this wavenumber range.

SAMPLE BASELINES AND ABSORPTION ARTIFACTS

An important test for a VCD measurement is the VCD spectrum of a racemic sample relative to that of the baseline of the empty instrument or the VCD spectrum of a nonchiral solvent, such as carbon tetrachloride. Because of possible baseline changes due to the sample cell, it is preferable to compare the VCD of the solvent with that of a solution of the racemic sample of the chiral molecule in the same solvent. Both should have zero VCD, but the racemic solution may have baseline artifacts (offsets) at the location of the absorption bands. Absorption bands change the index of refraction of the sample and can cause baseline deviations that are particular to the sample molecule. If such absorption artifacts are present, the only way to recover the artifact-free VCD spectrum is by subtraction of the racemic mixture (equal quantities of both enantiomers) from that of the measured VCD spectrum. Alternatively, subtraction of the VCD spectrum of the opposite enantiomer is divided by 2, because the VCD intensity doubles if the VCD spectrum of one enantiomer is subtracted from that of the other. The qualification standard used for measuring the VCD baseline with an absorbing sample present is racemic camphor in carbon tetrachloride solution versus the VCD spectrum of neat carbon tetrachloride in the range 900–1800 cm^{-1}, where there are only absorption bands from camphor and none for carbon tetrachloride. Both samples should have zero VCD and a baseline that is featureless with respect to the camphor absorption bands.

SIGNAL-TO-NOISE RATIO

The signal-to-noise ratio of a VCD measurement can be determined from the noise curve generated by subtracting one-half of the FT scans of a measurement from the other half, thus cancelling the signal and exposing the noise level. The noise level

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varies across the spectrum and increases when the light level reaching the detector decreases, either due to the overall throughput of the instrument in a particular spectral region or directly within the absorption bands of the sample. The level of noise in the noise curve can be reduced by increasing the measurement time of the spectrum. The noise level is inversely proportional to the square root of the measurement time. This is illustrated with a particular example in *Figures 9* and *10* of ⟨1782⟩.

Smoothing a VCD spectrum without increasing the measurement time will reduce the size of the peak-to-peak noise excursions but will not reduce the overall accuracy of a VCD measurement, because the VCD baseline, and hence the VCD intensity relative to the zero baseline, will undergo the same level of variations, although the VCD spectrum appears to have less noise after smoothing. To know the true level of the noise and hence the uncertainty level of a VCD spectrum, all smoothing functions, if any, should be turned off. Noise can legitimately be reduced by lowering the spectral resolution. In evaluating the noise level of the VCD measurement, the resolution and interferogram apodization must be specified.

2.3 Performance Qualification

PQ determines if the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement. Depending on typical use, the specifications for PQ may be different from the manufacturer's OQ specifications; however, for general use across a range of typical chiral molecules and sampling conditions, the specifications for OQ will be the same as those needed for PQ. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements.

Specific procedures, acceptance criteria, and time intervals for characterizing performance depend on the instrument and intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from test sample spectra.

Particular long-term stability tests that can be carried out for PQ evaluation beyond those carried out for IQ or OQ are as follows. It is recommended that a 60-min measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a 100-µm path-length cell, be performed between different applications of the VCD spectrometer to ensure that the signs, intensities, and signal-to-noise ratio of the VCD spectrum established as a qualification test during OQ are maintained. This is particularly important if there has been an interruption in power or a long hiatus between measurements.

If averaging over long periods of time, such as overnight, it is important that the stability of both the VCD baseline and the sample VCD spectrum are stable over time and that the signal-to-noise ratio improves as expected with the square-root of measurement time or number of interferometer scans, as illustrated by the example in ⟨1782⟩. In general, stability should be maintained to within the noise level of the VCD measurement. Because VCD intensities are generally three to four orders of magnitude smaller than the parent IR intensity, the instrument stability for the FT-IR performance of the instrument is typically larger over time relative to the noise level of the VCD spectrum.

3. PROCEDURE

The measurement of the VCD spectrum of a sample follows the same general rules as the measurement of an IR spectrum as for mid-IR spectroscopy described in ⟨854⟩.

3.1 Sample

A VCD spectrum can be measured in all types of standard IR transmission cells, either for neat liquids or solutions. For solids, such as in potassium bromide (KBr) pellets, mulls, or thin films, it is recommended that additional steps be taken to ensure that artifacts arising from sample linear birefringence are not present as described in ⟨1782⟩ for advanced instrumentation methods, such as dual PEM modulation or rotating sample cells.

Because of the importance of noise control in a VCD measurement, the absorbance of a sample should not exceed $A = 1.0$ in the region of measurement interest. For absorbance >1.0, insufficient radiation is present at the detector, and noise dominates the VCD spectrum. The optimum level of absorbance relative to the sample reference (empty beam or solvent) is approximately 0.4. One should adjust the path length and/or concentration of the sample such that the average absorbance is approximately 0.4–0.5 over the region of interest, with limits of 0.1–1.0. For samples with a wide range of absorbance, more than one measurement can be carried out with differing average absorbance values to ensure that a VCD spectrum of adequate quality is obtained over the entire range of interest.

To perform a solution-state VCD measurement, first find a solvent that dissolves an adequate amount of solute to achieve a desired absorbance level. Solvents without CH bonds are the most desirable for this purpose; these include carbon tetrachloride, deuterated chloroform ($CDCl_3$), or deuterated dimethyl sulfoxide (DMSO-d_6). The IR cell should have windows that are transparent in the mid-IR region, with generally low levels of linear birefringence. Commonly used materials are barium fluoride (BaF_2), which has good transmission to 800 cm^{-1}, and calcium fluoride (CaF_2), which is used if coverage to only 1100–1200 cm^{-1} is needed, such as for aqueous solutions.

3.2 Standard

To ensure that the analyst can use an IR cell for a transmission measurement of a solution, it is recommended that a standard OQ measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride using a 100-µm barium fluoride cell be performed. For different choices of concentration or path length, one can use the linear dependence of these quantities on the measured ΔA to ensure that an accurate measurement with the correct signs and intensities has been achieved.

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3.3 Analysis

VCD spectra, once measured, can be analyzed in a variety of ways, depending on the intended application. The principal application of VCD in the pharmaceutical industry is the determination of absolute configuration (AC) in conjunction with a VCD calculation using a quantum chemistry computation software package. A second application is the determination of EE of a sample, either for the purpose of determining the EE of a sample or for monitoring the EE of one or more species during the course of the chemical reaction involving chiral species. Achievement of the highest level of accuracy in the determination of EE requires the use of a chemometric software package that uses the entire range of the VCD spectrum, as opposed to a single band or wavenumber frequency. Other applications of VCD are its use with a standard database of VCD and IR spectra to simultaneously ensure the correct identity, AC, and EE of a chiral substance, for example as a chiral raw material identification. VCD can also be used to specify the AC and EE of a drug product in the presence of either achiral or chiral excipients, such as carbohydrates. These applications are described in more detail in ⟨1782⟩.

4. VALIDATION AND VERIFICATION

4.1 Validation

The objective of VCD procedure validation, as with the validation of any analytical process, is to demonstrate that the measurement is suitable for its intended purpose. The application of VCD spectroscopy is somewhat different from that of conventional vibrational spectroscopic techniques because it is not a primary technique. Rather, it is the polarization difference spectroscopy of the parent IR spectroscopy that has been used by itself for decades as a primary identification and quantitation technique as described for mid-IR spectroscopy in ⟨854⟩. The differential VCD spectrum supplements the parent IR spectrum with a new set of intensities at each position of an IR absorption band. The new set of intensities then reveals stereospecific properties of the sample molecules that must be chiral to have a non-zero VCD spectrum. There are two primary properties of a sample of a chiral molecule: one is the AC of the dominant chiral species (dominant enantiomer), and the second property is the EE of the dominant enantiomer over its lesser (mirror-image) enantiomer. In addition, the VCD spectrum carries additional information about the solution-state conformation, or conformational distribution, of the molecule that may be available in only lesser detail, if any, using the parent IR spectrum alone. Applications that require validation for the use of VCD spectroscopy also exist for the combined use of the measurement of the VCD of a substance for which there exists a reference spectrum with known AC and EE. Any subsequent measurement of the VCD spectrum, together with the simultaneous measurement of its parent IR spectrum, confirms in one measurement the molecular identity of the sample as well as the AC and EE. Such applications are useful, for example, for chiral raw-material identification of AC and EE, for which there is currently no established methodology for a single measurement. VCD can also confirm, in a single measurement, the AC and EE of a final drug product, including the chirality (if any) of the excipients.

Before validation of VCD for AC determination, an overall instrument validation should be carried out by the measurement of the VCD spectrum of neat (–)-*S*-α-pinene or (+)-*R*-α-pinene using a 75-µm cell. The calibrated, baseline-corrected VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1}. The noise level should be comparable to that established for the instrument during the OQ phase of instrumental qualification described above. Because the VCD spectrum of α-pinene is relatively large, sufficient signal-to-noise ratio is available with a 20-min collection to verify that the instrument is operating with correct VCD intensities, signs, and noise levels for the applications of VCD described below.

Validation for AC determination by VCD can be achieved by the successful comparison of the measured VCD spectrum of the validation standard, neat (–)-*S*-α-pinene, to its quantum-chemistry-calculated VCD spectrum. Success is achieved by measuring the baseline-corrected VCD spectrum with sufficient signal-to-noise ratio that the signs, frequencies, and relative intensities of the major VCD bands can be compared clearly to the corresponding VCD signs, frequencies, and relative intensities of the calculated VCD spectrum. If the signs, frequencies, and relative intensities agree, then the AC of the measured sample of (–)-α-pinene is the same as that for the model of *S*-α-pinene that has been constructed for the calculation of this standard molecule. If the frequencies and relative VCD intensities are opposite after instrument sign calibration, then the AC of the sample is opposite (the mirror image) of the enantiomer used for the calculation. A successful comparison requires pretreatment of the VCD data for baseline subtraction and intensity calibration. It also requires a computational procedure that is known to be accurate in calculating VCD spectra. Finally, a program should be used to impartially assess the degree of agreement between measured and calculated VCD spectra such that one relies on more than an analyst's visual assessment of the agreement, or lack of agreement, between a measured spectrum and a calculated spectrum. An example of this procedure can be found in ⟨1782⟩ as well as in the references cited in ⟨1782⟩.

Validation of VCD spectroscopy for the determination of EE is achieved by plotting the EE predicted by VCD versus the EE for a sample of neat (–)-*S*-α-pinene for a series of samples with differing values of %EE over the range from 100% EE to 0% EE. The EE predicted by VCD involves measurement of the VCD spectra at a chosen spectral resolution and time of collection to achieve a desired level of signal-to-noise ratio, followed by pretreatment of the measured VCD spectra for baseline subtraction. The prediction of EE could be based on a size of a single VCD band for the samples with differing VCD, normalized for constant IR intensity; but more generally and with better accuracy, the entire range of VCD bands across the spectrum is used for each spectrum by means of a chemometrics software package. Validation is accompanied by a measure of the %EE accuracy through a cross-correlation analysis, yielding a root mean square (RMS). An example of such a determination of %EE by VCD for neat (–)-*S*-α-pinene is provided, along with literature references, in ⟨1782⟩.

More generally, validation is required when a method is intended for use as an alternative to the official procedure for testing an official article. The objective is to demonstrate that the measurement of VCD is suitable for its intended purpose; for examples of various types of measurements, see *Validation of Compendial Procedures* ⟨1225⟩. For %EE determination, creation of a linear plot of actual versus VCD-predicted values of %EE establishes that the magnitude of IR-normalized VCD spectra, as evaluated

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with chemometrics, exhibits sufficient linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness with respect to the detection of %EE.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for VCD technology. For each particular application, tighter criteria may be needed to demonstrate suitability for the intended use.

ACCURACY

Accuracy can be determined by the measurement of a VCD spectrum of the established validation standard of neat (–)-α-pinene or (+)-α-pinene, as presented, for example, in ⟨1782⟩, and verifying that the measured signs and intensities of the VCD spectrum agree with the standard VCD spectrum to within the noise level of the measurement. In particular, the VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1}. This validation ensures that the VCD spectrometer is accurately calibrated and has the correct signs for the VCD bands.

4.2 Precision

REPEATABILITY

Validation criteria: Measurement of VCD spectra of the validation standard sample, neat (+)-α-pinene or (–)-α-pinene, in 20-min blocks with variations in the VCD spectra at or less than the noise level of each 20-min block that is approximately ΔA equal to or less than 1×10^{-5}

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the validation criteria of VCD measurement should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision. (For example, this could be two analysts on each of 3 days, or two analysts on two sets of equipment on 2 days for each analyst–equipment combination.)
Validation criteria: The *Validation criteria* are the same as those for *Repeatability*.

SPECIFICITY

Specificity is not a required metric for the determination of AC. For the measurement of EE for more than one chiral species in a single set of measurements, the analytical procedure, including chemometric analysis, must be able to unequivocally assess the EE of chiral species in the presence of components that may be expected to be present, i.e., solvent and other nonchiral species.
Validation criteria: The *Validation criteria* are demonstrated by meeting the *Accuracy* requirement.

QUANTITATION LIMIT

For the determination of AC, there is no quantitation limit (QL). The QL for EE can be determined by carrying out a determination of quantitation of %EE for the VCD validation standard of neat α-pinene for a series of EE values, performing a chemometric analysis of VCD-predicted EE values and performing a determination of RMS error, as illustrated, for example, in ⟨1782⟩.
Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to approximately 1% EE for neat α-pinene.

LINEARITY

A linear relationship between the analyte concentration and response should be demonstrated by preparing NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve should then be evaluated using appropriate statistical methods, such as a least-squares regression, for the area of peak height of a selected VCD band, or for the entire spectrum, using chemometric analysis such as partial least squares. For experiments that do not yield a linear relationship between analyte concentration and response, appropriate statistical methods must be applied to describe the analytical response.
Validation criteria: The *Validation criteria* find the correlation coefficient above 0.95 for a linear plot of actual EE versus VCD-predicted EE.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters, such as changes in spectral resolution, concentration, or path length for measurement of VCD.

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4.3 Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP–NF*, are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, can be executed by the analysts with suitable accuracy, specificity, and precision using the instruments, and sample solutions of chiral molecules available. If the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test (for additional information, see *Verification of Compendial Procedures* ⟨1226⟩). It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Verification of compendial methods should, at a minimum, include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in *4.1 Validation*.

⟨785⟩ OSMOLALITY AND OSMOLARITY

INTRODUCTION

Osmotic pressure plays a critical role in all biological processes that involve diffusion of solutes or transfer of fluids through membranes. Osmosis occurs when solvent but not solute molecules cross a semipermeable membrane from regions of lower to higher concentrations to produce equilibrium. The knowledge of osmotic pressures is important for practitioners in determining whether a parenteral solution is hypo-osmotic, iso-osmotic, or hyperosmotic. A quantitative measure of osmotic pressure facilitates the dilution required to render a solution iso-osmotic relative to whole blood.

OSMOTIC PRESSURE

The osmotic pressure of a solution depends on the number of particles in solution, and is therefore referred to as a colligative property. A particle can be a molecule or an ion or an aggregated species (e.g., a dimer) that can exist discretely in solution. A solution exhibits ideal behavior when no interaction occurs between solutes and solvent, except where solvent molecules are bound to solutes by hydrogen bonding or coordinate covalency. For such a solution containing a nondissociating solute, the osmotic pressure (π) is directly proportional to its molality (number of moles of solute per kilogram of the solvent):

$$\pi = (\rho RT/1000)m,$$

where ρ is the density of the solvent at the temperature T (in the absolute scale); R is the universal gas constant; and m is the molality of the solution. For a real solution containing more than one solute, the osmotic pressure is given by the formula:

$$\pi = (\rho RT/1000)\Sigma \nu_i m_i \Phi_{m,i}$$

where ν_i is the number of particles formed by the dissociation of one molecule of the i^{th} solute; $\nu_i = 1$ for nonionic (nondissociating) solutes; m_i is the molality of the i^{th} solute; and $\Phi_{m,i}$ is the molal osmotic coefficient of the i^{th} solute. The molal osmotic coefficient takes into account the deviation of a solution from ideal behavior. Its value depends upon the concentration of the solute(s) in solution, its chemical properties, and ionic characteristics. The value of the molal osmotic coefficient of a solute can be determined experimentally by measuring the freezing point depression at different molal concentrations. At concentrations of pharmaceutical interest, the value of the molal osmotic coefficient is less than one. The molal osmotic coefficient decreases with the increase in concentration of the solute *(Table 1)*.

OSMOLALITY

The osmolality of a solution ξ_m is given by

$$\xi_m = \Sigma \nu_i m_i \Phi_{m,i}.$$

The osmolality of a real solution corresponds to the molality of an ideal solution containing nondissociating solutes and is expressed in osmoles or milliosmoles per kilogram of solvent (Osmol per kg or mOsmol per kg, respectively), a unit that is similar to the molality of the solution. Thus, osmolality is a measure of the osmotic pressure exerted by a real solution across a semipermeable membrane. Like osmotic pressure, other colligative properties of a solution, such as vapor pressure lowering, boiling point elevation, and freezing point depression, are also directly related to the osmolality of the solution. Indeed, the osmolality of a solution is typically determined most accurately and conveniently by measuring freezing point depression (ΔT_f):

$$\Delta T_f = k_f \xi_m$$

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where k_f is the molal cryoscopic constant, which is a property of the solvent. For water, the value of k_f is 1.860° per Osmol. That is, 1 Osmol of a solute added to 1 kg of water lowers the freezing point by 1.860°.

OSMOLARITY

Osmolarity of a solution is a theoretical quantity expressed in osmoles per L (Osmol per L) of a solution and is widely used in clinical practice because it expresses osmoles as a function of volume. Osmolarity cannot be measured but is calculated theoretically from the experimentally measured value of osmolality.

Sometimes, osmolarity (ξ_c) is calculated theoretically from the molar concentrations:

$$\xi_c = \Sigma \nu_i c_i$$

where ν_i is as defined above, and c_i is the molar concentration of the i^{th} solute in solution. For example, the osmolarity of a solution prepared by dissolving 1 g of vancomycin in 100 mL of 0.9% sodium chloride solution can be calculated as follows:

$$[3 \times 10 \text{ g/L}/1449.25(\text{mol. wt. of vancomycin}) + 2 \times 9 \text{ g/L}/58.44(\text{mol. wt. of sodium chloride})] \times 1000 = 329 \text{ mOsmol/L}$$

The results suggest that the solution is slightly hyperosmotic because the osmolality of blood ranges between 285 and 310 mOsmol per kg. However, the solution is found to be hypo-osmotic and has an experimentally determined osmolality of 255 mOsmol per kg.[1] The example illustrates that osmolarity values calculated theoretically from the concentration of a solution should be interpreted cautiously and may not represent the osmotic properties of infusion solutions.

The discrepancy between theoretical (osmolarity) and experimental (osmolality) results is, in part, due to the fact that osmotic pressure is related to osmolality and not osmolarity. More significantly, the discrepancy between experimental results and the theoretical calculation is due to the fact that the osmotic pressure of a real solution is less than that of an ideal solution because of interactions between solute molecules or between solute and solvent molecules in a solution. Such interactions reduce the pressure exerted by solute molecules on a semipermeable membrane, reducing experimental values of osmolality compared to theoretical values. This difference is related to the molal osmotic coefficient ($\Phi_{m,i}$). The example also illustrates the importance of determining the osmolality of a solution experimentally, rather than calculating the value theoretically.

MEASUREMENT OF OSMOLALITY

The osmolality of a solution is commonly determined by the measurement of the freezing point depression of the solution.

Apparatus

The apparatus, an osmometer for freezing point depression measurement, consists of the following: a means of cooling the container used for the measurement; a resistor sensitive to temperature (thermistor), with an appropriate current- or potential-difference measurement device that may be graduated in temperature change or in osmolality; and a means of mixing the sample.

Osmometers that measure the vapor pressures of solutions are less frequently employed. They require a smaller volume of specimen (generally about 5 µL), but the accuracy and precision of the resulting osmolality determination are comparable to those obtained by the use of osmometers that depend upon the observed freezing points of solutions.

Standard Solutions

Prepare *Standard Solutions* as specified in *Table 1*, as necessary.[2]

Table 1. Standard Solutions for Osmometer Calibration*

Standard Solutions (Weight in g of sodium chloride per kg of water)	Osmolality (mOsmol/kg) (ξ_m)	Molal Osmotic Coefficient ($\Phi_{m,\,NaCl}$)	Freezing Point Depression (°) ΔT_f
3.087	100	0.9463	0.186
6.260	200	0.9337	0.372
9.463	300	0.9264	0.558
12.684	400	0.9215	0.744
15.916	500	0.9180	0.930
19.147	600	0.9157	1.116
22.380	700	0.9140	1.302

* Adapted from the *European Pharmacopoeia,* 4th Edition, 2002, p. 50.

[1] Kastango, E.S. and Hadaway, L. *International Journal of Pharmaceutical Compounding 5,* (2001) 465-469.

[2] Commercially available solutions for osmometer calibration, with osmolalities equal to or different from those listed in *Table 1* and standardized by methods traceable to NIST, may be used.

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Test Solution

For a solid for injection, constitute with the appropriate diluent as specified in the instructions on the labeling. For solutions, use the sample as is.

[NOTE—A solution can be diluted to bring it within the range of measurement of the osmometer, if necessary, but the results must be expressed as that of the diluted solution and must NOT be multiplied by a dilution factor to calculate the osmolality of the original solution, unless otherwise indicated in the monograph. The molal osmotic coefficient is a function of concentration. Therefore, it changes with dilution.]

Procedure

First, calibrate the instrument by the manufacturer's instructions. Confirm the instrument calibration with at least one solution from *Table 1* such that the osmolality of the *Standard Solution* lies within 50 mOsmol/kg of the expected value of the *Test Solution* or the center of the expected range of osmolality of the *Test Solution*. The instrument reading should be within ±4 mOsmol per kg from the *Standard Solution*. Introduce an appropriate volume of each *Standard Solution* into the measurement cell as in the manufacturer's instructions, and start the cooling system. Usually, the mixing device is programmed to operate at a temperature below the lowest temperature expected from the freezing point depression. The apparatus indicates when the equilibrium is attained. If necessary, calibrate the osmometer, using an appropriate adjustment device such that the reading corresponds to either the osmolality or freezing point depression value of the *Standard Solution* shown in *Table 1*.

[NOTE—If the instrument reading indicates the freezing point depression, the osmolality can be derived by using the appropriate formula under *Osmolality*.]

Repeat the procedure with each *Test Solution*. Read the osmolality of the *Test Solution* directly, or calculate it from the measured freezing point depression.

Assuming that the value of the osmotic coefficient is essentially the same whether the concentration is expressed in molality or molarity, the experimentally determined osmolality of a solution can be converted to osmolarity in the same manner in which the concentration of a solution is converted from molality to molarity. Unless a solution is very concentrated, the osmolarity of a solution (ξ_c) can be calculated from its experimentally determined osmolality (ξ_m):

$$\xi_c = 1000\xi_m/(1000/\rho + \Sigma w_i v_i)$$

where w_i is the weight in g; and v_i is the partial specific volume, in mL per g, of the i^{th} solute. The partial specific volume of a solute is the change in volume of a solution when an additional 1 g of solute is dissolved in the solution. This volume can be determined by the measurement of densities of the solution before and after the addition of the solute. The partial specific volumes of salts are generally very small, around 0.1 mL per g. However, those of other solutes are generally higher. For example, the partial specific volumes of amino acids are in the range of 0.6–0.9 mL per g. It can be shown from the above equation correlating osmolarity with osmolality that,

$$\xi_c = \xi_m (\rho - c)$$

where ρ is the density of the solution, and c is the total solute concentration, both expressed in g per mL. Thus, alternatively, the osmolarity can also be calculated from experimentally determined osmolality from the measurement of density of the solution by a suitable method and the total weight of the solute, after correction for water content, dissolved per mL of the solution.

⟨786⟩ PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about 75 µm. For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials, other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than 75 µm where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of the sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

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Estimate the particle size distribution as described under *Dry Sieving Method,* unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below 75 µm), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried out at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

PRINCIPLES OF ANALYTICAL SIEVING

Analytical test sieves are constructed from a woven-wire mesh, which is of simple weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75 µm. The size parameter involved in determining particle size distribution by analytical sieving is the length of the side of the minimum square aperture through which the particle will pass.

TEST SIEVES

Test sieves suitable for pharmacopeial tests conform to the most current edition of International Organization for Standardization Specification ISO 3310-1: Test Sieves—Technical Requirements and Testing (see *Table 1*). Unless otherwise specified in the monograph, use those ISO sieves listed as principal sizes in *Table 1*. Unless otherwise specified in the monograph, use those ISO sieves listed in *Table 1* as recommended in the particular region.

Table 1. Sizes of Standard Sieve Series in Range of Interest

ISO Nominal Aperture						
Principal Sizes	Supplementary Sizes		US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
R 20/3	R 20	R 40/3				
11.20 mm	11.20 mm	11.20 mm			11200	
	10.00 mm					
		9.50 mm				
	9.00 mm					
8.00 mm	8.00 mm	8.00 mm				
	7.10 mm					
		6.70 mm				
	6.30 mm					
5.60 mm	5.60 mm	5.60 mm			5600	3.5
	5.00 mm					
		4.75 mm				4
	4.50 mm					
4.00 mm	4.00 mm	4.00 mm	5	4000	4000	4.7
	3.55 mm					
		3.35 mm	6			5.5
	3.15 mm					
2.80 mm	2.80 mm	2.80 mm	7	2800	2800	6.5
	2.50 mm					
		2.36 mm	8			7.5
	2.24 mm					
2.00 mm	2.00 mm	2.00 mm	10	2000	2000	8.6
	1.80 mm					
		1.70 mm	12			10
	1.60 mm					

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Table 1. Sizes of Standard Sieve Series in Range of Interest *(continued)*

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
Principal Sizes	Supplementary Sizes					
R 20/3	R 20	R 40/3				
1.40 mm	1.40 mm	1.40 mm	14	1400	1400	12
	1.25 mm					
		1.18 mm	16			14
	1.12 mm					
1.00 mm	1.00 mm	1.00 mm	18	1000	1000	16
	900 µm					
		850 µm	20			18
	800 µm					
710 µm	710 µm	710 µm	25	710	710	22
	630 µm					
		600 µm	30			26
	560 µm					
500 µm	500 µm	500 µm	35	500	500	30
	450 µm					
		425 µm	40			36
	400 µm					
355 µm	355 µm	355 µm	45	355	355	42
	315 µm					
		300 µm	50			50
	280 µm					
250 µm	250 µm	250 µm	60	250	250	60
	224 µm					
		212 µm	70			70
	200 µm					
180 µm	180 µm	180 µm	80	180	180	83
	160 µm					
		150 µm	100			100
	140 µm					
125 µm	125 µm	125 µm	120	125	125	119
	112 µm					
		106 µm	140			140
	100 µm					
90 µm	90 µm	90 µm	170	90	90	166
	80 µm					
		75 µm	200			200
	71 µm					
63 µm	63 µm	63 µm	230	63	63	235
	56 µm					
		53 µm	270			282
	50 µm					
45 µm	45 µm	45 µm	325	45	45	330
	40 µm					
		38 µm			38	391

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Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a $\sqrt{2}$ progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings.

[NOTE—Mesh numbers are provided in the table for conversion purposes only.]

Test sieves are made from stainless steel or, less preferably, from brass or other suitable nonreactive wire.

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 µm, Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

Cleaning Test Sieves

Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

Test Specimen

If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200-mm diameter. For 76-mm sieves, the amount of material that can be accommodated is approximately 1/7th that which can be accommodated on a 200-mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker.

[NOTE—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.]

Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be redetermined. The use of test samples having a smaller mass (e.g., down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly isodiametrical shape, specimen weights below 5 g for a 200-mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

Agitation Method

Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), because changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

Endpoint Determination

The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76-mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve, intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

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SIEVING METHODS

Mechanical Agitation

DRY SIEVING METHOD

Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and replace the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, and a different particle size analysis method should be used.

Air Entrainment Methods

AIR JET and SONIC SIFTER SIEVING

Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as air jet sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method,* but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than those used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the sonic sifting method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic sifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 µm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

INTERPRETATION

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

⟨787⟩ SUBVISIBLE PARTICULATE MATTER IN THERAPEUTIC PROTEIN INJECTIONS

This chapter can be used as an alternative to *USP* general chapter *Particulate Matter in Injections* ⟨788⟩. It specifically addresses therapeutic protein injections and related preparations, allowing use of smaller test product volumes and smaller test aliquots to determine particulate matter content, with sample-handling instructions that take into account the issues associated with the analysis of these materials. Although the methodology and limits presented in this chapter are preferred for therapeutic protein injections, the use of regulatory-acceptable alternative analytical methods with adequately developed subvisible particulate limits is acceptable.

Therapeutic protein injections are biotechnology-derived products of protein or peptides and other therapeutic protein injections such as naturally sourced therapeutic proteins, including their final infusion preparations. Certain preparations that are not amenable to these tests, such as prophylactic vaccines, are not included in this guidance.

Particulate matter in therapeutic protein injections consists of mobile undissolved substances that may originate from various sources. The particles may be (a) truly foreign, or "extrinsic", e.g., unexpected foreign material, such as cellulose; (b) "intrinsic"

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resulting from addition or by insufficient cleaning during manufacturing, such as tank metals or gaskets, lubricants, filling hardware, or resulting from instability, e.g., changes over time, such as insoluble drug salt forms or package degradation; and (c) "inherent", such as particles of the protein or formulation components. All of these particle types may be detected and counted in the test method described in this chapter. In addition, for light obscuration (LO) methods, gas bubbles are commonly counted during particulate matter testing and are counting artifacts. For the determination of subvisible particles in therapeutic protein injections, the *Light Obscuration Particle Count Test* is the preferred method. The *Microscopic Particle Count Test* similar to that described in ⟨788⟩ may be useful in cases where the particles being analyzed are primarily nonproteinaceous, or in cases where examination of particle characteristics may be important. The results from this test are not equivalent to those from the LO test.

Not all therapeutic protein injections can be examined directly for subvisible particles by the LO method. When the test method is not directly applicable to specific test samples, as indicated by issues with method verification (reproducibility, linearity), a quantitative dilution with an appropriate diluent can be made to allow analysis by light obscuration. Method verification may be required to ensure the appropriateness of the sample handling procedures and the performance of the method for each drug product.

In the tests described below, the results obtained by examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, sampling plans based on known operational factors must be developed to support valid inferences from observed data in order to characterize the level of particulate matter in a large group of units. Sampling plans should be based on considerations of product volume, particle numbers historically found in comparison to limits, particle size distribution, and variability of particle counts between units. Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption.

LIGHT OBSCURATION PARTICLE COUNT TEST

Analysts should use a suitable instrument that is based on the principle of light blockage and that allows an automatic determination of particle size and number.

The sensor selected must be appropriate for the intended particle size range and anticipated particle count. Several standardization steps such as sample volume accuracy, sample flow rate, sensor resolution, calibration, and particle count accuracy are important for the proper operation of the apparatus and are further reviewed in general information chapter *Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions* ⟨1788⟩.[1]

For equipment calibration purposes, use of NIST-traceable particle size standards or equivalent traceable standards, see ⟨1788⟩. These standards generally are between 2 and 100 µm. Analysts should verify the performance of the apparatus using the USP Particle Count RS dispersed in particle-free water at an appropriate volume. Care must be taken to avoid agglomeration of particles during dispersion in the calibration process. The definition for *Particle-Free Water* is provided in the introductory section of *Reagents, Indicators, and Solutions*.

General Precautions

Carry out the test under conditions that limit ingress of particulate matter, preferably in a laminar-flow cabinet. The glassware and equipment used should be properly cleaned and handled to remain particle free. Care should be taken to minimize shaking and other stresses to the preparations (for liquids or reconstituted powders) and to prevent introduction of air bubbles into the preparation under examination. This is particularly important when preparations are combined or transferred to the container in which the determination will be carried out.

Preparatory Steps

Glassware selected for the method should accommodate the volume of blank and sample aliquots, allow easy mixing, and be amenable to repeated cleaning used to prevent any significant contribution to particle counts. Typically, separate vessels are utilized and retained specifically for this test only. Cleaning and rinsing of the method glassware should be in close proximity to the counting area.

The work area and equipment should be separated from general lab traffic. A good supply of filtered water (to comply with *Particle-Free Water*) and support equipment should be maintained and available for the routine work. Refer to general information chapter ⟨1788⟩.[1]

Two types of system controls are recommended: a *Blank Test* and *System Suitability Verification*.

1. The *Blank Test* involves the following:
   A. Run at the start of the day or analysis to ensure that proper care has been used in preparing the site and apparatus for use (*Environmental Blank*).
   B. Any additional blanks run throughout the day (such as between product families) should also comply with the *Blank Test* requirements (*Procedural Blank*).
2. The *System Suitability Verification* is run to verify proper count/mL and ratio for the current USP Particle Count RS or equivalent commerical standard. See general information chapter ⟨1788⟩.[1]

[1] For informational purposes only, analysts can refer to ⟨1788⟩, which may be a helpful but not mandatory resource.

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Blank Test

In order to check that (a) the environment is suitable for the test, (b) the glassware is properly cleaned, and (c) the water or suitable solvent that will be used is particle free (b and c constitute the "blank"), the following test is carried out. Determine the count in five aliquots of degassed particle-free water or suitable solvent, representative of the product test volume (but NLT 1 mL). If the number of particles 10 µm or greater in size exceeds 1 particle per mL for the combined aliquot volume, the precautions taken for the test are not sufficient. Repeat the preparatory steps until the environment, glassware, and water are suitable for the test determination. The *Blank Test* must be run initially each day (environment) and is recommended throughout the daily routine, especially between product families (procedural).

System Suitability Verification

To verify system suitability, *Particle-Free Water* and the USP Particle Count RS or equivalent commercial standard aqueous suspension are tested from the same or similar type of container as product samples, are degassed in the same manner, and generally are handled like the test article. Ensure that the system can meet the *Blank Test* requirement. Determine the particulate matter in a sample of the USP Particle Count RS that corresponds to the product test volume, according to the method described below. Results from the Standard preparation should fall within the stated specifications of the Reference Standard.

Method

Product samples are tested in the manner that most suitably represents delivery (e.g., expelled syringe contents). For parenterals that have a sufficient volume (i.e., volume large enough to facilitate testing), testing of individual units is often diagnostic. For parenteral products that do not have a sufficient volume, carefully and thoroughly mix each unit, then combine the contents of a suitable number of units in a separate container to obtain the volume required for a single test (generally 0.2–5.0 mL). If a dilution is performed, ensure that the blank container has sufficient volume for product and diluents and that few, if any, particles are introduced in the process. Open each unit cautiously, remove the sealing closure, and avoid contamination of the contents before sampling, diluting, or, if necessary, pooling.

When a product solvent is specified, e.g., for lyophilized solids or powders for parenteral use, the reconstitution or dilution must be performed with the appropriate amount of specified solvent. In this case, the solvent itself may be tested to ensure that it is not a significant source of particles. Subtraction of the solvent particle count from the total count is not allowed.

Eliminating gas bubbles is a key step, especially for proteinaceous products that readily entrain gas. Two methods are recommended: either allowing the product fluid to stand under ambient pressure or applying a gentle (e.g., 75 Torr) vacuum. Other methods may be used when demonstrated to be suitable. Sonication should be avoided.

Once the samples have been degassed, they must be remixed gently to suspend all particles by mixing the contents of the sample gently but thoroughly by an appropriate means, e.g., slow swirling of the container by hand. Inversion to mix the product fluid is not recommended at any time.

Immediately after mixing, withdraw NLT four aliquots, each of a volume appropriate for the instrument's capacity (generally 0.2–5.0 mL). Count the number of particles over the selected size range, including particles equal to or greater than 10 and 25 µm. Disregard the result obtained for the first aliquot, and calculate the mean number of particles at each size range for the remaining aliquots of the preparation being tested. A tare volume also can be applied to control sampling that should be representative of the sensor dynamic range and needle volume.

General Considerations

Dilution is allowed as long as the diluent and methods are demonstrated to be appropriate and the smallest level of dilution that allows for reproducible testing is used. Under certain circumstances dilution of samples may be required to obtain reliable results. This may be the case with high-concentration products and/or high-viscosity products that cannot be drawn into the instrument properly; products that cause saturation of the instrument sensor due to high counts, especially in the smaller than 10-µm size ranges; and cases where the high concentration results in very small differences in refractive index between the solution and the protein aggregates, etc. In situations where sample dilution is conducted, the underlying rationale for the dilution as well as the suitability of the selected dilution scheme including choice of diluent must be demonstrated. Suitability of the dilution scheme and of the diluent may be assessed by demonstrating consistency of results over a dilution range. Bridging studies with and without dilution at size ranges intended for particle counting should be considered. Studies exploring the ability of dilution to decrease the number of aggregates, or to increase particles due to changes in the ratio of protein to excipients, should also be performed. An orthogonal technique may be used to justify the suitability of the method by verifying the impact of dilution.

Evaluation

The values below are derived historically from general chapters ⟨788⟩ and ⟨789⟩. If specifications are different from those stated below, they will be indicated in an individual monograph where available.

For parenteral products that are therapeutic protein injections for infusion or injection supplied in containers with a nominal content of less than or equal to 100 mL:

The average number of particles present in the units tested should not exceed 6000 per container equal to or greater than 10 µm and should not exceed 600 per container equal to or greater than 25 µm.

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For therapeutic protein injections supplied in containers with a nominal content of more than 100 mL, and parenteral infusion preparations or injections with a nominal content of more than 100 mL:

The average number of particles present in the units tested should not exceed 25/mL equal to or greater than 10 µm and should not exceed 3/mL equal to or greater than 25 µm. Also, total particle load should not exceed 6000 per container equal to or greater than 10 µm and should not exceed 600 per container equal to or greater than 25 µm.

Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption. However, filtrates are expected to comply with the guideline. For products supplied or first reconstituted in <100 mL, and then diluted for infusion in a volume >100 mL, particle content should be assessed both before and after dilution and evaluated based on their final volume.

MICROSCOPIC PARTICLE COUNT TEST

As noted, the LO method is the preferred method for therapeutic protein injections and parenteral infusions. However, the microscopic method may be used when appropriate, such as determination of extrinsic and intrinsic particle types only. It should be demonstrated, however, that particular classes of particles (e.g., inherent) are also being counted when using this method. For the determination of product acceptability, apply the limits for the membrane microscopic test in general chapter ⟨788⟩. Because of the interference of some protein particles and their physical characteristics (fragile or translucent), the results of the *Microscopic Particle Count Test* are not equivalent to those of the *Light Obscuration Particle Count Test*, and the two methods cannot be considered interchangeable.

⟨788⟩ PARTICULATE MATTER IN INJECTIONS

This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter. Portions of the present general chapter text that are national USP text, and therefore not part of the harmonized text, are marked with symbols (•.) to specify this fact.

Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

•As stated in *Injections and Implanted Drug Products* ⟨1⟩, solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter in Injections* ⟨788⟩. This requirement has been indefinitely postponed for products for veterinary use. Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenteral products for which the labeling specifies use of a final filter prior to administration are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.•

For the determination of particulate matter, two procedures, *Method 1 (Light Obscuration Particle Count Test)* and *Method 2 (Microscopic Particle Count Test)*, are specified hereinafter. When examining injections and parenteral infusions for subvisible particles, *Method 1* is preferably applied. However, it may be necessary to test some preparations by the *Light Obscuration Particle Count Test* followed by the *Microscopic Particle Count Test* to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for subvisible particles by one or both of these methods. When *Method 1* is not applicable, e.g., in the case of preparations having reduced clarity or increased viscosity, the test should be carried out according to *Method 2*. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units.

•For the purpose of this chapter, small-volume parenteral is synonymous with small-volume injection, and large-volume parenteral is synonymous with large-volume injection.•

METHOD 1 LIGHT OBSCURATION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage that allows for an automatic determination of the size of particles and the number of particles according to size. The definition for *particle-free water* is provided in *Reagents, Indicators, and Solutions—Reagent Specifications*.

The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 µm and 25 µm. These standard particles are dispersed in *particle-free water*. Care must be taken to avoid aggregation of particles during dispersion. •System suitability can be verified by using the USP Particle Count RS.•

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General Precautions

The test is carried out under conditions limiting particulate matter, preferably in a laminar flow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution, and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water*.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned, and that the water to be used is particle-free, the following test is carried out. Determine the particulate matter in five samples of *particle-free water*, each of 5 mL, according to the method described below. If the number of particles of 10 µm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, and water are suitable for the test.

Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water*, and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 min or sonicating.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of NLT 25 mL; the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are reconstituted with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable.

•For pharmacy bulk packages for parenteral use labeled "Not for Direct Infusion", proceed as directed for small-volume parenterals when the volume is 25 mL or more. Calculate the test result on a portion that is equivalent to the maximum dose given in the labeling. For example, if the total bulk package volume is 100 mL and the maximum dose volume is 10 mL, then the average particle count per mL would be multiplied by 10 to obtain the test result based on the 10-mL maximum dose. [NOTE—For the calculation of test results, consider this maximum dose portion to be equivalent to the contents of one full container.]

Products packaged with dual compartments meant to hold a drug product and a solvent should be prepared and tested as directed for large-volume parenterals or small-volume parenterals, depending on container volume. Mix each unit as directed in the labeling, activating and agitating to ensure thorough mixing of the separate components and drug dissolution.•

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, using an appropriate sampling plan.

Remove four portions, NLT 5 mL each, and count the number of particles equal to or greater than 10 µm and 25 µm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of *Test 1.A*.
For preparations supplied in containers with a nominal volume of less than 100 mL, apply the criteria of *Test 1.B*.
For preparations supplied in containers with a nominal volume of 100 mL, apply the criteria of *Test 1.B*.
[NOTE—*Test 1.A* is used in the *Japanese Pharmacopoeia*.]
If the average number of particles exceeds the limits, test the preparation by the *Microscopic Particle Count Test*.

TEST 1.A

Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL: The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per mL equal to or greater than 10 µm and does not exceed 3 per mL equal to or greater than 25 µm.

TEST 1.B

Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL: The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 µm and does not exceed 600 per container equal to or greater than 25 µm.

METHOD 2 MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope, a filter assembly for retaining particulate matter, and a membrane filter for examination.

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The microscope is adjusted to 100 ± 10 magnifications and is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, and two suitable illuminators to provide episcopic illumination in addition to oblique illumination.

The ocular micrometer is a circular diameter graticule (see *Figure 1*) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 µm and 25 µm in diameter at 100 magnifications, and a linear scale graduated in 10-µm increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within ±2% is acceptable. The large circle is designated the graticule field of view (GFOV).

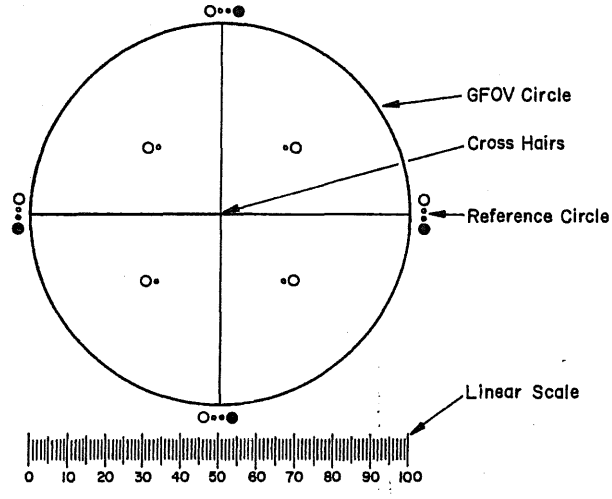


Figure 1. Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10-µm and 25-µm diameters at 100× are provided as comparison scales for particle sizing.

Two illuminators are required. One is an episcopic brightfield illuminator internal to the microscope, the other is an external, focusable auxiliary illuminator that can be adjusted to give reflected oblique illumination at an angle of 10°–20°.

The filter assembly for retaining particulate matter consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, nongridded or gridded, and 1.0 µm or finer in nominal pore size.

General Precautions

The test is carried out under conditions limiting particulate matter, preferably in a laminar flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution, and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with *particle-free water*.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned, and that the water to be used is particle-free, the following test is carried out. Determine the particulate matter of a 50-mL volume of *particle-free water* according to the method described below. If more than 20 particles 10 µm or larger in size or if more than five particles 25 µm or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter, and water are suitable for the test.

Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water*, and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container; the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, using an appropriate sampling plan.

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Wet the inside of the filter holder fitted with the membrane filter with several mL of *particle-free water*. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply a vacuum. If needed, add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of *particle-free water*. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the membrane filter in a Petri dish, and allow the membrane filter to air-dry with the cover slightly ajar. After the membrane filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are equal to or greater than 10 µm and the number of particles that are equal to or greater than 25 µm. Alternatively, partial membrane filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by estimating the equivalent diameter of the particle in comparison with the 10 µm and 25 µm reference circles on the graticule. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the *Microscopic Particle Count Test*, do not attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases, the interpretation of enumeration may be aided by testing a sample of the solution by the *Light Obscuration Particle Count Test*.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of *Test 2.A*.
For preparations supplied in containers with a nominal volume of less than 100 mL, apply the criteria of *Test 2.B*.
For preparations supplied in containers with a nominal volume of 100 mL, apply the criteria of *Test 2.B*.
[NOTE—*Test 2.A* is used in the *Japanese Pharmacopoeia*.]

TEST 2.A

Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL: The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per mL equal to or greater than 10 µm and does not exceed 2 per mL equal to or greater than 25 µm.

TEST 2.B

Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL: The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 µm and does not exceed 300 per container equal to or greater than 25 µm.

⟨789⟩ PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS

Particulate matter consists of mobile, randomly sourced, extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis because of the small amount of material they represent and because of their heterogeneous composition. Ophthalmic solutions should be essentially free from particles that can be observed on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating extraneous particles within specific size ranges.

Every ophthalmic solution is subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph. When higher limits are appropriate, they will be specified in the individual monograph. Ophthalmic preparations that are suspensions, emulsions, or gels are exempt from these requirements, as are medical devices. Refer to the specific monograph when a question of test applicability occurs.

Light obscuration and microscopic procedures for the determination of particulate matter in ophthalmic solutions are identical to those for injections; therefore, where appropriate, *USP* general test chapter *Particulate Matter in Injections* ⟨788⟩ is cross-referenced. This chapter provides a test approach in two stages. The ophthalmic solution is first tested by the light obscuration procedure (stage 1). If it fails to meet the prescribed limits, it must pass the microscopic procedure (stage 2) with its own set of test limits. Where for technical reasons the ophthalmic solution cannot be tested by light obscuration, microscopic testing may be used exclusively. Documentation is required, demonstrating that the light obscuration procedure is incapable of testing the ophthalmic solution or that it produces invalid results.

It is expected that most articles will meet the requirements on the basis of the light obscuration test alone; however, it may be necessary to test some articles by the light obscuration test followed by the microscopic test to reach a conclusion on conformance to requirements. Any product that is not a pure solution having a clarity and a viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Such materials may be analyzed by the microscopic counting method. In some instances, the viscosity of a material to be tested may be sufficiently high so as to preclude its analysis by either test method. In this event, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

In the tests described below, the results obtained by examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, sampling plans based on known operational factors must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units. Sampling plans need to be based on consideration of product volume, particle numbers historically found to be present in comparison to limits, size distribution of particles present, and variability of particle counts between units.

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LIGHT OBSCURATION PARTICLE COUNT TEST

This test applies to ophthalmic solutions, including solutions constituted from sterile solids, for which a test for *Particulate matter* is specified in the individual monograph. The test counts suspended particles that are solid or liquid.

Test Apparatus, Instrument Standardization, Test Environment, Test Procedure, and Calculations

Proceed as directed in *Particulate Matter in Injections* ⟨788⟩, *Method 1 Light Obscuration Particle Count Test.*

Interpretation

The ophthalmic solution meets the requirements of the test if the average number of particles present in the units tested does not exceed the appropriate value listed in *Table 1*. If the average number of particles exceeds the limit, test the article by the *Microscopic Particle Count Test.*

Table 1. Light Obscuration Particle Test Limits

	Diameter		
	≥10 µm	≥25 µm	≥50 µm
Number of particles	50/mL	5/mL	2/mL

MICROSCOPIC PARTICLE COUNT TEST

Some articles cannot be tested meaningfully by light obscuration. In such cases, individual monographs clearly specify that only a microscopic particle count is to be performed. The microscopic particle count test enumerates subvisible, essentially solid, particulate matter in ophthalmic solutions after collection on a microporous membrane filter. Some ophthalmic solutions, such as solutions that do not filter readily because of their high viscosity, may be exempted from analysis using the microscopic test.

When performing the microscopic test, do not attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane surface. These materials show little or no surface relief and present a gelatinous or film-like appearance. Because in solution this material consists of units on the order of 1 µm or less, which may be counted only after aggregation or deformation on an analytical membrane, interpretation of enumeration may be aided by testing a sample of the solution by the light obscuration particle count method.

Test Apparatus, Test Environment, Test Procedure, and Enumeration of Particles

Proceed as directed in *Particulate Matter in Injections* ⟨788⟩, *Method 2 Microscopic Particle Count Test.*

Interpretation

The ophthalmic solution meets the requirements of the test if the average number of particles present in the units tested does not exceed the appropriate value listed in *Table 2*.

Table 2. Microscopic Particle Test Limits

	Diameter		
	≥10 µm	≥25 µm	≥50 µm
Number of particles	50/mL	5/mL	2/mL

⟨790⟩ VISIBLE PARTICULATES IN INJECTIONS

INTRODUCTION

All products intended for parenteral administration must be visually inspected for the presence of particulate matter as specified in *Injections and Implanted Drug Products* ⟨1⟩. Dry solids, from which constituted solutions are prepared for injection, meet the requirements for *Completeness and clarity of solutions* in *Injections and Implanted Drug Products* ⟨1⟩ when they are prepared just before use. Where used in this chapter, the term *essentially free* means that when injectable drug products are inspected as described herein, no more than the specified number of units may be observed to contain visible particulates. Particulate matter is defined in *Particulate Matter in Injections* ⟨788⟩ as extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in solutions. See *Subvisible Particulate Matter in Therapeutic Protein Injections* ⟨787⟩ for additional information on inherent, intrinsic, and extrinsic particulates. Examples of such particulate matter include, but are not limited

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to, fibers, glass, metal, elastomeric materials, and precipitates. However, some products may contain inherent particles or agglomerates; in such cases, requirements for these particular visible particulates are specified in the individual monograph or in the approved regulatory application but the control and acceptance criteria for extraneous particles described in this chapter apply.

Where the nature of the contents or the container–closure system permits only limited capability for inspection of the total contents, the 100% inspection of a batch shall be supplemented with the inspection of constituted (e.g., dried) or withdrawn (e.g., dark amber container, suspensions, highly colored liquids) contents of a sample of containers from the batch. The destructive nature of these tests requires the use of a sample smaller[1] than those traditionally used for non-destructive acceptance sampling after 100% inspection. Although the tests described in this chapter may be useful during studies to examine product stability, this chapter is not intended to establish any new testing requirements for stability studies.

INSPECTION PROCEDURE

Used along with 100% inspection during the manufacturing process, this procedure is sufficient to demonstrate that the batch is essentially free of visible particulates. A complete program for the control and monitoring of particulate matter remains an essential prerequisite.

Inspected units must be free of visible particulates when examined without magnification (except for optical correction as may be required to establish normal vision) against a black background and against a white background. Illumination at the inspection point is maintained at a minimum intensity between 2000 and 3750 lux. This can be achieved through the use of two 13-W or 15-W fluorescent lamps (e.g., F13/T5 or F15/T8). The use of a high-frequency ballast to reduce flicker from the fluorescent lamps is recommended. Alternative light sources (e.g., incandescent, LED) that provide illumination at the point of inspection within the specified minimum intensity range are acceptable. Higher illumination intensity is recommended for examination of colored solutions or product in containers other than clear glass.

Before performing the inspection, remove any adherent labels from the container, and wash and dry the outside. The unit under inspection should be gently swirled and/or inverted, ensuring that no air bubbles are produced, and inspected for approximately 5 s against each of the backgrounds. The presence of any particles should be recorded.

Sampling at Batch Release (After 100% Manufacturing Inspection)

Sample and inspect the batch using ANSI/ASQ Z1.4 (or ISO 2859-1). General Inspection Level II, single sampling plans for normal inspection with an AQL of 0.65%. Alternative sampling plans with equivalent or better protection are acceptable. NMT the specified number of units contains visible particulates.

Product in Distribution[2]

If it becomes necessary to evaluate product that has been shipped to customers (e.g., because of a complaint or regulatory concern), sample and inspect 20 units. If no particles are observed in the sample, the batch is considered essentially free of visible particulates. If available, additional units may be inspected to gain further information on the risk of particulates in the batch.

⟨791⟩ pH

INTRODUCTION

For compendial purposes, pH is defined as the value given by a suitable, properly calibrated, potentiometric sensor and measuring system.

[NOTE—The measuring system has traditionally been referred to as the "pH meter." While the pH meter is still in common use, the measuring system can also be embedded inside the pH sensor, and the pH signal can be transmitted digitally to an external device such as a computer, Programmable Logic Controller (PLC), Distributed Control System (DCS), data acquisition system, terminal, or other microprocessor-controlled device.]

By definition, pH is equal to $-\log_{10}[a_{H^+}]$ where a_{H^+} is the activity of the hydrogen (H^+) or hydronium ion (H_3O^+), and the hydrogen ion activity very closely approximates the hydrogen ion concentration.

The practical pH scale is defined:

$$pH = pH_S + [(E - E_S)/k]$$

E = measured potential where the galvanic cell contains the solution under test (pH)
E_S = measured potential where the galvanic cell contains the appropriate buffer solution for calibration (pH_S)
k = change in potential/unit change in pH and is derived from the Nernst equation (as follows)

[1] The special level sampling plans described in ANSI/ASQ Z1.4–2008 or ISO 2859 are appropriate to guide the selection of sample size and acceptance criteria for this purpose.

[2] Testing outlined in *Product in Distribution* is permissible only if *Sampling at Batch Release (after 100% Manufacturing Inspection)* has been successfully completed.

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$$k = \log_e(10) \times (RT/nF)$$

R = 8.314 J/mole/°K
T = temperature (°K)
n = moles/half-reaction
F = Faraday constant, 96485 C/mole

The resulting equation is [0.05916 + 0.0001984(T – 25°)] volts at temperature T. Values of k from 15°–35° are provided in *Table 1*.

Table 1. Values of *k* for Various Temperatures

Temperature (°C)	*k* (V)
15.00	0.05718
20.00	0.05817
25.00	0.05916
30.00	0.06016
35.00	0.06115

Values of k at other temperatures can be determined from the equation above. For practical purposes, values of k are determined from pH sensor calibration.

pH MEASUREMENT SYSTEM

The measurement system consists of: (1) a measuring electrode sensitive to hydrogen-ion activity, typically a glass electrode, though other electrode types are possible, (2) a suitable reference electrode, for example, a silver–silver chloride electrode, and (3) a voltage measurement system with an input resistance capable of measuring at a high input impedance of the pH sensor. The measuring and reference electrode may be separated or combined. The voltage measurement system may be separated from the pH sensor or integrated into the sensor. For most applications, a temperature measurement will be necessary for compensation of the Nernst temperature influence described above. A temperature device may be embedded into the pH sensor, or an external temperature device may be used.

INSTRUMENT REQUIREMENTS

The measurement system shall be capable of performing a two-point (or more) pH calibration (see below). The accuracy of the pH measurement system is described in the *Calibration* section. The resolution of the pH measurement system shall be at least 0.01 pH. The instrument shall be capable of temperature-compensating the pH sensor measurement to convert the millivolt signal to pH units at any temperature, either automatically using a temperature device built into the sensor system or by manual entry of the sample temperature into the measurement system. The accuracy of the temperature measurement system shall be ±1°. The resolution of the temperature measurement system shall be at least 0.1°. Lab-based pH measurements are typically performed at 25 ± 2° unless otherwise specified in the individual monograph or herein. However, temperatures outside this range are acceptable if samples are more conveniently prepared at alternative temperatures. Examples of non-lab-based measurements include test samples inside process pipes, vessels, tanks, and other non-standard processing conditions.

[NOTE—The definitions of pH, the pH scale, and the values assigned to the buffer solutions for calibration are for the purpose of establishing a practical, operational system so that results may be compared between laboratories. The measured pH values do not necessarily correspond exactly to those obtained by the definition, pH = –log a_{H^+}, rather the values obtained are closely related to the activity of the hydrogen-ion in aqueous solutions.]

[NOTE—Where a pH measurement system is calibrated by use of an aqueous buffer and then used to measure the "pH" of nonaqueous systems, the ionization constant of the acid or base, the dielectric constant of the medium, the liquid-junction potential (which may give rise to errors of approximately 1 pH unit), and the hydrogen-ion response of the glass electrode are all changed. For these reasons, the values so obtained with solutions that are only partially aqueous in character can be regarded only as apparent pH values.]

BUFFER SOLUTIONS FOR CALIBRATION OF THE pH MEASUREMENT SYSTEM

Buffer solutions for calibration are prepared as directed in *Table 2*.[1] Buffer salts of requisite purity can be obtained from the National Institute of Standards and Technology, other national authorities, or other suppliers. Buffer solutions should be stored in appropriate containers that ensure stability of the pH through the expiry date, and fitted with a tight closure. For buffer

[1] Commercially available buffer solutions for pH measurement system, calibrated by methods traceable to the National Institute of Standards and Technology (NIST) or other national authorities, labeled with a pH value accurate to 0.02 pH unit may be used. Solutions prepared from ACS reagent grade materials or other suitable materials, may be used provided the pH of the resultant solution is the same as that of the solution prepared from the NIST (or other national authorities) certified material. Buffer solutions that are greater than 12 should be used immediately, or should be prepared using freshly boiled water, and stored under conditions to minimize carbon dioxide absorption and ingress.

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solutions greater than 11, the storage should be in containers that are resistant to or reduce carbon dioxide intrusion which would lower the pH of the buffer. For buffer solutions lower than 11, they should typically be prepared at intervals not to exceed 3 months. For buffer solutions greater than 11, they should typically be prepared and used fresh unless carbon dioxide ingress is restricted. All buffer solutions should be prepared using *Purified Water*. *Table 2* indicates the pH of the buffer solutions as a function of temperature. The instructions presented here are intended for the preparation of solutions having the designated molal (m) concentrations. However, in order to facilitate their preparation, the instructions are given in molarity. The difference in concentration between molality and molarity preparations for these buffer solutions is less than 1%, and the pH difference is negligible. Calibration using buffer solutions shall be done in the temperature range of the buffers listed in *Table 2*.

[NOTE—The Nernst temperature compensation corrects only for the electrode millivolt output change with temperature, not the actual pH change of the buffer solution with temperature which is unique for each buffer.]

Features such as automatic buffer recognition or buffer pH–temperature correction are available for convenience in accommodating the temperature influence on buffer solutions. The pH–temperature response can be determined from the values in *Table 2*.

Table 2. pH Values of Buffer Solutions for Calibration

Temperature (°C)	Potassium Tetraoxalate, 0.05 m	Potassium Hydrogen Tartrate Saturated at 25°	Potassium Dihydrogen Citrate, 0.05 M	Potassium Biphthalate, 0.05 m	Equimolal Phosphate, 0.05 m	Potassium Dihydrogen Phosphate, 0.0087 M, and Disodium Hydrogen Phosphate, 0.0303 M	Sodium Tetraborate, 0.01 m	Sodium Carbonate, 0.025 M, and Sodium Bicarbonate, 0.025 M	Calcium Hydroxide, Saturated at 25°
10	1.67	—	—	4.00	6.92	—	9.33	—	13.00
15	1.67	—	3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68	—	3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.86	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
40	1.69	—	—	4.04	6.84	—	9.07	—	11.98
45	1.70	—	—	4.05	6.83	—	9.04	—	11.84
50	1.71	—	—	4.06	6.83	—	9.01	—	11.71
55	1.72	—	—	4.08	6.83	—	8.99	—	11.57
60	1.72	—	—	4.09	6.84	—	8.96	—	11.45
ΔpH/Δ°C	0.0010	−0.0014	−0.0022	0.0018	−0.0016	−0.0028	−0.0074	−0.0096	−0.0310

Preparation of alternative volumes at the same concentrations to those indicated below is acceptable.

Potassium tetraoxalate, 0.05 m: Dissolve 12.61 g of $KH_3(C_2O_4)_2 \cdot 2H_2O$, and dilute with water to make 1000.0 mL.

Potassium hydrogen tartrate, saturated at 25°: Add $C_4H_5KO_6$ to water until saturation is exceeded at 25°. Then filter or decant.

Potassium dihydrogen citrate, 0.05 M: Dissolve 11.41 g of $C_6H_7KO_4$, and dilute with water to make 1000.0 mL.

Potassium biphthalate, 0.05 m: Dissolve 10.12 g of $KHC_8H_4O_4$, previously dried at 110° for 1 h, and dilute with water to make 1000.0 mL.

Equimolal phosphate, 0.05 m: Dissolve 3.53 g of disodium hydrogen phosphate (Na_2HPO_4) and 3.39 g of potassium dihydrogen phosphate (KH_2PO_4), each previously dried at 120° for 2 h, and dilute with water to make 1000.0 mL.

Potassium dihydrogen phosphate, 0.0087 M, and disodium hydrogen phosphate, 0.0303 M: Dissolve 1.18 g of KH_2PO_4 and 4.30 g Na_2HPO_4, both dried for 2 h at 120 ± 2°, and dilute with water to make 1000.0 mL.

Sodium tetraborate, 0.01 m: Dissolve 3.80 g of $Na_2B_4O_7 \cdot 10H_2O$, and dilute with water to make 1000.0 mL. Protect from absorption of carbon dioxide.

Sodium carbonate, 0.025 M, and sodium bicarbonate, 0.025 M: Dissolve 2.64 g of sodium carbonate (Na_2CO_3) and 2.09 g of sodium bicarbonate ($NaHCO_3$), and dilute with water to make 1000.0 mL.

Calcium hydroxide, saturated at 25°: Add $Ca(OH)_2$ to water until saturation is exceeded at 25°. Use water that has been recently boiled and protected from the atmosphere to limit carbon dioxide absorption. Then filter or decant.

CALIBRATION

Because of variations in the nature and operation of the available pH measurement systems, it is not practical to provide universal directions for the calibration of the measurement system. However, the general principles to be followed are set forth in the following paragraphs. Examine the electrodes, especially the reference electrode and electrolyte level, if a liquid electrolyte is used. If necessary, replenish electrolyte supply, and observe other precautions indicated by the instrument and electrode manufacturers.

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The calibration or verification of the pH measurement system should be periodically executed. The historical performance of the measurement system, the criticality of the pH measurement, the maintenance of the pH sensor, and the frequency of measurement operation is used to determine the frequency of the calibration/verification. In addition to the two-point calibration process described below, other multipoint methods may be available for use. These are acceptable if the pH sensor slope and the offset criteria (see step 10) as well as the calibration accuracy (see step 14) are met.

If the pH of the buffer is sensitive to ambient carbon dioxide, then use *Purified Water* that has been recently boiled, and subsequently stored in a container designed to minimize ingress of carbon dioxide.

1. To calibrate the pH measurement system, select three buffer solutions for calibration, preferably from those given in *Table 2*, such that the expected pH of the material under test falls within their range. Two of the buffers are used for the calibration process, and the third buffer is used for verification. The value of the verification buffer shall be between two of the calibration buffers. If the operational range of the pH sensor is beyond the pH range of the buffer solutions in *Table 2*, then either 1) select two nearby pH buffers from *Table 2* or 2) select one from *Table 2* and another documented prepared buffer that is outside the range.
2. Rinse the pH sensor several times with water, then with the first buffer solution.
3. Immerse the pH sensor in the first buffer solution at a temperature within the range of *Table 2*.
4. If automatic temperature measurement and compensation is not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument. For temperatures not listed in *Table 2*, use linear interpolation to determine the pH value as a function of temperature.
5. Initiate the two-point calibration sequence with the first buffer according to the manufacturer's instructions.
6. Remove the pH sensor from the first buffer and rinse the electrode(s) with water, and then with the second buffer solution.
7. Immerse the pH sensor in the second buffer at a temperature within the range of *Table 2*.
8. If automatic temperature measurement and compensation is not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument.
9. Continue the two-point calibration sequence with the second buffer according to the manufacturer's instructions.
10. After completion of the two-point calibration process, verify that the pH slope and offset are within acceptable parameters. Typical acceptable parameters are a slope of 90%–105% and an offset of 0 ± 30 mV (0.5 pH units at 25° C). Depending on the pH instrumentation, the pH slope and offset may be determined in software or by manual methods. If using manual methods, follow supplier instructions to calculate the pH sensor slope/offset. If these parameters are not within acceptable parameters, the sensor should be properly cleaned, replenished, serviced, or replaced, and the two-point calibration process shall be repeated.
11. Remove the pH sensor from the second buffer, and rinse thoroughly with water, and then the verification buffer.
12. Immerse the pH sensor in the verification buffer at a temperature within the range of *Table 2*.
13. If automatic temperature measurement and compensation are not included in the measuring system, manually enter the temperature of the buffer and pH value of the buffer solution at that temperature into the instrument.
14. The pH reading shall be within ±0.05 pH of the value in *Table 2* at the buffer solution temperature.

OPERATION

All test samples should be prepared using *Purified Water*, unless otherwise specified in the monograph. All test measurements should use manual or automated Nernst temperature compensation.

1. Prepare the test material according to requirements in the monograph or according to specific procedures. If the pH of the test sample is sensitive to ambient carbon dioxide, then use *Purified Water* that has been recently boiled, and subsequently stored in a container designed to minimize ingress of carbon dioxide.
2. Rinse the pH sensor with water, then with a few portions of the test material.
3. Immerse the pH sensor into the test material and record the pH value and temperature.

In all pH measurements, allow sufficient time for stabilization of the temperature and pH measurement.

Diagnostic functions such as glass or reference electrode resistance measurement may be available to determine equipment deficiencies. Refer to the electrode supplier for diagnostic tools to assure proper electrode function.

Where approximate pH values suffice, indicators and test papers (see *Indicators* and *Indicator and Test Papers*) may be suitable.

For a discussion of buffers, and for the composition of standard buffer solutions called for in compendial tests and assays, see *Buffer Solutions* in the section *Solutions*. This referenced section is not intended to replace the use of the pH calibration buffers in *Table 2*.

⟨795⟩ PHARMACEUTICAL COMPOUNDING—NONSTERILE PREPARATIONS

Change to read:

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1.1 Scope
2. PERSONNEL TRAINING AND EVALUATION
3. PERSONAL HYGIENE AND GARBING

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APPENDIX

1. INTRODUCTION AND SCOPE

This chapter describes the minimum standards to be followed when preparing compounded nonsterile preparations (CNSPs) for humans and animals. For purposes of this chapter, nonsterile compounding is defined as combining, admixing, diluting, pooling, reconstituting other than as provided in the manufacturer's labeling, or otherwise altering a drug or bulk drug substance to create a nonsterile medication.

The requirements in this chapter must be followed to minimize harm, including death, to human and animal patients that could result from 1) excessive microbial contamination, 2) variability from the intended strength of correct ingredients (e.g., ±10% of the labeled strength), 3) physical and chemical incompatibilities, 4) chemical and physical contaminants, and/or 5) use of ingredients of inappropriate quality.

Handling of nonsterile hazardous drugs (HDs) must additionally comply with *Hazardous Drugs—Handling in Healthcare Settings* ⟨800⟩.

1.1 Scope

CNSPS SUBJECT TO THE REQUIREMENTS IN THIS CHAPTER

CNSPs that must comply with this chapter include but are not limited to the following dosage forms:

- Solid oral preparations
- Liquid oral preparations
- Rectal preparations
- Vaginal preparations
- Topical preparations (i.e., creams, gels, ointments)
- Nasal and sinus preparations intended for local application (i.e., nasal sprays and nasal irrigation)
- Otic preparations

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PRACTICES NOT SUBJECT TO THE REQUIREMENTS IN THIS CHAPTER

The following practices are not considered compounding and are not required to meet the requirements of this chapter:
Administration: Preparation of a single dose for a single patient when administration will begin within 4 hours of beginning the preparation is not required to meet the standards in this chapter.
Nonsterile radiopharmaceuticals: Compounding of nonsterile radiopharmaceuticals is not required to meet the standards in this chapter and is subject to the requirements in *Radiopharmaceuticals—Preparation, Compounding, Dispensing, and Repackaging* ⟨825⟩.
Reconstitution: Reconstitution of a conventionally manufactured nonsterile product in accordance with the directions contained in the manufacturer approved labeling is not required to meet the standards in this chapter.
Repackaging: Repackaging of conventionally manufactured drug products is not required to meet the standards in this chapter (see *Good Repackaging Practices* ⟨1178⟩).
Splitting tablets: Breaking or cutting a tablet into smaller portions is not required to meet the standards in this chapter.

PERSONNEL AND SETTINGS AFFECTED

This chapter applies to all persons who prepare CNSPs and all places where CNSPs are prepared. This includes but is not limited to pharmacists, technicians, nurses, physicians, veterinarians, dentists, naturopaths, and chiropractors, in all places including but not limited to pharmacies, hospitals and other healthcare institutions, patient treatment sites, and physicians' or veterinarians' practice sites.

The compounding facility's leadership and all personnel involved in preparing, storing, packaging, and transporting CNSPs are responsible for 1) ensuring that the applicable practices and quality standards in this chapter are continually and consistently applied to their operations, and 2) proactively identifying and remedying potential problems within their operations. Personnel engaged in the compounding of CNSPs must also comply with laws and regulations of the applicable regulatory jurisdiction.

The compounding facility must designate one or more individuals to be responsible and accountable for the performance and operation of the facility and personnel for the preparation of CNSPs. The responsibilities of the designated person(s) include but are not limited to:

- Overseeing a training program to ensure competency of personnel involved in compounding, handling, and preparing of CNSPs
- Selecting components
- Monitoring and observing compounding activities and taking immediate corrective action if deficient practices are observed
- Ensuring that standard operating procedures (SOPs) are fully implemented. The designated person(s) must ensure that follow-up is carried out if problems, deviations, or errors are identified
- Establishing, monitoring, and documenting procedures for the handling and storage of CNSPs and/or components of CNSPs

The designated person(s) must be identified in an SOP. If the compounding facility has only one person responsible for all of the compounding in the facility, then that person is the designated person.

2. PERSONNEL TRAINING AND EVALUATION

All personnel involved in the preparation and handling of CNSPs must be initially trained, must demonstrate competency, and must undergo refresher training every 12 months. Training and competency of personnel must be documented as described in *15. Documentation*.

A designated person must oversee a training program that describes the required training, the frequency of training, and the process for evaluating the competency of personnel involved in nonsterile compounding and handling of CNSPs. This program must equip personnel with knowledge and training in the required skills necessary to perform their assigned tasks.

Before beginning to prepare CNSPs independently, all compounding personnel must complete training and be able to demonstrate proficiency in the principles and hands-on skills of nonsterile manipulations for the type of compounding they will be performing. Proficiency must be demonstrated every 12 months in at least the following core competencies:

- Hand hygiene
- Garbing
- Cleaning and sanitizing
- Handling and transporting components and CNSPs
- Measuring and mixing
- Proper use of equipment and devices selected to compound CNSPs
- Documentation of the compounding process (e.g., Master Formulation Records and Compounding Records)

Steps in the training procedure must include the following:

- Read and understand this chapter, other applicable standards, and other relevant literature
- Understand and interpret Safety Data Sheets (SDSs) and, if applicable, Certificates of Analysis (COA)
- Read and understand procedures related to their compounding duties

A designated person must oversee the training of personnel. Training and observation may be performed by the designated person(s) or an assigned trainer. Personnel must be observed and guided throughout the training process. The personnel will then be expected to repeat the procedures independently, but under the direct supervision of the designated person(s)

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and/or trainer. Personnel will be permitted to perform the procedure without direct supervision only after independently demonstrating understanding and competency. Upon completion of the training program, the designated person(s) and/or trainer must document that the personnel has been trained and successfully completed competency assessments (see *15. Documentation*).

In addition to the initial and annual competency training and evaluation described in this section, a designated person should monitor and observe compounding activities and must take immediate corrective action if deficient practices are observed. SOPs must describe procedures for the monitoring and observing of compounding activities and personnel.

If the facility has only one person in the compounding operation, that person must document that they have obtained training and demonstrated competency, and they must comply with the other requirements of this chapter.

3. PERSONAL HYGIENE AND GARBING

Individuals entering the compounding area must maintain personal hygiene. Individuals must evaluate whether they have a personal risk of potentially contaminating the compounding environment and CNSP (e.g., personnel with rashes, recent tattoos or oozing sores, conjunctivitis, or active respiratory infection). Individuals must report these conditions to the designated person(s). The designated person(s) is responsible for evaluating whether these individuals should be excluded from working in compounding areas before their conditions have resolved because of the risk of contaminating the CNSP and the environment.

3.1 Personnel Preparation

Personnel engaged in compounding must maintain hand hygiene and maintain cleanliness required for the type of compounding performed.

Before entering the compounding area, compounding personnel must remove any items that are not easily cleanable and that might interfere with garbing. At a minimum, personnel must:

- Remove personal outer garments (e.g., bandanas, coats, hats, jackets)
- Remove all hand, wrist, and other exposed jewelry, including piercings that could interfere with the effectiveness of garbing or hand hygiene (e.g., watches, rings that may tear gloves)
- Remove earbuds or headphones

The designated person(s) may permit accommodations as long as the quality of the environment and CNSP will not be affected.

3.2 Hand Hygiene

Personnel must perform hand hygiene when entering the compounding area to compound as described in *Box 3-1*. Alcohol hand sanitizers alone are not sufficient.

Box 3-1. Hand Hygiene Procedures

- Wash hands and forearms up to the elbows with soap and water for at least 30 seconds.
- Dry hands and forearms to the elbows completely with disposable towels or wipers.
- Allow hands and forearms to dry thoroughly before donning gloves.

To minimize the risk of cross-contaminating other CNSPs and contaminating other objects (e.g., pens and keyboards), gloves should be wiped or replaced before beginning a CNSP with different components.

All gloves must be inspected for holes, punctures, or tears and must be replaced immediately if such defects are detected.

3.3 Garb and Glove Requirements

Gloves must be worn for all compounding activities. Other garb (e.g., shoe covers, head and facial hair covers, face masks, gowns) should be worn as needed for the protection of personnel from chemical exposures and for prevention of preparation contamination and must be appropriate for the type of compounding performed. The garbing requirements and frequency of changing the garb must be determined by the facility and documented in the facility's SOPs.

Garb must be stored in a manner that minimizes contamination (e.g., away from sinks to avoid splashing). Visibly soiled garb or garb with tears or punctures must be changed immediately.

If gowns are worn, they may be re-used if not soiled. If used, gloves, shoe covers, hair covers, facial hair covers, face masks, or head coverings may not be re-used and must be replaced with new ones. If used, non-disposable garb, such as goggles, should be cleaned and sanitized with 70% isopropyl alcohol before re-use.

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4. BUILDINGS AND FACILITIES

4.1 Compounding Space

Space must be specifically designated for nonsterile compounding. The method of designation (e.g., visible perimeter) must be described in the facility's SOP. Other activities must not be occurring in the space at the same time as compounding. The compounding space must be well-lighted and must be maintained in a clean, orderly, and sanitary condition, and in a good state of repair. Carpet is not allowed in the compounding space. Surfaces should be resistant to damage by cleaning and sanitizing agents.

The space must provide for the orderly placement of equipment and materials to prevent mix-ups among components, containers, labels, in-process materials, and finished CNSPs. The space should be designed, arranged, and used in a way that minimizes cross-contamination from non-compounding areas.

4.2 Storage Area

Compounding personnel must monitor temperatures in storage area(s) either manually at least once daily on days that the facility is open or by a continuous temperature recording device to determine whether the temperature remains within the appropriate range for the CNSPs or components. The results of the temperature readings must be documented on a temperature log or stored in the continuous temperature recording device, and must be retrievable. All temperature monitoring equipment must be calibrated or verified for accuracy as recommended by the manufacturer or every 12 months if not specified by the manufacturer.

The compounding facility must adhere to SOPs to detect and prevent temperature excursions within storage area(s). When it is known that a CNSP or component has been exposed to temperatures either below or above the storage temperature limits for the CNSP or component, personnel must determine whether the CNSP or component integrity or quality has been compromised and, if so, the CNSP or component must be discarded.

All CNSPs, components, equipment, and containers must be stored off the floor and in a manner that prevents contamination and permits inspection and cleaning of the storage area(s).

4.3 Water Sources

A source of hot and cold water and an easily accessible sink must be available for compounding. The sink must be emptied of all items unrelated to compounding and cleaned when visibly soiled before being used to clean any equipment used in nonsterile compounding. The plumbing system must be free of defects that may contribute to the contamination of any CNSP. *Purified Water* (see *Water for Pharmaceutical Purposes* ⟨1231⟩), distilled water, or reverse osmosis water should be used for rinsing equipment and utensils.

5. CLEANING AND SANITIZING

Cleaning and sanitizing of the surfaces in the nonsterile compounding area(s) must occur on a regular basis at the minimum frequencies specified in *Table 1* or, if compounding is not performed daily, cleaning and sanitizing must be completed before initiating compounding. Cleaning and sanitizing must be repeated when spills occur and when surfaces are visibly soiled.

Cleaning and sanitizing agents must be selected and used with consideration of compatibilities, effectiveness, and to minimize the potential to leave residues.

If cleaning and sanitizing are performed as separate steps, cleaning must be performed first.

Table 1. Minimum Frequency for Cleaning and Sanitizing Surfaces in Nonsterile Compounding Area(s)

Site	Minimum Frequency
Work surfaces	• At the beginning and end of each shift, after spills, and when surface contamination is known or suspected • Clean and sanitize the work surfaces between compounding CNSPs with different components
Floors	Daily, after spills, and when surface contamination (e.g., splashes) is known or suspected
Walls	Every 3 months, after spills, and when surface contamination (e.g., splashes) is known or suspected
Ceilings	When visibly soiled and when surface contamination is known or suspected
Storage shelving	Every 3 months, after spills, and when surface contamination (e.g., splashes) is known or suspected

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6. EQUIPMENT AND COMPONENTS

6.1 Equipment

The equipment and supplies used for compounding a CNSP must be suitable for the specific compounding process. Equipment surfaces that contact components must not be reactive, additive, or sorptive, and must not alter the quality of the CNSPs. Disposable or dedicated equipment may be used to reduce the chance of bioburden and cross-contamination.

Equipment must be stored in a manner to minimize the risk of contamination and must be located to facilitate its use, maintenance, and cleaning. Equipment and devices used in the compounding or testing of compounded preparations must be inspected prior to use and, if appropriate, verified for accuracy as recommended by the manufacturer and at the frequency recommended by the manufacturer, or at least every 12 months, whichever is more frequent. After compounding, the equipment must be cleaned to prevent cross-contamination of the next preparation.

Weighing, measuring, or otherwise manipulating components that could generate airborne chemical particles [e.g., active pharmaceutical ingredients (APIs), added substances, conventionally manufactured products] must be assessed to determine if these activities must be performed in closed system processing device to reduce the potential exposure to personnel or contamination of the facility or CNSPs. Examples of closed system processing devices include containment ventilated enclosures (CVEs), biological safety cabinets (BSCs), or single-use containment glove bags. The process evaluation must be carried out in accordance with the facility SOP and the assessment must be documented.

If a BSC or CVE is used, it must be certified every 12 months according to requirements such as the current Controlled Environment Testing Association (CETA), NSF International, or American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) guidelines, or other laws and regulations of the applicable regulatory jurisdiction.

If a CVE or other non-disposable device is used, it must be cleaned as described in *Table 2*.

Table 2. Minimum Frequency for Cleaning and Sanitizing Equipment in Nonsterile Compounding Area(s)

Site	Minimum Frequency
CVE	• At the beginning and end of each shift, after spills, and when surface contamination is known or suspected • Clean and sanitize the horizontal work surface of the CVE between compounding CNSPs with different components
Other devices and equipment used in compounding operations	• Before first use and thereafter in accordance with the manufacturer's recommendations • If no recommendation is available, after compounding CNSPs with different components

6.2 Components

The compounding facility must have written SOPs for the selection and inventory control of all components from receipt to use in a CNSP.

SDSs must be readily accessible to all personnel working with APIs and added substances located in the compounding facility. Personnel must be instructed on how to retrieve and interpret needed information.

COMPONENT SELECTION

A designated person must be responsible for selecting components to be used in compounding.

APIs:

- Must comply with the criteria in the *USP–NF* monograph, if one exists
- Must have a COA that includes the specifications and test results and shows that the API meets the specifications
- In the United States, must be obtained from an FDA-registered facility
- Outside of the United States, must comply with laws and regulations of the applicable regulatory jurisdiction

All components other than APIs:

- Should be accompanied by a COA that verifies that the component meets the criteria in the *USP–NF* monograph, if one exists, and any additional specifications for the component
- In the United States, should be obtained from an FDA-registered facility
  - If it cannot be obtained from an FDA-registered facility, the designated person(s) must select a component that is suitable for the intended use
- Outside of the United States, must comply with laws and regulations of the applicable regulatory jurisdiction

COMPONENT RECEIPT

Upon receipt of components other than conventionally manufactured products, the COA must be reviewed to ensure that the component has met the acceptance criteria in a *USP-NF* monograph, if one exists. For components other than conventionally manufactured products, information including the receipt date, quantity received, supplier name, lot number, expiration date, and results of any in-house or third-party testing performed must be documented.

The date of receipt by the compounding facility must be clearly and indelibly marked on each component package that lacks a vendor expiration date. Packages of components (i.e., API and added substances) that lack a vendor's expiration date

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must not be used by the compounding facility after 3 years from the date of receipt. A shorter expiration date must be assigned according to *Pharmaceutical Compounding—Sterile Preparations ⟨797⟩, 9.3 Components, Component Receipt* if the same component container is also used in sterile compounding or if the ingredient is known to be susceptible to degradation.

For each use, the lot must be examined for evidence of deterioration and other aspects of unacceptable quality. Once removed from the original container, components not used in compounding (e.g., excess after weighing) should be discarded and not returned to the original container to minimize the risk of contaminating the original container.

Any component found to be of unacceptable quality must be promptly rejected, clearly labeled as rejected, and segregated from active stock to prevent use before appropriate disposal. Any other lots of that component from that vendor must be examined to determine whether the other lots have the same defect.

COMPONENT EVALUATION BEFORE USE

Before use, compounding personnel must visually re-inspect all components. Packages must be inspected to detect container breaks, looseness of the cap or closure, or deviation from the expected appearance or texture of the contents that might have occurred during storage.

Compounding personnel must ascertain before use that components are of the correct identity based on the labeling and have been stored under required conditions in the facility.

If the correct identity, strength, purity, and quality of components intended for preparation of CNSPs cannot be confirmed (e.g., containers with damaged or incomplete labeling), they must be immediately rejected. If they are not immediately discarded, they must be clearly labeled as rejected, and segregated to prevent their use before disposal.

COMPONENT HANDLING

All components must be handled in accordance with the manufacturer's instructions or per laws and regulations of the applicable regulatory jurisdiction. The handling must minimize the risk of contamination, mix-ups, and deterioration (e.g., loss of identity, strength, purity, and quality).

COMPONENT SPILL AND DISPOSAL

The facility must maintain chemical hazard and disposal information (e.g., SDSs) and must review and update its chemical hazard and disposal information every 12 months. The chemical hazard and disposal information (e.g., SDSs) must be made accessible to compounding personnel.

The facility must have an SOP for the management of nonhazardous component spills and disposal. If required by the SOP, these activities must be documented and corrective action taken.

The facility must have a readily accessible spill kit in the compounding area. The contents of the spill kit should be affixed to the packaging of the spill kit if not readily visible on the manufacturer's label.

All personnel who may be required to remediate a spill must receive training in spill management of chemicals used and stored at the compounding facility. Refresher training must be conducted every 12 months and documented for all personnel who may be required to clean up a spill.

Waste must be disposed of in accordance to laws and regulations of the applicable regulatory jurisdiction. The disposal of components must comply with laws and regulations of the applicable regulatory jurisdiction. For information on the handling of HDs, see ⟨800⟩.

7. MASTER FORMULATION AND COMPOUNDING RECORDS

7.1 Creating Master Formulation Records

A Master Formulation Record is a detailed record of procedures that describes how the CNSP is to be prepared. A Master Formulation Record must be created for each unique formulation of a CNSP. CNSPs are prepared according to the Master Formulation Record and the preparation information is documented on a Compounding Record (see *7.2 Creating Compounding Records*). Any changes or alterations to the Master Formulation Record must be approved and documented according to the facility's SOP. *Box 7-1* lists the information that must be included in a Master Formulation Record.

Box 7-1. Master Formulation Records

A Master Formulation Record must include at least the following information:

- Name, strength or activity, and dosage form of the CNSP
- Identities and amounts of all components
  - If applicable, relevant characteristics of components (e.g., particle size, salt form, purity grade, solubility)
- Container–closure system(s)
- Complete instructions for preparing the CNSP, including equipment, supplies, and a description of the compounding steps
- Physical description of the final CNSP
- Assigned beyond-use date (BUD) and storage requirements
- Reference source to support the assigned BUD and storage requirements
- If applicable, calculations to determine and verify quantities and/or concentrations of components and strength or activity of API
- Labeling requirements (e.g., shake well)
- Quality control (QC) procedures (e.g., pH testing, visual inspection) and expected results
- Other information needed to describe the compounding process and ensure repeatability (e.g., adjusting pH, temperature)

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7.2 Creating Compounding Records

A Compounding Record documents the compounding of each CNSP. A Compounding Record must be created for all CNSPs. Each Compounding Record must be reviewed for completeness before the CNSP is released. The identifier of the person completing the review and the date of review must be documented on the Compounding Record. The Compounding Record must permit traceability of all components in the case of a recall or known quality issue. The Master Formulation Record can be used as the basis for preparing the Compounding Record. For example, a copy of the Master Formulation Record can be made that contains spaces to record the information needed to complete the Compounding Record. *Box 7-2* lists the information that must be included in a Compounding Record.

Box 7-2. Compounding Records

Compounding Records must include at least the following information:

- Name, strength or activity, and dosage form of the CNSP
- Date and time of preparation of the CNSP
- Assigned internal identification number (e.g., prescription, order, or lot number)
- A method to identify the individuals involved in the compounding process and verifying the final CNSP
- Name, vendor or manufacturer, lot number, and expiration date of each component
- Weight or measurement of each component
- Total quantity compounded
- Assigned BUD and storage requirements
- If applicable, calculations to determine and verify quantities and/or concentrations of components and strength or activity of API
- Physical description of the final CNSP
- Results of quality control procedures (e.g., pH testing, visual inspection)
- Master Formulation Record reference for the CNSP

8. RELEASE INSPECTIONS

At the completion of compounding and before release and dispensing, the CNSP must be visually inspected to determine whether the physical appearance is as expected. Inspections must also confirm that the CNSP and its labeling match the Compounding Record and the prescription or medication order. Some CNSPs, as noted in their Master Formulation Record, also must be visually checked for certain characteristics (e.g., emulsions must be checked for phase separation). All checks and inspections, and if required, any other tests necessary to ensure the quality of the CNSP must be detailed in the facility's Master Formulation Records. Checks and inspections must be documented. Additional quality assurance (QA) and quality control activities are described in *12. Quality Assurance and Quality Control*. Pre-release inspection also must include a visual inspection of container–closure integrity (e.g., checking for leakage, cracks in the container, or improper seals). CNSPs with observed defects must be immediately discarded, or marked and segregated from acceptable units in a manner that prevents them from being released or dispensed.

9. LABELING

The term labeling designates all labels and other written, printed, or graphic matter on the immediate container or on, or in, any package or wrapper in which the article is enclosed, except any outer shipping container. The term label designates the part of the labeling on the immediate container. See *Labeling* ⟨7⟩.

Every dispensed CNSP must be labeled with adequate, legible identifying information to prevent errors during storage, dispensing, and use. All labeling must be in compliance with laws and regulations of the applicable regulatory jurisdiction.

The label on each immediate container of the CNSP must, at a minimum, display the following information:

- Assigned internal identification number (e.g., barcode, prescription, order, or lot number)
- Active component(s), and amounts, activities, or concentrations
- Dosage form
- Amount or volume in each container
- Storage conditions if other than controlled room temperature
- BUD

The labeling on the CNSP should display the following information:

- Route of administration
- Indication that the preparation is compounded
- Any special handling instructions
- Any warning statements that are applicable
- Name, address, and contact information of the compounding facility if the CNSP is to be sent outside of the facility or healthcare system in which it was compounded

Labeling operations must be controlled to prevent labeling errors and CNSP mix-ups. A final check must be conducted to verify that the correct label has been affixed to the finished CNSP. All labels must also comply with laws and regulations of the applicable regulatory jurisdiction.

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10. ESTABLISHING BEYOND-USE DATES

10.1 Terminology

Each CNSP label must state the date, or the hour and date, beyond which the preparation cannot be used and must be discarded (i.e., the BUD). BUDs for CNSPs are calculated in terms of hours, days, or months.

BUDs and expiration dates are not the same. An expiration date identifies the time during which a conventionally manufactured drug product, active ingredient, or added substance can be expected to meet the requirements of a compendial monograph, if one exists, or maintain expected quality provided it is kept under the specified storage conditions. The expiration date limits the time during which a conventionally manufactured product, API, or added substance may be dispensed or used (see *Labeling* ⟨7⟩, *Labels and Labeling for Products in Other Categories, Expiration Date and Beyond-Use Date*). Expiration dates are assigned by manufacturers based on analytical and performance testing of the sterility, chemical and physical stability, and packaging integrity of the product. Expiration dates are specific for a particular formulation in its container and at stated exposure conditions of illumination and temperature.

10.2 Parameters to Consider in Establishing a BUD

BUDs for CNSPs should be established conservatively to ensure that the preparation maintains its required characteristics to minimize the risk of contamination or degradation.

When establishing a BUD for a CNSP, it is critical that personnel carefully consider the possible ways that the physical or chemical characteristics of the CNSP could change over time. The following factors must be considered:

- The chemical and physical stability properties of the API and any added substances in the preparation (e.g., if the API and added substances in the preparation are known to degrade over time and/or under certain storage conditions, which would reduce the strength of the preparation and/or produce harmful impurities)
- The compatibility of the container–closure system with the finished preparation (e.g., leachables, interactions, adsorption, and storage conditions)
- Degradation of the container–closure system, which can lead to a reduction in integrity of the CNSP
- The potential for microbial proliferation in the CNSP

10.3 Establishing a BUD for a CNSP

The BUDs indicate the days after the CNSP is prepared and beyond which the CNSP must not be used. The day that the preparation is compounded is considered Day 1. The BUDs in *Table 3* are based on the ability of the CNSP to maintain chemical and physical stability and to suppress microbial growth. *Table 3* represents the maximum BUDs for CNSPs that are packaged in tight, light-resistant containers unless conditions under *10.4 CNSPs Requiring Shorter BUDs* or *10.5 Extending BUDs for CNSPs* apply.

The aqueous and nonaqueous dosage forms in *Table 3* are defined based on the water activity (Aw) of the most similar drug product described in *Application of Water Activity Determination to Nonsterile Pharmaceutical Products* ⟨1112⟩. In general, the use of Aw aids in assessing the susceptibility of CNSPs to microbial contamination and the potential for API degradation due to hydrolysis. Reduced Aw greatly assists in the prevention of microbial proliferation in conventionally manufactured products and is expected to convey the same benefit to CNSPs. The list of manufactured products in *Application of Water Activity Determination to Nonsterile Pharmaceutical Products* ⟨1112⟩, *Table 2* is not exhaustive. However, it provides guidance on the Aw value of a particular CNSP and can assist personnel in determining the BUD by dosage form based on *Table 3*.

CNSPs with an Aw > 0.6 should contain suitable antimicrobial agents to protect against bacteria, yeast, and mold contamination from proliferation if inadvertently introduced during or after the compounding process. When antimicrobial preservatives are clinically contraindicated in a CNSP, storage of the preparation in a refrigerator is required if such storage does not change the physical or chemical properties of the CNSP (i.e., precipitation).

Table 3. Maximum BUD by Type of Preparation in the Absence of a *USP-NF* Compounded Preparation Monograph or CNSP-Specific Stability Information

Type of Preparation	BUDs (days)	Storage Temperature[a]
Non-preserved aqueous dosage forms[b]	14	Refrigerator
Preserved aqueous dosage forms[b]	35	Controlled room temperature or refrigerator
Nonaqueous dosage forms[c]	90	Controlled room temperature or refrigerator
Solid dosage forms[d]	180	Controlled room temperature or refrigerator

[a] See *Packaging and Storage Requirements* ⟨659⟩.
[b] An aqueous preparation is one that has an Aw of > 0.6 (e.g., emulsions, gels, creams, solutions, sprays, or suspensions).
[c] Any preparation other than solid dosage forms that have a reduced Aw of ≤0.6 (e.g., suppositories, ointments, fixed oils, or waxes).
[d] Capsules, tablets, granules, powders.

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10.4 CNSPs Requiring Shorter BUDs

A shorter BUD must be established under the following circumstances:

- If the API or any other components in the CNSP have an expiration date that is earlier than the BUD that could be assigned from *Table 3*, the expiration date supersedes the BUD and must be the assigned shortest date
- If the CNSP includes components from conventionally manufactured product(s), the BUD of the CNSP must not exceed the shortest remaining expiration date of any of those conventionally manufactured product(s)
- If the CNSP includes components from other compounded preparations, the BUD of the final CNSP must not exceed the shortest remaining BUD of any of those compounded preparations
- If the formulation is known to require a shorter BUD

10.5 Extending BUDs for CNSPs

CNSPS WITH A *USP–NF* MONOGRAPH

If there is a *USP–NF* compounded preparation monograph for the CNSP, the BUD must not exceed the BUD specified in the monograph.

CNSPS WITH STABILITY INFORMATION

The BUDs specified in *Table 3* for aqueous dosage forms and nonaqueous dosage forms may be extended up to maximum of 180 days if there is a stability study (published or unpublished) using a stability-indicating assay for the API(s), CNSP, and type of container–closure that will be used.

If the BUD of the CNSP is extended beyond the BUDs in *Table 3*, an aqueous CNSP should be tested for antimicrobial effectiveness (see *Antimicrobial Effectiveness Testing* ⟨51⟩). The compounder may rely on 1) antimicrobial effectiveness testing that is conducted (or contracted for) once for each formulation in the particular container–closure system in which it will be packaged or 2) antimicrobial effectiveness testing results provided by an FDA-registered facility or published in peer-reviewed literature sources if the CNSP formulation (including any preservative) and container–closure system are exactly the same as those tested unless a bracketing study is performed. Antimicrobial effectiveness testing may be performed on a low concentration and a high concentration of the active ingredient in the formulation to establish preservative effectiveness across various strengths of the same formulation (e.g., bracketing). The concentration of all other ingredients (including preservatives) must be the same throughout the bracketing study.

11. SOPS

Facilities preparing CNSPs must develop SOPs on all aspects of the compounding operation. All personnel who conduct or oversee compounding activities must be trained in the SOPs and are responsible for ensuring that they are followed.

One or more person(s) must be designated to ensure that SOPs are fully implemented. The designated person(s) must ensure that follow-up occurs if problems, deviations, or errors are identified.

12. QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control programs are necessary to ensure that consistently high-quality CNSPs are prepared. QA is a system of procedures, activities, and oversight that ensures that the compounding process consistently meets quality standards. QC is the sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the CNSP. See *Quality Assurance in Pharmaceutical Compounding* ⟨1163⟩.

A facility's QA and QC programs must be formally established and documented in SOPs that ensure that all aspects of the preparation of CNSPs are conducted in accordance with this chapter and laws and regulations of the applicable regulatory jurisdiction. A designated person must ensure that the facility has formal, written QA and QC programs that establish a system of:

1. Adherence to procedures
2. Prevention and detection of errors and other quality problems
3. Evaluation of complaints and adverse events
4. Appropriate investigations and corrective actions

The SOPs must describe the roles, duties, and training of the personnel responsible for each aspect of the QA program. Designated person(s) responsible for the QA program must have the training, experience, responsibility, and authority to perform these duties. The overall QA and QC program must be reviewed at least once every 12 months by the designated person(s). The results of the review must be documented and appropriate action must be taken if needed.

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13. CNSP PACKAGING AND TRANSPORTING

13.1 Packaging of CNSPs

SOPs must describe packaging of CNSPs. Personnel should select and use packaging materials that will maintain the physical and chemical integrity and stability of the CNSPs. Packaging materials must protect CNSPs from damage, leakage, contamination, and degradation, while simultaneously protecting personnel from exposure.

13.2 Transporting CNSPs

If transporting CNSPs, the facility must have written SOPs to describe the mode of transportation, any special handling instructions, and whether temperature monitoring devices are needed.

14. COMPLAINT HANDLING AND ADVERSE EVENT REPORTING

Compounding facilities must develop and implement SOPs for complaint and adverse event report receipt, acknowledgment, and handling and designate one or more person(s) to be responsible for handling them. Complaints may include concerns or reports on the quality and labeling of, or possible adverse reactions to, a specific CNSP.

14.1 Complaint Handling

The designated person(s) must ensure that all complaints are reviewed to determine whether the complaint indicates a potential quality problem with the CNSP. If it does, a thorough investigation into the cause of the problem must be initiated and completed. The investigation must consider whether the quality problem extends to other CNSPs. Corrective action, if necessary, must be implemented for all potentially affected CNSPs. Consider whether to initiate a recall of potentially affected CNSPs and whether to cease nonsterile compounding processes until all underlying problems have been identified and corrected.

A readily retrievable written or electronic record of each complaint must be kept by the facility, regardless of the source of the complaint (e.g., e-mail, telephone, mail). The record must contain the name of the complainant or unique identifier, the date the complaint was received, the nature of the complaint, and the response to the complaint. In addition, to the extent that the information is known, the following should be recorded: the name and strength of the CNSP, the prescription or medication order number, and the lot number, if one is assigned.

The record must also include the findings of any investigation and any follow-up. Records of complaints must be easily retrievable for review and evaluation for possible trends and must be retained in accordance with the record-keeping requirements in *15. Documentation*. A CNSP that is returned in connection with a complaint must be quarantined until it is destroyed after completion of the investigation and in accordance with laws and regulations of the applicable regulatory jurisdiction.

14.2 Adverse Event Reporting

The designated person(s) must ensure that reports of potential adverse events involving a CNSP are reviewed. If the investigation into an adverse event reveals a quality problem with a CNSP that is likely to affect other patients, those patients and prescribers potentially affected must be informed. The designated person(s) must review all adverse event reports as part of the QA and QC programs (see *12. Quality Assurance and Quality Control*). Adverse events must be reported in accordance with facility SOPs and all laws and regulations of the applicable regulatory jurisdiction. In addition, adverse events associated with a CNSP should be reported to the FDA through the MedWatch program for human drugs and through Form FDA 1932a for animal drugs.

15. DOCUMENTATION

All facilities where CNSPs are prepared must have and maintain written or electronic documentation to demonstrate compliance with the requirements in this chapter. This documentation must include, but is not limited to, the following:

- Personnel training, competency assessments, and corrective actions for any failures
- Equipment records (e.g., calibration, verification, and maintenance reports)
- COA
- Receipt of components
- SOPs, Master Formulation Records, and Compounding Records
- Release inspection and testing records
- Information related to complaints and adverse events including corrective actions taken
- Results of investigation and corrective actions

Documentation must comply with all laws and regulations of the applicable regulatory jurisdiction. Records must be legible and stored in a manner that prevents their deterioration and/or loss. All required compounding records for a particular CNSP (e.g., Master Formulation Record, Compounding Record, and release inspection and testing results) must be readily retrievable

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for at least 3 years after preparation or as required by the laws and regulations of the applicable regulatory jurisdiction, whichever is longer.

GLOSSARY

Active pharmaceutical ingredient (API): Any substance or mixture of substances intended to be used in the compounding of a preparation, thereby becoming the active ingredient in that preparation and furnishing pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or affecting the structure and function of the body.

Added substances: Ingredients that are necessary to compound a preparation but are not intended or expected to cause a pharmacologic response if administered alone in the amount or concentration contained in a single dose of the compounded preparation. The term is used synonymously with the terms inactive ingredients, excipients, and pharmaceutical ingredients.

Biological safety cabinet (BSC): A ventilated cabinet which may be used for compounding. These cabinets divided into three general classes (Class I, Class II, and Class III). Class II BSCs are further divided into types (Type A1, Type A2, Type B1, and Type B2).

Certificate of Analysis (COA): A report from the supplier of a component, container, or closure that accompanies the supplier's material and contains the specifications and results of all analyses and a description of the material.

Cleaning: The process of removing soil (e.g., organic and inorganic material) from objects and surfaces, normally accomplished by manually or mechanically using water with detergents or enzymatic products.

Component: Any ingredient used in the compounding of a preparation, including any active ingredient, added substance, or conventionally manufactured product.

Compounded nonsterile preparation (CNSP): A preparation intended to be nonsterile created by combining, admixing, diluting, pooling, reconstituting other than as provided in the manufacturer's labeling, or otherwise altering of a drug or bulk drug substance.

Compounder: Personnel trained to compound preparations.

Compounding: The process of combining, admixing, diluting, pooling, reconstituting other than as provided in the manufacturer's labeling, or otherwise altering a drug or bulk drug substance to create a nonsterile medication.

Compounding area: A space that is specifically designated for nonsterile compounding. A visible perimeter should establish the boundaries of the nonsterile compounding area.

Container–closure system: Packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection.

Containment glove bag: A single-use disposable glove bag that is capable of containing airborne chemical particles.

Containment ventilated enclosure (CVE): A full or partial enclosure that uses ventilation principles to capture, contain, and remove airborne contaminants through high-efficiency particulate air (HEPA) filtration and to prevent their release into the work environment.

Conventionally manufactured product: A pharmaceutical dosage form, usually the subject of an FDA-approved application that is manufactured under current good manufacturing practice conditions.

Designated person(s): One or more individuals assigned to be responsible and accountable for the performance and operation of the facility and personnel for the preparation of CNSPs.

Hazardous drug (HD): Any drug identified by at least one of the following six criteria: carcinogenicity, teratogenicity or developmental toxicity, reproductive toxicity in humans, organ toxicity at low dose in humans or animals, genotoxicity, or new drugs that mimic existing HDs in structure or toxicity. See ⟨800⟩.

Label: A display of written, printed, or graphic matter on the immediate container of any article.

Labeling: All labels and other written, printed, or graphic matter that are 1) on any article or any of its containers or wrappers, or 2) accompanying such an article.

Purified Water: The minimal quality of source water for the production of Purified Water is drinking water whose attributes are prescribed by the US Environmental Protection Agency (EPA), the EU, Japan, or the World Health Organization (WHO). This source water may be purified using unit operations that include deionization, distillation, ion exchange, reverse osmosis, filtration, or other suitable purification procedures. (See *Water for Pharmaceutical Purposes* ⟨1231⟩, *3. Waters Used for Pharmaceutical Manufacturing and Testing Purposes, 3.1 Bulk Monographed Waters and Steam, 3.1.1 Purified Water.*)

Preservative: A substance added to inhibit microbial growth.

Quality assurance (QA): A system of procedures, activities, and oversight that ensures that the compounding process consistently meets quality standards.

Quality control (QC): The sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the CNSP.

Reconstitution: The process of adding a diluent to a conventionally manufactured product to prepare a solution or suspension.

Release inspection and testing: Visual inspection and testing performed to ensure that a preparation meets appropriate quality characteristics.

Sanitizing agent: An agent for reducing, on inanimate surfaces, the number of all forms of microbial life including fungi, viruses, and bacteria.

Specification: The tests, analytical methods, and acceptance criteria to which an API or other components, CNSP, container–closure system, equipment, or other material used in compounding CNSPs must conform to be considered acceptable for its intended use.

Stability: The extent to which a product or preparation retains physical and chemical properties and characteristics within specified limits throughout its expiration or BUD.

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APPENDIX

Acronyms

API(s)	Active pharmaceutical ingredient(s)
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers
Aw	Water activity
BSC(s)	Biological safety cabinet(s)
BUD(s)	Beyond-use date(s)
CETA	Controlled Environment Testing Association
CNSP(s)	Compounded nonsterile preparation(s)
COA	Certificate(s) of Analysis
CVE	Containment ventilated enclosure
FDA	Food and Drug Administration
HD(s)	Hazardous drug(s)
QA	Quality assurance
QC	Quality control
SDS(s)	Safety Data Sheet(s)
SOP(s)	Standard operating procedure(s) ▲ (USP 1-Dec-2019)

⟨797⟩ PHARMACEUTICAL COMPOUNDING—STERILE PREPARATIONS

Change to read:

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1. INTRODUCTION AND SCOPE

This chapter describes the minimum standards to be followed when preparing compounded sterile human and animal drugs [compounded sterile preparations (CSPs)]. Sterile compounding is defined as combining, admixing, diluting, pooling, reconstituting, repackaging, or otherwise altering a drug or bulk drug substance to create a sterile medication.

The requirements in this chapter must be followed to minimize harm, including death, to human and animal patients that could result from 1) microbial contamination (nonsterility), 2) excessive bacterial endotoxins, 3) variability from the intended strength of correct ingredients, 4) physical and chemical incompatibilities, 5) chemical and physical contaminants, and/or 6) use of ingredients of inappropriate quality.

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Aseptic technique must be followed for preparing any sterile medication. Procedures must be in place to minimize the potential for contact with nonsterile surfaces, introduction of particulate matter or biological fluids, and mix-ups with other products or CSPs.

Pursuant to *General Notices, 2.30 Legal Recognition*, assuring compliance with *USP* standards is the responsibility of regulatory bodies. Accreditation or credentialing organizations may adopt and enforce *USP* standards. USP has no role in enforcement.

1.1 Scope

CSPS AFFECTED

The requirements in this chapter must be met to ensure the sterility of any CSP. Although the list below is not exhaustive, the following must be sterile:

- Injections, including infusions
- Irrigations for internal body cavities (i.e., any space that does not normally communicate with the environment outside of the body such as the bladder cavity or peritoneal cavity). [NOTE—Irrigations for the mouth, rectal cavity, and sinus cavity are not required to be sterile.]
- Ophthalmic dosage forms
- Preparations for pulmonary inhalation. [NOTE—Nasal dosage forms intended for local application are not required to be sterile.]
- Baths and soaks for live organs and tissues
- Implants

SPECIFIC PRACTICES

Repackaging: Repackaging of a sterile product or preparation from its original container into another container must be performed in accordance with the requirements in this chapter.

Allergenic extracts: Licensed allergenic extracts are mixed and diluted to prepare prescription sets for administration to patients. A prescription set is a vial or set of vials of premixed licensed allergenic extracts for subcutaneous immunotherapy diluted with an appropriate diluent for an individual patient. Because of certain characteristics of allergenic extracts and allergy practice, preparation of allergenic extract prescription sets is not subject to the requirements in this chapter that are applicable to other sterile CSPs. The standards for compounding allergenic extracts are in *21. Compounding Allergenic Extracts* and are applicable only when:

1. The compounding process involves transfer via sterile needles and syringes of conventionally manufactured sterile allergen products and appropriate conventionally manufactured sterile added substances, and
2. Manipulations are limited to penetrating stoppers on vials with sterile needles and syringes, and transferring sterile liquids in sterile syringes to sterile vials

Otherwise, compounding of allergenic extracts prescription sets must meet the requirements for Category 1 or Category 2 CSPs, which are described in this chapter.

Hazardous drugs: Compounding of sterile hazardous drugs (HDs) must additionally comply with *Hazardous Drugs—Handling in Healthcare Settings* ⟨800⟩.

Blood-derived and other biological materials: When compounding activities require the manipulation of a patient's blood-derived or other biological material (e.g., autologous serum), the manipulations must be clearly separated from other compounding activities and equipment used in CSP preparation activities, and they must be controlled by specific standard operating procedures (SOPs) in order to avoid any cross-contamination. Handling of blood components must additionally comply with jurisdictional standards and guidelines.

Sterile radiopharmaceuticals: Compounding of radiopharmaceuticals is not required to meet the standards of this chapter for Category 1 and Category 2 CSPs and is subject to the requirements in *Radiopharmaceuticals—Preparation, Compounding, Dispensing, and Repackaging* ⟨825⟩.

PERSONNEL AND SETTINGS AFFECTED

This chapter describes the minimum requirements that apply to all persons who prepare CSPs and all places where CSPs are prepared. This includes, but is not limited to, pharmacists, technicians, nurses, physicians, veterinarians, dentists, naturopaths, and chiropractors in all places including, but not limited to, hospitals and other healthcare institutions, medical and surgical patient treatment sites, infusion facilities, pharmacies, and physicians' or veterinarians' practice sites. Any person, whether preparing a CSP or not, entering a sterile compounding area must meet the requirements in *3. Personal Hygiene and Garbing*.

The compounding facility must designate one or more individuals [i.e., the designated person(s)] to be responsible and accountable for the performance and operation of the facility and personnel in the preparation of CSPs and for performing other functions as described in this chapter.

1.2 Administration

For the purposes of this chapter, administration means the direct application of a sterile medication to a single patient by injecting, infusing, or otherwise providing a sterile medication in its final form. Administration of medication is out of the scope of this chapter. Standard precautions such as the Centers for Disease Control and Prevention's (CDC's) safe injection practices apply to administration.

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1.3 Immediate Use CSPs

Compounding of CSPs for direct and immediate administration to a patient is not subject to the requirements for Category 1 or Category 2 CSPs when all of the following are met:

1. Aseptic processes are followed and written procedures are in place to minimize the potential for contact with nonsterile surfaces, introduction of particulate matter or biological fluids, and mix-ups with other conventionally manufactured products or CSPs.
2. The preparation is performed in accordance with evidence-based information for physical and chemical compatibility of the drugs (e.g., FDA-approved labeling, stability studies).
3. The preparation involves not more than 3 different sterile products.
4. Any unused starting component from a single-dose container must be discarded after preparation for the individual patient is complete. Single-dose containers must not be used for more than 1 patient.
5. Administration begins within 4 hours following the start of preparation. If administration has not begun within 4 hours following the start of preparation, it must be promptly, appropriately, and safely discarded.
6. Unless administered by the person who prepared it or administration is witnessed by the preparer, the CSP must be labeled with the names and amounts of all active ingredients, the name or initials of the person who prepared the preparation, and the exact 4-hour time period within which administration must begin.

1.4 Preparation Per Approved Labeling

Compounding does not include mixing, reconstituting, or other such acts that are performed in accordance with directions contained in approved labeling provided by the product's manufacturer and other manufacturer directions consistent with that labeling [21 USC 353a (e)].

Preparing a conventionally manufactured sterile product in accordance with the directions in the manufacturer's approved labeling is out of scope of this chapter only if:

1. The product is prepared as a single dose for an individual patient, and
2. The approved labeling includes information for the diluent, the resultant strength, the container closure system, and storage time.

PROPRIETARY BAG AND VIAL SYSTEMS

Docking and activation of proprietary bag and vial systems (e.g., addEASE, ADD-Vantage, Mini Bag Plus) in accordance with the manufacturer's labeling for *immediate* administration to an individual patient is not considered compounding and may be performed outside of an International Organization for Standardization (ISO) 5 environment.

Docking of the proprietary bag and vial systems for *future activation* and administration is considered compounding and must be performed in accordance with this chapter, with the exception of *14. Establishing Beyond-Use Dates*. Beyond-use dates (BUDs) for proprietary bag and vial systems must not be longer than those specified in the manufacturer's labeling.

1.5 CSP Categories

This chapter distinguishes two categories of CSPs, Category 1 and Category 2, primarily based on the conditions under which they are made, the probability for microbial growth, and the time period within which they must be used. Category 1 CSPs are those assigned a BUD of 12 hours or less at controlled room temperature or 24 hours or less when refrigerated if made in accordance with all of the applicable requirements for Category 1 CSPs in this chapter. Category 2 CSPs are those that may be assigned a BUD of greater than 12 hours at controlled room temperature or greater than 24 hours if refrigerated (see *14. Establishing Beyond-Use Dates*) if made in accordance with all of the applicable requirements for Category 2 CSPs in this chapter.

The requirements that are not specifically described as applicable to Category 1 or Category 2, such as training, competency testing, and personal hygiene for personnel, are applicable to the compounding of all CSPs.

CSPs can be compounded either by using only sterile starting ingredients or by using some or all nonsterile starting ingredients. If all of the components used to compound a drug are sterile to begin with, the sterility of the components must be maintained during compounding to produce a CSP. If one or more of the starting components being used to compound is not sterile, the sterility of the compounded preparation must be achieved through a sterilization process (e.g., terminal sterilization in the final sealed container) or sterilizing filtration, and then maintained if the CSP is subsequently manipulated. When compounding with nonsterile starting components, supplies, or equipment, the quality of the components and the effectiveness of the sterilization step are critical to achieving a sterile preparation.

2. PERSONNEL TRAINING AND EVALUATION

All personnel involved in the compounding of CSPs must be initially trained and qualified by demonstrating proficiency in compounding CSPs. A designated person must oversee the training of personnel. Training and observation may be performed by the designated person(s) or an assigned trainer. Personnel must complete training every 12 months in appropriate sterile compounding principles and practices.

Each compounding facility must develop a written training program that describes the required training, the frequency of training, and the process for evaluating the performance of individuals involved in preparing CSPs. This program should equip

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personnel with the appropriate knowledge and train them in the required skills necessary to perform their assigned tasks. Training and evaluation of personnel must be documented.

2.1 Demonstrating Proficiency in Core Competencies

Before beginning to prepare CSPs independently, all compounding personnel must complete training and be able to demonstrate knowledge of principles and proficiency of skills for performing sterile manipulations and achieving and maintaining appropriate environmental conditions. Competency must be demonstrated every 12 months in at least the following:

- Hand hygiene
- Garbing
- Cleaning and disinfection
- Calculations, measuring, and mixing
- Aseptic technique
- Achieving and/or maintaining sterility and apyrogenicity
- Use of equipment
- Documentation of the compounding process (e.g., master formulation and compounding records)
- Principles of high-efficiency particulate air (HEPA)-filtered unidirectional airflow within the ISO Class 5 area
- Proper use of primary engineering controls (PECs)
- Principles of movement of materials and personnel within the compounding area

All compounding personnel must complete written or electronic testing every 12 months. Any other personnel handling CSPs and/or accessing the compounding area must complete training and demonstrate competency in maintaining the quality of the environment in which they are performing their assigned task. The designated person(s) must ensure that any person who enters the sterile compounding area maintains the quality of the environment.

If the facility has only one person in the compounding operation, that person must document that they have obtained training and demonstrated competency, and they must comply with the other requirements of this chapter.

2.2 Demonstrating Competency in Garbing and Hand Hygiene

All compounding personnel must be visually observed initially and every 6 months while performing hand hygiene and garbing procedures (see *3. Personal Hygiene and Garbing*). The visual audit must be documented and the documentation maintained to provide a record of personnel competency.

Initial gloved fingertip and thumb sampling evaluates a compounder's competency in correctly performing hand hygiene and garbing (see *Box 2-1*). Before being allowed to independently compound, all compounders must successfully complete an initial competency evaluation, including visual observation and gloved fingertip and thumb sampling on both hands, no fewer than 3 separate times. Each fingertip and thumb evaluation must occur after performing a separate and complete hand hygiene and full garbing procedure. After the initial competency evaluation, compounding personnel must successfully complete gloved fingertip and thumb sampling at least every 6 months after completing the media-fill test (see *2.3 Competency Testing in Aseptic Manipulation*).

Initial gloved fingertip and thumb sampling must be performed on donned sterile gloves in a classified area or segregated compounding area (SCA). Subsequent gloved fingertip and thumb sampling must be performed on donned sterile gloves inside of an ISO Class 5 PEC. If conducting gloved fingertip and thumb sampling in a compounding aseptic isolator (CAI), compounding aseptic containment isolator (CACI), or a pharmaceutical isolator, samples must be taken from the sterile gloves placed over the gloves attached to the restricted-access barrier system (RABS) sleeves.

Successful completion of initial gloved fingertip and thumb sampling is defined as zero colony-forming units (cfu). Successful completion of subsequent gloved fingertip and thumb sampling after media-fill testing is defined as ≤3 cfu (total from both hands). Action levels for gloved fingertip and thumb sampling results are shown in *Table 1*.

Failure is indicated by visual observation of improper hand hygiene and garbing procedures and/or gloved fingertip and thumb sampling results that exceed the action levels in *Table 1*. Results of the evaluation and corrective actions, in the event of failure, must be documented and the documentation maintained to provide a record and long-term assessment of personnel competency. Documentation must at a minimum include the name of the person evaluated, evaluation date/time, media and components used including manufacturer, expiration date and lot number, starting temperature for each interval of incubation, dates of incubation, the results, and the identification of the observer and the person who reads and documents the results.

Box 2-1. Gloved Fingertip and Thumb Sampling Procedures

- Use one sampling device per hand (e.g., plates, paddles, or slides) containing general microbial growth agar [e.g., trypticase soy agar (TSA)] supplemented with neutralizing additives (e.g., lecithin and polysorbate 80) as this agar supports both bacterial and fungal growth.
- Label each sampling device with a personnel identifier, whether it was from the right or left hand, and the date and time of sampling.
- Do not apply sterile 70% isopropyl alcohol (IPA) to gloves immediately before touching the sampling device because this could cause a false-negative result.
- Using a separate sampling device for each hand, collect samples from all gloved fingers and thumbs from both hands by rolling finger pads and thumb pad over the agar surface.
- Incubate the sampling device at a temperature of 30°–35° for no less than 48 hours and then at 20°–25° for no less than 5 additional days. Store media devices during incubation to prevent condensate from dropping onto the agar and affecting the accuracy of the cfu reading (e.g., invert plates).
- Record the number of cfu per hand (left hand, right hand).
- Determine whether the cfu action level is exceeded by counting the total number of cfu from both hands.

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Table 1. Action Levels for Gloved Fingertip and Thumb Sampling[a]

Gloved Fingertip and Thumb Sampling	Action Levels (total number of cfu from both hands)
Initial sampling after garbing	>0
Subsequent sampling after media-fill testing (every 6 months)	>3

[a] Action levels are based on the total cfu count from both hands.

2.3 Competency Testing in Aseptic Manipulation

All compounding personnel must perform media-fill testing to assess their sterile technique and related practices (see *Box 2-2*) initially and every 6 months thereafter. Gloved fingertip and thumb sampling must be performed inside of an ISO Class 5 PEC following media-fill tests to evaluate the ability of the compounder to demonstrate acceptable aseptic processing.

When performing a media-fill test, simulate the most difficult and challenging compounding procedures and processing conditions encountered by the person replacing all the components used in the CSPs with soybean–casein digest media.

If using commercial sterile microbial growth media, a certificate of analysis (COA) must be obtained from the supplier stating that the lot of the growth media will support the growth of microorganisms. Store microbial growth media in accordance with manufacturer instructions and initiate the media-fill test before the expiration date of the media. If preparing sterile microbial growth media in-house for sterile-to-sterile media-fill testing, the growth promotion capability of the media must be demonstrated for each batch and documented as described in *Sterility Tests* ⟨71⟩, *Culture Media and Incubation Temperatures, Growth Promotion Test of Aerobes, Anaerobes, and Fungi*.

Failure is indicated by visible turbidity or other visual manifestations of growth in the media in one or more container–closure unit(s) on or before the end of the incubation period.

Results of the evaluation and corrective actions, in the event of failure, must be documented and the documentation maintained to provide a record and long-term assessment of personnel competency. Documentation must at a minimum include the name of the person evaluated, evaluation date/time, media and components used including manufacturer, expiration date and lot number, starting temperature for each interval of incubation, dates of incubation, the results, and the identification of the observer and the person who reads and documents the results.

Box 2-2. Media-Fill Testing Procedures

- If all of the starting components are sterile to begin with, manipulate them in a manner that simulates sterile-to-sterile compounding activities, and transfer the sterile soybean–casein digest media into the same types of container–closure systems commonly used at the facility. Do not further dilute the media unless specified by the manufacturer.
- If some of the starting components are nonsterile to begin with, use a nonsterile soybean–casein digest powder to make a solution. Dissolve nonsterile commercially available soybean–casein digest medium in nonbacteriostatic water to make a 3% nonsterile solution. Manipulate it in a manner that simulates nonsterile-to-sterile compounding activities. Prepare at least 1 container as the positive control to demonstrate growth promotion, which is indicated by visible turbidity upon incubation.
- Once the compounding simulation is completed and the final containers are filled with the test media, incubate them in an incubator for 7 days at 20°–25° followed by 7 days at 30°–35° to detect a broad spectrum of microorganisms.
- Failure is indicated by visible turbidity or other visual manifestations of growth in the media in one or more container–closure unit(s) on or before 14 days.

3. PERSONAL HYGIENE AND GARBING

Personal hygiene and garbing are essential to maintain microbial control of the environment. Most microorganisms detected in cleanrooms are transferred from individuals. Squamous cells are normally shed from the human body at a rate of 10^6 or more per hour, and those skin particles are covered with microorganisms.[1, 2] Individuals entering a compounding area must be properly garbed and must maintain proper personal hygiene to minimize the risk of contamination to the environment and/or CSPs.

Individuals that may have a higher risk of contaminating the CSP and the environment (e.g., personnel with rashes, recent tattoos, oozing sores, conjunctivitis, or active respiratory infection) must report these conditions to the designated person(s). The designated person(s) is responsible for evaluating whether these individuals should be excluded from working in compounding areas before their conditions have resolved because of the risk of contaminating the CSP and the environment.

3.1 Personnel Preparation

Individuals entering a compounding area must take appropriate steps to minimize microbial contamination of the environment and the CSPs, including hand hygiene (*3.2 Hand Hygiene*), garbing (*3.3 Garbing Requirements*), and consideration of needed materials to be brought into the compounding area. Before entering a compounding area, individuals must remove any items that are not easily cleanable or that are not necessary for compounding. At a minimum, individuals must:

- Remove personal outer garments (e.g., bandanas, coats, hats, jackets, sweaters, vests).
- Remove all cosmetics because they shed flakes and particles.

[1] Agalloco J, Akers JE. Aseptic processing: a vision of the future. *Pharm Technol.* 2005; Aseptic Processing supplement, s16.
[2] Eaton T. Microbial risk assessment for aseptically prepared products. *Am Pharm Rev.* 2005;8(5):46–51.

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- Remove all hand, wrist, and other exposed jewelry including piercings that could interfere with the effectiveness of garbing (e.g., the fit of gloves, cuffs of sleeves, and eye protection) or otherwise increase the risk of contamination of the CSP. Cover any jewelry that cannot be removed.
- Not wear earbuds or headphones.
- Not bring electronic devices that are not necessary for compounding or other required tasks into the compounding area.
- Keep nails clean and neatly trimmed to minimize particle shedding and avoid glove punctures. Nail products (e.g., polish, artificial nails, and extenders) must not be worn.
- Wipe eyeglasses, if worn.

The designated person(s) may permit accommodations as long as the quality of the CSP and environment will not be affected.

3.2 Hand Hygiene

Personnel must wash hands and forearms up to the elbows with soap and water before initiating compounding activities *Box 3-1*). Brushes must not be used for hand hygiene. Hand dryers must not be used. A closed system of soap (i.e., non-refillable container) to minimize the risk of extrinsic contamination must be readily available or in close proximity to the sink.

Box 3-1. Hand Washing Procedures

- Remove visible debris from underneath fingernails under warm running water using a disposable nail cleaner.
- Wash hands and forearms up to the elbows with soap and water for at least 30 seconds.
- Dry hands and forearms to the elbows completely with low-lint disposable towels or wipers.

The order of hand washing and garbing depends on the placement of the sink (see *4.4 Water Sources*). The order of garbing must be determined by the facility and documented in the facility's SOP. Hands must be sanitized with alcohol-based hand rub before donning sterile gloves (see *Box 3-2*). Sterile gloves must be donned in a classified room or SCA.

Box 3-2. Hand Sanitizing Procedures

- Apply an alcohol-based hand rub to dry skin following the manufacturer's instructions for the volume of product to use.
- Apply product to one hand and rub hands together, covering all surfaces of hands and fingers, until hands are dry.
- Allow hands to dry thoroughly before donning sterile gloves.

3.3 Garbing Requirements

Any person entering a compounding area must be properly garbed in accordance with the facility's SOPs. Garb must be donned and doffed in an order that reduces the risk of contamination. The order of garbing must be determined by the facility and documented in the facility's SOP. Sterile gloves must be donned in a classified room or SCA. Skin must not be exposed inside the ISO Class 5 PEC (e.g., gloves must not be donned or doffed inside the ISO Class 5 PEC exposing bare hands). Donning and doffing garb should not occur in the ante-room or the SCA at the same time. The minimum garbing requirements include:

- Low-lint garment with sleeves that fit snugly around the wrists and that is enclosed at the neck (e.g., gowns or coveralls)
- Low-lint, disposable covers for shoes
- Low-lint, disposable covers for head that cover the hair and ears, and if applicable, disposable cover for facial hair
- Face mask
- Sterile powder-free gloves
- If using a RABS, such as a CAI or CACI, disposable gloves (e.g., cotton, nonsterile, sterile) should be worn inside gloves attached to the RABS sleeves. Sterile gloves must be worn over gloves attached to the RABS sleeve

Garb must be replaced immediately if it becomes visibly soiled or if its integrity is compromised. Gowns and other garb must be stored in a manner that minimizes contamination (e.g., away from sinks to avoid splashing). When personnel exit the compounding area, garb except for gowns cannot be reused and must be discarded. Gowns may be re-used within the same shift if the gown is maintained in a classified area or inside the perimeter of an SCA.

If compounding a HD, appropriate personal protective equipment (PPE) must be worn and disposed of in accordance with ⟨800⟩.

GLOVES

Gloves must be sterile and powder free. Application of sterile 70% IPA to gloves must occur regularly throughout the compounding process and whenever nonsterile surfaces (e.g., vials, counter tops, chairs, or carts) are touched.

All gloves must be inspected for holes, punctures, or tears and must be replaced immediately if such defects are detected. The RABS sleeves and gloves and the pharmaceutical isolator gauntlet sleeves and gloves should be changed per the manufacturer's recommendations and as defined in the facility's SOP.

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4. FACILITIES AND ENGINEERING CONTROLS

Sterile compounding facilities must be designed, outfitted, and maintained properly to minimize the risk of contamination of CSPs. The required air quality must be achieved and maintained through PECs and secondary engineering controls (SECs). The ante-room, buffer room, and SCA must be separated from areas not directly related to compounding. The ante-room and buffer room must be appropriately controlled to achieve and maintain the required air quality classifications. The design of the facility should take into account the number of personnel and their movements, and the equipment, supplies, and components to maintain and facilitate the maintenance of air quality. The number of operations being performed, the equipment (e.g., PECs, carts, computers), the personnel in the compounding area (and in adjacent areas), and the complexity of the compounding procedures are critical considerations for maintaining control of environmental conditions in the facility.

4.1 Protection from Airborne Contaminants

Sterile compounding facilities must be designed to minimize the risk of airborne contamination of the area in which sterile compounding occurs. Proper design and controls are required to minimize the risk of exposure of CSPs to airborne contaminants.

AIR QUALITY STANDARDS

The ISO standards for air quality in controlled environments are provided in *Table 2* and referenced throughout this chapter.

Table 2. ISO Classification of Particulate Matter in Room Air[a]

ISO Class	Particle Count[b]/m³
3	35.2
4	352
5	3520
6	35,200
7	352,000
8	3,520,000

[a] Adapted from ISO 14644-1, Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration.
[b] Limits for number of particles ≥0.5 µm measured under dynamic operating conditions.

DESIGN REQUIREMENTS TO MAINTAIN AIR QUALITY

Facilities used for compounding CSPs must be designed so that air quality improves with movement through separate operational areas to the PEC. Classified areas in which the air quality is controlled (see *Table 2*) include ante-rooms, buffer rooms, and PECs.

- Ante-rooms providing access to positive pressure buffer rooms must meet at least ISO Class 8 classification. Ante-rooms providing access to negative pressure buffer rooms must meet at least ISO Class 7 classification (see ⟨800⟩). Typically, personnel hand hygiene and garbing procedures, staging of components, and other activities that potentially generate higher levels of particulates are performed in the ante-room. Ante-rooms are also transition areas to ensure that proper air classification and pressure relationships are maintained between classified and unclassified areas.
- A buffer room must meet at least ISO Class 7 air quality. Activities in the buffer room must be controlled to minimize any effects on air quality in the area where CSPs are prepared.
- Category 1 and Category 2 CSPs must be prepared in an ISO Class 5 or better PEC.

If compounding only Category 1 CSPs, the PEC may be placed in an unclassified SCA.

4.2 Facility Design and Environmental Controls

In addition to minimizing airborne contamination, sterile compounding facilities must be designed and controlled to provide a well-lighted and comfortable working environment (see *Physical Environments That Promote Safe Medication Use* ⟨1066⟩). The cleanroom suite should be maintained at a temperature of 20° or cooler and a relative humidity below 60% to minimize the risk for microbial proliferation and provide comfortable conditions for compounding personnel attired in the required garb. The temperature and humidity must be monitored in each room of the cleanroom suite each day that compounding is performed, either manually or by a continuous recording device. The results of the temperature and humidity readings must be documented at least once daily or stored in the continuous recording device, and must be retrievable. The temperature and humidity readings must be reviewed as described in the facility's SOPs. Temperature and humidity in the cleanroom suite must be controlled through a heating, ventilation, and air conditioning (HVAC) system. Free-standing humidifiers/dehumidifiers and air conditioners must not be used within the classified area or within the perimeter of the SCA. Temperature and humidity monitoring devices must be verified for accuracy at least every 12 months or as required by the manufacturer.

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The designated person(s) is responsible for ensuring that each area related to CSP preparation meets the classified air quality standard appropriate for the activities to be conducted in that area. The designated person(s) must also ensure that the ISO Class 5 areas are located, operated, maintained, monitored, and certified to have appropriate air quality.

TYPES OF SECS AND DESIGN

The PEC must be located in the buffer room of the cleanroom suite or the SCA in a manner that minimizes conditions that could increase the risk of microbial contamination. For example, strong air currents from opened doors, personnel traffic, or air streams from the HVAC system(s) can disrupt the unidirectional airflow of an open-faced PEC such as a laminar airflow workbench (LAFW). Access to the SEC must be restricted to authorized personnel and required materials.

Cleanroom suite: The ISO-classified ante-room and buffer room must be separated from the surrounding unclassified areas of the facility by fixed walls and doors, and controls must be in place to minimize the flow of lower-quality air into the more controlled areas. Air supplied to the cleanroom suite must be introduced through HEPA filters that are located in the ceiling of the buffer and ante-rooms.

Air returns in the cleanroom suite must be low on the wall unless a visual smoke study demonstrates an absence of stagnant airflow where particulate will accumulate. This smoke study along with environmental monitoring must be repeated whenever a change to the placement of equipment within the room is made or any other alteration is performed within the cleanroom suite that affects the quality of the air (e.g., HVAC alterations, change of HEPA filter units).

The classified rooms must be equipped with a pressure-differential monitoring system. The ante-room must have a line of demarcation to separate the clean side from the dirty side. Alternatively, facilities may be designed with two separate ante-rooms, a clean ante-room and a dirty ante-room. The ante-room is entered through the dirty side/room, and the clean side/room is the area closest to the buffer room. Required garb must be donned prior to entering the clean side/room of the ante-room (see *3. Personal Hygiene and Garbing*).

It is also critical to control materials (e.g., supplies and equipment) as they move from classified areas of lower quality to those of higher quality (e.g., ISO Class 8 ante-room to ISO Class 7 buffer room to ISO Class 5 PEC) to minimize the influx of contaminants. Airlocks and interlocking doors may be used to facilitate better control of air balance between areas of differing ISO classification (e.g., between the buffer room and ante-room), or between a classified area and an unclassified area (e.g., between the ante-room and an unclassified area such as a hallway). If a pass-through is used, both doors must never be opened at the same time, and doors should be interlocking.

Due to the interdependence of the various rooms or areas that make up a sterile compounding facility, it is essential to carefully define and control the dynamic interactions permitted between areas and rooms. Consider the placement of door closures, door surfaces, and the movement of the doors, all of which can affect airflow. Seals and sweeps should not be installed at doors between buffer and ante-rooms. Access doors should be hands-free. Tacky mats must not be placed within ISO-classified areas.

Segregated compounding area (SCA): A PEC may be located within an unclassified area, without an ante-room or buffer room. This type of design is called an SCA. Only Category 1 CSPs can be compounded in an SCA. The SCA must be located away from unsealed windows, doors that connect to the outdoors, and traffic flow, all of which may adversely affect the air quality in the PEC. An SCA must not be located where environmental control challenges (e.g., restrooms, warehouses, or food preparation areas) could negatively affect the air quality of the PEC within the SCA. The impact of activities (e.g., patient care activities) that will be conducted around or adjacent to the SCA must be considered carefully when designing such an area. A visible perimeter must establish the boundaries of the SCA.

THE CSP COMPOUNDING ENVIRONMENT

The PEC must be certified to meet ISO Class 5 or better conditions (see *Table 2*) during dynamic operating conditions and must be designed to prevent contamination during compounding of CSPs.

Unidirectional airflow must be maintained in the PEC. HEPA-filtered air must be supplied by the PEC at a velocity sufficient to sweep particles away from critical sites and maintain unidirectional airflow during operations. Proper design, control, and use minimizes turbulence and creation of eddies or stagnant air in the PEC.

TYPES OF PECS AND PLACEMENT

Proper placement of the PEC is critical to ensuring an ISO Class 5 environment for preparing CSPs. Placement of the PEC must allow for cleaning around the PEC. See *Table 3* for a summary of minimum requirements for the placement of PECs for preparing non-HD CSPs.

Types of PECs and their placement include the following.

Laminar airflow system (LAFS): An LAFS provides an ISO Class 5 or better environment for sterile compounding. The LAFS provides unidirectional HEPA-filtered airflow that is designed to prevent contamination of a sterile compounding environment. The unidirectional airflow within the LAFS helps protect the direct compounding area (DCA) from process-generated contamination (e.g., opening wrappings of sterile containers, compounder movement) as well as from outside sources.

Types of LAFS: Examples of LAFS include LAFWs, integrated vertical laminar flow zones (IVLFZs), and biological safety cabinets (BSCs).

LAMINAR AIRFLOW WORKBENCH (LAFW): An LAFW is a device that provides an ISO Class 5 or better environment for sterile compounding. The LAFW provides either horizontal or vertical unidirectional HEPA-filtered airflow. [NOTE—An LAFW must not be used for preparation of antineoplastic and/or active pharmaceutical ingredient (API) HDs (see ⟨800⟩).]

INTEGRATED VERTICAL LAMINAR FLOW ZONE (IVLFZ): An IVLFZ is a designated ISO Class 5 area serving as the PEC within an ISO Class 7 or cleaner buffer room. In the IVLFZ, unidirectional airflow is created by placing HEPA filters over the entire surface of the work tables and effective placement of air returns. The unidirectional HEPA-filtered zone must be separated from the ISO

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Class 7 area with a physical barrier to direct the airflow downward over the work area to separate the DCA from potential sources of contamination. Strategic location of air returns in addition to full coverage of HEPA filters above the work surface is required. Both static and dynamic smoke studies verifying a continuous flow of HEPA-filtered air void of turbulence, dead air zones, and refluxing from the HEPA filters to and across the entire work area and to the air returns must be documented (e.g., with video). [NOTE—Dynamic airflow smoke pattern tests have shown that it is difficult to achieve this type of design and also achieve and maintain unidirectional airflow under dynamic operating conditions.] [NOTE—A IVLFZ must not be used for preparation of antineoplastic and/or API HDs (see ⟨800⟩).]

CLASS II BIOLOGICAL SAFETY CABINET (BSC): A Class II BSC is a ventilated cabinet with an open front and inward and downward unidirectional HEPA-filtered airflow and HEPA-filtered exhaust. The BSC is designed to provide worker protection from exposure to airborne drugs and to provide an ISO Class 5 or better environment for preparing CSPs. [NOTE—The exhaust air from the BSC must be externally vented for preparation of antineoplastic and/or API HDs (see ⟨800⟩).]

Placement of LAFS: The LAFS must be located out of traffic patterns and away from room air currents that could disrupt the intended airflow patterns inside the PEC. If used to prepare only Category 1 CSPs, the ISO Class 5 PEC may be located in an unclassified SCA. If used to prepare Category 2 CSPs, the LAFS must be located within a cleanroom suite with an ISO Class 7 or better buffer room with an ISO Class 8 or better ante-room. A dynamic airflow smoke pattern test must be performed in the PEC initially and at least every 6 months to ensure that 1) the LAFS is properly placed into the facility and 2) compounders understand how to utilize the unidirectional airflow to maintain first air in the DCA.

Restricted-access barrier system (RABS): A RABS is an enclosure that provides HEPA-filtered ISO Class 5 unidirectional air. It allows for the ingress and/or egress of materials through defined openings that have been designed and validated to preclude the transfer of environmental air contamination, and that generally are not to be opened during compounding operations.

Types of RABS: Examples of RABS include CAIs and CACIs. In a CAI or CACI, glove ports are used to provide physical separation between the surrounding area and the aseptic manipulations.

COMPOUNDING ASEPTIC ISOLATOR (CAI): A CAI is designed for compounding non-HD CSPs. It is designed to maintain an ISO Class 5 environment throughout the compounding and material transfer processes. Air exchange into the CAI from the surrounding environment must not occur unless the air has first passed through a HEPA filter. [NOTE—A CAI must not be used for preparation of antineoplastic and/or API HDs (see ⟨800⟩).]

COMPOUNDING ASEPTIC CONTAINMENT ISOLATOR (CACI): A CACI is designed to provide worker protection from exposure to undesirable levels of airborne drug throughout the compounding and material transfer processes, and to maintain an ISO Class 5 environment for compounding sterile HD preparations (see ⟨800⟩).

Placement of RABS: If used to prepare only Category 1 CSPs, the ISO Class 5 environment may be achieved by placing the RABS in an unclassified SCA. If used to prepare Category 2 CSPs, the RABS must be located within a cleanroom suite with an ISO Class 7 or better buffer room with an ISO Class 8 or better ante-room. For placement of a CACI used for the preparation of antineoplastic and/or API HDs, see ⟨800⟩.

When a RABS is used, the recovery time after opening the transfer chamber to achieve ISO Class 5 air quality must be documented (e.g., by the manufacturer), and internal procedures must be developed to ensure that adequate recovery time is allowed after opening and closing the RABS, both before and during compounding operations. A dynamic airflow smoke pattern test must be performed in the PEC under dynamic operating conditions initially and at least every 6 months to ensure that 1) the RABS is properly integrated into the facility and 2) compounders understand how to utilize the unidirectional airflow to maintain first air in the DCA.

Pharmaceutical isolator: A pharmaceutical isolator provides isolation from the surrounding area and maintains ISO Class 5 air quality during dynamic operating conditions. [NOTE—A CAI or CACI is not a pharmaceutical isolator.] A pharmaceutical isolator comprises four elements:

1. Controlled workspace
2. Transfer device(s)
3. Access device(s)
4. Integral decontamination system

Placement of pharmaceutical isolators: A pharmaceutical isolator used to prepare only Category 1 CSPs can be placed in an unclassified SCA. If the pharmaceutical isolator is used to prepare Category 2 CSPs, the pharmaceutical isolator must be placed in an ISO Class 8 or better room. [NOTE—An ante-room is not required when using a pharmaceutical isolator.] A dynamic airflow smoke pattern test must be performed in the PEC initially and at least every 6 months to ensure that 1) the pharmaceutical isolator is properly placed into the facility and 2) compounders understand how to utilize the unidirectional airflow to maintain first air in the work zone. For placement of a pharmaceutical isolator used for the preparation of HDs, see ⟨800⟩.

Table 3. Summary of Minimum Requirements for Placement of PEC for Compounding Non-HD CSPs[a]

PEC Type	Device Type	Placement for Compounding Category 1 CSPs	Placement for Compounding Category 2 CSPs
LAFS	LAFW	Unclassified SCA	ISO Class 7 positive pressure buffer room with an ISO Class 8 positive pressure ante-room
	IVLFZ	N/A[b]	ISO Class 7 positive pressure buffer room with an ISO Class 8 positive pressure ante-room
	BSC	Unclassified SCA	ISO Class 7 positive pressure buffer room with an ISO Class 8 positive pressure ante-room

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Table 3. Summary of Minimum Requirements for Placement of PEC for Compounding Non-HD CSPs[a] *(continued)*

PEC Type	Device Type	Placement for Compounding Category 1 CSPs	Placement for Compounding Category 2 CSPs
RABS	CAI or CACI	Unclassified SCA	ISO Class 7 positive pressure buffer room with an ISO Class 8 positive pressure ante-room
Pharmaceutical isolator	Pharmaceutical isolator	Unclassified SCA	ISO Class 8 positive pressure room

[a] For compounding HDs, refer to ⟨800⟩.
[b] An IVLFZ must not be used in an unclassified area.

If a robotic enclosure is used as the PEC, a dynamic airflow smoke pattern test must be performed initially and every 6 months thereafter to ensure 1) that it is properly integrated into the facility, 2) that there is no turbulence or refluxing at any critical site, 3) that room air does not enter the PEC where sterile products and/or preparations may be exposed, and 4) that all processes can be performed without introducing contamination to the DCA(s).

AIR EXCHANGE REQUIREMENTS

For cleanroom suites, adequate HEPA-filtered airflow to the buffer room(s) and ante-room(s) is required to maintain the appropriate ISO classification during compounding activities. Airflow is measured in terms of the number of air changes per hour (ACPH). The ACPH may need to be higher to maintain the required ISO classification and microbial state of control depending on the following factors:

- number of personnel permitted to work in the area
- number of particulates that may be generated from activities and processes in the area
- the equipment located in the room
- the room pressure
- the effects of temperature

See *Table 4* for a summary of ACPH requirements for non-HD sterile compounding areas.

A minimum of 30 total HEPA-filtered ACPH must be supplied to ISO Class 7 rooms:

- The total HEPA-filtered air change rate must be adequate to maintain ISO Class 7 during dynamic operating conditions considering the factors listed above
- At least 15 ACPH of the total air change rate in a room must come from the HVAC through HEPA filters located in the ceiling
- The HEPA-filtered air from the PEC, when added to the HVAC-supplied HEPA-filtered air, increases the total HEPA-filtered ACPH to at least 30 ACPH
- If the PEC is used to meet the minimum total ACPH requirements, the PEC must not be turned off except for maintenance
- Rooms where activity levels are high may require more HEPA-filtered ACPH to maintain ISO Class 7 air quality under dynamic operating conditions
- The ACPH from HVAC, ACPH contributed from the PEC, and the total ACPH must be documented on the certification report

A minimum of 20 total HEPA-filtered ACPH must be supplied to ISO Class 8 rooms:

- The total HEPA-filtered air change rate must be adequate to maintain ISO Class 8 under dynamic operating conditions considering the factors listed above
- At least 15 ACPH of the total air change rate in a room must come from the HVAC through HEPA filters located in the ceiling
- Rooms where activity levels are high may require more HEPA-filtered ACPH to maintain ISO Class 8 air quality under dynamic operating conditions
- The total ACPH must be documented on the certification report

Table 4. Summary of ACPH Requirements for Non-HD Sterile Compounding Areas

Compounding Area	ACPH Requirement
Unclassified SCA	No requirement
ISO Class 7 room(s)	≥30 ACPH
ISO Class 8 room(s)	≥20 ACPH

ESTABLISHING AND MAINTAINING PRESSURE DIFFERENTIALS

Continuous differential positive pressure is required to minimize airflow from an area with lower air-quality classification to an area of higher air-quality classification. In a cleanroom suite, a minimum differential positive pressure of 0.020-inch water column is required between each ISO classified area (e.g., between the buffer room and ante-room). The pressure differential between the ante-room and the unclassified area must not be less than 0.020-inch water column. No pressure differential is required between the SCA and the surrounding area. See ⟨800⟩ for pressure requirements for compounding HD CSPs.

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Where pressure differentials are required, a pressure differential monitoring device must be used to continuously monitor the pressure differentials. The quantitative results from the pressure monitoring device must be reviewed and documented at least daily on the days when compounding is occurring.

FACILITIES PREPARING CSPS FROM NONSTERILE STARTING INGREDIENT(S) OR COMPONENT(S)

Weighing, measuring, or otherwise manipulating components could generate airborne chemical particles (e.g., API, added substances). If preparing a Category 2 CSP from nonsterile component(s), presterilization procedures, such as weighing and mixing, must be completed in no worse than an ISO Class 8 environment (e.g., ante-room, buffer room). Presterilization procedures must be performed in single-use containment glove bags, containment ventilated enclosure (CVE), BSC, or CACI to minimize the risk of airborne contamination. CVE, BSC, or CACI used for presterilization procedures must be certified at least every 6 months.

Presterilization procedures must not adversely affect the required air quality of the SEC as demonstrated during certification under dynamic operating conditions. Personnel must follow the hygiene and garbing requirements as described in *3. Personal Hygiene and Garbing* during presterilization procedures.

4.3 Creating Areas to Achieve Easily Cleanable Conditions

CLEANROOM SUITE

The surfaces of ceilings, walls, floors, doors, door frames, fixtures, shelving, work surfaces, counters, and cabinets in the classified area must be smooth, impervious, free from cracks and crevices, and non-shedding so they can be cleaned and disinfected and to minimize spaces in which microorganisms and other contaminants can accumulate. Surfaces should be resistant to damage by cleaning agents, disinfectants, sporicidal agents, and tools used to clean. Junctures between the ceiling and the walls and between the walls and the floor must be sealed to eliminate cracks and crevices where dirt can accumulate. If ceilings consist of inlaid panels, the panels must be caulked around each panel to seal them to the support frame.

Walls must be constructed of, or may be covered with, durable material (e.g., epoxy painted walls or heavy-gauge polymer) and the integrity of the surface must be maintained. Panels must be joined together and sealed to each other and the support structure. Floors must include coving to the sidewall, or the juncture between the floor and the wall must be caulked. Classified areas should minimize dust-collecting overhangs such as utility pipes and ledges such as windowsills. If overhangs or ledges are present, they must be easily cleanable. The exterior lens surface of ceiling light fixtures must be smooth, mounted flush, and sealed. Any other penetrations through the ceiling or walls must be sealed.

SCA

The SCA and all surfaces (e.g., walls, floors, counters, and equipment) in the SCA must be clean, uncluttered, and dedicated to compounding. Surfaces in the SCA should be smooth, impervious, free from cracks and crevices, and non-shedding so they can be easily cleaned and disinfected and to minimize spaces in which microorganisms and other contaminants can accumulate. Surfaces should be resistant to damage by cleaning agents, disinfectants, sporicidal agents, and tools used to clean. Dust-collecting overhangs such as utility pipes and ledges such as windowsills should be minimized. If overhangs or ledges are present, they must be easily cleanable.

4.4 Water Sources

The facility where CSPs are prepared must be designed so that activities such as hand hygiene and garbing will not adversely affect the ability of the PEC to function as designed. Sinks should enable hands-free use. Surfaces of sink(s) must be cleaned and disinfected at least daily and a sporicidal agent must be applied at least monthly (see *7.1 Cleaning, Disinfecting, and Sporicidal Agents*). If compounding is not performed daily, cleaning and disinfecting of the sink must be completed before initiating compounding.

In facilities with a cleanroom suite, the sink used for hand hygiene may be placed either inside or outside of the ante-room. If the sink is located outside of the ante-room, it must be located in a clean space to minimize the risk of bringing in contaminants into the ante-room. If the sink is located inside the ante-room, it may be placed on either the clean side or the dirty side of the ante-room. [NOTE—The order of hand washing and garbing depends on the placement of the sink (see *3.2 Hand Hygiene*).] The buffer room must not contain plumbed water sources [e.g., sink(s), eyewash(es), shower(s), or floor drain(s)]. The ante-room must not contain floor drain(s). If installed, sprinkler systems should be recessed and covered, and the covers should be easily cleanable.

In a facility with an SCA design, the sink must be accessible but located at least 1 meter away from the PEC. The sink must not be located inside the perimeter of the SCA.

4.5 Placement and Movement of Materials

Only furniture, equipment, and other materials necessary for performing compounding activities are permitted in a classified area or SCA, and they should be low-shedding and easily cleaned and disinfected. Their number, design, location, and manner of installation must not impact environmental air quality and must promote effective cleaning and disinfecting. No shipping carton(s) or other corrugated or uncoated cardboard are allowed in a classified area or SCA.

Carts used to transport components or equipment into classified areas must be constructed from nonporous materials with cleanable casters and wheels to promote mobility and ensure ease of cleaning and disinfection. In a cleanroom suite, carts must

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not be moved from the dirty side to the clean side of the ante-room unless the entire cart, including casters, is cleaned and disinfected.

Only equipment necessary for performing compounding activities is permitted in the PEC. Proper placement of equipment in a PEC must be initially verified by a dynamic airflow smoke pattern test to demonstrate minimal disruption in airflow. The dynamic airflow smoke pattern test must be repeated if equipment is placed in a different location. Equipment and other items used in a classified area or an SCA should not be removed except for calibration, servicing, cleaning, or other activities associated with maintenance. If removed, these items must be cleaned and wiped with sterile 70% IPA or a suitable disinfectant before they are returned to the classified area or inside the perimeter of the SCA.

5. CERTIFICATION AND RECERTIFICATION

Before a compounding area is used to compound either Category 1 or Category 2 CSPs, it must be certified using procedures in the current Controlled Environment Testing Association (CETA) certification guide for *Sterile Compounding Facilities* or an equivalent guideline. Certification indicates that the compounding area is meeting its design and air quality specifications (see *Table 2*). It is important to place special emphasis on certifying the ISO Class 5 areas.

Certification of the classified areas including the PEC must be performed initially, and recertification must be performed at least every 6 months and must include:

- Airflow testing: Airflow testing is performed to determine acceptability of the air velocity and volume, the air exchange rate, and the room pressure differential in doorways between adjacent rooms to ensure consistent airflow and that the appropriate quality of air is maintained under dynamic operating conditions. The ACPH from HVAC, ACPH contributed from the PEC, and the total ACPH must be documented on the certification report.
- HEPA filter integrity testing: HEPA filters must be leak tested at the factory and then leak tested again after installation and as part of recertification.
- Total particle count testing (see *5.1 Total Airborne Particle Sampling*): Total particle count testing must be performed under dynamic operating conditions using calibrated electronic equipment.
- Dynamic airflow smoke pattern test: Smoke pattern tests must be performed for each PEC during dynamic operating conditions to demonstrate unidirectional airflow and sweeping action over and away from the preparation(s).

Classified areas additionally must be recertified if there are changes to the area such as redesign, construction, replacement or relocation of any PEC, or alteration in the configuration of the room that could affect airflow or air quality.

All certification and recertification records must be reviewed by the designated person(s) to ensure that the classified environments meet the minimum requirements in this chapter. The number of personnel present in each PEC and SEC during total particle count tests and dynamic airflow smoke pattern tests must be documented. Records must be maintained in accordance with the requirements in *20. Documentation.*

A corrective action plan must be implemented and documented in response to any out-of-range results. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective.

5.1 Total Airborne Particle Sampling

It is imperative that all engineering control equipment function as designed and that the levels of total airborne particles remain within acceptable limits during compounding (see *Table 2*). A monitoring program for total airborne particles must be developed and implemented to measure the performance of the engineering controls that are being used to provide the specified levels of air cleanliness (e.g., in the ISO Class 5 PEC and ISO Class 7 and 8 rooms).

Total airborne particle count testing must be conducted in all classified areas during dynamic operating conditions at least every 6 months.

Total airborne particle sampling sites must be selected in all classified areas. Measurements of total airborne particles must be taken in each PEC at locations where there is greatest risk to the exposed CSPs, containers, and closures. When conducting sampling of the PEC, care should be taken to avoid disturbing the unidirectional airflow within the PEC. All sampling sites and procedures must be described in the facility's SOP. Measurements of total airborne particles in other classified areas, including the buffer room(s) and ante-room(s), should be taken at representative locations that reflect the quality of air in the room(s).

DATA EVALUATION AND ACTION LEVELS

If levels measured during the total air sampling program exceed the criteria in *Table 2* for the ISO classification of the area sampled, the cause must be investigated and corrective action taken and documented. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective. Some examples of corrective action include process or facility improvements or HEPA filter replacement or repair. The extent of the investigation should be consistent with the deviation and should include an evaluation of trends.

6. MICROBIOLOGICAL AIR AND SURFACE MONITORING

An effective microbiological air and surface monitoring program provides information on the environmental quality of the compounding area. In addition, an effective microbiological air and surface monitoring program identifies environmental quality trends over time, identifies potential routes of contamination, and allows for implementation of corrective actions to minimize the risk of CSP contamination. Sterile compounding facilities must develop and implement written procedures for microbiological air and surface monitoring (see *17. SOPs*). All microbiological air and surface monitoring procedures, the test

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results, and the corrective actions must be documented, and the records must be maintained in accordance with the requirements in *20. Documentation*. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective.

6.1 General Monitoring Requirements

The microbiological air and surface monitoring program must include 1) viable impact volumetric airborne particulate sampling and 2) surface sampling. The goals of a microbiological air and surface monitoring program are to determine whether contamination is present at unacceptable levels and to assess whether proper personnel practices are being followed, cleaning and disinfecting agents are effective, and environmental quality is maintained.

The microbiological air and surface monitoring program involves the collection and evaluation of samples from various air and surface locations to detect airborne and surface contaminants. The data from microbiological airborne and surface sampling are then used to assess risks for contamination, potential routes of contamination, and the adequacy of cleaning and disinfecting agents and procedures. Regular review of the sampling data must be performed to detect trends and the results of the review must be documented.

In addition, results from microbiological air and surface sampling must be reviewed in conjunction with personnel data (i.e., training records, visual observations, competency assessments) to assess the state of control and to identify potential risks of contamination. Corrective action in response to any adverse findings is required to maintain the necessary environmental quality for preparation of CSPs. Data must also be reviewed following corrective actions to confirm that the actions taken have been effective in achieving the required microbiological air and surface quality levels (see *Table 2*, *Table 5*, and *Table 6*).

Microbiological air and surface monitoring must be performed initially for sterile compounding facilities to establish a baseline level of environmental quality. After initial sampling, the environment in which sterile compounding activities are performed must be monitored according to the minimum frequencies described in this section to ensure that the environment remains suitable for sterile compounding. Evaluating results collected over a period of time can be useful in identifying trends or determining that a significant change has occurred, even when the results fall within the specified levels.

Microbiological air and surface monitoring must be conducted in all classified areas during dynamic operating conditions to confirm that the required environmental quality is maintained. In addition to the specific sampling frequencies described in this section, sampling must be performed in the following circumstances:

- In conjunction with the certification of new facilities and equipment
- After any servicing of facilities or equipment (see *4. Facilities and Engineering Controls*)
- In response to identified problems (e.g., positive growth in sterility tests of CSPs)
- In response to identified trends (e.g., repeated positive gloved fingertip and thumb sampling results, failed media fill testing, or repeated observations of air or surface contamination)
- In response to changes that could impact the sterile compounding environment (e.g., change in cleaning agents)

The microbiological air and surface monitoring program must be clearly described in the facility's SOPs, which must include a diagram of the sampling locations, procedures for collecting samples, frequency of sampling, size of samples (e.g., surface area, volume of air), time of day of sampling in relation to activities in the compounding area, and action levels that will trigger corrective action.

The times and locations of sampling should be carefully selected based on their relationship to the activities performed in the area. It is important to obtain samples from locations that pose the highest possible risk of contamination to the CSP and that are likely to be representative of the conditions throughout the area. To obtain air and surface samples that are representative of the typical compounding conditions at the facility, in all PECs and classified rooms, air sampling must be conducted during dynamic operating conditions and surface sampling must be performed at the end of a compounding activity or shift, but before the area has been cleaned and disinfected. The monitoring program must be designed and conducted in a manner that minimizes the chance that the sampling itself will contribute to contamination of the CSP or the environment.

It is important that personnel are trained in the proper operation of the air and surface sampling equipment to ensure accurate and reproducible sampling. All active air sampling devices must be serviced and calibrated as recommended by the manufacturer.

6.2 Monitoring Air Quality for Viable Airborne Particles

A monitoring program for viable airborne particles must be developed and implemented to assess microbiological air quality in all classified areas.

VIABLE AIR SAMPLING—TIMING AND LOCATIONS

Volumetric active air sampling of all classified areas using an impaction device must be conducted in each classified area [e.g., ISO Class 5 PEC and ISO Class 7 and 8 room(s)] during dynamic operating conditions at least every 6 months. Air sampling sites must be selected in all classified areas.

SAMPLING PROCEDURES

When conducting sampling of the PEC, care should be taken to avoid disturbing unidirectional airflow. See *Box 6-1* for active air sampling procedures. A general microbiological growth media that supports the growth of bacteria and fungi must be used (e.g., TSA). COAs from the manufacturer must verify that the media meets the expected growth promotion, pH, and sterilization requirements. Samples must be incubated in an incubator at temperatures that will promote growth of bacteria and fungi. The

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incubator temperature must be monitored during incubation, either manually or by a continuous recording device, and the results must be reviewed and documented as described in the facility's SOPs. The incubator must be placed in a location outside of the sterile compounding area.

Box 6-1. Active Air Sampling Procedures for Viable Airborne Monitoring

- Follow the manufacturer's instructions for operation of the active air sampling device, including placement of media.
- Using the sampling device, test at least 1 cubic meter or 1000 liters of air from each location sampled.
- At the end of the sampling, retrieve the media devices and cover them.
- Invert the media and incubate at 30°–35° for no less than 48 hours. Examine for growth. Record the total number of discrete colonies of microorganisms on each media device as cfu per cubic meter of air on an environmental sampling form based on sample type (i.e., viable air), sample location, and sample date.
- Then incubate the inverted media at 20°–25° for no less than 5 additional days. Examine the media devices for growth. Record the total number of discrete colonies of microorganisms on each media device as cfu per cubic meter of air on an environmental sampling form based on sample type (i.e., viable air), sample location, and sample date.
- Alternatively, to shorten the overall incubation period, two samples may be collected for each sample location and incubated concurrently.
  - Both samples could be TSA or one sample could be TSA and the other fungal media (e.g., malt extract agar (MEA) or sabouraud dextrose agar (SDA)).
  - Incubate each sample in a separate incubator. Incubate one sample at 30°–35° for no less than 48 hours, and incubate the other sample at 20°–25° for no less than 5 days.
    - If fungal media are used as one of the samples, incubate the fungal media sample at 20°–25° for no less than 5 days.
  - Count the total number of discrete colonies of microorganisms on each sample, and record these results as cfu per cubic meter of air.
  - Record the results of the sampling on an environmental sampling form based on sample type (i.e., viable air), and include the sample location, and sample date.

DATA EVALUATION AND ACTION LEVELS

Evaluate cfu counts against the action levels in *Table 5*, and examine counts in relation to previous data to identify adverse results or trends. If two devices of media are collected at a single location, all recovered growth on each must be documented and action levels applied to each media device. If levels measured during the viable air monitoring program exceed the levels in *Table 5* for the ISO classification levels of the area sampled, the cause must be investigated and corrective action must be taken. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective. The corrective action plan must be dependent on the cfu count and the microorganism recovered. Some examples of corrective action include process or facility improvements, personnel training, cleaning and disinfecting, or HEPA filter replacement and/or repair. The extent of the investigation should be consistent with the deviation and should include an evaluation of trends. The corrective action plan must be documented. If levels measured during viable air sampling exceed the levels in *Table 5*, an attempt must be made to identify any microorganisms recovered to the genus level (see *Microbial Characterization, Identification, and Strain Typing* ⟨1113⟩) with the assistance of a microbiologist.

Table 5. Action Levels for Viable Airborne Particle Air Sampling[a]

ISO Class	Air Sampling Action Levels [cfu per cubic meter (1000 liters) of air per plate]
5	>1
7	>10
8	>100

[a] Adapted from *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice.* U.S. Department of Health and Human Services, FDA, September 2004.

6.3 Monitoring Surfaces for Viable Particles

Surface sampling is an important tool used to assist in maintenance of a suitably controlled environment for compounding CSPs. Surface sampling is useful for evaluating facility cleaning and material handling procedures, work surface cleaning and disinfecting procedures, and personnel competency in work practices such as cleaning and disinfecting of component and/or vial surfaces. All sampling sites and procedures must be described in the facility's SOP.

SURFACE SAMPLING: TIMING AND LOCATIONS

Surface sampling of all classified areas and pass-through chambers connecting to classified areas for microbial contamination must be conducted at least monthly (see *Microbiological Control and Monitoring of Aseptic Processing Environments* ⟨1116⟩). Each classified area must be sampled, including the following:

- The interior of the PEC and the equipment contained in it
- Staging or work area(s) near the PEC
- Frequently touched surfaces

When conducted, surface sampling must be performed at the end of compounding activity or shift, but before the area has been cleaned and disinfected.

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SAMPLING PROCEDURES

See *Box 6-2* for the procedures for surface sampling on flat surfaces. Surface sampling devices (e.g., plates, paddles, or slides) containing microbial growth media must be used for sampling flat surfaces. COAs from the manufacturer must verify that the devices meet the expected growth promotion, pH, and sterilization requirements. Surface sampling devices must contain general microbial growth media (e.g., TSA) supplemented with neutralizing additives (e.g., lecithin and polysorbate 80) to neutralize the effects of any residual disinfecting agents. Surface sampling devices must have a raised convex surface. Sterile swabs wetted with sterile water or a sterile neutralizing buffer may be used when sampling irregular surfaces and difficult-to-reach locations, such as crevices, corners, and spaces between surfaces. After sampling, the sampled area must be thoroughly cleaned and disinfected (see *7. Cleaning, Disinfecting, and Applying Sporicidal Agents in Compounding Areas*).

Samples must be incubated in a calibrated incubator at temperatures that will promote growth of bacteria and fungi. The incubator temperature must be monitored during incubation, either manually or by a continuous recording device, and the results must be reviewed and documented. The incubator must be placed in a location outside of the sterile compounding area.

Box 6-2. Surface Sampling Procedures

- Remove the cover from the surface sampling device. Using a rolling motion, firmly press the media surface onto the surface to be sampled. The surface sampling device will leave a residue of growth media on the sample site. After sampling, remove the residue from the surface using sterile 70% IPA.
- Cover each surface sampling device. Store media devices during incubation to prevent condensate from dropping onto the agar and affecting the accuracy of the cfu reading (e.g., invert plates).
- Incubate the surface sampling devices at 30°–35° for no less than 48 hours. Examine for growth. Record the total number of discrete colonies of microorganisms on each device as cfu per sample on an environmental sampling form based on sample type (i.e., surface), sample location, and sample date.
- Incubate the surface sampling device at 20°–25° for no less than 5 additional days. Examine the device for growth. Record the total number of discrete colonies of microorganisms on each media device (cfu per sample) on the environmental sampling record based on sample type (i.e., surface), sample location, and sample date.
- Alternatively, to shorten the overall incubation period, two samples may be collected for each sample location and incubated concurrently.
  - Both samples could be TSA or one sample could be TSA and the other fungal media (e.g., MEA or SDA).
  - Incubate each sample in a separate incubator. Incubate one sample at 30°–35° for no less than 48 hours, and incubate the other sample at 20°–25° for no less than 5 days.
    - If fungal media are used as one of the samples, incubate the fungal media sample at 20°–25° for no less than 5 days.
  - Count the total number of discrete colonies of microorganisms on each sample, and record these results as cfu per sample.
  - Record the results of the sampling on an environmental sampling form based on sample type (i.e., surface), and include the sample location, and sample date.

DATA EVALUATION AND ACTION LEVELS

Evaluate cfu counts against the action levels in *Table 6*, and examine counts in relation to previous data to identify adverse results or trends. If two devices were collected at a single location, all recovered growth on each must be documented and action levels are applied to each device of media. If levels measured during surface sampling exceed the levels in *Table 6* for the ISO classification levels of the area sampled, the cause must be investigated and corrective action must be taken. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective. The corrective action plan must be dependent on the cfu count and the microorganism recovered. Some examples of corrective action include process or facility improvements, personnel training, cleaning and disinfecting, or HEPA filter replacement and/or repair. The extent of the investigation should be consistent with the deviation and should include an evaluation of trends. The corrective action plan must be documented. If levels measured during surface sampling exceed the levels in *Table 6*, an attempt must be made to identify any microorganism recovered to the genus level (see ⟨1113⟩) with the assistance of a microbiologist.

Table 6. Action Levels for Surface Sampling

ISO Class	Surface Sampling Action Levels (cfu/device or swab)
5	>3
7	>5
8	>50

7. CLEANING, DISINFECTING, AND APPLYING SPORICIDAL AGENTS IN COMPOUNDING AREAS

Cleaning, disinfecting, and applying a sporicidal agent are important because surfaces in classified areas and SCA are a potential source of microbial contamination of CSPs. The process of cleaning involves removing organic and inorganic materials from surfaces, usually with a manual or mechanical process and a cleaning agent. The process of disinfecting involves destruction of microorganisms, usually with a chemical agent.

Surfaces must be cleaned prior to being disinfected unless an Environmental Protection Agency (EPA)-registered (or equivalent) one-step disinfectant cleaner is used to accomplish both the cleaning and disinfection in one step. A sporicidal agent must be applied to destroy bacterial and fungal spores. Some EPA-registered (or equivalent) one-step disinfectant cleaners may have sporicidal properties. After cleaning and disinfecting or the application of a one-step disinfectant cleaner, or the application of a sporicidal agent in a PEC, apply sterile 70% IPA to remove any residue. See *Table 7* for a summary of the purposes of the cleaning, disinfectant, and sporicidal agents.

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Table 7. Purpose of Cleaning, Disinfecting, and Sporicidal Agents

Type of Agent	Purpose
Cleaning agent	An agent used for the removal of residues (e.g., dirt, debris, microbes, and residual drugs or chemicals) from surfaces.
Disinfectant	A chemical or physical agent used on inanimate surfaces and objects to destroy fungi, viruses, and bacteria.
Sporicidal agent	A chemical or physical agent that destroys bacterial and fungal spores when used at a sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

Cleaning and disinfecting surfaces and applying a sporicidal agent must occur at the minimum frequencies specified in *Table 8* or, if compounding is not performed daily, cleaning and disinfecting must be completed before initiating compounding.

All cleaning and disinfecting activities must be performed by trained and appropriately garbed personnel using facility-approved agents and procedures, which must be described in written SOPs. Personnel must be trained if there are any changes in the cleaning and disinfecting procedures. Cleaning must be performed in the direction of clean to dirty areas. The frequency, method(s), and location(s) of cleaning, disinfecting, and sporicidal agent use must be established in written SOPs, in accordance with the manufacturer's instructions, and must be followed by all cleaning personnel. The manufacturer's directions or published data for the minimum contact time must be followed for the cleaning, disinfecting, and sporicidal agents used. When sterile 70% IPA is used, it must be allowed to dry. All cleaning, disinfecting, and application of sporicidal agents must be documented according to facility SOPs.

Table 8. Minimum Frequency for Cleaning and Disinfecting Surfaces and Applying Sporicidal Agents in Classified Areas and within the Perimeter of the SCA[a]

Site	Cleaning	Disinfecting	Applying Sporicidal
PEC(s) and equipment inside the PEC(s)	Equipment and all interior surfaces of the PEC daily and when surface contamination is known or suspected.	• Equipment and all interior surfaces of the PEC daily and when surface contamination is known or suspected. • Apply sterile 70% IPA to the horizontal work surface at least every 30 minutes if the compounding process takes 30 minutes or less. If the compounding process takes more than 30 minutes, compounding must not be disrupted and the work surface of the PEC must be disinfected immediately after compounding.	Monthly
Removable work tray of the PEC	• Work surface of the tray daily • All surfaces and the area underneath the work tray monthly	• Work surface of the tray daily • All surfaces and the area underneath the work tray monthly	• Work surface of the tray monthly • All surfaces and the area underneath the work tray monthly
Pass-through(s)	Daily	Daily[b]	Monthly
Work surface(s) outside the PEC	Daily	Daily[b]	Monthly
Floor(s)	Daily	Daily[b]	Monthly
Wall(s), door(s), and door frame(s)	Monthly	Monthly[b]	Monthly
Ceiling(s)[c]	Monthly	Monthly[b]	Monthly
Storage shelving and bins	Monthly	Monthly[b]	Monthly
Equipment outside the PEC(s)	Monthly	Monthly[b]	Monthly

[a] Cleaning of sinks is described in *4.4 Water Sources*.
[b] Many disinfectants registered by the EPA are one-step cleaning and disinfecting agents, which means that the disinfectant has been formulated to be effective in the presence of light to moderate soiling without a separate cleaning step.
[c] Ceilings of the SCA are required to be cleaned, disinfected, and applied with sporicidal agent only when visibly soiled and when surface contamination is known or suspected.

7.1 Cleaning, Disinfecting, and Sporicidal Agents

Cleaning and disinfecting agents must be selected and used with careful consideration of compatibilities, effectiveness, and user safety. Considerations when selecting and using disinfectants include their antimicrobial activity, inactivation by organic matter, residue, shelf life, preparation requirements of the agent, and suitability for surfaces being disinfected. After the disinfectant or sporicidal agent is applied to the surface, the agent must be allowed to dwell for the minimum contact time specified by the manufacturer.

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7.2 Cleaning Supplies

All cleaning supplies (e.g., wipers, sponges, and mop heads) with the exception of tool handles and holders must be low-lint. Wipers, pads, and mop heads should be disposable. If disposable cleaning supplies are used, they must be discarded after each cleaning activity. Reusable cleaning tools must be made of cleanable materials (e.g., no wooden handles) and must be cleaned and disinfected before and after each use. Reusable cleaning tools must be dedicated for use in the classified areas or SCA and must not be removed from these areas except for disposal. They must be discarded as determined based on the condition of the tools. Dispose of cleaning supplies used in the classified areas and SCAs in a manner that minimizes the potential for dispersing contaminants into the air (e.g., with minimal agitation, away from work surfaces).

7.3 Cleaning, Disinfecting, and Applying Sporicidal Agents in the PEC

Clean, disinfect, and apply a sporicidal agent to equipment and all interior surfaces in the PEC at the minimum frequencies specified in *Table 8*. See *Box 7-1* and *Box 7-2* for procedures for cleaning, disinfecting, and applying a sporicidal agent in the PEC.

Box 7-1. Procedures for Cleaning and Disinfecting the PEC

- Remove visible particles, debris, or residue with an appropriate solution (e.g., *Sterile Water for Injection* or *Sterile Water for Irrigation*) using sterile, low-lint wipers.
- Using a low-lint wiper, apply a cleaning agent, followed by a disinfecting agent, or apply an EPA-registered (or equivalent) one-step disinfectant cleaner to equipment and all interior surfaces of the PEC.
- Ensure the contact time specified by the manufacturer is achieved.
- Using a low-lint wiper, apply sterile 70% IPA to equipment and all interior surfaces in the PEC.
- Allow the surface to dry completely before beginning compounding.

Box 7-2. Procedures for Applying a Sporicidal Agent in the PEC

- Remove visible particles, debris, or residue with an appropriate solution (e.g., *Sterile Water for Injection* or *Sterile Water for Irrigation*) using sterile, low-lint wipers.
- After cleaning and disinfecting (*Box 7-1*), apply the sporicidal agent using a low-lint wiper to all surfaces and the area underneath the work tray. If the sporicidal agent is an EPA-registered (or equivalent) one-step disinfectant sporicidal cleaner, separate cleaning and disinfecting steps are not required.
- Ensure the contact time specified by the manufacturer is achieved.
- Using a low-lint wiper, apply sterile 70% IPA to all interior surfaces, including underneath the work tray.
- Allow the surface to dry completely before beginning compounding.

8. INTRODUCING ITEMS INTO THE SEC AND PEC

8.1 Introducing Items into the Cleanroom Suite and SCAs

Before any item is introduced into the clean side of ante-room(s), placed into pass-through(s), or brought inside the perimeter SCA and when packaging integrity will not be compromised, it must be wiped with a sporicidal agent, EPA-registered disinfectant, or sterile 70% IPA using low-lint wipers by personnel wearing gloves. If an EPA-registered disinfectant or sporicidal agent is used, the agent must be allowed to dwell for the minimum contact time specified by the manufacturer. If sterile 70% IPA is used, it must be allowed to dry. The wiping procedure must not render the product label unreadable.

8.2 Introducing Items into the PEC

Just before any item is introduced into the PEC, it must be wiped with sterile 70% IPA using low-lint wipers and allowed to dry before use. When sterile items are received in sealed containers designed to keep them sterile until opening, the sterile items may be removed from the covering as the supplies are introduced into the ISO Class 5 PEC without the need to wipe the individual sterile supply items with sterile 70% IPA. The wiping procedure must not render the product label unreadable.

8.3 Use of Sterile 70% IPA on Critical Sites within the PEC

Critical sites (e.g., vial stoppers, ampule necks, and intravenous bag septums) must be wiped with sterile 70% IPA in the PEC to provide both chemical and mechanical actions to remove contaminants. The sterile 70% IPA must be allowed to dry before entering or puncturing stoppers/septums or breaking the necks of ampules.

9. EQUIPMENT, SUPPLIES, AND COMPONENTS

9.1 Equipment

PECs are described in *4.2 Facility Design and Environmental Controls, Types of PECs and Placement*. Other equipment used in compounding CSPs [e.g., automated compounding devices (ACDs) and balances] should be of suitable composition such that

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the surfaces that contact components are not reactive or sorptive. Equipment that must be brought into classified areas must be wiped with a sporicidal agent, EPA-registered disinfectant, or sterile 70% IPA using low-lint wipers.

Equipment must be placed in a manner that facilitates sterile compounding operations. The equipment must be capable of operating properly and within required performance parameters. Compounding personnel must follow established SOPs for the calibration, maintenance, cleaning, and use of the equipment based on the manufacturer's recommendations. Personnel must maintain records from equipment calibration, verification, and maintenance in accordance with the requirements in *20. Documentation*.

ACDs and other similar equipment are designed to assist in the compounding of preparations by delivering specific volumes of solution(s) automatically under computerized control.

Before using ACDs or other similar equipment, compounding personnel must conduct an accuracy assessment before the first use and again each day the equipment is used to compound CSPs. The precision of the equipment can be monitored based on an assessment of day-to-day variations in its accuracy measures. Compounding personnel must maintain a daily record of the accuracy measurements on the days the equipment is in use. Corrective actions must be implemented if accuracy measurements are outside the manufacturer's specification.

9.2 Supplies

Supplies (e.g., beakers, utensils, needles, syringes, filters, and tubing sets) should be of suitable composition such that the surfaces that contact components are not reactive or sorptive. Supplies in direct contact with the CSP must be sterile and depyrogenated.

9.3 Components

Compounding personnel must follow facility SOPs, which must address the selection, receipt, evaluation, handling, storage, and documentation of all CSP components, including all ingredients, containers, and closures.

COMPONENT SELECTION

Conventionally manufactured sterile products should be used when available and appropriate for the intended CSP.

APIs:

- Must comply with the criteria in the *USP–NF* monograph, if one exists
- Must have a COA that includes the specifications and test results and shows that the API meets the specifications
- Must be obtained from an FDA-registered facility

All components other than APIs:

- Must comply with the criteria in the *USP–NF* monograph, if one exists
- Must be accompanied by documentation (e.g., COA, labeling) that includes the specifications and test results and shows that the component meets the specifications
- Should be obtained from an FDA-registered facility
    - If it cannot be obtained from an FDA-registered facility, the designated person(s) must select an acceptable and reliable source (see *Good Distribution Practices for Bulk Pharmaceutical Excipients* ⟨1197⟩). The compounding facility must establish the identity, strength, purity, and quality of the ingredients obtained from that supplier by reasonable means. Reasonable means may include, but is not limited to, visual inspections, evaluation of a COA supplied by the manufacturer, and/or verification by analytically testing a sample to determine conformance with the COA or other specifications.

All APIs and other components used must be evaluated for suitability for use in sterile drug preparation. Components labeled with "not for pharmaceutical use", "not for injectable use", "not for human use" or an equivalent statement must not be used to compound for these purposes.

Each lot of commercially available sterile, depyrogenated containers and container–closure systems must be accompanied by a COA or other documentation showing conformance with established specifications (i.e., sterility and depyrogenation requirements). If sterilization and depyrogenation of supplies or container–closure systems are performed on site, the efficacy of each process must be established and documented (see *Sterilization of Compendial Articles* ⟨1229⟩).

COMPONENT RECEIPT

Upon receipt of each lot of a component, the external packaging must be examined for evidence of deterioration and other aspects of unacceptable quality. Facility personnel must verify the labeling and condition of the component [e.g., whether the outer packaging is damaged and whether temperature-sensing indicators show that the component has been exposed to excessive temperature(s)].

Any component found to be of unacceptable quality must be promptly rejected, clearly labeled as rejected, and segregated to prevent use before appropriate disposal. Any other lots of that component from that vendor must be examined to determine whether other lots have the same defect.

The date of receipt by the compounding facility must be clearly marked on each API or added substance package that lacks a vendor expiration date. Packages of components (i.e., API and added substances) that lack a vendor's expiration date must be assigned a conservative expiration date, not to exceed 1 year after receipt by the compounding facility.

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COMPONENT EVALUATION BEFORE USE

Compounding personnel must ascertain before use that components for CSPs are of the correct identity, appropriate quality, within expiry date, and have been stored under appropriate conditions. The following information should be used to make this determination: prescription or medication order, compounding record, master formulation record (if used), vendor labels, COAs of API(s) and other component(s), product labeling of conventionally manufactured sterile products, labeling of CSPs, and documentation of the compounding facility storage conditions and practices.

All components must be re-inspected before use. All packages must be re-inspected to detect container breaks, looseness of the cap or closure, and deviation from the expected appearance, aroma, and texture of the contents that might have occurred during storage. Sterile container–closures must be visually re-inspected to ensure that they are free from defects that could compromise sterility and are otherwise suitable for their intended use.

Any component found to be of unacceptable quality must be promptly rejected, clearly labeled as rejected, and segregated to prevent use before appropriate disposal. Any other lots of that component from that vendor must be examined to determine whether other lots have the same defect.

COMPONENT HANDLING AND STORAGE

All components must be handled and stored in a manner that prevents contamination, mix-ups, and deterioration. Components must be stored in closed containers under temperature, humidity, and lighting conditions consistent with those indicated in official monographs or specified by the suppliers and/or manufacturer.

Personnel must monitor temperature in the area(s) where components are stored either manually at least once daily on days that the facility is open or by a continuous temperature recording device to determine whether the temperature remains within the appropriate range. The results of the temperature readings must be documented on a temperature log or stored in the continuous recording device, and must be retrievable. All monitoring equipment must be calibrated or verified for accuracy as recommended by the manufacturer or every 12 months if not specified by the manufacturer.

10. STERILIZATION AND DEPYROGENATION

When selecting the sterilization method for CSPs prepared from one or more nonsterile starting components or using nonsterile supplies or devices, personnel must take into consideration the nature of the component(s), their physical and chemical properties, and the intended container–closure system. The sterilization method used must sterilize the CSP without degrading its physical and chemical stability (e.g., affecting its strength, purity, and quality) or the packaging integrity. See also the ⟨1229⟩ family of chapters.

The following must be considered when selecting an appropriate sterilization method:

- Terminal sterilization (e.g., dry heat, steam, or irradiation) is the preferred method unless the specific CSP or container–closure system cannot tolerate terminal sterilization.
- Steam sterilization is not an option if moisture, pressure, or the temperatures used would degrade the CSP or if there is insufficient moisture to sterilize the CSP within the final, sealed container–closure system.
- Filtration is not an option when compounding a suspension if the suspended drug particles are removed by the filter being used.

CSPs that are terminally sterilized (e.g., dry heat, steam, or irradiation) must use a process intended to achieve a probability of a nonsterile unit (PNSU) of 10^{-6}. [NOTE—This is also called the sterility assurance level (SAL).] A PNSU of 10^{-6} is equivalent to a probability that 1 unit in a million is nonsterile. A PNSU value cannot be applied to CSPs that are aseptically filled into a sterile container following sterilization by filtration because sterilization by filtration is not terminal sterilization.

Injectable compounded preparations that contain nonsterile components or that come into contact with nonsterile devices (e.g., containers, tubing) during any phase of the compounding procedure must be sterilized within 6 hours after completing the preparation to minimize the generation of bacterial endotoxins in CSPs.

A description of the terminal sterilization and depyrogenation process, including the temperature, pressure (if applicable), duration, permissible load conditions for each cycle, and the use of biological indicators and endotoxin challenge vials (ECVs) must be included in the facility's SOPs.

SOPs must include training and competency of personnel on all sterilization methods and equipment used by the facility. In addition, the SOPs must include a schedule and method for establishing and verifying the effectiveness of the terminal sterilization and depyrogenation methods selected, as well as the methods for maintaining and cleaning the sterilizing and depyrogenation equipment.

10.1 Depyrogenation

See *Dry Heat Depyrogenation* ⟨1228.1⟩. Dry heat depyrogenation must be used to render glassware, metal, and other thermostable containers and components pyrogen-free. Depyrogenation processes typically operate at a range of temperatures, from approximately 170° up to about 400°, depending on the exposure time (e.g., a cycle might hold the items at 250° for 30 minutes to achieve sterility and depyrogenation). The duration of the exposure period must include sufficient time for the items to reach the depyrogenation temperature. The items must remain at the depyrogenation temperature for the duration of the depyrogenation period.

The effectiveness of the dry heat depyrogenation cycle must be established initially and verified annually using ECVs to demonstrate that the cycle is capable of achieving a ≥3-log reduction in endotoxins (see *Bacterial Endotoxins Test* ⟨85⟩). The

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effectiveness of the depyrogenation cycle must be re-established if there are changes to the depyrogenation cycle described in SOPs (e.g., changes in load conditions, duration, temperature). This verification must be documented.

Items that are not thermostable must be depyrogenated by rinsing with sterile, non-pyrogenic water (e.g., *Sterile Water for Injection*, *Sterile Water for Irrigation*) and then thoroughly drained or dried immediately before use in compounding.

10.2 Sterilization by Filtration

See *Sterilizing Filtration of Liquids* ⟨1229.4⟩. Sterilizing filters must be sterile, depyrogenated, have a nominal pore size of 0.22 µm or smaller, and include labeling for pharmaceutical use. Sterilizing filters with labeling that states "for laboratory use only" or an equivalent statement must not be used for compounding CSPs. Sterilizing filters must be certified by the manufacturer to retain at least 10^7 microorganisms of a strain of *Brevundimonas diminuta* per square centimeter of upstream filter surface area under conditions similar to those in which the CSPs will be filtered (i.e., pressure, flow rate, and volume filtered).

The designated person(s) must ensure—from available published information, from supplier documentation, or through direct challenge (e.g., filtering the CSP)—that the filters 1) are chemically and physically compatible with all ingredients in the CSP (e.g., water-miscible alcohols may damage filter integrity); 2) are chemically stable at the pressure and temperature conditions that will be used; and 3) have enough capacity to filter the required volumes. The filter dimensions and the CSP to be sterilized by filtration should permit the sterilization process to be completed without the need for replacement of the filter during the process. Filter units used to sterilize CSPs must be subjected to the manufacturers' recommended integrity testing, such as a post-use bubble point test. If multiple filters are required for the compounding process, each of the filters must pass a filter-integrity test.

When CSPs are known to contain excessive particulate matter, a prefiltration step must be performed using a filter of larger nominal pore size (e.g., 1.2 µm) or a separate filter of larger nominal pore size should be placed upstream of (i.e., prior to) the sterilizing filter to remove gross particulate contaminants before the CSP is passed through the sterilizing-grade filter. Excessive particulate matter requiring a prefiltration step could potentially be a signal of an inappropriate formulation, and therefore the formulation and the process should be assessed and, if necessary, modified. CSPs that were prepared using a filter that failed integrity tests must be discarded or, after investigating the cause of the failure and selection of an appropriate filter, refiltered for sterilization no more than one additional time.

10.3 Sterilization by Steam Heat

Temperatures used to achieve sterilization by steam heat are lower than those used to achieve depyrogenation. The process of thermal sterilization using saturated steam under pressure (i.e., autoclaving) is the preferred method for terminal sterilization of aqueous CSPs in their final, sealed container–closure system (see *Steam Sterilization by Direct Contact* ⟨1229.1⟩). Steam sterilization is not an option if moisture, pressure, or the temperatures used would degrade the CSP.

To achieve sterility when steam sterilization is used, all materials must be directly exposed to steam under adequate pressure for the length of time necessary, as determined by use of appropriate biological indicators, to render the items sterile (e.g., between 20 and 60 minutes at 121° saturated steam under a pressure of 15 psi, depending on the volume or size of the CSP being sterilized). The duration of the exposure period must include sufficient time for the entire contents of the CSP and other items to reach the sterilizing temperature. The CSP and other items must remain at the sterilizing temperature for the duration of the sterilization period.

CSPs must be placed in the autoclave to allow steam to reach the CSPs without entrapment of air. Flat, stainless steel trays with low sides or ventilated bottoms will permit steam contact. When preparing items for steam sterilization that must be wrapped, wrap them in low-lint protective fabric or paper or sealed in envelopes that will permit steam penetration and that are designed to prevent post-sterilization microbial contamination. For CSPs, immediately before filling ampules and vials that will be steam sterilized, solutions must be passed through a filter with a nominal pore size of not larger than 1.2 µm for removal of particulate matter.

Sealed containers must be able to generate steam internally. Stoppered and crimped empty vials must contain a small amount of sterile water to generate steam. Deep containers, such as beakers and graduated cylinders, must be inverted or placed on their sides at a downward-sloping angle to minimize air entrapment and to facilitate condensate drainage, or must have a small amount of sterile water placed in them before steam sterilization. Porous materials and those items with occluded pathways (e.g., tubing) must only be sterilized by steam if the autoclave chamber has suitable cycles for dry goods, such as a pre-vacuum process to remove air before steam is sent into the chamber. Elastomeric closures and many other dry goods will need a drying cycle after steam exposure to remove condensed or absorbed moisture.

The effectiveness of steam sterilization must be verified and documented with each sterilization run or load by using appropriate biological indicators, such as spores of *Geobacillus stearothermophilus*, ATCC 12980, ATCC 7953, or equivalent (see *Biological Indicators for Sterilization* ⟨1229.5⟩), and other confirmation methods such as physicochemical indicators and integrators (see *Physicochemical Integrators and Indicators for Sterilization* ⟨1229.9⟩).

The steam supplied must be free of contaminants and generated using water per the manufacturer's recommendation. A calibrated data recorder or chart must be used to monitor each cycle and to examine for cycle irregularities (e.g., deviations in temperature or pressure). The date, run, and load numbers of the steam sterilizer used to sterilize a CSP must be documented in the compounding record.

10.4 Sterilization by Dry Heat

Dry heat may be used for those items that cannot be sterilized by steam or other means, when either the moisture would damage the material or the wrapping material is impermeable (see *Dry Heat Sterilization* ⟨1229.8⟩). Sterilization by dry heat

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requires higher temperatures and longer exposure times than sterilization by steam. The duration of the exposure period must include sufficient time for the entire contents of CSPs and other items to reach the sterilizing temperature. The CSP and other items must remain at the sterilizing temperature for the duration of the sterilization period.

Dry heat sterilization is usually performed in an oven designed for sterilization at a temperature of 160° or higher. If lower temperatures are used, they must be shown to achieve effective sterilization (see *Dry Heat Sterilization* ⟨1229.8⟩, *Validation of Dry Heat Sterilization, Biological Indicators*).

Heated air must be evenly distributed throughout the chamber, which is typically accomplished by an air blower. The calibrated oven must be equipped with temperature controls and a timer. During sterilization, sufficient space must be left between materials to allow for circulation of the hot air. A calibrated data recorder or chart must be used to monitor each cycle and the data must be reviewed to identify cycle irregularities (e.g., deviations in temperature or exposure time).

The effectiveness of the dry heat sterilization method must be verified and documented with each sterilization run or load using appropriate biological indicators such as spores of *Bacillus atrophaeus*, ATCC 9372 (see ⟨1229.5⟩), and other confirmation methods (e.g., temperature-sensing devices). The date, run, and load numbers of the dry heat oven used to sterilize a CSP must be documented in the compounding record.

11. MASTER FORMULATION AND COMPOUNDING RECORDS

11.1 Creating Master Formulation Records

A Master Formulation Record is a detailed record of procedures that describes how the CSP is to be prepared. A Master Formulation Record must be created for CSPs prepared for more than 1 patient and for CSPs prepared from nonsterile ingredient(s). Any changes or alterations to the Master Formulation Record must be approved and documented according to the facility's SOP. *Box 11-1* lists the information that must be included in a Master Formulation Record.

Box 11-1. Master Formulation Records

A Master Formulation Record must include at least the following information:

- Name, strength or activity, and dosage form of the CSP
- Identities and amounts of all ingredients
- Type and size of container–closure system(s)
- Complete instructions for preparing the CSP, including equipment, supplies, a description of the compounding steps, and any special precautions
- Physical description of the final CSP
- BUD and storage requirements
- Reference source to support the stability of the CSP
- Quality control (QC) procedures (e.g., pH testing, filter integrity testing)
- Other information as needed to describe the compounding process and ensure repeatability (e.g., adjusting pH and tonicity, sterilization method (e.g., steam, dry heat, irradiation, or filter)

11.2 Creating Compounding Records

A Compounding Record documents the compounding of each CSP. A Compounding Record must be created for all CSPs. The Compounding Record must be created to document the compounding process or repackaging process. A prescription or medication order or label may serve as the compounding record. If an ACD, workflow management system, or other similar equipment is used, the required information in the compounding record may be stored electronically as long as it is retrievable and contains the required information (see *Box 11-2*). A Master Formulation Record can serve as the basis for preparing the Compounding Record. For example, a copy of the Master Formulation Record can be made that contains spaces for recording the information needed to complete the Compounding Record. *Box 11-2* lists the information that must be included in a Compounding Record.

Box 11-2. Compounding Records

Compounding Records must include at least the following information:

- Name, strength or activity, and dosage form of the CSP
- Date and time of preparation of the CSP
- Assigned internal identification number (e.g., prescription, order, or lot number)
- A method to identify the individuals involved in the compounding process and verifying the final CSP
- Name of each component
- Vendor, lot number, and expiration date for each component for CSPs prepared for more than 1 patient and for CSPs prepared from nonsterile ingredient(s)
- Weight or volume of each component
- Strength or activity of each component
- Total quantity compounded
- Assigned BUD and storage requirements
- Results of QC procedures (e.g., visual inspection, filter integrity testing, pH testing)

If applicable, the Compounding Record must also include:

- Master Formulation Record reference for the CSP
- Calculations made to determine and verify quantities and/or concentrations of components

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12. RELEASE INSPECTIONS AND TESTING

All release testing procedures (e.g., visual inspections and testing) must be included in the facility's documentation (see *11. Master Formulation and Compounding Records* and *17. SOPs*). Any out-of-specification results must be investigated, and a corrective action plan must be implemented and documented as part of the quality assurance (QA) and QC program (see *18. Quality Assurance and Quality Control*).

12.1 Visual Inspection

At the completion of compounding, before release and dispensing, the CSP must be visually inspected to determine whether the physical appearance of the CSP is as expected (e.g., it is inspected for evidence of inappropriate visible particulates or other foreign matter, discoloration, or other defects). The CSP must be visually inspected to confirm that the CSP and its labeling match the prescription or medication order. The inspection also must include a visual inspection of container–closure integrity (e.g., checking for leakage, cracks in the container, or improper seals). CSPs with observed defects must be discarded, or marked and segregated from acceptable units in a manner that prevents them from being released or dispensed.

When a CSP will not be released or dispensed on the day of preparation, a visual inspection must be conducted immediately before it is released or dispensed to make sure that the CSP does not exhibit any defects, such as precipitation, cloudiness, or leakage, which could develop during storage. A CSP with such defects must be immediately discarded, or marked and segregated from acceptable units in a manner that prevents it from being released or dispensed. Any defect may indicate sterility or stability problems, which should be investigated to determine the cause (see *18. Quality Assurance and Quality Control*).

12.2 Sterility Testing

Sterility testing is not required for Category 1 CSPs (see *Table 10*). If a Category 2 CSP is assigned a BUD that requires sterility testing (see *Table 11*), the testing must be performed according to ⟨71⟩ or a validated alternative method (see *Validation of Alternative Microbiological Methods* ⟨1223⟩) that is non-inferior to ⟨71⟩ testing.

If sterility testing is performed, the minimum quantity of each container to be tested for each media is specified in *Sterility Tests* ⟨71⟩, *Table 2*, and the number of containers required to be tested in relation to the batch size is specified in *Sterility Tests* ⟨71⟩, *Table 3*, except as described below.

If the number of CSPs to be compounded in a single batch is less than the number of CSPs needed for testing as specified in *Sterility Tests* ⟨71⟩, *Table 3*, additional units must be compounded to be able to perform sterility testing as follows:

- If between 1 and 39 CSPs are compounded in a single batch, the sterility testing must be performed on a number of units equal to 10% of the number of CSPs prepared, rounded up to the next whole number. For example:
  - If 1 CSP is compounded, 10% of 1 rounded up to the next whole number would indicate that 1 additional CSP must be prepared for sterility testing.
  - If 39 CSPs are compounded, 10% of 39 rounded up to the next whole number would indicate that 4 additional CSPs must be prepared for sterility testing.

If more than 40 CSPs are prepared in a single batch, the sample sizes specified in *Sterility Tests* ⟨71⟩, *Table 3* must be used.

If sterility testing is performed according to ⟨71⟩, a *Sterility Tests* ⟨71⟩, *Method Suitability Test* must be performed to ensure that contamination can be recovered. If performing sterility testing according to ⟨71⟩, the *Sterility Tests* ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration* method is the method of choice when the CSP formulation permits. The preferred alternative is the ⟨71⟩, *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium* method. If an alternative method is used for sterility testing, the method must be validated (see ⟨1223⟩) and demonstrated to be suitable for that CSP formulation.

Sterility tests resulting in failures must prompt an investigation into the possible causes and must include identification of the microorganism, as well as an evaluation of the sterility testing procedure, compounding facility, process, and/or personnel that may have contributed to the failure. The source(s) of the contamination, if identified, must be corrected, and the facility must determine whether the conditions causing the sterility failure affect other CSPs. The investigation and resulting corrective actions must be documented.

12.3 Bacterial Endotoxins Testing

Category 2 injectable CSPs made from one or more nonsterile component(s) and assigned a BUD that requires sterility testing (see *Table 11*) must be tested to ensure that they do not contain excessive bacterial endotoxins (see ⟨85⟩). Category 2 injectable CSPs made from one or more nonsterile component(s) and assigned a BUD that does not require sterility testing should be tested for bacterial endotoxins. In the absence of a bacterial endotoxins limit in an official monograph or other CSP formula source, the CSP must not exceed the endotoxins limit calculated as described in ⟨85⟩ for the appropriate route of administration for humans. CSPs for non-human species must not exceed the endotoxin reference limits calculated as described in ⟨85⟩ based on the weight of the target animal unless a different limit is scientifically supported. CSPs administered epidurally should have the same endotoxin limit as that of intrathecally administered CSPs. See also *Guidelines on the Endotoxins Test* ⟨1085⟩.

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13. LABELING

CSPs must be labeled with legible identifying information to prevent errors during storage, dispensing, and use. The term labeling designates all labels and other written, printed, or graphic matter on the immediate container or on, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term label designates that part of the labeling that is on the immediate container. See *Labeling* ⟨7⟩.

The label on the immediate container of the CSP must, at a minimum, display prominently and legibly the following information:

- Assigned internal identification number (e.g., barcode, prescription, order, or lot number)
- Active ingredient(s) and their amounts, activities, or concentrations
- Storage conditions if other than controlled room temperature
- BUD
- Route of administration
- Total amount or volume if it is not obvious from the container
- If it is a single-dose container, a statement stating such when space permits
- If it is a multiple-dose container, a statement stating such

The labeling on the CSP should indicate that the preparation is compounded.

If the CSP is to be sent outside of the facility in which it was compounded, the labeling must include the contact information of the compounding facility. The labeling of the CSP must also provide any applicable special handling instructions or warning statements.

Labeling procedures must be followed as described in the facility's SOPs to prevent labeling errors and CSP mix-ups. The label of the CSP must be verified to ensure that it conforms with the:

1. Prescription or medication order;
2. Master Formulation Record, if required (see *11.1 Creating Master Formulation Records*); and
3. Compounding Record (see *11.2 Creating Compounding Records*)

All labels must also comply with laws and regulations of the applicable regulatory jurisdiction.

14. ESTABLISHING BEYOND-USE DATES

14.1 Terminology

Each CSP label must state the BUD, which is the date, or the hour and date, beyond which the preparation must not be used and must be discarded. The BUD is determined from the date/time that preparation of the CSP is initiated. The BUD is not intended to limit the time during which the CSP is administered (e.g., infused).

BUDs and expiration dates are not the same. An expiration date identifies the time during which a conventionally manufactured product, API, or added substance can be expected to meet the requirements of a compendial monograph, if one exists, or maintain expected quality provided it is kept under the specified storage conditions. The expiration date limits the time during which the conventionally manufactured product, API, or added substance may be dispensed or used (see *Labeling* ⟨7⟩, *Labels and Labeling for Products in Other Categories, Expiration Date and Beyond-Use Date*). Expiration dates are assigned by manufacturers based on analytical and performance testing of the sterility, chemical and physical stability, and packaging integrity of the product. Expiration dates are specific for a particular formulation in its container and at stated exposure conditions of illumination and temperature. See *Table 9* for a summary of terms.

Table 9. Summary of Terms

Term	Definition	Applicability
BUD	Either the date, or hour and date, after which a CSP must not be used. The BUD is determined from the date/time that preparation of the CSP is initiated.	Applies to all CSPs
Expiration Date	The time during which a product can be expected to meet the requirements of the compendial monograph, if one exists, or maintain expected quality provided it is kept under the specified storage conditions.	Applies to all conventionally manufactured products, APIs, and added substances

14.2 Parameters to Consider in Establishing a BUD

Multiple factors that affect sterility and chemical and physical stability must be considered when establishing BUDs for CSPs. BUDs should be established conservatively for CSPs to ensure that the drug maintains its required characteristics (i.e., stability and sterility) until its BUD.

When establishing a BUD for a CSP, compounders must consider factors that may affect stability, including but not limited to:

- The chemical and physical properties of the drug and/or its formulation

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- The compatibility of the container–closure system with the finished preparation (e.g., leachables, interactions, and storage conditions)

The BUDs for CSPs in *Table 10* and *Table 11* are based primarily on factors that affect the achievement and maintenance of sterility, which include, but are not limited to, the following:

- Environment in which the CSP is prepared (e.g., PEC in a cleanroom suite or SCA)
- Aseptic processing and sterilization method
- Starting components (e.g., sterile or nonsterile starting ingredients)
- Whether or not sterility testing is performed
- Storage conditions (e.g., packaging and temperature)

ASEPTIC PROCESSING AND STERILIZATION METHODS

A CSP may be prepared by the following methods (see *10. Sterilization and Depyrogenation*):

1. **Aseptic processing**, which includes either 1) compounding with only sterile starting ingredient(s), or 2) compounding with nonsterile ingredient(s) followed by sterilization by filtration. [NOTE—Sterilization by filtration is not a form of terminal sterilization.]
2. **Terminal sterilization**, which includes compounding with sterile and/or nonsterile starting ingredient(s) and subsequent sterilization with a process intended to achieve a PNSU of 10^{-6} (e.g., dry heat, steam, irradiation).

Terminal sterilization is the preferred method of sterilization, unless the specific CSP or container–closure system cannot tolerate terminal sterilization. *Table 11* allows for longer BUDs for CSPs that are terminally sterilized than for aseptically processing CSPs because terminal sterilization using a verified method provides reasonable assurance that a CSP will be sterile.

STARTING COMPONENTS

The use of one or more nonsterile starting component(s) is a risk factor to be considered when preparing a CSP. A longer BUD is permitted in *Table 11* for CSPs that are aseptically processed from conventionally manufactured sterile starting component(s) than from one or more nonsterile starting component(s).

STERILITY TESTING

Sterility testing (see *12.2 Sterility Testing*) of a CSP can provide additional assurance of the absence of contamination, although passing a sterility test does not guarantee that all units of a batch of CSPs are sterile because contamination may not be uniformly distributed throughout the batch. A longer BUD is permitted in *Table 11* if sterility testing results are within acceptable limits.

STORAGE CONDITIONS

Storage in colder conditions [i.e., in a refrigerator or freezer (see *Packaging and Storage Requirements* ⟨659⟩)] has been shown to slow the growth of most microorganisms. However, the chemical and physical stability of the CSP and its components must be considered when storing in colder conditions (e.g., some formulations may precipitate when stored in a refrigerator or freezer). A longer BUD is permitted in *Table 10* and *Table 11* for CSPs stored in colder conditions than for CSPs stored at controlled room temperature.

If the CSP will be stored in a frozen state, the container–closure system must be able to withstand the physical stress (i.e., without breaking or cracking) during storage in a freezer. The CSP must be thawed in appropriate conditions to avoid compromising the physical and chemical stability of the preparation and its components (e.g., do not heat in a microwave). Once the CSP is thawed, the CSP must not be re-frozen.

CSPs may be stored under different storage conditions before they are used (e.g., CSPs may first be frozen, and then thawed in the refrigerator, and finally kept at controlled room temperature before administration). The storage time of a CSP must not exceed the original BUD placed on the CSP for its labeled storage condition, and BUDs must not be additive. For example, an aseptically processed CSP prepared from one or more nonsterile starting component(s) cannot be stored for 45 days in a freezer, then 4 days refrigerated, and then 1 day at controlled room temperature for a total of 50 days. Once a CSP has been stored under a condition that would require a shorter BUD (i.e., controlled room temperature), the CSP must be used within the time frame for that storage condition (in this example, 1 day).

14.3 Establishing a BUD for a CSP

BUDs for CSPs must be established in accordance with *Table 10* for Category 1 CSPs and *Table 11* for Category 2 CSPs. One day is equivalent to 24 hours.

The BUDs in *Table 10* and *Table 11* for CSPs are based on the risk of microbial contamination or not achieving sterility despite implementation of the requirements in this chapter. Therefore, it is assumed that the CSP formulation will remain chemically and physically stable, and its packaging will maintain its integrity for the duration of the BUD.

A shorter BUD must be assigned when the stability of the CSP or its components is less than the hours or days stated in *Table 10* or *Table 11*. Additionally, the BUD must not exceed the shortest remaining expiration date or BUD of any of the starting components, regardless of the source.

Table 10 establishes the longest permitted BUDs for Category 1 CSPs. Category 1 CSPs may be prepared in an SCA or cleanroom suite (see *4.2 Facility Design and Environmental Controls*).

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Table 10. BUDs for Category 1 CSPs

	Storage Conditions	
	Controlled Room Temperature (20°–25°)	Refrigerator (2°–8°)
BUD	≤12 hours	≤24 hours

Table 11 establishes the longest permitted BUDs for Category 2 CSPs. Category 2 CSPs must be prepared in a cleanroom suite (see *4.2 Facility Design and Environmental Controls*).

Table 11. BUDs for Category 2 CSPs

Preparation Characteristics		Storage Conditions		
Compounding Method	Sterility Testing Performed and Passed	Controlled Room Temperature (20°–25°)	Refrigerator (2°–8°)	Freezer (–25° to –10°)
Aseptically processed CSPs	No	Prepared from one or more nonsterile starting component(s): 1 day	Prepared from one or more nonsterile starting component(s): 4 days	Prepared from one or more nonsterile starting component(s): 45 days
		Prepared from only sterile starting components: 4 days	Prepared from only sterile starting components: 10 days	Prepared from only sterile starting components: 45 days
	Yes	30 days	45 days	60 days
Terminally sterilized CSPs	No	14 days	28 days	45 days
	Yes	45 days	60 days	90 days

14.4 Multiple-Dose CSPs

A compounded multiple-dose container is designed to contain more than 1 dose, intended to be entered or penetrated multiple times, and usually contains a preservative. A preservative is intended to inhibit the growth of microorganisms and minimize the risk of contamination. The use of preservatives must be appropriate for the CSP formulation and the route of administration. For example, the preservative must not be inactivated by any ingredients in the CSP and some preservatives are not always appropriate for the patient (e.g., neonates) or route of administration (e.g., intrathecal or ophthalmic injections). The use of preservatives, however, must not be considered a substitute for aseptic technique.

A multiple-dose CSP must be prepared as a Category 2 CSP. A multiple-dose CSP must additionally pass antimicrobial effectiveness testing in accordance with *Antimicrobial Effectiveness Testing* ⟨51⟩. The compounder may rely on 1) antimicrobial effectiveness testing that is conducted (or contracted for) once for each formulation in the particular container–closure system in which it will be packaged or 2) antimicrobial effectiveness testing results provided by an FDA-registered facility or published in peer-reviewed literature sources if the CSP formulation (including any preservative) and container–closure system are exactly the same as those tested unless a bracketing study is performed. Antimicrobial effectiveness testing may be performed on a low concentration and a high concentration of the active ingredient in the formulation to establish preservative effectiveness across various strengths of the same formulation (e.g., bracketing). The concentration of all other ingredients (including preservatives) must be the same throughout the bracketing study.

After a multiple-dose container is initially entered or punctured, the multiple-dose container must not be used for longer than the assigned BUD or 28 days if supported by antimicrobial effectiveness testing results (see ⟨51⟩) on the CSP, whichever is shorter.

The container–closure system used to package the multiple-dose CSP must be evaluated for and conform to container–closure integrity (see *Package Integrity Evaluation—Sterile Products* ⟨1207⟩). The container–closure integrity test needs to be conducted only once on each formulation and fill volume in the particular container–closure system in which the multiple-dose CSP will be packaged.

15. USE OF CONVENTIONALLY MANUFACTURED PRODUCTS AS COMPONENTS

This section addresses the time within which an entered or punctured conventionally manufactured product must be used.

15.1 Use of Conventionally Manufactured Single-Dose Containers

A conventionally manufactured single-dose container is a container–closure system that holds a sterile medication for parenteral administration (injection or infusion) that is not required to meet the antimicrobial effectiveness testing requirements. If a single-dose vial is entered or punctured only in an ISO Class 5 or cleaner air, it may be used up to 12 hours after initial entry or puncture as long as the storage requirements during that 12-hour period are maintained. Opened single-dose ampules must not be stored for any time period.

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15.2 Use of Conventionally Manufactured Multiple-Dose Containers

A conventionally manufactured product in a multiple-dose container is intended to contain more than 1 dose of a drug product (see *Packaging and Storage Requirements ⟨659⟩, General Definitions, Injection Packaging Systems*). Once initially entering or puncturing the multiple-dose container, the multiple-dose container must not be used for more than 28 days (see ⟨51⟩) unless otherwise specified by the manufacturer on the labeling.

15.3 Use of Conventionally Manufactured Pharmacy Bulk Packages

A conventionally manufactured pharmacy bulk package is a container of a sterile product for parenteral use that contains many single doses. The contents are intended for use in a pharmacy admixture program and are restricted to the sterile preparation of admixtures for infusion or, through a sterile transfer device, for the filling of empty sterile containers. The pharmacy bulk package must be used according to the manufacturer's labeling (see *Packaging and Storage Requirements ⟨659⟩, General Definitions, Injection Packaging Systems*). The pharmacy bulk package must be entered or punctured only in an ISO Class 5 PEC.

16. USE OF CSPs AS COMPONENTS

This section addresses the use of CSPs (e.g., multiple-dose CSPs, single-dose CSPs, and compounded stock solutions) as components to prepare finished CSPs.

When a CSP is used as a component, care must be taken to minimize the risk of contamination of both the starting component CSP and the finished CSP(s). The BUD of a CSP prepared from one or more compounded components may not exceed the shortest BUD of any of the individual starting components (see *14. Establishing Beyond-Use Dates*).

16.1 Use of Compounded Multiple-Dose CSPs

A multiple-dose CSP is designed to contain more than 1 dose of medication, intended to be entered or punctured multiple times, and usually contains a preservative. Multiple-dose CSPs are required to meet the criteria for antimicrobial effectiveness testing (see ⟨51⟩) and the requirements in *14.4 Multiple-Dose CSPs*. Multiple-dose CSPs must be stored under the conditions upon which its BUD is based (e.g., refrigerator, controlled room temperature). After a multiple-dose CSP is initially entered or punctured, the multiple-dose CSP must not be used for longer than the assigned BUD or 28 days, whichever is shorter.

16.2 Use of Compounded Single-Dose CSPs and CSP Stock Solutions

When a compounded single-dose CSP or CSP stock solution is used as a component to compound additional CSPs, the original compounded single-dose CSP or CSP stock solution must be entered or punctured in ISO Class 5 or cleaner air, and must be stored under the conditions upon which its BUD is based (e.g., refrigerator, controlled room temperature). The component CSP may be used for sterile compounding for up to 12 hours or its assigned BUD, whichever is shorter, and any remainder must be discarded.

17. SOPs

Facilities that prepare CSPs must develop SOPs for the compounding process and other support activities. A designated person must ensure that SOPs are appropriate and are implemented, which includes ensuring that personnel demonstrate competency in performing every procedure that relates to their job function. A designated person must follow up to ensure that corrective actions are taken if problems, deviations, failures, or errors are identified. The corrective action must be documented.

All personnel who perform or oversee compounding or support activities must be trained in the SOPs. All compounding personnel must:

- Be able to recognize potential problems, deviations, failures, or errors associated with preparing a CSP (e.g., those related to equipment, facilities, materials, personnel, the compounding process, or testing) that could potentially result in contamination or other adverse impact on CSP quality
- Report any problems, deviations, failures or errors to the designated person(s)

SOPs must be reviewed at least every 12 months by the designated person(s) to ensure that they reflect current practices, and the review must be documented. Any changes or alterations to an SOP must be made only by a designated person and must be documented. Revisions to SOPs must be communicated to all personnel involved in these processes and procedures, and personnel should document acknowledgment of the communication.

18. QUALITY ASSURANCE AND QUALITY CONTROL

QA is a system of procedures, activities, and oversight that ensures that the compounding process consistently meets quality standards. QC is the sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the CSP. See *Quality Assurance in Pharmaceutical Compounding* ⟨1163⟩.

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A facility's QA and QC programs must be formally established and documented in SOPs that ensure that all aspects of the preparation of CSPs are conducted in accordance with the requirements in this chapter and laws and regulations of the applicable regulatory jurisdiction. A designated person must ensure that the facility has formal, written QA and QC programs that establish a system of:

1. Adherence to procedures
2. Prevention and detection of errors and other quality problems
3. Evaluation of complaints and adverse events
4. Appropriate investigations and corrective actions

The SOPs must describe the roles, duties, and training of the personnel responsible for each aspect of the QA program. The overall QA and QC program must be reviewed at least once every 12 months by the designated person(s). The results of the review must be documented and appropriate action must be taken if needed.

18.1 Notification About and Recall of Out-of-Specification Dispensed CSPs

If a CSP is dispensed or administered before the results of release testing are known, the facility must have procedures in place to:

1. Immediately notify the prescriber of a failure of specifications with the potential to cause patient harm (e.g., sterility, strength, purity, bacterial endotoxin, or other quality attributes), and
2. Determine whether a recall is necessary

An SOP for recall of out-of-specification dispensed CSPs must contain:

- Procedures to determine the severity of the problem and the urgency for implementation and completion of the recall
- Procedures to determine the distribution of any affected CSP, including the date and quantity of distribution
- Procedures to identify patients who have received the CSP
- Procedures for disposition and reconciliation of the recalled CSP

The sterile compounding facility must document the implementation of the recall procedures. The recall must be reported to appropriate regulatory bodies as required by laws and regulations of the applicable regulatory jurisdiction (e.g., state board of pharmacy, state health department).

18.2 Complaint Handling

Compounding facilities must develop and implement SOPs for handling complaints. Complaints may include, but are not limited to, concerns or reports on the quality, labeling, or possible adverse reactions related to a specific CSP.

A designated person must review all complaints to determine whether the complaint indicates a potential quality problem with the CSP. If it does, a thorough investigation into the cause of the problem must be initiated and completed. The investigation must consider whether the quality problem extends to other CSPs. Corrective action, if necessary, must be implemented for all potentially affected CSPs. Consider whether to initiate a recall of potentially affected CSPs and whether to cease sterile compounding processes until all underlying problems have been identified and corrected.

A readily retrievable written or electronic record of each complaint must be kept by the facility, regardless of the source of the complaint (e.g., email, telephone, mail). The record must contain the name of the complainant or unique identifier, the date the complaint was received, the nature of the complaint, and the response to the complaint. In addition, to the extent that the information is known, the following should be recorded: the name and strength of the CSP and the assigned internal identification number (e.g., prescription, order, or lot number).

The record must also include the findings of any investigation and any follow-up. Records of complaints must be easily retrievable for review and evaluation for possible trends and must be retained in accordance with the record-keeping requirements in *20. Documentation*. A CSP that is returned in connection with a complaint must be quarantined until it is destroyed after completion of the investigation and in accordance with laws and regulations of the applicable regulatory jurisdiction.

18.3 Adverse Event Reporting

Adverse events potentially associated with the quality of CSPs must be reported in accordance with facility SOPs and all laws and regulations of the applicable regulatory jurisdiction. In addition, adverse events potentially associated with the quality of the CSP should be reported to the applicable jurisdictional regulatory body (e.g., state boards of pharmacy, state health departments, FDA's MedWatch program for human drugs, or FDA Form 1932a for animal drugs).

19. CSP HANDLING, STORAGE, PACKAGING, SHIPPING, AND TRANSPORT

Processes and techniques for handling, storing, packaging, and transporting CSPs must be outlined in SOPs. Personnel who will be handling, storing, packaging, and transporting CSPs within the facility must be trained in accordance with the relevant SOPs, and the training must be documented.

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19.1 Handling and Storing CSPs

CSPs must be handled in a manner that maintains CSP quality and packaging integrity. To help ensure that CSP quality is maintained during storage at the compounding facility, personnel must monitor conditions in the storage areas. A controlled temperature area (see ⟨659⟩) must be established and monitored to ensure that the temperature remains within the appropriate range for the CSP. The temperature must be monitored each day, either manually or by a continuous recording device. The results of the temperature readings must be documented in a temperature log at least once daily or stored in the continuous temperature recording device, and must be retrievable. Temperature monitoring devices must be verified for accuracy at least every 12 months or as required by the manufacturer.

The compounding facility must detect and minimize temperature excursions that are outside the temperature limits within the controlled temperature areas. When it is known that a CSP has been exposed to temperatures either below or above the storage temperature limits for the CSP, a designated person must determine (e.g., by consulting literature or analytical testing) whether the CSP is expected to retain its integrity or quality. If this cannot be determined, it must be discarded.

19.2 Packaging of CSPs

Packaging materials should protect CSPs from damage, leakage, contamination, degradation, and adsorption while preventing inadvertent exposure to transport personnel. The facility must select appropriate shipping containers and packaging materials based on the product specifications, information from vendors, and the mode of transport.

Alternative modes of transport and/or special packaging (e.g., tamper-evident closures) may be needed to protect the quality of CSPs. If the CSP is sensitive to light, light-resistant packaging materials must be used. In some cases, the CSP must be packaged in a special container (e.g., a cooler) to protect it from temperature fluctuations.

19.3 Shipping and Transporting CSPs

Compounding personnel must select modes of transport that are expected to deliver properly packed CSPs in an undamaged, sterile, and stable condition. Inappropriate transport can adversely affect the quality of CSPs. For example, preparation-specific considerations should be given to physical shaking that might occur during pneumatic tube transport or undue exposure to heat, cold, or light. When shipping or transporting CSPs that require special handling (e.g., CSPs with stability concerns), personnel must include specific handling instructions on the exterior of the container.

20. DOCUMENTATION

All facilities where CSPs are prepared must have and maintain written or electronic documentation to demonstrate compliance with the requirements in this chapter. This documentation must include, but is not limited to, the following:

- Personnel training, competency assessments, and qualification records including corrective actions for any failures
- Certification reports, including corrective actions for any failures
- Environmental air and surface monitoring procedures and results
- Equipment records (e.g., calibration, verification, and maintenance reports)
- Receipt of components
- SOPs, Master Formulation Records (when used), and Compounding Records
- Release inspection and testing records
- Information related to complaints and adverse events
- Results of investigations and corrective actions

Documentation must comply with all laws and regulations of the applicable jurisdiction. Records must be legible and stored in a manner that prevents their deterioration and/or loss. All required compounding records for a particular CSP (e.g., Master Formulation Record, Compounding Record, and release testing results) must be readily retrievable for at least 3 years after preparation or as required by laws and regulations of the applicable regulatory jurisdiction, whichever is longer.

21. COMPOUNDING ALLERGENIC EXTRACTS

Licensed allergenic extracts are mixed and diluted into prescription sets for an individual patient, even though these allergenic extract combinations are not specified in the approved licenses for the licensed biological products [e.g., Biological License Applications (BLA)]. Because patients must be maintained on a maintenance dose of prepared concentrated allergenic extracts for a period of time longer than the BUDs specified for Category 1 and Category 2, longer BUDs are required for prescription sets to achieve effective therapy.

Allergenic extracts prescription sets must follow standards at least as stringent as those in this section:

Personnel Qualifications

1. A designated person with training and expertise in allergen immunotherapy is responsible for ensuring that personnel who will be preparing allergen immunotherapy are trained, evaluated, and supervised.
2. Before beginning to independently prepare allergenic extracts, all compounding personnel must complete training and be able to demonstrate knowledge of principles and skills for sterile compounding.

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3. Annual personnel training and competency must be documented. Personnel must demonstrate proficiency in these procedures by passing written or electronic testing before they can be allowed to compound allergenic extract prescription sets.

4. Before being allowed to independently compound, all compounders must successfully complete gloved fingertip and thumb sampling on both hands (see *Box 2-1* and *Table 1*), no fewer than 3 separate times. Each fingertip and thumb evaluation must occur after performing separate and complete hand hygiene and garbing procedure. After the initial competency evaluation, compounding personnel must successfully complete gloved fingertip and thumb sampling at least every 12 months thereafter.

5. Compounding personnel must have their sterile technique and related practices evaluated every 12 months as demonstrated by successful completion of a media-fill test (see *Box 2-2*).

6. Personnel who fail competency evaluations must successfully pass reevaluations in the deficient area(s) before they can resume compounding of allergenic extract prescription sets. The designated person(s) must identify the cause of failure and determine appropriate retraining requirements.

7. Personnel who have not compounded an allergenic extract prescription set in more than 6 months must be evaluated in all core competencies before resuming compounding duties.

Personnel Hygiene and Garbing

8. Before beginning compounding of allergen immunotherapy prescription sets, personnel must perform hand hygiene (see *Box 3-1*) and garbing procedures according to facility SOPs.

9. The minimum garb requirements include:

- Low-lint garment with sleeves that fit snugly around the wrists and that is enclosed at the neck (e.g., gowns or coveralls)
- Low-lint, disposable covers for head that cover the hair and ears and, if applicable, disposable cover for facial hair
- Face mask
- Sterile powder-free gloves

10. Compounding personnel must rub sterile 70% IPA onto all surfaces of the gloves and allow them to dry thoroughly throughout the compounding process.

Facilities

11. The compounding process must occur in an ISO Class 5 PEC or in a dedicated allergenic extracts compounding area (AECA). The PEC or AECA used to compound prescription sets must be located away from unsealed windows, doors that connect to the outdoors, and traffic flow, all of which may adversely affect the air quality. Neither a PEC nor an AECA may be located where environmental control challenges (e.g., restrooms, warehouses, or food preparation areas) could negatively affect the air quality. The PEC or the work surfaces in the AECA must be located at least 1 meter away from a sink. The impact of activities that will be conducted around or adjacent to the PEC or AECA must be considered carefully when designing such an area.

- If used, the PEC must be certified every 6 months (see *5. Certification and Recertification*).
- If used, a visible perimeter must establish the boundaries of the AECA.
  - Access to the AECA during compounding must be restricted to authorized personnel.
  - During compounding activities, no other activity is permitted in the AECA.
  - The surfaces of walls, floors, fixtures, shelving, counters, and cabinets in the AECA must be cleanable.
  - Carpet is not allowed in the AECA.
  - Surfaces should be resistant to damage by cleaning and sanitizing agents.
  - The surfaces in the AECA upon which the allergenic extract prescription sets are prepared must be smooth, impervious, free from cracks and crevices, and non-shedding to allow for easy cleaning and disinfecting.
  - Dust-collecting overhangs such as utility pipes, ledges, and windowsills should be minimized. If overhangs or ledges are present, they must be easily cleanable.
  - The AECA must be designed and controlled to provide a well-lighted working environment, with temperature and humidity controls for the comfort of compounding personnel wearing the required garb.

Cleaning and Disinfecting

12. In a PEC, all interior surfaces of the PEC must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.

13. In an AECA, all work surfaces in the AECA where direct compounding is occurring must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.

- If present, walls, doors, and door frames within the perimeter of the AECA must be cleaned and disinfected monthly and when surface contamination is known or suspected.
- Ceilings within the perimeter of the AECA must be cleaned and disinfected when visibly soiled and when surface contamination is known or suspected.

14. Vial stoppers on packages of conventionally manufactured sterile ingredients must be wiped with sterile 70% IPA to ensure that the critical sites are wet and allowed to dry before they are used to compound allergenic extracts prescription sets.

Establishing BUDs

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15. The BUD for the prescription set must be no later than the earliest expiration date of any allergenic extract or any diluent that is part of the prescription set, and the BUD must not exceed 1 year from the date the prescription set is mixed or diluted.

Labeling

16. The label of each vial of an allergenic extract prescription set must display the following prominently and understandably:
- Patient name
- Type and fractional dilution of each vial, with a corresponding vial number
- BUD
- Storage conditions

Shipping and Transport

17. If shipping or transporting allergenic extract prescription sets, compounding personnel must select modes of transport that are expected to deliver properly packed prescription sets in an undamaged, sterile, and stable condition. Inappropriate transport can adversely affect the quality of allergenic extract prescription sets.

18. When shipping or transporting allergenic extract prescription sets that require special handling, personnel must include specific handling instructions on the exterior of the container.

Documentation

19. All facilities where allergenic extract prescription sets are prepared must have and maintain written or electronic documentation to include, but not limited to, the following:
- SOPs describing all aspects of the compounding process
- Personnel training records, competency assessments, and qualification records including corrective actions for any failures
- Certification reports of the PEC, if used, including corrective actions for any failures
- Temperature logs for the refrigerator(s)
- Compounding records for individual allergenic extract prescription sets (see *Box 21-1*)
- Information related to complaints and adverse events
- Investigations and corrective actions

Box 21-1. Compounding Records for Individual Allergenic Extract Prescription Sets

Compounding Records must include at least the following information:
- Name, concentration, volume, vendor or manufacturer, lot number, and expiration date for each component
- Date and time of preparation of the allergenic extract
- Assigned internal identification number
- A method to identify the individuals involved in the compounding process and verifying the final CSP
- Total quantity compounded
- Assigned BUD and storage requirements
- Results of QC procedures (e.g., visual inspection, second verification of quantities)

GLOSSARY

Active pharmaceutical ingredient (API): Any substance or mixture of substances intended to be used in the compounding of a preparation, thereby becoming the active ingredient in that preparation and furnishing pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or affecting the structure and function of the body.

Added substances: Ingredients that are necessary to compound a preparation but are not intended or expected to cause a pharmacologic response if administered alone in the amount or concentration contained in a single dose of the compounded preparation. The term is used synonymously with the terms inactive ingredients, excipients, and pharmaceutical ingredients.

Administration: The direct application of a sterile medication to a single patient by injecting, infusing, or otherwise providing a sterile medication in its final form.

Airlock: A space with interlocked doors, constructed to maintain air pressure control when items move between two adjoining areas (generally with different air cleanliness standards). The intent of an airlock is to prevent ingress of particulate matter and microbial contamination from a lesser-controlled area.

Allergenic extract prescription set: Combinations of licensed allergenic extracts which would be mixed and diluted to provide subcutaneous immunotherapy to an individual patient, even though these allergenic extract combinations are not specified in the approved BLAs for the licensed biological products.

Allergenic extracts: Biological substances used for the diagnosis and/or treatment of allergic diseases such as allergic rhinitis, allergic sinusitis, allergic conjunctivitis, bee venom allergy, and food allergy.

Allergenic extracts compounding area (AECA): A designated, unclassified space, area, or room with a visible perimeter that is suitable for preparation of allergenic extract prescription sets.

Ante-room: An ISO Class 8 or cleaner room with fixed walls and doors where personnel hand hygiene, garbing procedures, and other activities that generate high particulate levels may be performed. The ante-room is the transition room between the unclassified area of the facility and the buffer room.

Aseptic processing: A method by which separate, sterile components (e.g., drugs, containers, or closures) are brought together under conditions that maintain their sterility. The components can either be purchased as sterile or, when starting with nonsterile components, can be separately sterilized prior to combining (e.g., by membrane filtration, autoclave).

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Aseptic technique: A set of methods used to keep objects and areas free of microorganisms and thereby minimize infection risk to the patient. It is accomplished through practices that maintain the microbe count at an irreducible minimum.

Biological safety cabinet (BSC), Class II: A ventilated cabinet with an open front and inward and downward unidirectional HEPA-filtered airflow and HEPA-filtered exhaust. A BSC used to prepare a CSP must be capable of providing an ISO Class 5 or better environment for preparation of the CSPs.

Blood components: Any therapeutic constituent of blood separated by physical or mechanical means (e.g., white cells, red cells, platelets, plasma, serum). It is not intended to include plasma-derived products (e.g., albumin, coagulation factors, immunoglobulins) manufactured under an approved BLA or equivalent.

Buffer room: An ISO Class 7 or cleaner room with fixed walls and doors where PEC(s) that generate and maintain an ISO Class 5 environment are physically located. The buffer room may only be accessed through the ante-room.

Category 1 CSP: A CSP that is assigned a BUD of 12 hours or less at controlled room temperature or 24 hours or less refrigerated that is compounded in accordance with all applicable requirements for Category 1 CSPs in this chapter.

Category 2 CSP: A CSP that is assigned a BUD of greater than 12 hours at controlled room temperature or greater than 24 hours refrigerated that is compounded in accordance with all applicable requirements for Category 2 CSPs in this chapter.

Certificate of analysis (COA): A report from the supplier of a component, container, or closure that accompanies the supplier's material and contains the specifications and results of all analyses and a description of the material.

Classified area: An area that maintains an air quality classification based on the ISO standards (see also the definition for *ISO class*).

Cleaning agent: An agent for the removal of residues (e.g., dirt, debris, microbes, and residual drugs or chemicals) from surfaces.

Cleanroom suite: A classified area that consists of both an ante-room and buffer room.

Component: Any ingredient used in the compounding of a preparation, including any active ingredient, added substance, or conventionally manufactured product.

Compounded sterile preparation (CSP): A preparation intended to be sterile that is created by combining, admixing, diluting, pooling, reconstituting, repackaging, or otherwise altering a drug product or bulk drug substance.

Compounded stock solution: A sterile mixture of components that is used to compound additional CSPs.

Compounding: The process of combining, admixing, diluting, pooling, reconstituting, repackaging, or otherwise altering a drug or bulk drug substance to create a sterile medication.

Compounding area: The area where compounding is occurring (i.e., a cleanroom suite, inside the perimeter of the SCA, or AECA).

Compounding aseptic containment isolator (CACI): A type of RABS that uses HEPA filtration to provide an ISO Class 5 unidirectional air environment designed for the compounding of sterile HDs.

Compounding aseptic isolator (CAI): A type of RABS that uses HEPA filtration to provide an ISO Class 5 unidirectional air environment designed for compounding of sterile non-HDs.

Container–closure system: Packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection.

Containment ventilated enclosure: A full or partial enclosure that uses ventilation principles to capture, contain, and remove airborne contaminants through HEPA filtration and prevent their release into the work environment.

Conventionally manufactured product: A pharmaceutical dosage form, usually the subject of an FDA-approved application, and manufactured under current good manufacturing practice conditions.

Critical site: A location that includes any component or fluid pathway surfaces (e.g., vial septa, injection ports, and beakers) or openings (e.g., opened ampules and needle hubs) that are exposed and at risk of direct contact with air (e.g., ambient room or HEPA filtered), moisture (e.g., oral and mucosal secretions), or touch contamination.

Designated person(s): One or more individuals assigned to be responsible and accountable for the performance and operation of the compounding facility and personnel in the preparation of CSPs.

Direct compounding area (DCA): A critical area within the ISO Class 5 PEC where critical sites are exposed to unidirectional HEPA-filtered air, also known as first air.

Disinfectant: A chemical or physical agent used on inanimate surfaces and objects to destroy fungi, viruses, and bacteria. Sporicidal disinfectant agents are considered a special class of disinfectants that also are effective against bacterial and fungal spores.

Dynamic airflow smoke pattern test: A PEC test in which a visible source of smoke, which is neutrally buoyant, is used to observe air patterns within the unidirectional space (i.e., the DCA) under dynamic operating conditions (see *Dynamic operating conditions*). This test is not appropriate for ISO Class 7 or ISO Class 8 cleanrooms that do not have unidirectional airflow (see *Visual smoke study*).

Dynamic operating conditions: Conditions in the compounding area in which operating personnel are present and simulating or performing compounding. The conditions should reflect the largest number of personnel and highest complexity of compounding expected during routine operations as determined by the designated person(s).

Excipients: See *Added substances*.

Filter integrity test: A test (e.g., bubble point test) of the integrity of a sterilizing grade filter performed after the filtration process to detect whether the integrity of the filter has been compromised.

First air: The air exiting the HEPA filter in a unidirectional air stream.

Formulation: The specific qualitative and quantitative composition of the final CSP.

Garb: Items such as gloves, garments (e.g., gowns, coveralls), shoe covers, head and facial hair covers, masks, and other items designed to reduce particle-shedding from personnel and minimize the risk of contamination of CSP(s).

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Hazardous drug (HD): Any drug identified by at least one of the following six criteria: carcinogenicity, teratogenicity or developmental toxicity, reproductive toxicity in humans, organ toxicity at low dose in humans or animals, genotoxicity, or new drugs that mimic existing HDs in structure or toxicity.

High-efficiency particulate air (HEPA) filtration: Being, using, or containing a filter designed to remove 99.97% of airborne particles measuring 0.3-micron or greater in diameter passing through it.

Integrated vertical laminar flow zone (IVLFZ): A designated ISO Class 5 area serving as the PEC within an ISO Class 7 or cleaner buffer room. In the IVLFZ, unidirectional airflow is created by placing HEPA filters over the entire surface of the work tables and effective placement of air returns.

ISO class: An air-quality classification from the International Organization for Standardization.

Laminar airflow system (LAFS): A device or zone within a buffer area that provides an ISO Class 5 or better air quality environment for sterile compounding. The system provides a unidirectional HEPA-filtered airflow.

Laminar airflow workbench (LAFW): A device that is a type of LAFS that provides an ISO Class 5 or better air quality environment for sterile compounding. The device provides a unidirectional HEPA-filtered airflow.

Line of demarcation: A visible line on the floor that separates the clean and dirty sides of the ante-room.

Low-lint wiper: A wiper exhibiting few, if any, fibers or other contamination, visible without magnification, which is separate from, or easily removed from, the wiper material in a dry condition.

Media-fill test: A simulation used to qualify processes and personnel engaged in sterile compounding to ensure that the processes and personnel are able to prepare CSPs without contamination.

Multiple-dose container: A container of sterile medication for parenteral administration (e.g., injection or infusion) that is designed to contain more than 1 dose of the medication. A multiple-dose container is usually required to meet the antimicrobial effectiveness testing criteria. See *Packaging and Storage Requirements ⟨659⟩, Injection Packaging Systems, Multiple-dose container.*

One-step disinfectant cleaner: A product with an EPA-registered (or equivalent) claim that it can clean and disinfect a non-porous surface in the presence of light to moderate organic soiling without a separate cleaning step.

Pass-through: An enclosure with sealed doors on both sides that should be interlocked. The pass-through is positioned between two spaces for the purpose of minimizing particulate transfer while moving materials from one space to another.

Perimeter: A visible demarcation that defines the boundaries of the SCA or AECA (e.g. a visible line or wall).

Pharmacy bulk package: A conventionally manufactured sterile product for parenteral use that contains many single doses intended for use in a pharmacy admixture program. A pharmacy bulk package may either be used to prepare admixtures for infusion or, through a sterile transfer device, for filling sterile containers. See *Packaging and Storage Requirements ⟨659⟩, Injection Packaging Systems, Pharmacy bulk package.*

Pharmaceutical isolator: An enclosure that provides HEPA-filtered ISO Class 5 unidirectional air operated at a continuously higher pressure than its surrounding environment and is decontaminated using an automated system. It uses only decontaminated interfaces or rapid transfer ports for materials transfer. [NOTE—A CAI or CACI is not a pharmaceutical isolator.]

Positive-pressure room: A room that is maintained at higher pressure than the adjacent spaces, and therefore the net airflow is out of the room.

Preservative: A substance added to inhibit microbial growth.

Primary engineering control (PEC): A device or zone that provides an ISO Class 5 air quality environment for sterile compounding.

Probability of a nonsterile unit (PNSU): The probability of an item being nonsterile after it has been exposed to a verified sterilization process. A PNSU value can only be applied to terminal sterilization. [NOTE—This is also called the sterility assurance level (SAL).]

Pyrogen: A substance that induces a febrile reaction in a patient.

Quality assurance (QA): A system of procedures, activities, and oversight that ensures that the compounding process consistently meets quality standards.

Quality control (QC): The sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the CSP.

Reconstitution: The process of adding a diluent to a conventionally manufactured product to prepare a sterile solution or suspension.

Release inspection and testing: Visual inspection and testing performed to ensure that a preparation meets appropriate quality characteristics.

Repackaging: The act of removing a sterile product or preparation from its original primary container and placing it into another primary container, usually of smaller size without further manipulation.

Restricted-access barrier system (RABS): An enclosure that provides HEPA-filtered ISO Class 5 unidirectional air that allows for the ingress and/or egress of materials through defined openings that have been designed and validated to preclude the transfer of contamination, and that generally are not to be opened during operations. Examples of RABS include CAIs and CACIs.

Secondary engineering control (SEC): The area where the PEC is placed (e.g., a cleanroom suite or an SCA). It incorporates specific design and operational parameters required to minimize the risk of contamination within the compounding area.

Segregated compounding area (SCA): A designated, unclassified space, area, or room with a defined perimeter that contains a PEC and is suitable for preparation of Category 1 CSPs only.

Single-dose containers: A container of sterile medication for parenteral administration (e.g., injection or infusion) that is designed for use with a single patient as a single injection/infusion. A single-dose container usually does not contain a preservative. See *Packaging and Storage Requirements ⟨659⟩, Injection Packaging Systems, Single-dose container.*

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Specification: The tests, analytical methods, and acceptance criteria to which an API or other components, CSP, container–closure system, equipment, or other material used in compounding CSPs must conform to be considered acceptable for its intended use.

Sporicidal agent: A chemical or physical agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

Stability: The extent to which a product or preparation retains physical and chemical properties and characteristics within specified limits throughout its expiration or BUD.

Sterility: The absence of viable microorganisms.

Sterility assurance level (SAL): See *Probability of a nonsterile unit (PNSU)*.

Sterilization by filtration: Passage of a gas or liquid through a sterilizing-grade membrane to yield filtrates that are sterile.

Sterilizing-grade membranes: Filter membranes that are documented to retain 100% of a culture of 10^7 microorganisms of a strain of *Brevundimonas diminuta* per square centimeters of membrane surface under a pressure of not less than 30 psi. Such filter membranes are nominally 0.22-µm or 0.2-µm pore size.

Terminal sterilization: The application of a lethal process (e.g., dry heat, steam, irradiation) to sealed containers for the purpose of achieving a predetermined PNSU of greater than 10^{-6} or a probability of less than one in one million of a nonsterile unit.

Unclassified space: A space not required to meet any air cleanliness classification based on the ISO.

Unidirectional airflow: Air within a PEC moving in a single direction in a uniform manner and at sufficient velocity to sweep particles away from the DCA.

Workflow management system: Technology comprised of hardware and software that allows for automation to assist in the verification of components of, and preparation of, CSPs and to document components and processes.

Verify: To confirm that a method, process, system, or equipment will perform as expected under the conditions of actual use.

Visual smoke study: A test, used in ISO Class 7 and ISO Class 8 rooms that do not have unidirectional airflow, in which a visible source of smoke, which is neutrally buoyant, is used to verify an absence of stagnant airflow where particulates can accumulate. This test does not need to be performed under dynamic operating conditions and is not appropriate for PECs (see *Dynamic airflow smoke pattern test*).

APPENDIX

Acronyms

ACD	Automated compounding device
ACPH	Air changes per hour
AECA	Allergenic extracts compounding area
APIs	Active pharmaceutical ingredient(s)
BLA	Biological License Application
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC(s)	Biological safety cabinet(s)
BUD(s)	Beyond-use date(s)
CACI	Compounding aseptic containment isolator
CAI	Compounding aseptic isolator
CDC	Centers for Disease Control and Prevention
CETA	Controlled Environment Testing Association
cfu	Colony-forming units
COA(s)	Certificate(s) of analysis
CSP(s)	Compounded sterile preparation(s)
CVE	Containment ventilated enclosure
DCA(s)	Direct compounding area(s)
ECV(s)	Endotoxin challenge vial(s)
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
HD(s)	Hazardous drug(s)
HEPA	High-efficiency particulate air
HVAC	Heating, ventilation, and air conditioning
IPA	Isopropyl alcohol

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Acronyms *(continued)*

ISO	International Organization for Standardization
IVLFZ	Integrated vertical laminar flow zone
LAFS	Laminar airflow system
LAFW(s)	Laminar airflow workbench(es)
MEA	Malt extract agar
PEC(s)	Primary engineering control(s)
PNSU	Probability of a nonsterile unit
PPE	Personal protective equipment
QA	Quality assurance
QC	Quality control
RABS	Restricted-access barrier system
SAL	Sterility assurance level
SCA	Segregated compounding area
SDA	Sabouraud dextrose agar
SEC(s)	Secondary engineering control(s)
SOP(s)	Standard operating procedure(s)
TSA	Trypticase soy agar ▲(USP 1-Dec-2019)

Add the following:

▲⟨800⟩ HAZARDOUS DRUGS—HANDLING IN HEALTHCARE SETTINGS

Change to read:

▲▲(RB 1-Dec-2019)

1. INTRODUCTION AND SCOPE

This chapter describes practice and quality standards for handling hazardous drugs (HDs) to promote patient safety, worker safety, and environmental protection. Handling HDs includes, but is not limited to, the receipt, storage, compounding, dispensing, administration, and disposal of sterile and nonsterile products and preparations.

This chapter applies to all healthcare personnel who handle HD preparations and all entities that store, prepare, transport, or administer HDs (e.g., pharmacies, hospitals and other healthcare institutions, patient treatment clinics, physicians' practice facilities, or veterinarians' offices). Personnel who may potentially be exposed to HDs include, but are not limited to: pharmacists, pharmacy technicians, nurses, physicians, physician assistants, home healthcare workers, veterinarians, and veterinary technicians.

Entities that handle HDs must incorporate the standards in this chapter into their occupational safety plan. The entity's health and safety management system must, at a minimum, include:

- A list of HDs
- Facility and engineering controls
- Competent personnel
- Safe work practices
- Proper use of appropriate Personal Protective Equipment (PPE)
- Policies for HD waste segregation and disposal

The chapter is organized into the following main sections:

1. Introduction and Scope
2. List of Hazardous Drugs
3. Types of Exposure
4. Responsibilities of Personnel Handling Hazardous Drugs
5. Facilities and Engineering Controls
6. Environmental Quality and Control
7. Personal Protective Equipment
8. Hazard Communication Program

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9. Personnel Training
10. Receiving
11. Labeling, Packaging, Transport, and Disposal
12. Dispensing Final Dosage Forms
13. Compounding
14. Administering
15. Deactivating, Decontaminating, Cleaning, and Disinfecting
16. Spill Control
17. Documentation and Standard Operating Procedures
18. Medical Surveillance
Glossary
Appendices
Appendix 1: Acronyms
Appendix 2: Examples of Designs for Hazardous Drug Compounding Areas
Appendix 3: Types of Biological Safety Cabinets
References

2. LIST OF HAZARDOUS DRUGS

The National Institute for Occupational Safety and Health (NIOSH) maintains a list of antineoplastic and other HDs used in healthcare. An entity must maintain a list of HDs, which must include any items on the current NIOSH list that the entity handles. The entity's list must be reviewed at least every 12 months. Whenever a new agent or dosage form is used, it should be reviewed against the entity's list.

The NIOSH list of antineoplastic and other HDs provides the criteria used to identify HDs. These criteria must be used to identify HDs that enter the market after the most recent version of the NIOSH list, or that the entity handles as an investigational drug. If the information available on a drug is deemed insufficient to make an informed decision, consider the drug hazardous until more information is available.

Box 1: Containment Requirements

- Drugs on the NIOSH list that must follow the requirements in this chapter include:
  - Any HD API
  - Any antineoplastic requiring HD manipulation
- Drugs on the NIOSH list that do not have to follow all the containment requirements of this chapter if an assessment of risk is performed and implemented include:
  - Final dosage forms of compounded HD preparations and conventionally manufactured HD products, including antineoplastic dosage forms that do not require any further manipulation other than counting or repackaging (unless required by the manufacturer)
- For dosage forms of other HDs on the NIOSH list, the entity may perform an assessment of risk to determine alternative containment strategies and/work practices

Some dosage forms of drugs defined as hazardous may not pose a significant risk of direct occupational exposure because of their dosage formulation (e.g., tablets or capsules—solid, intact medications that are administered to patients without modifying the formulation). However, dust from tablets and capsules may present a risk of exposure by skin contact and/or inhalation. An assessment of risk may be performed for these dosage forms to determine alternative containment strategies and/or work practices. If an assessment of risk is not performed, all HDs must be handled with all containment strategies defined in this chapter.

The assessment of risk must, at a minimum, consider the following:

- Type of HD (e.g., antineoplastic, non-antineoplastic, reproductive risk only)
- Dosage form
- Risk of exposure
- Packaging
- Manipulation

If an assessment of risk approach is taken, the entity must document what alternative containment strategies and/or work practices are being employed for specific dosage forms to minimize occupational exposure. If used, the assessment of risk must be reviewed at least every 12 months and the review documented.

3. TYPES OF EXPOSURE

Routes of unintentional entry of HDs into the body include dermal and mucosal absorption, inhalation, injection, and ingestion (e.g., contaminated foodstuffs, spills, or mouth contact with contaminated hands). Containers of HDs have been shown to be contaminated upon receipt. Both clinical and nonclinical personnel may be exposed to HDs when they handle HDs or touch contaminated surfaces. *Table 1* lists examples of potential routes of exposure based on activity.

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Table 1. Examples of Potential Opportunities of Exposure Based on Activity

Activity	Potential Opportunity of Exposure
Receipt	• Contacting HD residues present on drug containers, individual dosage units, outer containers, work surfaces, or floors
Dispensing	• Counting or repackaging tablets and capsules
Compounding and other manipulations	• Crushing or splitting tablets or opening capsules • Pouring oral or topical liquids from one container to another • Weighing or mixing components • Constituting or reconstituting powdered or lyophilized HDs • Withdrawing or diluting injectable HDs from parenteral containers • Expelling air or HDs from syringes • Contacting HD residue present on PPE or other garments • Deactivating, decontaminating, cleaning, and disinfecting areas contaminated with or suspected to be contaminated with HDs • Maintenance activities for potentially contaminated equipment and devices
Administration	• Generating aerosols during administration of HDs by various routes (e.g., injection, irrigation, oral, inhalation, or topical application) • Performing certain specialized procedures (e.g., intraoperative intraperitoneal injection or bladder instillation) • Priming an IV administration set
Patient-care activities	• Handling body fluids (e.g., urine, feces, sweat, or vomit) or body-fluid-contaminated clothing, dressings, linens, and other materials
Spills	• Spill generation, management, and disposal
Transport	• Moving HDs within a healthcare setting
Waste	• Collection and disposal of hazardous waste and trace contaminated waste

4. RESPONSIBILITIES OF PERSONNEL HANDLING HAZARDOUS DRUGS

Each entity must have a designated person who is qualified and trained to be responsible for developing and implementing appropriate procedures; overseeing entity compliance with this chapter and other applicable laws, regulations, and standards; ensuring competency of personnel; and ensuring environmental control of the storage and compounding areas. The designated person must thoroughly understand the rationale for risk-prevention policies, risks to themselves and others, risks of non-compliance that may compromise safety, and the responsibility to report potentially hazardous situations to the management team. The designated person must also be responsible for the oversight of monitoring the facility and maintaining reports of testing/sampling performed in facilities, and acting on the results.

All personnel who handle HDs are responsible for understanding the fundamental practices and precautions and for continually evaluating these procedures and the quality of final HDs to prevent harm to patients, minimize exposure to personnel, and minimize contamination of the work and patient-care environment.

Change to read:

5. FACILITIES AND ENGINEERING CONTROLS

HDs must be handled under conditions that promote patient safety, worker safety, and environmental protection. Signs designating the hazard must be prominently displayed before the entrance to the HD handling areas. Access to areas where HDs are handled must be restricted to authorized personnel to protect persons not involved in HD handling. HD handling areas must be located away from breakrooms and refreshment areas for personnel, patients, or visitors to reduce risk of exposure.

Designated areas must be available for:

- Receipt and unpacking
- Storage of HDs
- Nonsterile HD compounding (if performed by the entity)
- Sterile HD compounding (if performed by the entity)

Certain areas are required to have negative pressure from surrounding areas to contain HDs and minimize risk of exposure. Consideration should be given to uninterrupted power sources (UPS) for the ventilation systems to maintain negative pressure in the event of power loss.

5.1 Receipt

Antineoplastic HDs and all HD APIs must be unpacked (i.e., removal from external shipping containers) in an area that is neutral/normal or negative pressure relative to the surrounding areas. HDs must not be unpacked from their external shipping containers in sterile compounding areas or in positive pressure areas.

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5.2 Storage

HDs must be stored in a manner that prevents spillage or breakage if the container falls. Do not store HDs on the floor. In areas prone to specific types of natural disasters (e.g., earthquakes) the manner of storage must meet applicable safety precautions, such as secure shelves with raised front lips.

Antineoplastic HDs requiring manipulation other than counting or repackaging of final dosage forms and any HD API must be stored separately from non-HDs in a manner that prevents contamination and personnel exposure. These HDs must be stored in an externally ventilated, negative-pressure room with at least 12 air changes per hour (ACPH). Non-antineoplastic, reproductive risk only, and final dosage forms of antineoplastic HDs may be stored with other inventory if permitted by entity policy.

Sterile and nonsterile HDs may be stored together, but HDs used for nonsterile compounding should not be stored in areas designated for sterile compounding to minimize traffic into the sterile compounding area.

Refrigerated antineoplastic HDs must be stored in a dedicated refrigerator in a negative pressure area with at least 12 ACPH [e.g., storage room, buffer room, or containment segregated compounding area (C-SCA)]. If a refrigerator is placed in a negative pressure buffer room, an exhaust located adjacent to the refrigerator's compressor and behind the refrigerator should be considered.

5.3 Compounding

Engineering controls are required to protect the preparation from cross-contamination and microbial contamination (if preparation is intended to be sterile) during all phases of the compounding process. Engineering controls for containment are divided into three categories representing primary, secondary, and supplementary levels of control. A containment primary engineering control (C-PEC) is a ventilated device designed to minimize worker and environmental HD exposure when directly handling HDs. The containment secondary engineering control (C-SEC) is the room in which the C-PEC is placed. Supplemental engineering controls [e.g., closed-system drug-transfer device (CSTD)] are adjunct controls to offer additional levels of protection. *Appendix 2* provides examples for designs of HD compounding areas.

Sterile and nonsterile HDs must be compounded within a C-PEC located in a C-SEC. The C-SEC used for sterile and nonsterile compounding must:

- Be externally vented
- Be physically separated (i.e., a different room from other preparation areas)
- Have an appropriate air exchange (e.g., ACPH)
- Have a negative pressure between 0.01 and 0.03 inches of water column relative to all adjacent areas

The C-PEC must operate continuously if it supplies some or all of the negative pressure in the C-SEC or if it is used for sterile compounding. If there is any loss of power to the C-PEC, or if repair or moving occurs, all activities occurring in the C-PEC must be suspended immediately. If necessary, protect the unit by covering it appropriately per the manufacturer's recommendations. Once the C-PEC can be powered on, decontaminate, clean, and disinfect (if used for sterile compounding) all surfaces and wait the manufacturer-specified recovery time before resuming compounding.

A sink must be available for hand washing. An eyewash station and/or other emergency or safety precautions that meet applicable laws and regulations must be readily available. Care must be taken to locate water sources and drains in areas where their presence will not interfere with required ISO classifications. Water sources and drains must be located at least 1 meter away from the C-PEC.

For entities that compound both nonsterile and sterile HDs, the respective C-PECs must be placed in separate rooms, unless those C-PECs used for nonsterile compounding are sufficiently effective that the room can continuously maintain ISO 7 classification throughout the nonsterile compounding activity. If the C-PECs used for sterile and nonsterile compounding are placed in the same room, they must be placed at least 1 meter apart and particle-generating activity must not be performed when sterile compounding is in process.

5.3.1 NONSTERILE COMPOUNDING

In addition to this chapter, nonsterile compounding must follow standards in *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩. A C-PEC is not required if manipulations are limited to handling of final dosage forms (e.g., counting or repackaging of tablets and capsules) that do not produce particles, aerosols, or gasses.

The C-PECs used for manipulation of nonsterile HDs must be either externally vented (preferred) or have redundant–HEPA filters in series. Nonsterile HD compounding must be performed in a C-PEC that provides personnel and environmental protection, such as a Class I Biological Safety Cabinet (BSC) or Containment Ventilated Enclosure (CVE). A Class II BSC or a compounding aseptic containment isolator (CACI) may also be used. For occasional nonsterile HD compounding, a C-PEC used for sterile compounding (e.g., Class II BSC or CACI) may be used but must be decontaminated, cleaned, and disinfected before resuming sterile compounding in that C-PEC. A C-PEC used only for nonsterile compounding does not require unidirectional airflow because the critical environment does not need to be ISO classified.

The C-PEC must be placed in a C-SEC that has at least 12 ACPH. *Table 2* summarizes the engineering controls required for nonsterile HD compounding.

Due to the difficulty of cleaning HD contamination, surfaces of ceilings, walls, floors, fixtures, shelving, counters, and cabinets in the nonsterile compounding area must be smooth, impervious, free from cracks and crevices, and non-shedding.

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Table 2. Engineering Controls for Nonsterile HD Compounding

C-PEC	C-SEC Requirements
• Externally vented (preferred) or redundant–HEPA filtered in series • Examples: CVE, Class I or II BSC, CACI	• Externally vented • 12 ACPH • Negative pressure between 0.01 and 0.03 inches of water column relative to adjacent areas

5.3.2 STERILE COMPOUNDING

In addition to this chapter, sterile compounding must follow standards in ⟨797⟩.

All C-PECs used for manipulation of sterile HDs must be externally vented. Sterile HD compounding must be performed in a C-PEC that provides an ISO Class 5 or better air quality, such as a Class II or III BSC or CACI. Class II BSC types A2, B1, or B2 are acceptable. For most known HDs, type A2 cabinets offer a simple and reliable integration with the ventilation and pressurization requirements of the C-SEC. Class II type B2 BSCs are typically reserved for use with volatile components. *Appendix 3* describes the different types of BSCs.

A laminar airflow workbench (LAFW) or compounding aseptic isolator (CAI) must not be used for the compounding of an antineoplastic HD. A BSC or CACI used for the preparation of HDs must not be used for the preparation of a non-HD unless the non-HD preparation is placed into a protective outer wrapper during removal from the C-PEC and is labeled to require PPE handling precautions.

The C-PEC must be located in a C-SEC, which may either be an ISO Class 7 buffer room with an ISO Class 7 ante-room (preferred) or an unclassified containment segregated compounding area (C-SCA). If the C-PEC is placed in a C-SCA, the beyond-use date (BUD) of all compounded sterile preparations (CSPs) prepared must be limited as described in ⟨797⟩ for CSPs prepared in a segregated compounding area. *Table 3* summarizes the engineering controls required for sterile HD compounding.

Table 3. Engineering Controls for Sterile HD Compounding

Configuration	C-PEC	C-SEC	Maximum BUD
ISO Class 7 buffer room with an ISO Class 7 ante-room	• Externally vented • Examples: Class II BSC or CACI	• Externally vented • 30 ACPH • Negative pressure between 0.01 and 0.03 inches of water column relative to adjacent areas	As described in ⟨797⟩
Unclassified C-SCA	• Externally vented • Examples: Class II BSC or CACI	• Externally vented • 12 ACPH • Negative pressure between 0.01 and 0.03 inches of water column relative to adjacent areas	As described in ⟨797⟩ for CSPs prepared in a segregated compounding area

ISO Class 7 buffer room with an ISO class 7 ante-room: The C-PEC is placed in an ISO Class 7 buffer room that has fixed walls, HEPA-filtered supply air, a negative pressure between 0.01 and 0.03 inches of water column relative to all adjacent areas and a minimum of 30 ACPH.

The buffer room must be externally vented. Because the room through which entry into the HD buffer room (e.g., ante-room or non-HD buffer room) plays an important role in terms of total contamination control, the following is required:

- Minimum of 30 ACPH of HEPA-filtered supply air
- Maintain a positive pressure of at least 0.02 inches of water column relative to all adjacent unclassified areas
- Maintain an air quality of ISO Class 7 or better

An ISO Class 7 ante-room with fixed walls is necessary to provide inward air migration of equal cleanliness classified air into the negative pressure buffer room to contain any airborne HD. A hand-washing sink must be placed in the ante-room at least 1 meter from the entrance to the HD buffer room to avoid contamination migration into the negative pressure HD buffer room.

Although not a recommended facility design, if the negative-pressure HD buffer room is entered though the positive-pressure non-HD buffer room, the following is also required:

- A line of demarcation must be defined within the negative-pressure buffer room for donning and doffing PPE
- A method to transport HDs, HD CSPs, and HD waste into and out of the negative pressure buffer room to minimize the spread of HD contamination. This may be accomplished by use of a pass-through chamber between the negative-pressure buffer area and adjacent space. The pass-through chamber must be included in the facility's certification to ensure that particles are not compromising the air quality of the negative-pressure buffer room. A refrigerator pass-through must not be used. Other methods of containment (such as sealed containers) may be used.

HD CSPs prepared in an ISO Class 7 buffer room with an ISO Class 7 ante-room may use the BUDs described in ⟨797⟩, based on the categories of CSP, sterility testing, and storage temperature.

Containment segregated compounding area (C-SCA): The C-PEC is placed in an unclassified C-SCA that has fixed walls, a negative pressure between 0.01 and 0.03 inches of water column relative to all adjacent areas, and a minimum of 12 ACPH. The C-SCA must be externally vented. A hand-washing sink must be placed at least 1 meter from C-PEC and may be either inside the C-SCA or directly outside the C-SCA.

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Only ▲Category 1▲ (CN 1-Dec-2019) HD CSPs may be prepared in a C-SCA. HD CSPs prepared in the C-SCA must not exceed the BUDs described in ⟨797⟩ for CSPs prepared in a segregated compounding area.

5.4 Containment Supplemental Engineering Controls

Containment supplemental engineering controls, such as CSTDs, provide adjunct controls to offer an additional level of protection during compounding or administration. Some CSTDs have been shown to limit the potential of generating aerosols during compounding. However, there is no certainty that all CSTDs will perform adequately. Until a published universal performance standard for evaluation of CSTD containment is available, users should carefully evaluate the performance claims associated with available CSTDs based on independent, peer-reviewed studies and demonstrated contamination reduction.

A CSTD must not be used as a substitute for a C-PEC when compounding. CSTDs should be used when compounding HDs when the dosage form allows. CSTDs must be used when administering antineoplastic HDs when the dosage form allows. CSTDs known to be physically or chemically incompatible with a specific HD must not be used for that HD.

6. ENVIRONMENTAL QUALITY AND CONTROL

Environmental wipe sampling for HD surface residue should be performed routinely (e.g., initially as a benchmark and at least every 6 months, or more often as needed, to verify containment). Surface wipe sampling should include:

- Interior of the C-PEC and equipment contained in it
- Pass-through chambers
- Surfaces in staging or work areas near the C-PEC
- Areas adjacent to C-PECs (e.g., floors directly under C-PEC, staging, and dispensing area)
- Areas immediately outside the HD buffer room or the C-SCA
- Patient administration areas

There are currently no studies demonstrating the effectiveness of a specific number or size of wipe samples in determining levels of HD contamination. Wipe sampling kits should be verified before use to ensure the method and reagent used have been tested to recover a specific percentage of known marker drugs from various surface types found in the sampled area. There are currently no certifying agencies for vendors of wipe sample kits.

There is currently no standard for acceptable limits for HD surface contamination. Common marker HDs that can be assayed include cyclophosphamide, ifosfamide, methotrexate, fluorouracil, and platinum-containing drugs. An example of measurable contamination would be cyclophosphamide levels >1.00 ng/cm^2, which were shown in some studies to result in uptake of the drug in exposed workers. If any measurable contamination is found, the designated person must identify, document, and contain the cause of contamination. Such action may include reevaluating work practices, re-training personnel, performing thorough deactivation, decontamination, cleaning, and improving engineering controls. Repeat the wipe sampling to validate that the deactivation/decontamination and cleaning steps have been effective.

7. PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment (PPE) provides worker protection to reduce exposure to HD aerosols and residues. Additional PPE may be required to handle the HDs outside of a C-PEC, such as treating a patient or cleaning a spill. The NIOSH list of antineoplastic and other HDs provides general guidance on PPE for possible scenarios that may be encountered in healthcare settings. Disposable PPE must not be re-used. Reusable PPE must be decontaminated and cleaned after use.

Gowns, head, hair, shoe covers, and two pairs of chemotherapy gloves are required for compounding sterile and nonsterile HDs. Two pairs of chemotherapy gloves are required for administering injectable antineoplastic HDs. Gowns shown to resist permeability by HDs are required when administering injectable antineoplastic HDs. For all other activities, the entity's SOP must describe the appropriate PPE to be worn based on its occupational safety plan and assessment of risk (if used). The entity must develop SOPs for PPE based on the risk of exposure (see *Types of Exposure*) and activities performed.

Appropriate PPE must be worn when handling HDs including during:

- Receipt
- Storage
- Transport
- Compounding (sterile and nonsterile)
- Administration
- Deactivation/decontamination, cleaning, and disinfecting
- Spill control
- Waste disposal

7.1 Gloves

When chemotherapy gloves are required, they must meet American Society for Testing and Materials (ASTM) standard D6978 (or its successor). Chemotherapy gloves should be worn for handling all HDs including non-antineoplastics and for reproductive risk only HDs. Chemotherapy gloves must be powder-free because powder can contaminate the work area and can adsorb and retain HDs. Gloves must be inspected for physical defects before use. Do not use gloves with pin holes or weak spots.

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When used for sterile compounding, the outer chemotherapy gloves must be sterile. Chemotherapy gloves should be changed every 30 minutes unless otherwise recommended by the manufacturer's documentation and must be changed when torn, punctured, or contaminated. Hands must be washed with soap and water after removing gloves.

7.2 Gowns

When gowns are required, they must be disposable and shown to resist permeability by HDs. Gowns must be selected based on the HDs handled. Disposable gowns made of polyethylene-coated polypropylene or other laminate materials offer better protection than those made of uncoated materials. Gowns must close in the back (i.e., no open front), be long sleeved, and have closed cuffs that are elastic or knit. Gowns must not have seams or closures that could allow HDs to pass through.

Cloth laboratory coats, surgical scrubs, isolation gowns, or other absorbent materials are not appropriate protective outerwear when handling HDs because they permit the permeation of HDs and can hold spilled drugs against the skin, thereby increasing exposure. Clothing may also retain HD residue from contact, and may transfer to other healthcare workers or various surfaces. Washing of non-disposable clothing contaminated with HD residue should only be done according to facility policy as drug residue may be transferred to other clothing. Potentially contaminated clothing must not be taken home under any circumstances.

Gowns must be changed per the manufacturer's information for permeation of the gown. If no permeation information is available for the gowns used, change them every 2–3 hours or immediately after a spill or splash. Gowns worn in HD handling areas must not be worn to other areas in order to avoid spreading HD contamination and exposing other healthcare workers.

7.3 Head, Hair, Shoe, and Sleeve Covers

Head and hair covers (including beard and moustache, if applicable), shoe covers, and sleeve covers provide protection from contact with HD residue. When compounding HDs, a second pair of shoe covers must be donned before entering the C-SEC and doffed when exiting the C-SEC. Shoe covers worn in HD handling areas must not be worn to other areas to avoid spreading HD contamination and exposing other healthcare workers.

Disposable sleeve covers may be used to protect areas of the arm that may come in contact with HDs. Disposable sleeve covers made of polyethylene-coated polypropylene or other laminate materials offer better protection than those made of uncoated materials.

7.4 Eye and Face Protection

Many HDs are irritating to the eyes and mucous membranes. Appropriate eye and face protection must be worn when there is a risk for spills or splashes of HDs or HD waste materials when working outside of a C-PEC (e.g., administration in the surgical suite, working at or above eye level, or cleaning a spill). A full-facepiece respirator provides eye and face protection. Goggles must be used when eye protection is needed. Eye glasses alone or safety glasses with side shields do not protect the eyes adequately from splashes. Face shields in combination with goggles provide a full range of protection against splashes to the face and eyes. Face shields alone do not provide full eye and face protection.

7.5 Respiratory Protection

Personnel who are unpacking HDs that are not contained in plastic should wear an elastomeric half-mask with a multi-gas cartridge and P100-filter until assessment of the packaging integrity can be made to ensure no breakage or spillage occurred during transport. If the type of drug can be better defined, a more targeted cartridge can be used.

Surgical masks do not provide respiratory protection from drug exposure and must not be used when respiratory protection from HD exposure is required. A surgical N95 respirator provides the respiratory protection of an N95 respirator, and like a surgical mask, provides a barrier to splashes, droplets, and sprays around the nose and mouth.

For most activities requiring respiratory protection, a fit-tested NIOSH-certified N95 or more protective respirator is sufficient to protect against airborne particles. However, N95 respirators offer no protection against gases and vapors and little protection against direct liquid splashes (see the Centers for Disease Control and Prevention's (CDC's) Respirator Trusted-Source Information).

Fit test the respirator and train workers to use respiratory protection. Follow all requirements in the Occupational Safety and Health Administration (OSHA) respiratory protection standard (29 CFR 1910.134). An appropriate full-facepiece, chemical cartridge-type respirator or powered air-purifying respirator (PAPR) should be worn when there is a risk of respiratory exposure to HDs, including when:

- Attending to HD spills larger than what can be contained with a spill kit
- Deactivating, decontaminating, and cleaning underneath the work surface of a C-PEC
- There is a known or suspected airborne exposure to powders or vapors

7.6 Disposal of Used Personal Protective Equipment

Consider all PPE worn when handling HDs to be contaminated with, at minimum, trace quantities of HDs. PPE must be placed in an appropriate waste container and further disposed of per local, state, and federal regulations. PPE worn during compounding should be disposed of in the proper waste container before leaving the C-SEC. Chemotherapy gloves and sleeve

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covers (if used) worn during compounding must be carefully removed and discarded immediately into a waste container approved for trace contaminated waste inside the C-PEC or contained in a sealable bag for discarding outside the C-PEC.

8. HAZARD COMMUNICATION PROGRAM

Entities are required to establish policies and procedures that ensure worker safety during all aspects of HD handling. The entity must develop SOPs to ensure effective training regarding proper labeling, transport, storage, and disposal of the HDs and use of Safety Data Sheets (SDS), based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Elements of the hazard communication program plan must include:

- A written plan that describes how the standard will be implemented
- All containers of hazardous chemicals must be labeled, tagged, or marked with the identity of the material and appropriate hazard warnings
- Entities must have an SDS for each hazardous chemical they use (29 CFR 1910.1200)
- Entities must ensure that the SDSs for each hazardous chemical used are readily accessible to personnel during each work shift and when they are in their work areas
- Personnel who may be exposed to hazardous chemicals when working must be provided information and training before the initial assignment to work with a hazardous chemical, and also whenever the hazard changes
- Personnel of reproductive capability must confirm in writing that they understand the risks of handling HDs

9. PERSONNEL TRAINING

All personnel who handle HDs must be trained based on their job functions (e.g., in the receipt, storage, compounding, repackaging, dispensing, administrating, and disposing of HDs). Training must occur before the employee independently handles HDs. The effectiveness of training for HD handling competencies must be demonstrated by each employee. Personnel competency must be reassessed at least every 12 months. Personnel must be trained prior to the introduction of a new HD or new equipment and prior to a new or significant change in process or SOP. All training and competency assessment must be documented.

The training must include at least the following:

- Overview of entity's list of HDs and their risks
- Review of the entity's SOPs related to handling of HDs
- Proper use of PPE
- Proper use of equipment and devices (e.g., engineering controls)
- Response to known or suspected HD exposure
- Spill management
- Proper disposal of HDs and trace-contaminated materials

10. RECEIVING

The entity must establish SOPs for receiving HDs. HDs should be received from the supplier in impervious plastic to segregate them from other drugs and to allow for safety in the receiving and internal transfer process. HDs must be delivered to the HD storage area immediately after unpacking.

PPE, including chemotherapy gloves, must be worn when unpacking HDs (see *Personal Protective Equipment*). A spill kit must be accessible in the receiving area.

The entity must enforce policies that include a tiered approach, starting with visual examination of the shipping container for signs of damage or breakage (e.g., visible stains from leakage, sounds of broken glass). *Table 4* summarizes the steps for receiving and handling of damaged shipping containers.

Table 4. Summary of Requirements for Receiving and Handling Damaged HD Shipping Containers

If the shipping container appears damaged	• Seal container without opening and contact the supplier • If the unopened package is to be returned to the supplier, enclose the package in an impervious container and label the outer container "Hazardous" • If the supplier declines return, dispose of as hazardous waste
If a damaged shipping container must be opened	• Seal the container in plastic or an impervious container • Transport it to a C-PEC and place on a plastic-backed preparation mat • Open the package and remove undamaged items • Wipe the outside of the undamaged items with a disposable wipe • Enclose the damaged item(s) in an impervious container and label the outer container "Hazardous" • If the supplier declines return, dispose of as hazardous waste • Deactivate, decontaminate, and clean the C-PEC (see *Deactivating, Decontaminating, Cleaning, and Disinfecting*) and discard the mat and cleaning disposables as hazardous waste

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When opening damaged shipping containers, they should preferably be transported to a C-PEC designated for nonsterile compounding. If a C-PEC designated for sterile compounding is the only one available, it must be disinfected after the decontamination, deactivation, and cleaning step before returning to any sterile compounding activity.

Damaged packages or shipping cartons must be considered spills that must be reported to the designated person and managed according to the entity's SOPs. Segregate HDs waiting to be returned to the supplier in a designated negative pressure area. Clean-up must comply with established SOPs.

11. LABELING, PACKAGING, TRANSPORT AND DISPOSAL

The entity must establish SOPs for the labeling, packaging, transport, and disposal of HDs. The SOPs must address prevention of accidental exposures or spills, personnel training on response to exposure, and use of a spill kit. Examples of special exposure-reducing strategies include small-bore connectors (such as Luer Lock) and syringes, syringe caps, CSTDs, the capping of container ports, sealed impervious plastic bags, impact-resistant and/or water-tight containers, and cautionary labeling.

11.1 Labeling

HDs identified by the entity as requiring special HD handling precautions must be clearly labeled at all times during their transport. Personnel must ensure that the labeling processes for compounded preparations do not introduce contamination into the non-HD handling areas.

11.2 Packaging

Personnel must select and use packaging containers and materials that will maintain physical integrity, stability, and sterility (if needed) of the HDs during transport. Packaging materials must protect the HD from damage, leakage, contamination, and degradation, while protecting healthcare workers who transport HDs. The entity must have written SOPs to describe appropriate shipping containers and insulating materials, based on information from product specifications, vendors, and mode of transport.

11.3 Transport

HDs that need to be transported must be labeled, stored, and handled in accordance with applicable federal, state, and local regulations. HDs must be transported in containers that minimize the risk of breakage or leakage. Pneumatic tubes must not be used to transport any liquid HDs or any antineoplastic HDs because of the potential for breakage and contamination.

When shipping HDs to locations outside the entity, the entity must consult the Transport Information on the SDS. The entity must ensure that labels and accessory labeling for the HDs include storage instructions, disposal instructions, and HD category information in a format that is consistent with the carrier's policies.

11.4 Disposal

All personnel who perform routine custodial waste removal and cleaning activities in HD handling areas must be trained in appropriate procedures to protect themselves and the environment to prevent HD contamination. Disposal of all HD waste, including, but not limited to, unused HDs and trace-contaminated PPE and other materials, must comply with all applicable federal, state, and local regulations.

12. DISPENSING FINAL DOSAGE FORMS

HDs that do not require any further manipulation, other than counting or repackaging of final dosage forms, may be prepared for dispensing without any further requirements for containment unless required by the manufacturer or if visual indicators of HD exposure hazards are present (e.g., HD dust or leakage).

Counting or repackaging of HDs must be done carefully. Clean equipment should be dedicated for use with HDs and should be decontaminated after every use. Tablet and capsule forms of antineoplastic HDs must not be placed in automated counting or packaging machines, which subject them to stress and may create powdered contaminants.

13. COMPOUNDING

Entities and personnel involved in compounding HDs must be compliant with the appropriate USP standards for compounding including ⟨795⟩ and ⟨797⟩. Compounding must be done in proper engineering controls as described in *Compounding*. When compounding HD preparations in a C-PEC, a plastic-backed preparation mat should be placed on the work surface of the C-PEC. The mat should be changed immediately if a spill occurs and regularly during use, and should be discarded at the end of the daily compounding activity. Disposable or clean equipment for compounding (such as mortars and pestles, and spatulas) must be dedicated for use with HDs.

Bulk containers of liquid and API HD must be handled carefully to avoid spills. If used, APIs or other powdered HDs must be handled in a C-PEC to protect against occupational exposure, especially during particle-generating activities (such as crushing tablets, opening capsules, and weighing powder).

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14. ADMINISTERING

HDs must be administered safely using protective medical devices and techniques. Examples of protective medical devices include needleless and closed systems. Examples of protective techniques include spiking or priming of IV tubing with a non-HD solution in a C-PEC and crushing tablets in a plastic pouch.

Appropriate PPE must be worn when administering HDs. After use, PPE must be removed and disposed of in a waste container approved for trace-contaminated HD waste at the site of drug administration. Equipment (such as tubing and needles) and packaging materials must be disposed of properly, such as in HD waste containers, after administration.

CSTDs must be used for administration of antineoplastic HDs when the dosage form allows. Techniques and ancillary devices that minimize the risk posed by open systems must be used when administering HDs through certain routes. Administration into certain organs or body cavities (e.g., the bladder, eye, peritoneal cavity, or chest cavity) often requires equipment for which locking connections may not be readily available or possible.

Healthcare personnel should avoid manipulating HDs such as crushing tablets or opening capsules if possible. Liquid formulations are preferred if solid oral dosage forms are not appropriate for the patient. If HD dosage forms do require manipulation such as crushing tablet(s) or opening capsule(s) for a single dose, personnel must don appropriate PPE and use a plastic pouch to contain any dust or particles generated.

15. DEACTIVATING, DECONTAMINATING, CLEANING, AND DISINFECTING

All areas where HDs are handled and all reusable equipment and devices must be deactivated, decontaminated, and cleaned. Additionally, sterile compounding areas and devices must be subsequently disinfected.

The entity must establish written procedures for decontamination, deactivation, and cleaning, and for sterile compounding areas disinfection. Additionally, cleaning of nonsterile compounding areas must comply with ⟨795⟩ and cleaning of sterile compounding areas must comply with ⟨797⟩. Written procedures for cleaning must include procedures, agents used, dilutions (if used), frequency, and documentation requirements.

All personnel who perform deactivation, decontamination, cleaning, and disinfection activities in HD handling areas must be trained in appropriate procedures to protect themselves and the environment from contamination. All personnel performing these activities must wear appropriate PPE resistant to the cleaning agents used, including two pairs of chemotherapy gloves and impermeable disposable gowns (see *Personal Protective Equipment*). Additionally, eye protection and face shields must be used if splashing is likely. If warranted by the activity, respiratory protection must be used.

The deactivating, decontaminating, cleaning, and disinfecting agents selected must be appropriate for the type of HD contaminant(s), location, and surface materials. The products used must be compatible with the surface material. Consult manufacturer or supplier information for compatibility with cleaning agents used. Agents used for deactivation, decontamination, and cleaning should be applied through the use of wipes wetted with appropriate solution and not delivered by a spray bottle to avoid spreading HD residue. All disposable materials must be discarded to meet EPA regulations and the entity's policies. Perform cleaning in areas that are sufficiently ventilated. *Table 5* summarizes the purpose and example agents for each step.

Table 5. Cleaning Steps

Cleaning Step	Purpose	Example Agents
Deactivation	Render compound inert or inactive	As listed in the HD labeling or other agents which may incorporate Environmental Protection Agency (EPA)-registered oxidizers (e.g., peroxide formulations, sodium hypochlorite, etc.)
Decontamination	Remove HD residue	Materials that have been validated to be effective for HD decontamination, or through other materials proven to be effective through testing, which may include alcohol, water, peroxide, or sodium hypochlorite
Cleaning	Remove organic and inorganic material	Germicidal detergent
Disinfection (for sterile manipulations)	Destroy microorganisms	EPA-registered disinfectant and/or sterile alcohol as appropriate for use

15.1 Deactivation

Deactivation renders a compound inert or inactive. Residue from deactivation must be removed by decontaminating the surface.

There is no one proven method for deactivating all compounds. The ultimate goal should be complete surface decontamination. Products that have known deactivation properties (EPA-registered oxidizing agents that are appropriate for the intended use) should be used when possible. Care should be taken when selecting materials for deactivation due to potential adverse effects (hazardous byproducts, respiratory effects, and caustic damage to surfaces). Damage to surfaces is exhibited by corrosion to stainless steel surfaces caused by sodium hypochlorite if left untreated. To prevent corrosion, sodium hypochlorite must be neutralized with sodium thiosulfate or by following with an agent to remove the sodium hypochlorite (e.g., sterile alcohol, sterile water, germicidal detergent, or sporicidal agent).

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15.2 Decontamination

Decontamination occurs by inactivating, neutralizing, or physically removing HD residue from non-disposable surfaces and transferring it to absorbent, disposable materials (e.g., wipes, pads, or towels) appropriate to the area being cleaned. When choosing among various products available for decontaminating HDs, consideration should be given to surface compatibility and facility requirements. It is imperative to adhere to manufacturer's use instructions. Because of the growing number of assays available for HDs, additional surface wipe sampling is now possible and should be done to document the effectiveness of any agent used for decontamination of HD residue from work surfaces (see *Environmental Quality and Control*).

The amount of HD contamination introduced into the C-PEC may be reduced by wiping down HD containers. The solution used for wiping HD packaging must not alter the product label. The work surface of the C-PEC must be decontaminated between compounding of different HDs. The C-PEC must be decontaminated at least daily (when used), any time a spill occurs, before and after certification, any time voluntary interruption occurs, and if the ventilation tool is moved.

C-PECs may have areas under the work tray where contamination can build up. These areas must be deactivated, decontaminated, and cleaned at least monthly to reduce the contamination level in the C-PEC. Accessing this area may be difficult. Deactivate, decontaminate, and clean as much as possible of the C-PEC surfaces before accessing the area under the work tray. When deactivating, decontaminating, and cleaning the area under the work tray of a C-PEC, the containment airflows are compromised by opening the cabinets. To provide protection to the worker performing this task, respiratory protection may be required.

15.3 Cleaning

Cleaning is a process that results in the removal of contaminants (e.g., soil, microbial contamination, HD residue) from objects and surfaces using water, detergents, surfactants, solvents, and/or other chemicals. Cleaning agents used on compounding equipment should not introduce microbial contamination. No cleaning step may be performed when compounding activities are occurring.

15.4 Disinfection

Disinfection is a process of inhibiting or destroying microorganisms. Before disinfection can be adequately performed, surfaces must be cleaned. Disinfection must be done for areas intended to be sterile, including the sterile compounding areas.

16. SPILL CONTROL

All personnel who may be required to clean up a spill of HDs must receive proper training in spill management and the use of PPE and NIOSH-certified respirators (see *Personal Protective Equipment*). Spills must be contained and cleaned immediately only by qualified personnel with appropriate PPE. Qualified personnel must be available at all times while HDs are being handled. Signs must be available for restricting access to the spill area. Spill kits containing all of the materials needed to clean HD spills must be readily available in all areas where HDs are routinely handled. If HDs are being prepared or administered in a non-routine healthcare area, a spill kit and respirator must be available. All spill materials must be disposed of as hazardous waste.

The circumstances and management of spills must be documented. Personnel who are potentially exposed during the spill or spill clean up or who have direct skin or eye contact with HDs require immediate evaluation. Non-employees exposed to an HD spill should follow entity policy, which may include reporting to the designated emergency service for initial evaluation and completion of an incident report or exposure form.

SOPs must be developed to prevent spills and to direct the clean up of HD spills. SOPs must address the size and scope of the spill and specify who is responsible for spill management and the type of PPE required. The management of the spill (e.g., decontamination, deactivation, and cleaning) may be dependent on the size and type of spill. The SOP must address the location of spill kits and clean-up materials as well as the capacity of the spill kit. Written procedures should address use of appropriate full-facepiece, chemical cartridge-type respirators if the capacity of the spill kit is exceeded or if there is known or suspected airborne exposure to vapors or gases.

17. DOCUMENTATION AND STANDARD OPERATING PROCEDURES

The entity must maintain SOPs for the safe handling of HDs for all situations in which these HDs are used throughout a facility. The SOPs must be reviewed at least every 12 months by the designated person, and the review must be documented. Revisions in forms or records must be made as needed and communicated to all personnel handling HDs.

The SOPs for handling of HDs should include:

- Hazard communication program
- Occupational safety program
- Designation of HD areas
- Receipt
- Storage
- Compounding
- Use and maintenance of proper engineering controls (e.g., C-PECs, C-SECs, and CSTDs)

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- Hand hygiene and use of PPE based on activity (e.g., receipt, transport, compounding, administration, spill, and disposal)
- Deactivation, decontamination, cleaning, and disinfection
- Dispensing
- Transport
- Administering
- Environmental monitoring (e.g., wipe sampling)
- Disposal
- Spill control
- Medical surveillance

Personnel who transport, compound, or administer HDs must document their training according to OSHA standards (see OSHA Standard 1910.120 Hazardous Waste Operations and Emergency Response) and other applicable laws and regulations.

18. MEDICAL SURVEILLANCE

Medical surveillance is part of a comprehensive exposure control program complementing engineering controls, safe work processes, and use of PPE. Healthcare workers who handle HDs as a regular part of their job assignment should be enrolled in a medical surveillance program. The general purpose of surveillance is to minimize adverse health effects in personnel potentially exposed to HDs. Medical surveillance programs involve assessment and documentation of symptom complaints, physical findings, and laboratory values (such as a blood count) to determine whether there is a deviation from the expected norms.

Medical surveillance can also be viewed as a secondary prevention tool that may provide a means of early detection if a health problem develops. Tracking personnel through medical surveillance allows the comparison of health variables over time in individual workers, which may facilitate early detection of a change in a laboratory value or health condition. Medical surveillance programs also look for trends in populations of workers. Examining grouped data compared with data from unexposed workers may reveal a small alteration or increase in the frequency of a health effect that would be obscured if individual workers' results alone were considered.

Medical surveillance evaluates the protection afforded by engineering controls, other administrative controls, safe work processes, PPE, and worker education about the hazards of the materials they work with in the course of their duties. The data-gathering elements of a medical surveillance program are used to establish a baseline of workers' health and then to monitor their future health for any changes that may result from exposure to HDs.

Elements of a medical surveillance program should be consistent with the entity's Human Resource policies and should include:

- Development of an organized approach to identify workers who are potentially exposed to HDs on the basis of their job duties
- Use of an entity-based or contracted employee health service to perform the medical surveillance while protecting the confidentiality of the employees' personal medical information
- Initial baseline assessment (pre-placement) of a worker's health status and medical history. Data elements collected include a medical (including reproductive) history and work history to assess exposure to HDs, physical examination, and laboratory testing. Methods used to assess exposure history include a review of:
  - Records of HDs handled, with quantities and dosage forms
  - Estimated number of HDs handled per week
  - Estimates of hours spent handling HDs per week and/or per month
  - Performance of a physical assessment and laboratory studies linked to target organs of commonly used HDs, such as a baseline complete blood count. Biological monitoring to determine blood or urine levels of specific HDs is not currently recommended in surveillance protocols, but may have a role in the follow-up of acute spills with a specific agent.
- Medical records of surveillance should be maintained according to OSHA regulation concerning access to employee exposure and medical records
- Monitoring workers' health prospectively through periodic surveillance using the elements of data gathering described above (updated health and exposure history, physical assessment, and laboratory measures, if appropriate)
- Monitoring of the data to identify prevention failure leading to health effects; this monitoring may occur in collaboration with the employee health service
- Development of a follow-up plan for workers who have shown health changes suggesting toxicity or who have experienced an acute exposure. This follow-up should include evaluation of current engineering and administrative controls and equipment to ensure that all systems are appropriately and accurately implemented (see *Follow-Up Plan*)
- Completion of an exit examination when a worker's employment at the entity ends, to document the information on the employee's medical, reproductive, and exposure histories. Examination and laboratory evaluation should be guided by the individual's history of exposures and follow the outline of the periodic evaluation

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18.1 Follow-Up Plan

The occurrence of exposure-related health changes should prompt immediate re-evaluation of primary preventive measures (e.g., administrative and engineering controls, PPE, and others). In this manner, medical surveillance acts as a check on the effectiveness of controls already in use.

The entity should take the following actions:

- Perform a post-exposure examination tailored to the type of exposure (e.g., spills or needle sticks from syringes containing HDs). An assessment of the extent of exposure should be conducted and included in a confidential database and in an incident report. The physical examination should focus on the involved area as well as other organ systems commonly affected (i.e., the skin and mucous membranes for direct contact or inhalation; the pulmonary system for aerosolized HDs). Treatment and laboratory studies will follow as indicated and be guided by emergency protocols
- Compare performance of controls with recommended standards; conduct environmental sampling when analytical methods are available
- Verify and document that all engineering controls are in proper operating condition
- Verify and document that the worker complied with existing policies. Review policies for the use of PPE and employee compliance with PPE use and policies. Review availability of appropriate PPE (see *Personal Protective Equipment*)
- Develop and document a plan of action that will prevent additional exposure of workers
- Ensure confidential, two-way communication between the worker and the employee health unit(s) regarding notification, discussions about a change in health condition, or detection of an adverse health effect
- Provide and document a follow-up medical survey to demonstrate that the plan implemented is effective
- Ensure that any exposed worker receives confidential notification of any adverse health effect. Offer alternative duty or temporary reassignment
- Provide ongoing medical surveillance of all workers at risk for exposure to HDs to determine whether the plan implemented is effective

GLOSSARY

Active pharmaceutical ingredient (API): Any substance or mixture of substances intended to be used in the compounding of a drug preparation, thereby becoming the active ingredient in that preparation and furnishing pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans and animals or affecting the structure and function of the body.

Alternative duty: Performance of other tasks that do not include the direct handling of HDs.

Ante-room: An ISO Class 7 or cleaner room where personnel hand hygiene, garbing procedures, and other activities that generate high particulate levels are performed. The ante-room is the transition room between the unclassified area of the facility and the buffer room.

Assessment of risk: Evaluation of risk to determine alternative containment strategies and/or work practices.

Beyond-use date (BUD): The date or time beyond which a compounded preparation cannot not be used and must be discarded (see ⟨795⟩ and ⟨797⟩). The date or time is determined from the date or time when the preparation was compounded.

Biological safety cabinet (BSC): A ventilated cabinet often used for preparation of hazardous drugs. These cabinets are divided into three general classes (Class I, Class II, and Class III). Class II BSCs are further divided into types (Type A1, Type A2, Type B1, and Type B2). See *Appendix 3* for details.

Buffer room: A type of C-SEC under negative pressure that meets ISO Class 7 or better air quality where the C-PEC that generates and maintains an ISO Class 5 environment is physically located. Activities that occur in this area are limited to the preparation and staging of components and supplies used when compounding HDs.

Chemotherapy glove: A medical glove that meets the ASTM Standard Practice for Assessment of Resistance of Medical Gloves to Permeation by Chemotherapy Drugs (D6978) or its successor.

Classified space: An area that maintains an air cleanliness classification based on the International Organization for Standardization (ISO).

Cleaning: The process of removing soil (e.g., organic and inorganic material) from objects and surfaces, normally accomplished by manually or mechanically using water with detergents or enzymatic products.

Closed-system drug-transfer device (CSTD): A drug-transfer device that mechanically prohibits the transfer of environmental contaminants into the system and the escape of HD or vapor concentrations outside the system.

Compounded preparation: A nonsterile or sterile drug or nutrient preparation that is compounded in a licensed pharmacy or other healthcare-related facility in response to or anticipation of a prescription or a medication order from a licensed prescriber.

Compounding aseptic containment isolator (CACI): A specific type of CAI that is designed for the compounding of sterile HDs. The CACI is designed to provide worker protection from exposure to undesirable levels of airborne drugs throughout the compounding and material transfer processes and to provide an aseptic environment with unidirectional airflow for compounding sterile preparations.

Compounding aseptic isolator (CAI): An isolator specifically designed for compounding sterile, non-hazardous pharmaceutical ingredients or preparations. The CAI is designed to maintain an aseptic compounding environment throughout the compounding and material transfer processes.

Compounding personnel: Individuals who participate in the compounding process.

Containment primary engineering control (C-PEC): A ventilated device designed and operated to minimize worker and environmental exposures to HDs by controlling emissions of airborne contaminants through the following:

- The full or partial enclosure of a potential contaminant source

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- The use of airflow capture velocities to trap and remove airborne contaminants near their point of generation
- The use of air pressure relationships that define the direction of airflow into the cabinet
- The use of HEPA filtration on all potentially contaminated exhaust streams

Containment secondary engineering control (C-SEC): The room with fixed walls in which the C-PEC is placed. It incorporates specific design and operational parameters required to contain the potential hazard within the compounding room.

Containment segregated compounding area (C-SCA): A type of C-SEC with nominal requirements for airflow and room pressurization as they pertain to HD compounding.

Containment ventilated enclosure (CVE): A full or partial enclosure that uses ventilation principles to capture, contain, and remove airborne contaminants through HEPA filtration and prevent their release into the work environment.

Deactivation: Treatment of an HD contaminant on surfaces with a chemical, heat, ultraviolet light, or another agent to transform the HD into a less hazardous agent.

Decontamination: Inactivation, neutralization, or removal of HD contaminants on surfaces, usually by chemical means.

Doff: To remove PPE.

Don: To put on PPE.

Disinfection: The process of inhibiting or destroying microorganisms.

Engineering control: Primary, secondary, and supplemental devices designed to eliminate or reduce worker exposure to HDs.

EPA-registered disinfectant: Antimicrobial products registered with the Environmental Protection Agency (EPA) for healthcare use against pathogens specified in the product labeling.

Externally vented: Exhausted to the outside

Final dosage form: Any form of a medication that requires no further manipulation before administration.

Globally Harmonized System of Classification and Labeling of Chemicals (GHS): A system for standardizing and harmonizing the classification and labeling of chemicals.

Goggles: Tight-fitting eye protection that completely covers the eyes, eye sockets, and facial area that immediately surrounds the eyes. Goggles provide protection from impact, dust, and splashes. Some goggles fit over corrective lenses.

Hazardous drug (HD): Any drug identified by at least one of the following criteria:

- Carcinogenicity, teratogenicity, or developmental toxicity
- Reproductive toxicity in humans
- Organ toxicity at low dose in humans or animals
- Genotoxicity or new drugs that mimic existing HDs in structure or toxicity

High-efficiency particulate air (HEPA) filtration: An extended-medium, dry-type filter in a rigid frame, having a minimum particle collection efficiency of 99.97% for particles with a mass median diameter of 0.3 µm when tested at a rated airflow in accordance with MIL STD 282 using IEST Recommended Standard RP-CC001.5.

Negative-pressure room: A room that is maintained at a lower pressure than the adjacent areas; therefore the net flow of air is into the room.

Pass-through: An enclosure with interlocking doors that is positioned between two spaces for the purpose of reducing particulate transfer while moving materials from one space to another. A pass-through serving negative-pressure rooms needs to be equipped with sealed doors.

Personal protective equipment (PPE): Items such as gloves, gowns, respirators, goggles, faceshields, and others that protect individual workers from hazardous physical or chemical exposures.

Positive-pressure room: A room that is maintained at a higher pressure than the adjacent areas; therefore, the net flow of air is out of the room.

Repackaging: The act of removing a product from its original primary container and placing it into another primary container, usually of smaller size.

Safety data sheet (SDS): An informational document that provides written or printed material concerning a hazardous chemical. The SDS is prepared in accordance with the HCS [previously known as a Material Safety Data Sheet (MSDS)].

Spill kit: A container of supplies, warning signage, and related materials used to contain the spill of an HD.

Standard operating procedure (SOP): Written procedures describing operations, testing, sampling, interpretation of results, and corrective actions that relate to the operations that are taking place.

Supplemental engineering control: An adjunct control (e.g., CSTD) that may be used concurrently with primary and secondary engineering controls. Supplemental engineering controls offer additional levels of protection and may facilitate enhanced occupational protection, especially when handling HDs outside of primary and secondary engineering controls (e.g., during administering).

Unclassified space: A space not required to meet any air cleanliness classification based on the International Organization for Standardization (ISO).

APPENDICES

Appendix 1: Acronyms

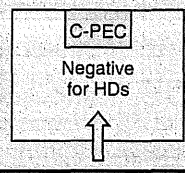
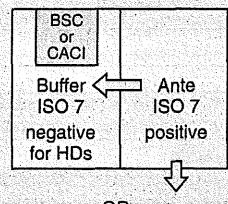
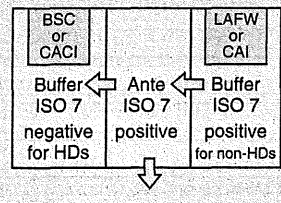
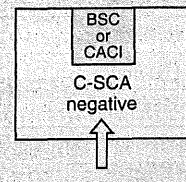
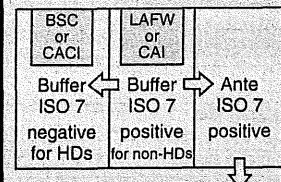
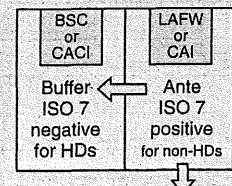
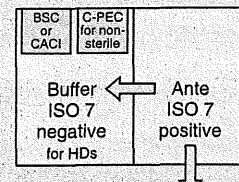
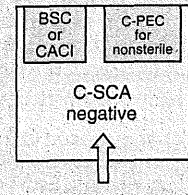
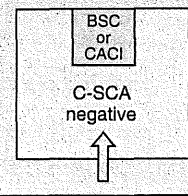
ACPH	Air changes per hour
API	Active pharmaceutical ingredient
ASTM	American Society for Testing and Materials

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BSC	Biological safety cabinet
BUD	Beyond-use date
CACI	Compounding aseptic containment isolator
CAI	Compounding aseptic isolator
CDC	Centers for Disease Control and Prevention
C-PEC	Containment primary engineering control
C-SCA	Containment segregated compounding area
C-SEC	Containment secondary engineering control
CSP	Compounded sterile preparation
CSTD	Closed-system drug-transfer device
CVE	Containment ventilated enclosure
EPA	Environmental Protection Agency
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
HCS	Hazard Communication Standard
HD	Hazardous drug
HEPA	High-efficiency particulate air
IV	Intravenous
LAFW	Laminar airflow workbench
NIOSH	National Institute for Occupational Safety and Health
ONS	Oncology Nursing Society
OSHA	Occupational Safety and Health Administration
PAPR	Powered air-purified respirator
PPE	Personal protective equipment
SDS	Safety Data Sheet
SOP	Standard operating procedure
ULPA	Ultra-low particulate air
UPS	Uninterrupted power source

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Appendix 2: Examples of Designs for Hazardous Drug Compounding Areas[a]

Use	Optimal Primary and Secondary Control	Minimum ACPH	Limitations Primary and Secondary Control	Minimum ACPH	Notes for limitations
Nonsterile HD compounding		12			
Sterile HD compounding	 OR 	30	 OR  **This design is not recommended** OR  Typically used in oncology clinic settings.	12 30 30	Maximum BUD as described in <797> for segregated compounding area. If this design is in place, measures must be taken to avoid contamination of the positive-pressure buffer room. Maximum BUD as described in <797>.
Both sterile HD and nonsterile HD compounding	A separate room for sterile and nonsterile compounding is recommended		 OR  OR 	30 12 12	For rooms used for both sterile and nonsterile compounding, particle-generating activity must not be performed when sterile compounding is in process. C-PECs must be at least 1 meter apart. Maximum BUD as described in <797> for segregated compounding area. Maximum BUD as described in <797> for segregated compounding area.

[a] The arrows indicate direction of airflow.

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Appendix 3: Types of Biological Safety Cabinets

Class I: A BSC that protects personnel and the environment but does not protect the product/preparation. A minimum velocity of 75 linear feet/minute of unfiltered room air is drawn through the front opening and across the work surface, providing personnel protection. The air is then passed through a HEPA/ULPA (ultra-low particulate air) filter, either into the room or to the outside in the exhaust plenum, providing environmental protection.

Class II: Class II (Types A1, A2, B1, and B2) BSCs are partial barrier systems that rely on the movement of air to provide personnel, environmental, and product/preparation protection. Personnel and product/preparation protection are provided by the combination of inward and downward airflow captured by the front grille of the cabinet. Side-to-side cross-contamination of products/preparations is minimized by the internal downward flow of HEPA/ULPA filtered air moving toward the work surface and then drawn into the front and rear intake grilles. Environmental protection is provided when the cabinet exhaust air is passed through a HEPA/ULPA filter.

Type A1 (formerly, Type A): These Class II BSCs maintain a minimum inflow velocity of 75 feet/minute; have HEPA-filtered, down-flow air that is a portion of the mixed down-flow and inflow air from a common plenum; may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and may have positive-pressure contaminated ducts and plenums that are not surrounded by negative-pressure plenums. Type A1 BSCs are not suitable for use with volatile toxic chemicals and volatile radionuclides.

Type A2 (formerly, Type B3): These Class II BSCs maintain a minimum inflow velocity of 100 feet/minute; have HEPA-filtered, down-flow air that is a portion of the mixed down-flow and inflow air from a common exhaust plenum; may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and have all contaminated ducts and plenums under negative pressure or surrounded by negative-pressure ducts and plenums. If these cabinets are used for minute quantities of volatile toxic chemicals and trace amounts of radionuclides, they must be exhausted through properly functioning exhaust canopies.

Type B1: These Class II BSCs maintain a minimum inflow velocity of 100 feet/minute; have HEPA-filtered, down-flow air composed largely of uncontaminated, recirculated inflow air; exhaust most of the contaminated down-flow air through a dedicated duct exhausted to the atmosphere after passing it through a HEPA filter; and have all contaminated ducts and plenums under negative pressure or surrounded by negative-pressure ducts and plenums. If these cabinets are used for work involving minute quantities of volatile toxic chemicals and trace amounts of radionuclides, the work must be done in the directly exhausted portion of the cabinet.

Type B2 (total exhaust): These Class II BSCs maintain a minimum inflow velocity of 100 feet/minute; have HEPA-filtered, down-flow air drawn from the laboratory or the outside; exhaust all inflow and down-flow air to the atmosphere after filtration through a HEPA filter without recirculation inside the cabinet or return to the laboratory; and have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted negative-pressure ducts and plenums. These cabinets may be used with volatile toxic chemicals and radionuclides.

Class III: The Class III BSC is designed for work with highly infectious microbiological agents and other hazardous operations. It provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a viewing window that is secured with locks and/or requires the use of tools to open. Both supply and exhaust air are HEPA/ULPA filtered. Exhaust air must pass through two HEPA/ULPA filters in series before discharge to the outdoors.

REFERENCES

1. American College of Occupational and Environmental Medicine: ACOEM Task Force on Reproductive Toxicology. Reproductive and developmental hazard management guidance. April 26, 2011.
2. American Society of Clinical Oncology. Worker safety when handling hazardous drugs is focus of statement by oncology societies. March 3, 2015.
3. American Society of Health-System Pharmacists. ASHP guidelines on compounding sterile preparations. *Am J Health-Syst Pharm.* 2014;71(2):145–166.
4. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. *Am J Health-Syst Pharm.* 2006; 63:1172–1193.
5. Baker EL, editor. Surveillance in occupational health and safety. *Am J Public Health.* 1989;79(Suppl.):1–63.
6. Chaffee BW, Armitstead JA, Benjamin BE, Cotugno MC, Forrey RA, Hintzen BL, et al. Guidelines for the safe handling of hazardous drugs: consensus recommendations. *Am J Health-Syst Pharm.* 2010;67(18):1545–1546.
7. Connor TH. Permeability of nitrile rubber, latex, polyurethane, and neoprene gloves to 18 antineoplastic drugs. *Am J Health-Syst Pharm.* 1999;56(23):2450–2453.
8. Connor TH, Mackenzie BA, DeBord DG. Clarification about hazardous drugs. *Am J Health-Syst Pharm.* 2012;69(22):1949–1950.
9. Controlled Environment Testing Association. CETA application guide for informational notes to meet the NSF/ANSI 49:2010a standard requirements CAG-010-2011. July 1, 2011. Raleigh, NC: CETA; 2011.
10. Controlled Environment Testing Association. CETA application guide for the use of surface decontaminants in biosafety cabinets CAG-004-2007. January 30, 2007. Raleigh, NC:ETA;2007.
11. Controlled Environment Testing Association. CETA compounding isolator testing guide, CAG-002-2006. Revised December 8, 2008.Raleigh, NC: CETA; 2008.
12. Controlled Environment Testing Association. CETA CAG-005-2007 servicing hazardous drug compounding primary engineering controls. Raleigh, NC: CETA; 2007.

www.webofpharma.com

13. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories (BMBL) 5th edition. Page last updated: March 13, 2015. Atlanta, GA: CDC, 2015. http://www.cdc.gov/biosafety/publications/bmbl5.
14. Centers for Disease Control and Prevention. Workplace safety and health: *Chemotherapy drug exposures at an oncology clinic—Florida.* June 2012. Atlanta, GA: CDC; 2012 http://www.cdc.gov/niosh/hhe/reports/pdfs/2009-0148-3158.pdf.
15. Centers for Disease Control and Prevention. Hand hygiene in healthcare settings. Page last updated: May 1, 2015. Atlanta, GA: CDC; 2015. http://www.cdc.gov/handhygiene/.
16. Centers for Disease Control and Prevention. NIOSH Alert: preventing occupational exposures to antineoplastic and other hazardous drugs in health care settings Cincinnati, OH: Department of Health and Human Services, CDC; 2004.
17. NIOSH list of antineoplastic and other hazardous drugs in healthcare settings, 2014 Cincinnati, OH: Department of Health and Human Services, CDC; 2014.
18. Centers for Disease Control and Prevention. The National Personal Protective Technology Laboratory (NPPTL). Respirator trusted-source information. Section 3: ancillary respirator information. Atlanta, GA: CDC; 2014. http://www.cdc.gov/niosh/npptl/topics/respirators/disp_part/respsource3.html.
19. Centers for Disease Control and Prevention. Workplace solutions: medical surveillance for healthcare workers exposed to hazardous drugs. Department of Health and Human Services, CDC; 2012.
20. Centers for Disease Control and Prevention. Workplace solutions: personal protective equipment for health care workers who work with hazardous drugs. Cincinnati, OH: Department of Health and Human Services, CDC; 2009.
21. Centers for Disease Control and Prevention. Workplace solutions: safe handling of hazardous drugs for veterinary healthcare workers. Cincinnati, OH: Department of Health and Human Services, CDC; 2010.
22. Goodin S, Griffith N, Chen B, Chuk K, Daouphars M, Doreau C, et al. Safe handling of oral chemotherapeutic agents in clinical practice: recommendations from an international pharmacy panel. *J Oncol Pract.* 2011;7(1):7–12.
23. Hematology/Oncology Pharmacy Association. Ensuring healthcare worker safety when handling hazardous drugs: the joint position statement from the Oncology Nursing Society, the American Society of Clinical Oncology, and the Hematology/Oncology Pharmacy Association. 2015. http://www.hoparx.org/uploads/HOPA_ASCO_ONS_Joint_Position.pdf
24. International Society of Oncology Pharmacy Practitioners Standards Committee. ISOPP standards of practice. Safe handling of cytotoxics. *J Oncol Pharm Pract.* 2007;13 Suppl:1–81.
25. Massoomi F. The evolution of the CSTD. *Oncology Safety* 2015;12(2 suppl).
26. McDiarmid MA, Emmett EA. Biological monitoring and medical surveillance of workers exposed to antineoplastic agents. *Semin Occup Med.* 1987;2(2):109–117.
27. NST International. NSF/ANSI 49-2011. Biosafety cabinetry: design, construction, performance, and field certification. Annex E. 2011.
28. Neuss MN, Polovich M, McNiff K, Esper P, Gilmore TR, LeFebvre KB, et al. 2013 updated American Society of Clinical Oncology/Oncology Nursing Society chemotherapy administration safety standards including standards for the safe administration and management of oral chemotherapy, *Oncol Nursing Forum.* 2013;40(3):225–233.
29. Oncology Nursing Society. Safe handling of hazardous drugs. In: Polovich M, editor. 2nd ed. Pittsburgh, PA: *Oncology Nursing Society;* 2011.
30. Oncology Nursing Society, American Society of Clinical Oncology, and Hematology/Oncology Pharmacy Association. Joint position statement: ensuring healthcare worker safety when handling hazardous drugs. 2015. https://www.ons.org/advocacy-policy/positions/practice/hazardous-drugs
31. Occupational Safety and Health Administration, The Joint Commission, and NIOSH letter to hospitals, April 4, 2011. Washington, DC: United States Department of Labor. http://www.osha.gov/ooc/drug-letter.pdf.
32. Occupational Safety and Health Administration. Hospital respiratory protection program toolkit. OSHA publication number 3767-05 2015.
33. Occupational Safety and Health Administration. Occupational Safety and Health Standards: hazard communication. Washington, DC: United States Department of Labor; 2012. http://www.osha.gov/dsg/hazcom/standards.html.
34. Occupational Safety and Health Administration. Occupational Safety and Health Standards: access to employee exposure and medical records [29 CFR 1910.1020]. Washington, DC: United States Department of Labor.
35. Occupational Safety and Health Administration. Occupational Safety and Health Standards: hazardous waste operations and emergency response [29 CFR 1910.120]. Washington, DC: United States Department of Labor.
36. OSHA Technical Manual Controlling occupational exposure to hazardous drugs. Section VI, Chapter 2. Washington, DC: United States Department of Labor;1999.
37. Occupational Safety and Health Administration. Personal protective equipment. Washington, DC: United States Department of Labor; 2003.
38. Occupational Safety and Health Administration. *Respiratory protection eTool* [29 CFR 1910.134]. Washington, DC: United States Department of Labor.
39. Occupational Safety and Health Administration. Occupational Safety and Health Standards: toxic and hazardous substances [CFR 1910.1200]. Washington, DC: United States Department of Labor; 2013. https://www.osha.gov/SLTC/hazardoustoxicsubstances/index.html
40. Power LA. Closed-system transfer devices for safe handling of injectable hazardous drugs. *Pharm Pract News.* New York: McMahon Publishing Group; June 2013;1-16.
41. Power LA, Sessink PJM, Gesy K, Charbonneau F. Hazardous drug residue on exterior vial surfaces: evaluation of a commercial manufacturing process. *Hosp Pharm.* 2014;49(4):355–362.

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42. Sessink PJM, Trahan J, Coyne JW. Reduction in surface contamination with cyclophosphamide in 30 US hospital pharmacies following implementation of a closed-system drug transfer device. *Hosp Pharm.* 2013;48(3):204–212.
43. University HealthSystem Consortium. UHC consensus statement: model hazardous drug safety plan for institutions. Chicago, IL: UHC; October 2009, revised January 2011.
44. Watts D. Gloves as PPE: standards for permeation and penetration. *Clean Air Containment Rev.* Issue 2;April 2010:16-20.
45. Wesdock JC, Sokas RK. Medical surveillance in work-site safety and health programs. *Am Fam Physician.* 2000;61(9):2785–2790.▲ (RB 1-Dec-2019)

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⟨801⟩ POLAROGRAPHY

Polarography is an electrochemical method of analysis based on the measurement of the current flow resulting from the electrolysis of a solution at a polarizable microelectrode, as a function of an applied voltage. The polarogram (see *Figure 1*) obtained by this measurement provides qualitative and quantitative information on electro-reducible and electro-oxidizable substances. The normal concentration range for substances being analyzed is from 10^{-2} molar to 10^{-5} molar.

In direct current (dc) polarography, the microelectrode is a dropping mercury electrode (DME) consisting of small reproducible drops of mercury flowing from the orifice of a capillary tube connected to a mercury reservoir. A saturated calomel electrode (SCE) with a large surface area is the most commonly employed reference electrode. As the voltage applied to the cell increases, only a very small residual current flows until the substance under assay undergoes reduction or oxidation. Then the current increases, at first gradually, then almost linearly with voltage, and it gradually reaches a limiting value as is shown in *Figure 1*.

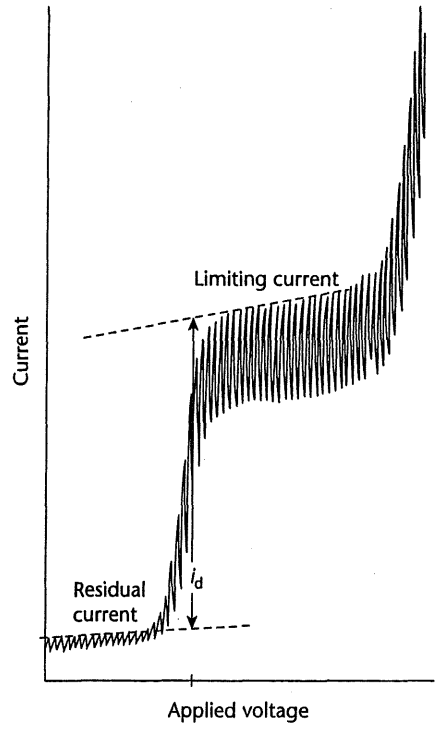


Fig. 1. Typical Polarogram Showing Change in Current Flow with Increasing Potential Applied to the Dropping Mercury Electrode.

On the initial rising portion of the polarographic wave, the increased flow of current results in a decrease in the concentration of the electro-active species at the electrode surface. As the voltage and current increase, the concentration of the reactive species decreases further to a minimal value at the electrode surface. The current is then limited by the rate at which the reacting species can diffuse from the bulk of the solution to the surface of the microelectrode. The final current rise is caused by the reaction of the supporting electrolyte. This large concentration of electrolyte is inert within the potential range used in the analysis, and it prevents the reactive species from reaching the electrode by electrical migration, thus assuring that the limiting current is diffusion-controlled.

Since, in the case of the DME, the electrode surface is being constantly renewed in a cyclic fashion, the current increases from a small value as the drop begins to form to a maximum value as the drop falls. By the use of a suitable recorder to measure the current, the characteristic saw-toothed record is obtained. The limiting current is the sum of the residual and the diffusion currents. The residual current is subtracted from the limiting current to give the wave height.

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ILKOVIC EQUATION

The linear relationship between the diffusion current (i_d) and the concentration of electro-active species is shown by the Ilkovic equation:

$$i_d = 708nD^{1/2}Cm^{2/3}t^{1/6}$$

in which i_d is the maximum current in microamperes; n is the number of electrons required per molecule of electro-active substance; D is its diffusion coefficient, in square cm per second; C is the concentration, in millimoles per L; m is the rate of mercury flow from the DME, in mg per second; and t is the drop time, in seconds.

Modern polarographs are equipped with recorders capable of following the current during the latter portion of the drop life; consequently, the maximum of the oscillations is the measure of the current. When the current is measured only at the end of the drop life, the technique is termed sampled dc polarography. In this case, only the maximum currents are recorded and oscillations due to drop growth are not observed.

For instruments equipped with galvanometers to measure the current or recorders operated in a damped mode, the saw-toothed waves correspond to oscillations about the average current. In the latter case, the average of the oscillations is the measure of the current. For polarograms obtained in this manner, the i_d given by the Ilkovic equation is the average current in microamperes observed during the life of the drop, when the coefficient 708 is replaced by 607.

CONTROL OF THE DIFFUSION CURRENT

The Ilkovic equation identifies the variables that must be controlled to ensure that the diffusion current is directly proportional to the concentration of electro-active material. At 25° the diffusion coefficients for aqueous solutions of many ions and organic molecules increase 1% to 2% per degree rise in temperature. Thus the temperature of the polarographic cell must be controlled to within ±0.5°. The quantities m and t depend upon the dimensions of the capillary and the height of the mercury column above the electrode. Although results obtained with different capillaries can be compared if the product $m^{2/3}t^{1/6}$ is known, it is advisable to use the same capillary with a constant head of mercury during a series of analyses. The diffusion current is proportional to the square root of the height of the mercury column. A mercury reservoir with a diameter greater than 4 cm prevents any significant drop in the mercury level during a series of runs.

The capillary for the DME has a bore of approximately 0.04 mm and a length of 6 cm to 15 cm. The height of the mercury column, measured from the tip of the capillary to the top of the mercury pool, ranges from 40 cm to 80 cm. The exact length of the capillary and the height of the mercury column are adjusted to give a drop-time of between 3 and 5 seconds at open circuit with the capillary immersed in the test solution.

Equipment is available that allows controlled drop-times of fractions of a second to several seconds. As detail within a polarogram is related to the number of drops delivered during a given potential change, such short drop-times allow more rapid recording of the polarogram.

The current flowing through the test solution during the recording of a polarogram is in the microampere range. Thus, the current flow produces negligible changes in the test solution and several polarograms can be run on the same test solution without significant differences.

HALF-WAVE POTENTIAL

The half-wave potential ($E_{1/2}$) occurs at the point on the polarogram one-half the distance between the residual current and the limiting current plateau. This potential is characteristic of the electro-active species and is largely independent of its concentration or the capillary used to obtain the wave. It is dependent upon the solution composition and may change with variations in the pH or in the solvent system or with the addition of complexing agents. The half-wave potential thus serves as a criterion for the qualitative identification of a substance.

The potential of the DME is equal to the applied voltage versus the reference electrode after correction for the iR drop (that potential need to pass the current, i, through the solution with a resistance R). It is especially important to make this correction for nonaqueous solutions, which ordinarily possess high resistance, if an accurate potential for the DME is needed. Correction of the half-wave potential is not required for quantitative analysis. Unless otherwise indicated, it is to be understood that potentials represent measurements made against the SCE.

REMOVAL OF DISSOLVED OXYGEN

Inasmuch as oxygen is reduced at the DME in two steps, first to hydrogen peroxide and then to water, it interferes where polarograms are to be made at potentials more negative than about 0 volt versus SCE, and must be removed. This may be accomplished by bubbling oxygen-free nitrogen through the solution for 10 to 15 minutes immediately before recording the wave, the nitrogen first having been "conditioned" to minimize changes due to evaporation, by being passed through a separate portion of the solution.

It is necessary that the solution be quiet and vibration-free during the time the wave is recorded, to ensure that the current is diffusion-controlled. Therefore, the nitrogen aeration should be stopped and the gas be directed to flow over the surface of the solution before a polarogram is recorded.

In alkaline media, sodium bisulfite may be added to remove oxygen, provided the reagent does not react with other components of the system.

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MEASUREMENT OF WAVE HEIGHT

To use a polarogram quantitatively, it is necessary to measure the height of the wave. Since this is a measure of the magnitude of the diffusion current, it is measured vertically. To compensate for the residual current, the segment of the curve preceding the wave is extrapolated beyond the rise in the wave. For a well-formed wave where this extrapolation parallels the limiting current plateau, the measurement is unambiguous. For less well-defined waves, the following procedure may be used unless otherwise directed in the individual monograph. Both the residual current and the limiting current are extrapolated with straight lines, as shown by the graph (*Figure 1*). The wave height is taken as the vertical distance between these lines measured at the half-wave potential.

PROCEDURE

[**CAUTION**—Mercury vapor is poisonous, and metallic mercury has a significant vapor pressure at room temperature. The work area in which mercury is used should be constructed in such a way that any spilled or spattered droplets of mercury can be completely recovered with relative ease. Scrupulously clean up mercury after each use of the instrument. Work in a well-ventilated laboratory, taking care to clean up any spilled mercury.]

Transfer a portion of the final dilution of the substance being assayed to a suitable polarographic cell immersed in a water bath regulated to 25 ± 0.5°. Pass a stream of nitrogen through the solution for 10 to 15 minutes to remove dissolved oxygen. Start the mercury dropping from the capillary, insert the capillary into the test solution, and adjust the height of the mercury reservoir. Switch the flow of nitrogen to pass over the surface of the solution, and record the polarogram over the potential range indicated in the individual monograph, using the appropriate recorder or galvanometer sensitivity to give a suitable wave. Measure the height of the wave, and unless otherwise directed in the monograph, compare this with the wave height obtained with the appropriate USP Reference Standard, measured under the same conditions.

PULSE POLAROGRAPHY

In conventional dc polarography, the current is measured continuously as potential is applied as a linear ramp (see *Figure 2*).

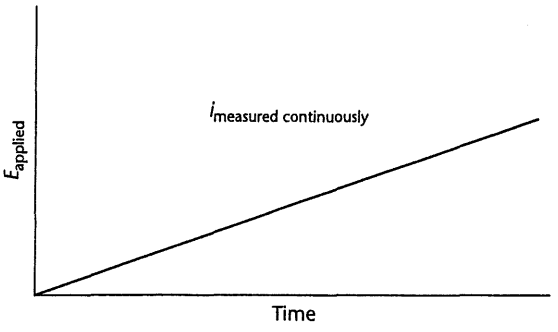


Fig. 2. Direct Current (dc) Polarography.

This current is composed of two elements. The first, the diffusion (faradaic) current, is produced by the substance undergoing reduction or oxidation at the working electrode, and is directly proportional to the concentration of this substance. The second element is the capacitative current (charging of the electrochemical double layer). The changes in these currents as the mercury drop varies in size produce the oscillations present in typical dc polarograms.

In normal pulse polarography, a potential pulse is applied to the mercury electrode near the end of the drop life, with the drop being held at the initial potential during growth period (see *Figure 3*).

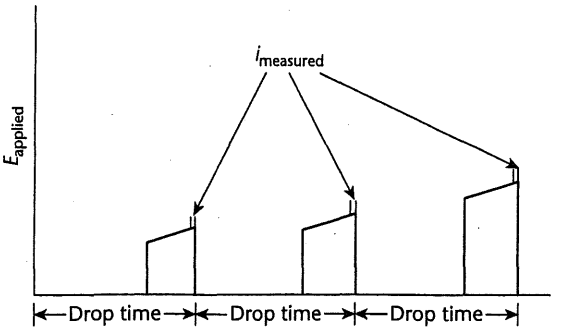


Fig. 3. Pulse Polarography.

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Each succeeding drop has a slightly higher pulse applied to it, with the rate of increase being determined by the selected scan rate. The current is measured at the end of the pulse where the capacitative current is nearly zero, and thus primarily faradaic current is measured (see *Figure 4*).

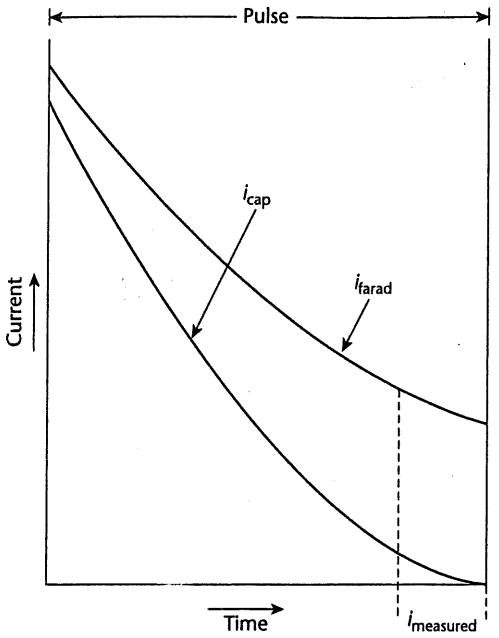


Fig. 4. Plot of Current Versus Time in Pulse Polarography.

In addition, since the pulse is applied for only a short duration, the diffusion layer is not depleted as extensively as in dc polarography and larger current levels are obtained for equivalent concentrations. Concentrations as low as 10^{-6} M can be measured, providing approximately a ten-fold increase in sensitivity over that with dc polarography. Limiting current values are more easily measured, since the waves are free from oscillations.

Differential pulse polarography is a technique whereby a fixed-height pulse applied at the end of the life of each drop is superimposed on a linear increasing dc ramp (see *Figure 5*).

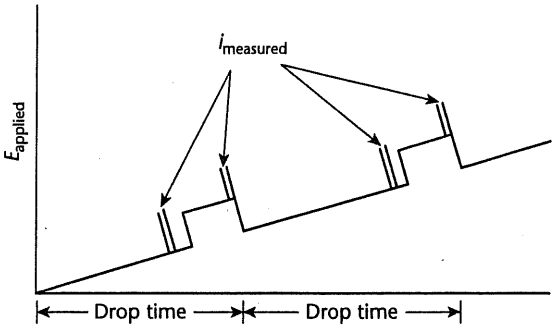


Fig. 5. Differential Pulse Polarography.

Current flow is measured just before application of the pulse and again at the end of the pulse. The difference between these two currents is measured and presented to the recorder. Such a differential signal provides a curve approximating the derivative of the polarographic wave, and gives a peak presentation. The peak potential is equivalent to:

$$E_{1/2} - \Delta E/2$$

where ΔE is the pulse height. The peak height is directly proportional to concentration at constant scan rates and constant pulse heights. This technique is especially sensitive (levels of 10^{-7}M may be determined) and affords improved resolution between closely spaced waves.

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ANODIC STRIPPING VOLTAMMETRY

Anodic stripping voltammetry is an electrochemical technique whereby trace amounts of substances in solution are concentrated (reduced) onto an electrode and then stripped (oxidized) back into solution by scanning the applied voltage anodically. The measurement of the current flow as a function of this voltage and scanning rate provides qualitative and quantitative information on such substances. The concentration step permits analyses at 10^{-7}M to 10^{-9}M levels.

Basic instrumentation includes a voltage ramp generator; current-measuring circuitry; a cell with working, reference, and counter electrodes; and a recorder or other read-out device. Instruments having dc or pulse-polarographic capabilities are generally quite adequate for stripping application. The working electrode commonly used is the hanging mercury drop electrode (HMDE), although the mercury thin-film electrode (MTFE) has acquired acceptance. For analysis of metals such as silver, platinum, and gold, whose oxidation potentials are more positive than mercury, and mercury itself, the use of solid electrodes such as platinum, gold, or carbon is required. A saturated calomel electrode or a silver–silver chloride electrode serves as the reference except for the analysis of mercury or silver. A platinum wire is commonly employed as the counter electrode.

Test specimens containing suitable electrolyte are pipeted into the cell. Dissolved oxygen is removed by bubbling nitrogen through the cell for 5 to 10 minutes.

Generally, an electrolysis potential equivalent to 200 to 300 mV more negative than the half-wave potential of the material to be analyzed is applied (although this potential is to be determined experimentally), with stirring for 1 to 10 minutes. For reproducible results, maintain constant conditions (i.e., deposition time, stirring rate, temperature, specimen volume, and drop size if HMDE is used).

After deposition, the stirring is discontinued and the solution and electrode are allowed to equilibrate for a short period. The potential is then rapidly scanned anodically (10 mV/second or greater in dc polarography and 5 mV/second in differential pulse polarography). As in polarography, the limiting current is proportional to concentration of the species (wave height in dc and pulse; peak height in differential pulse), while the half-wave potential (dc, pulse) or peak potential (differential pulse) identifies the species. It is imperative that the choice of supporting electrolyte be made carefully in order to obtain satisfactory behavior. Quantitation is usually achieved by a standard addition or calibration method.

This technique is appropriate for trace-metal analysis, but has limited use in organic determinations, since many of these reactions are irreversible. In analyzing substances such as chloride, cathodic stripping voltammetry may be used. The technique is the same as anodic stripping voltammetry, except that the substance is deposited anodically and then stripped by a cathodic voltage scan.

⟨811⟩ POWDER FINENESS

The particle size distribution should be estimated by *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩ or by application of other methods where practical. A simple descriptive classification of powder fineness is provided in this chapter. For practical reasons, sieves are commonly used to measure powder fineness. Sieving is most suitable where a majority of the particles are larger than about 75 µm, although it can be used for some powders having smaller particle sizes where the method can be validated. Light diffraction is also a widely used technique for measuring the size of a wide range of particles.

CLASSIFICATION OF POWDER FINENESS

Where the cumulative distribution has been determined by analytical sieving or by application of other methods, powder fineness may be classified in the following manner:

x_{90} = particle dimension corresponding to 90% of the cumulative undersize distribution

x_{50} = median particle dimension (i.e., 50% of the particles are smaller and 50% of the particles are larger)

x_{10} = particle dimension corresponding to 10% of the cumulative undersize distribution

It is recognized that the symbol d is also widely used to designate these values. Therefore, the symbols d_{90}, d_{50}, and d_{10} may be used.

The following parameters may be defined based on the cumulative distribution. $Q_R(x)$ = cumulative distribution of particles with a dimension less than or equal to x where the subscript R reflects the distribution type.

R	Distribution Type
0	Number
1	Length
2	Area
3	Volume

Therefore, by definition:

1. $Q_R(x) = 0.90$ when $x = x_{90}$
2. $Q_R(x) = 0.50$ when $x = x_{50}$
3. $Q_R(x) = 0.10$ when $x = x_{10}$

An alternative but less informative method of classifying powder fineness is by use of the terms in the following table.

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Classification of Powders by Fineness

Descriptive Term	X_{50} (μm)	Cumulative Distribution by Volume Basis, $Q_3(x)$
Coarse	>355	$Q_3(355) < 0.50$
Moderately Fine	180–355	$Q_3(180) < 0.50$ and $Q_3(355) \geq 0.50$
Fine	125–180	$Q_3(125) < 0.50$ and $Q_3(180) \geq 0.50$
Very Fine	≤125	$Q_3(125) \geq 0.50$

⟨821⟩ RADIOACTIVITY

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1. INTRODUCTION

Radiopharmaceuticals and radioactive devices require specialized techniques in their production, testing, handling, dispensing, and administration in order to ensure optimal effectiveness and maintain safety for workers, patients, and the public. Hence, all operations involving these articles should be carried out by or under the supervision of personnel who have been appropriately trained in the handling of radioactive materials and appropriate instruments.

The facilities for the production, storage, and use of radioactive drugs and radioactive devices are generally subject to licensing by the U.S. Nuclear Regulatory Commission or Agreement State agency, or similar governmental agency outside the United States. Most radioactive drugs and radioactive devices are subject to other regulations relating to transportation, environmental release, and workplace safety.

USP monographs for radioactive articles include specifications for radionuclide identification and assay for radioactivity. Moreover, radioactivity counting assemblies or radiation detectors are required instruments for the determination of radionuclidic purity and radiochemical purity. The purpose of this general chapter is to provide standards for measurement of radioactivity, including instrument qualification, performance checks, identification of radionuclides and radionuclidic impurities, and assay of radionuclides.

Information regarding radioactivity is described in *Radioactivity—Theory and Practice* ⟨1821⟩, which includes definitions, types of decay, general considerations relating to radioactive decay, counting, radionuclide production, purity, labeling, and instrumentation for detection and measurement of radioactive emissions, as well as a glossary.

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2. PERSONNEL TRAINING AND DOCUMENTATION

Personnel training should be completed in accordance with written policies and/or procedures. Documentation of the personnel training should be included in the Quality Assurance Program. The length of time to retain the documentation varies depending on the appropriate regulatory requirements.

3. QUALIFICATION OF INSTRUMENTS

Qualification of instruments should be completed in accordance with written policies and/or procedures. Documentation of the qualification of instruments should be included in the Quality Assurance Program. The length of time to retain the documentation varies depending on the applicable regulatory requirements.

3.1 Installation Qualification

Installation qualification (IQ) is to be performed upon installation of an instrument and after major repair. There are two categories in an IQ—visual inspection and operating environment. Visual inspection involves a check of the condition of the instrument to determine if it is ready to use. The inspection confirms that all cables are intact, the instrument is intact with no broken pieces/parts, and all components of the equipment are in working order. The operating environment for the device is checked for proper temperature, humidity, and power requirements. The operating environment should also be checked to avoid high background radioactivity. Temperatures are typically 10°–30° C and relative humidity ranges are generally 20%–90%. Shielding may be necessary to ensure that the background radiation has no negative effect on the instrument performance.

3.2 Operational Qualification

Operational qualification (OQ) is a check of operational specifications for the equipment, including equipment set-up, functional testing of subsystems, and proper overall operation. OQ should describe operational procedures for the equipment and is performed after IQ and after major repairs. OQ should include all appropriate tests to show that the instrument is capable of functioning properly. Typical examples of OQ include clock accuracy, high voltage, zero adjust, background response, and contamination check.

3.3 Performance Qualification

Performance qualification (PQ) demonstrates that the equipment is capable of performing tasks required in the operating environment and provides the intended results. PQ should describe the required performance tasks for the equipment and include all appropriate tests to demonstrate that the instrument is performing within its operating parameters. These PQ tests are done following the OQ tests. Additionally, some or all the PQ tests are performed routinely (e.g., daily, quarterly, semiannually, annually) to demonstrate the instruments continual acceptance for use. Typical PQ tests include constancy and relative response, accuracy, reproducibility (precision), system linearity, determination of "quench" curves in liquid scintillation counters, and supplier equivalence.

3.4 Geometry Testing

The purpose of the geometry test is to demonstrate correct readings of the test sample, accounting for differences in the container, volume, or position of the test sample compared to the radioactive calibration standard. Geometry testing should be performed at installation of the dose calibrator and for each type of vial and syringe used to contain the test sample. Testing should include the test sample holder. Corrections factors may be developed as needed.

4. ONGOING PERFORMANCE TESTS OF INSTRUMENTS

Performance and operational tests are completed periodically to ensure the instrument is capable of meeting specified criteria for accurate assays and that no changes have occurred since initial testing. Tests include physical inspection, measurement of system parameters, and operational tests with radioactive calibration sources. These tests should also be completed after repair. Regulatory agencies may require specific tests at a regular frequency. The following sections describe the performance tests using a dose calibrator as an example. Ongoing performance tests for other instruments used for radiation measurements should be designed as appropriate to each instrument, testing the parameters that are pertinent to ensuring the performance of the instrument, considering its application and the level of precision of measurement required. The same principles will apply to other instruments used for radiation measurements.

4.1 Physical Inspections

Ensure that the dose calibrator and associated accessories, such as source holders and well liners, are undamaged and that foreign objects are not present in the chamber. Prior to use, repair or replace any damaged components as required and remove all foreign objects.

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4.2 System Parameters

The system electronics should be tested according to the manufacturer's diagnostic testing procedures. The results should meet the acceptance criteria detailed in the manufacturer's manual. These parameters typically include the high voltage on the chamber, the zero adjustment on the electrometer, verification of communication between components of the system, and the accuracy of the system clock.

If the manufacturer's diagnostic procedures are not available, then at a minimum the user should check, record, and trend the chamber high voltage and the measured value for the electrometer zero check. Small changes in the high voltage will identify system malfunctions that might not be apparent with the use of radioactive calibration sources, but will begin impacting higher activity readings. The system clock should be checked and should be accurate within one minute.

4.3 Operational Tests

BACKGROUND AND CONTAMINATION TEST

The purpose of this test is to determine the background radiation level and to ensure the absence of contamination that may affect measurements on the dose calibrator. Background radiation may fluctuate due to movement of sources near the chamber, as well as by contamination in or near the chamber, the liner, or the source holder. For this reason, this test should be performed with the liner and the source holder in the chamber. All radioactive sources should be well shielded or at a sufficient distance from the chamber. The background radiation level should be determined and recorded at initial installation, and periodically verified. The background should be recorded at least once on each day of use, or more frequently if necessary, using the most common radionuclide setting. It is especially important to verify if the minimum assayed quantity of radioactivity decreases.

CONSTANCY TEST

The purpose of this test is to ensure the constancy of the dose calibrator over time. This test should be performed on each day of use, and completed after the system tests outlined above. Typical radioactive calibration sources used in this test are Cesium–137 or Cobalt–60 contained within a solid matrix. Cobalt–57 might not be suitable because of its nominal half-life of less than one year. The radioactive calibration source is not required to have the same geometry as routine test samples. A single radioactive calibration source is sufficient for this check. The source is placed in the source holder and the reading recorded for the dedicated radionuclide setting, as well as for all other commonly used radionuclide settings. This ensures no drift in response for any setting. Results should be recorded and be within the appropriate range of the decay corrected value recorded during IQ. Control charts or other data representations should be used to identify and document trends.

ACCURACY TEST

The purpose of this test is to demonstrate the stability of the dose calibrator over the energy range used. This test should be performed annually or after the dose calibrator is repaired or moved. If the dose calibrator is used to measure the quantity of multiple radionuclides, two or three different radioactive calibration sources with different energies should be included to cover the expected energy range of the radionuclides. These calibration sources are typically contained in a solid matrix and do not necessarily match the geometry of routine test samples. If the dose calibrator is only used for a single radionuclide, or only for positron emission tomography (PET) radionuclides, a single calibration source may be adequate. The quantity of radioactivity in the calibration source should be greater than 100 μCi (3.7×10^6 Bq). Sources used for this test should be traceable to a National Metrology Institute (NMI). Measurements of the calibration source activity are recorded and the average measured value is compared to the expected value. The measured value should be within the appropriate range of the expected value.

REPRODUCIBILITY TEST

The purpose of this test is to measure the precision of the dose calibrator and should be performed at least annually. The radioactive calibration source should have a half-life such that decay corrections are not required over the period of the test. The quantity of radioactivity in the calibration source should be greater than 100 μCi (3.7×10^6 Bq). An appropriate number of consecutive readings are recorded using the same geometry. Each of the measurements taken should be within an appropriate range of the mean of all the measurements.

LINEARITY TEST

The purpose of this test is to show that the response of the dose calibrator is linear across the range of radioactivity levels to be measured. This is especially critical at high levels of radioactivity where the measured radioactivity may differ from the true radioactivity due to saturation effects, and at low levels of radioactivity due to variations on the background radiation and changes in the source positioning. This test should be performed annually or after the dose calibrator has been repaired or moved.

The test is considered successful if the ratio of the measured radioactivity to the expected activity is within the appropriate range of the expected values. Three techniques may be employed for this test: decay, graded shield, and graded source. Use of graded shields is acceptable once an initial linearity test using the decay method is successfully completed. Refer to the manufacturer's recommendations on the use of the graded shields. The graded source method requires accurate measurements of volume or mass, and is not generally recommended.

Table 1 is an example of ongoing performance checks for dose calibrators.

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Table 1. List of Performance Tests

Parameter	Daily[a]	Annually (at a minimum)
Physical inspection	Yes	N/A
System electronics	Yes	N/A
Clock accuracy	Yes	N/A
High voltage	Yes	N/A
Zero adjust	Yes	N/A
Background response/contamination check	Yes	N/A
Radioactive calibration source (constancy and relative response)	Yes	N/A
Accuracy	See footnote[b]	Yes
Reproducibility (precision)	See footnote[b]	Yes
System linearity	See footnote[b]	Yes

[a] At the beginning of each day of use.
[b] This is required after repair.

5. IDENTIFICATION OF RADIONUCLIDES

5.1 Half-Life Determination

The half-life is an identifying characteristic of a radionuclide. The half-life is determined by measuring the quantity of radioactivity in the test sample as a function of time. The approximate half-life may be used to confirm the radionuclidic identity. Perform the measurements using an appropriately calibrated instrument, such as a quantitative radioactivity detector, provided the quantity of radioactivity is within the linear range of the instrument throughout the measurements and the geometry of the test sample is not changed during the measurements.

The test sample may be used directly, or diluted and/or dried in a capsule as needed. The test sample is prepared in a manner that avoids losses during handling. If the test sample is a liquid or solution, the sample is held in a closed container. If the test sample is a residue from drying in a capsule, the sample is protected by a cover consisting of a sheet of adhesive cellulose acetate or other material. The quantity of radioactivity in the test sample should be sufficient to allow measurements over the time frame of the test. If necessary, correction for dead-time losses may be applied. The measured half-life should be within the range of the expected half-life as defined in each particular application of this test.

5.2 Gamma-Ray Spectrometry

The gamma-ray emission spectrum may be used to identify and quantify gamma-emitting radionuclides. The detector should be calibrated with a radioactive calibration source traceable to a NMI. The calibration source used to determine the efficiency of the detector should have a sufficient quantity of radioactivity to produce an adequate number of counts for each photopeak used in the calibration. The calibration source and the test sample should be measured with the same geometry and distance from the detector. It is critical that the geometry, including vial type and volume, be the same because these factors affect the amount of incident radiation on the detector. Photopeaks in the test sample should be comparable to those in the calibration source, both in terms of energy and intensity. The presence of unknown peaks or changes in peak abundance is indicative of impurities. Ensure that background radiation levels remain constant during the measurement, as changes may result in unknown peaks. Counting time or shielding should be optimized to achieve the necessary required minimum detectable activity to meet test specifications. It may be necessary to allow the radioactivity in the test sample to decay before it is possible to detect impurities at the required levels. There may also be short-lived impurities present, so an analysis should be completed to identify impurities and their half-lives to ensure the sample is assayed prior to their decay. Positron-emitting radionuclides typically cannot be differentiated because their emitted energy (511 keV) is the same for each radionuclide; thus, gamma-ray spectrometry is not a recommended prerelease test for radionuclidic identity for PET radionuclides. Some positron-emitting radionuclides have characteristic gamma-ray emissions in addition to 511 keV, which may be used for purposes of identification (e.g., Ge-69 and Na-22). Performance tests of the gamma-spectroscopy system should be completed on each day of use to ensure the functionality of the system.

5.3 Beta-Emitter Analysis and Identification

The purpose of the beta-emitter analysis and identification is to determine the maximum energy of the beta particle, which is unique and can be used for identification. Beta-emitting radionuclides can be identified either by evaluation of their spectrum or by measuring their mass attenuation coefficients in a series of absorption foils and constructing an attenuation curve. Performance tests of the beta counting system should be completed on each day of use to ensure the functionality of the system.

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5.4 Alpha Spectrometry

The alpha emission spectrum may be used to identify and quantify alpha-emitting radionuclides. Pure alpha emitters present unique challenges due to their low penetration distance and their toxicity, especially if they are volatile compounds that could create airborne radioactivity and therefore internal radiation exposure to the experimenter or operator. Alpha particles can be detected either by the use of proportional counters, scintillation detection using a silver-activated zinc sulfide phosphor, or by the techniques of liquid scintillation counting (LSC). Performance tests of the alpha spectroscopy system should be completed on each day of use to ensure the functionality of the system.

6. ASSAY OF RADIONUCLIDES

6.1 Measurement of Radioactivity Using a Dose Calibrator

Using a holder, the test sample is placed in the chamber of the dose calibrator at a given position. Once the response is stable, the radioactivity reading is taken. Performing measurements on the test sample with the same geometry as the calibration source provides the most accurate results; however, geometry correction factors may be developed and used as necessary. Refer to the manufacturer's recommendations for the maximum radioactivity that can be determined in the dose calibrator.

6.2 Measurement of Radioactivity Using a Solid-State Detector

The test sample is positioned in front of the detector or into the well of a well-type detector. The detector must be adequately shielded and calibrated using a radioactive calibration source traceable to an NMI.

6.3 Measurement of Radioactivity Using Chromatographic Systems

A scintillation detector can be used for dynamic radioactivity measurements (e.g., the eluate of a liquid chromatograph is directed over or through a detector). The quantity of radioactivity in the test sample should provide a count rate that is within the linear range of the detector.

6.4 Measurement of Radioactivity Using a Liquid Scintillation Counter

LSC is the standard laboratory method to quantify radioactivity of particle-emitting radionuclides, mostly beta- and alpha-emitting radionuclides. The sample to be analyzed may require a liquid scintillation fluid (cocktail) depending on the energy of the beta particle. The LSC detection method uses liquid scintillation cocktails to transform emitted radiation into detectable light photons. The sample to be analyzed is placed into a liquid scintillation vial and the cocktail is added in the required amount. While a sample that can be dissolved into the fluid is preferred, other samples can be assayed if the impact on the light output is quantified. The detector should be calibrated using an appropriate radioactive calibration source traceable to an NMI. The absorption of scintillation photons is called quench, and it is necessary to develop a "quench curve" during PQ and additional quench curves may be necessary for different types of samples to correct the counting efficiency. Performance tests of the LSC should be completed on each day of use to ensure the functionality of the system.

GLOSSARY

The following definitions apply to commonly encountered words and phrases when dealing with radioactivity.

Alpha particles (α)**:** Positively charged particles emitted from nuclei during radioactive decay. Alpha particles are Helium-4 nuclei, consisting of two protons and two neutrons, but no electrons.

Beta particles (β^-)**:** Negatively charged particles that are emitted from nuclei during radioactive decay. Beta particles are electrons.

Calibration factor: The coefficient used to convert the measured ionization chamber current to a nominal radioactivity. This term is often referred to as the "calibration coefficient".

Calibration time: Arbitrary time and date, if appropriate at which the specified amount of radioactivity is present.

Counting assembly: Consists of a sensing unit and an electronic scaling device. The sensing unit may be a Geiger-Müller tube, a proportional counter, a scintillation detector in which a photomultiplier tube is employed in conjunction with a scintillator, or a solid-state semiconductor.

Dose calibrator: A well-type ionization chamber commonly used to assay radiopharmaceuticals. Display units are typically in curies (μCi/mCi/Ci) or becquerels (kBq/MBq/GBq).

Gamma rays (γ-rays)**:** Electromagnetic radiation emitted from nuclei during radioactive decay. Gamma rays have a wide range of energies. The gamma rays emitted from a given radionuclide are always at the same energy(ies) providing a unique signature that enables the identification of a gamma-emitting radionuclide.

Geiger-Müller counter: Often referred to as "G-M counter" or "Geiger counter". An instrument in which a high voltage potential is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The negative electrons are internally multiplied to produce a readily detectable current pulse. Display units are typically counts per minute (cpm) or milliroentgen per hour (mR/hr).

Geometry: The characteristics of a radioactive source (i.e., container type, container wall thickness, volume, and position of the container in the well chamber) that, along with the physical characteristics of the ionization chamber, affect the magnitude of the calibration coefficient for a specific radionuclide. The principal geometry considerations that may affect the

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accuracy of a source measurement in a dose calibrator are container configuration, source volume, position of the source in the chamber well, and the radionuclide itself.

[NOTE—It is customary to compare a standardized preparation and radiopharmaceutical drug or preparation using identical geometry conditions for assay, identification, and other parameters. The validity of the result is critically dependent upon the reproducibility of the spatial relationships of the source to the detector and its surroundings and upon the accuracy of the standardized preparation.]

Ionization chamber: An instrument in which an electric field is applied across a volume of inert gas for the purpose of collecting ions produced by a radiation field. The positive ions and negative electrons drift along the lines of force of the electric field, and are collected on electrodes, producing an ionization current. The most commonly used form of ionization chambers for measurement of the activities of radiopharmaceuticals is a well-type ionization chamber known as a dose calibrator.

Isobars: Nuclides with the same mass number (protons + neutrons).

Isomers: Atoms with the same number of protons and neutrons, but a different nuclear energy configuration. Short-lived radioactive isomers are generally referred to as metastable. Different isomers are different nuclides based on their nuclear energy configurations.

Isotones: Nuclides with the same number of neutrons and a different number of protons. Isotones are different elements with different atomic masses.

Isotopes: Nuclides with the same number of protons and a different number of neutrons. Isotopes are the same element with a different atomic mass.

Isotopic carrier (also referred to as carrier)**:** A stable isotope of the element concerned either present in or added to the radioactive preparation in the same chemical form as the radionuclide.

Liquid scintillation counter (LSC): An instrument which detects scintillation light from the absorption of radiation energy in a scintillation liquid. This instrument is used primarily for beta-emitting radionuclides that do not also emit gamma photons. For best results, the radioactive sample should be soluble in the scintillation liquid.

Minimum detectable activity: Smallest quantity of radioactivity that can be detected above the background with a specified level of confidence.

National Metrology Institute (NMI)**:** A measurement standards body, which is a laboratory of metrology that establishes standards for a country or organization. For example, National Institute of Standards and Technology (NIST) is the NMI for the United States.

Nuclide: An atom with a specific number of protons and neutrons in a given nuclear energy state.

Positrons (β^+)**:** Positively charged particles emitted from a nucleus during radioactive decay. Positrons are anti-electrons.

Radioactivity: (1) The spontaneous transformation of nuclei characterized by the emission of particles or photons. Radioactivity is typically described as atoms undergoing radioactive decay per unit time (or disintegrations per unit time). (2) The quantity of radioactive material, as measured in units of curies (U.S. units) or becquerels (SI units). The quantity of radioactive material may also be referred to as activity.

Radiochemical identity: The molecular structure of the intended active radiopharmaceutical ingredient, which is present in the radiopharmaceutical preparation.

Radiochemical purity: The ratio, expressed as a percentage, of the radioactivity of the intended active radiopharmaceutical ingredient to the total radioactivity of all radioactive ingredients and impurities present in the radiopharmaceutical preparation.

Radioisotope: A radioactive atom, generally used in the context of an isotope of an element.

Radionuclide: An unstable nuclide that undergoes radioactive decay; a radioactive nucleus. The terms radionuclide and radioisotope are commonly used interchangeably.

Radionuclidic identity: The intended radionuclide in the radiopharmaceutical preparation.

Radionuclidic purity: The ratio, expressed as a percentage, of the radioactivity of the intended radionuclide to the total radioactivity of all radionuclides in the radiopharmaceutical preparation.

Radiopharmaceutical (radiopharmaceutical preparation/radioactive drug)**:** A finished dosage form that contains a radioactive substance in association with one or more other ingredients and that is intended to diagnose, stage a disease, monitor treatment, or provide therapy. A radiopharmaceutical includes any nonradioactive reagent kit or radionuclide generator that is intended to be used in the preparation of any such substance. The terms radiopharmaceutical and radioactive drug are commonly used interchangeably.

Scintillation crystal counter: An instrument consisting of a crystal scintillator, such as NaI (Tl), with attached photomultiplier tube and associated electronics. Scintillation light produced from the absorption of gamma and X-rays in the crystal is converted to electrons and amplified in the photomultiplier tube. The resultant current pulse may be further analyzed with regard to photon energy. A commonly used form of this instrument that has a hole in the crystal of sufficient size to allow placement of a test tube or similar container is known as a "well counter".

Semiconductor detector: An instrument consisting of a semiconductor material, such as silicon or germanium crystals, that detects ionizing radiation through generation of charge carriers (passage of electrons through holes). The current pulse produced by migration of these charge carriers, under the influence of a voltage potential across the material, can be further amplified and analyzed to determine the quantity and energy of the incident radiation.

Solid-state detector: A crystal-based detector, in contrast to a gas-based detector. Often is used as a synonym for a semiconductor detector.

Strength: The radioactivity concentration of the radiopharmaceutical at the calibration time. The unit of strength is the amount of radioactivity on a volume basis (e.g., mCi/mL or MBq/mL).

Total radioactivity: The radioactivity of the radionuclide, expressed per unit (e.g., vial, capsule, ampoule, generator, and others) at the calibration time.

Validation: Establishment of documented evidence that a method, process, or system meets its intended requirements.

Verification: Confirmation that an established method, process, or system meets predetermined acceptance criteria.

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X-rays: A type of electromagnetic radiation emitted from the electron orbitals. While they do not arise from the nucleus they are often present immediately following a decay event if there are interactions between the emitted radiation and the orbital electrons.

REFERENCES

1. American Society for Testing and Materials (ASTM). Active Standard ASTM D3648. Standard practices for the measurement of radioactivity. West Conshohocken, PA: ASTM International; 2011.
2. American Association of Physicists in Medicine (AAPM). AAPM Report No. 181. The selection, use, calibration, and quality assurance of radionuclide calibrators used in nuclear medicine. College Park, MD: AAPM; June 2012.

⟨823⟩ POSITRON EMISSION TOMOGRAPHY DRUGS FOR COMPOUNDING, INVESTIGATIONAL, AND RESEARCH USES

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GLOSSARY

1. INTRODUCTION

Radionuclides used in positron emission tomography (PET) typically possess short physical half-lives, $T_{1/2}$ (e.g., $T_{1/2}$ of ^{15}O = 2.03 min, ^{62}Cu = 9.67 min, ^{13}N = 9.96 min, ^{11}C = 20.4 min, ^{68}Ga = 67.7 min, ^{18}F = 109.8 min, ^{64}Cu = 12.7 h). As a result, these radionuclides usually are produced using particle acceleration techniques (e.g., cyclotrons) or from generators, and then are processed into the final PET drug product in close proximity to the site where the PET procedure will be conducted.

The short half-lives of PET radionuclides create unique constraints for the preparation and testing of PET drug products. This chapter describes guidelines for making and testing PET drug products based on the following constraints:

- It is not possible to complete all testing before the use of PET drug products.
- An entire batch or sub-batch of a PET drug product may be contained in a single vial. Samples withdrawn for quality control (QC) testing are representative of the entire batch or sub-batch.
- An entire batch or sub-batch may be administered to a single patient.
- The mass of the PET drug in a PET drug product usually ranges from nanogram to microgram quantities.
- PET drug products do not enter a traditional drug distribution chain. Instead, PET drug products are used in-house or are delivered to the point of use by dedicated couriers.
- Small-scale facilities for the preparation of PET drug products have limited personnel and resources, which require the following:
    - Allowance for multiple operations in one area with adequate controls;

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- Allowance for the making and testing of multiple PET drug products using shared equipment;
- Appropriate requirements for aseptic operations;
- Appropriate requirements for system suitability and other day-of-use activities;
- QC requirements for components, materials, and supplies;
- Self-verification of significant steps in radionuclide production, PET drug production, or compounding and testing; and
- Single-person oversight of production and compounding, review of batch records, and release authorization.

The scope of this chapter includes the production and compounding of PET drug products for human administration as used (a) according to state-regulated practice of medicine and pharmacy, (b) according to an approved investigational new drug (IND) application (see 21 CFR 312), and (c) according to research uses under the supervision of a Radioactive Drug Research Committee (RDRC; see 21 CFR 361). The scope of this chapter does not include dispensing activities as defined in other USP general chapters.

2. PERSONNEL

Sufficient numbers of personnel with the appropriate education, training, and experience are needed for the preparation and testing of PET drug products. The number depends on the size and complexity of the operations executed at each facility.

2.1 Training Requirements

Personnel should be trained before they begin to make and test PET drug products. Training can be performed by various methods, including live instruction, audio-video instruction, and study of publications. Training should address but is not limited to radionuclide production techniques, synthetic and purification methods, materials, components, reagents, stock solutions, automated and manual apparatus used to make PET drug products, and QC methods, including equipment, software, and documentation. Training must be documented.

2.2 Aseptic Operations Training

Training should address aseptic manipulations as well as the techniques and equipment used to achieve and maintain International Organization for Standardization (ISO) Class 5 environmental conditions. Training also should address all aseptic operations, including the assembly of sterile components, compounding, and filtration. Manipulations of sterile solutions should be performed by operators who are qualified to use aseptic techniques (see *Facilities and Equipment* below).

Personnel involved in aseptic operations should be evaluated periodically by aseptic simulations in which a microbiological growth medium is used to assess the quality of the aseptic operation. Aseptic simulations should provide the following:

- Include all manipulations required for the aseptic assembly of the PET drug product vial assembly (e.g., vial, filter, and syringe assembly, etc.).
- Represent worst-case scenarios for aseptic operations.
- Be performed in triplicate to qualify a new operator. Each operator should be requalified annually by conducting at least one media fill.
- Be performed any time procedures are changed significantly.

After the simulation process, the media should show the absence of contamination after incubation at a suitable temperature for 14 days. An operator who fails written assessments or whose aseptic simulations result in microbial growth should be immediately re-instructed and re-evaluated to ensure correction of aseptic practice deficiencies.

3. QUALITY ASSURANCE

QA is a broad concept that covers all matters that influence identity, strength, quality, and purity of a PET drug product. QC is a subset of QA that deals with testing of materials and PET drug products to determine if they meet acceptance criteria. The QA function typically consists of oversight activities, and the QC function consists of execution activities.

QC functions include the following.

- Evaluate each lot of incoming material to ensure that it meets its established specifications before use in the preparation or testing of PET drug products.
- Evaluate each batch of a PET drug product to ensure the batch meets its established specifications before authorizing the final release of the batch.

The oversight functions associated with QA include the following:

- Review completed batch records for accuracy and completeness.
- Approve procedures, specifications, processes, and methods.
- Ensure that personnel are properly trained and qualified, as appropriate.
- Ensure that PET drug products have adequately defined identity, strength, quality, and purity.
- Ensure that changes to component quality, suppliers, changes to production procedures, and changes to testing procedures and specifications are appropriate and implemented properly.
- Investigate errors and ensure that appropriate corrective and preventive actions are taken to prevent their recurrence.
- Handle complaints.

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- Ensure that the PET drug products are produced, tested, labeled, released, and distributed according to the facility's established procedures and practices for PET drug products.
- Conduct periodic audits to monitor compliance with established procedures and practices for PET drug products.

Personnel at the facility may perform both QA and QC functions.

4. FACILITIES AND EQUIPMENT

Facilities should be adequate for the production, compounding, and testing of PET drug products. Work areas should be organized to prevent cross-contamination, mix-ups, and errors, especially in areas used for making multiple PET drug products. Work areas should be periodically cleaned to prevent the contamination of equipment, materials, components, or PET drug products by personnel or environmental conditions that could reasonably be expected to adversely affect PET drug product quality. These requirements should be described in written procedures, and their routine execution should be documented.

4.1 Environmental Controls for Parenteral PET Drug Products

Because the sterility test results for parenteral PET drug products are obtained after release, facilities and equipment should ensure a sterile PET drug product.

ASEPTIC WORKSTATION

The primary environmental control for aseptic operations is a high-efficiency particulate air (HEPA) filter that is capable of producing air with a cleanliness rating of ISO Class 5. This can be achieved with a laminar airflow workstation, aseptic isolator, biological safety cabinet, or other suitable device (generically, aseptic workstations). The aseptic workstation should be protected from sources of microbial contamination and should be located in an area where personnel traffic is limited. The area around the aseptic workstation should not be used for storage of materials that shed large quantities of particulate matter (e.g., corrugated boxes).

The proper operation of the aseptic workstation must be certified by measurement of airborne particles, HEPA filter integrity testing, pressure differential testing, or other means. The specific tests depend on the type of aseptic workstation. Certification should be performed at the inception of operation and at least annually thereafter or after repair or replacement of the HEPA filter. These requirements supersede those in other USP general information chapters (e.g., *Microbiological Control and Monitoring of Aseptic Processing Environments* ⟨1116⟩).

The work area inside the aseptic workstation should be clean. The internal surfaces should allow easy cleaning and disinfection. The internal surfaces should be cleaned and disinfected with appropriate disinfectants that are sterile filtered or certified sterile with a manufacturer's certification.

MICROBIOLOGICAL TESTING

Microbiological testing of the environment should be performed to assess air quality and surface disinfection of the aseptic areas. This can be achieved by either settling plates or active air-sampling plates. Surface disinfection of critical surfaces (e.g., the work surface of the aseptic workstation or operators' fingers) should be assessed with swab or contact plates. For microbiological testing of the aseptic workstation, the air should be tested as part of the workstation qualification (e.g., every six months) and the surface (using swab or contact plates) should be assessed after use, each day of use. Nonviable particle counts may be determined less frequently following certification of the *Aseptic workstation* (see above).

Alert and action limits should be established for samples obtained during microbiological testing. Typical alert levels are set at less than three colony-forming units (cfu) per plate. More than three cfu require corrective actions that may include operator retraining, recertification of the aseptic workstation, or other actions. The results of microbiological testing also should be used in the investigation of positive sterility tests.

4.2 Equipment

Equipment used to make and test PET drug products should be appropriate for its intended purpose and should be installed, cleaned, and maintained in an appropriate manner. Equipment should be capable of producing consistent results.

The following requirements should be described in written procedures, and performance of these procedures should be documented.

1. *Installation of New Equipment*—Newly installed equipment should be qualified before it is used to make or test PET drug products at an appropriate level of detail based on complexity. All qualification activities should be properly documented, including the date and the name of the person who performed the qualification. For more complex equipment, qualification consists of three phases:
   - Installation Qualification (IQ)—IQ is a check of items required for proper installation of the equipment, including physical location, required utilities and supplies, communications, and environmental conditions. IQ should describe the installation procedure for the equipment.
   - Operational Qualification (OQ)—OQ is a check of operational specifications for the equipment, including equipment set-up, functional testing of subsystems, and proper overall operation. OQ should describe operational procedures for the equipment.
   - Performance Qualification (PQ)—PQ demonstrates that the equipment is capable of performing tasks required to make and test PET drug products in the operating environment and that the equipment provides the intended results. PQ should describe the required performance tasks for the equipment.

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2. *Calibration of Equipment*—Analytical equipment calibration should be performed before use, as appropriate. A schedule should be developed for recalibration and should have a sufficient frequency to ensure accurate results. Calibration activities should be properly documented, including the date and the name of the person who performed the calibration.
3. *Preventive Maintenance of Equipment*—A preventive maintenance schedule should be developed for major production and testing equipment, including automated chemistry modules, gas chromatographs, high-performance liquid chromatographs, and others. The schedule should have a sufficient frequency to minimize equipment downtime. Major repairs may require recalibration and requalification. Preventative maintenance activities should be properly documented, including the date of such performance and the name of the person who performed them.

4.3 Cleaning Equipment and Components

Equipment used in production or compounding of PET drug products includes automated, computer-controlled devices, as well as manually operated apparatus. Before it is used in making PET drug products, equipment should be properly cleaned to ensure that the resulting PET drug product meets established specifications for identity, strength, quality, and purity (see *Controls and Acceptance Criteria for Finished PET Drug Products* below). Once cleaned, equipment should be maintained in a state of cleanliness before use.

Equipment may be used to make multiple batches of one or more PET drug products. Documented studies should demonstrate the effectiveness of the cleaning process between batches. All impurities should be controlled at levels that conform to established specifications for identity, strength, quality, and purity. Written procedures for line clearance between batches of different PET drug products should describe routine execution of cleaning processes.

4.4 Day-of-Use Checks

Day-of-use checks are necessary for processing equipment to ensure proper function. Written procedures for the day-of-use checks should be established and followed. These procedures should be designed to check key parameters at the beginning of each operational cycle (e.g., temperature, pressure integrity, gas supply, vacuum supply, proper delivery line selection, reagent delivery volumes, gas flow rates, radiation monitors, and other process sensors). Some parameters may be periodically checked as part of the calibration and preventive maintenance schedules as described above.

4.5 System Suitability for QC Equipment

System suitability tests are necessary for QC equipment to ensure that the equipment, components, and personnel (i.e., the system) function as a whole to execute the desired analytical method. System suitability tests should be performed prior to using the equipment according to established procedures. Written procedures should be established and followed for system suitability tests, and the test results should be documented.

The system suitability tests required for chromatographic methods include tailing factor, replicate injections, and resolution. When the test chromatogram used for system suitability contains only a single peak, then tailing factor, replicate injections, and column efficiency (theoretical plates) are adequate. The use of internal or external standards with a known concentration is necessary for these determinations. Standards should be prepared from well-characterized materials or from materials that have a manufacturer's certification. Two acceptable approaches that may be used for chromatographic methods are the following:

1. Create a calibration curve from a range of standards with known concentrations. The concentrations of the standards should bracket the conditions of use for the chromatographic method. The calibration curve should be used over a suitably specified period of time (e.g., six months), after which time a new one should be created. A new calibration curve should be created each time an alteration is made to the chromatographic system. Routine system suitability for replicate injections consists of a single injection of a known standard and a measurement of the concentration based on the calibration curve. If the measured concentration agrees with the known concentration within a predefined range (e.g., 10% for manual injections and 5% for automated injections), this demonstrates the suitability of the system for replicate injections and ensures that the calibration curve is appropriate for use in subsequent sample injections. The tailing factor and resolution (or column efficiency, as appropriate) should be determined from the same chromatogram.
2. At the beginning of each testing cycle, create a single-point calibration from two injections of a known standard. The measured area of the peaks for these injections should agree within a predefined range (e.g., 10% for manual injections and 5% for automated injections). Then the results are averaged and used with the standard concentration to provide a calibration factor that is used in subsequent sample injections for that day. The tailing factor and resolution (or column efficiency as appropriate) should be determined from one of the two chromatograms.

Other chromatographic parameters such as signal-to-noise ratio, limit of detection, and limit of quantitation can be determined as part of routine system suitability testing.

System suitability tests also may be appropriate for other QC equipment, including dose calibrators, scanners for radio–thin layer chromatography (radio–TLC), and multichannel analyzers. When used, these tests should be performed at installation, relocation, and appropriate intervals thereafter. These tests should use known standards to demonstrate the proper function of the equipment, for example:

1. *Dose Calibrator*—Accuracy, geometry, and linearity should be assessed at installation and at appropriate intervals thereafter. The instrument should be calibrated in accordance with nationally recognized standards or the manufacturer's instructions. Routine system suitability testing should include a constancy check with a suitable high-energy radionuclide source.
2. *Radio–TLC Scanner*—Uniformity, positional accuracy, detector linearity, and resolution should be assessed with a suitable radionuclide source. Routine system suitability testing should include checks for these parameters.

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3. *Multichannel Analyzer*—Sensitivity and resolution should be assessed at installation and at appropriate intervals thereafter. Routine system suitability testing should include a constancy check with a suitable high-energy radionuclide source.

5. CONTROL OF COMPONENTS, MATERIALS, AND SUPPLIES

Components, materials, and supplies that are used in the preparation of PET drug products should be controlled to avoid contamination, mix-ups, and errors. A designated person should be responsible for ensuring that these activities are carried out and completed properly. Records of completed examinations and tests for components, materials, and supplies should be maintained for one year after their expiration or for one year after batch release, whichever is longer. The following activities should be established and performed:

1. Establish written specifications for the identity, strength, quality, and purity of ingredients, reagents, target materials, and gases.
2. Establish written specifications for the identity and quality of sterile empty vials, transfer lines, sterile stopcocks, sterile needles, sterile membrane filters, and other components used in the PET drug product vial assembly.
3. Establish written specifications for the identity, strength, quality, and purity of analytical supplies (e.g., solvents, chromatography columns, and authentic standards), sterility test media, and endotoxin test reagents used in the testing of PET drug products.
4. Establish appropriate storage conditions (based on heat, light, humidity, and other factors) for components, materials, and supplies used to make and test PET drug products.
5. Store components, materials, and supplies in a controlled-access area according to established storage conditions. Segregate components, materials, and supplies as appropriate to avoid mix-ups and errors.
6. Log each lot of shipment of components, materials, and supplies, and record the date of receipt, quantity received, manufacturer, manufacturer's lot number, and expiration date. If no expiration date is designated by the manufacturer, assign one based on knowledge of its physical and chemical properties and previous experience with its use. For organic substrates and reagents that are potentially susceptible to degradation or to a change in composition, the expiration date should be based on the material's stability.
7. Determine that each lot of components, materials, and supplies complies with established written specifications. Compliance with specifications can be demonstrated by inspection of the labeling or inspection of the manufacturer's certification. The identity of each lot of components, materials, and supplies should be verified by defined procedures, tests, or documented manufacturer's certification, as appropriate. Perform an identity test for precursors (e.g., melting point determination or other appropriate tests). Alternatively, the manufacturer's certification can be used as the only acceptance criterion for a precursor if final testing of the PET drug product ensures that the correct precursor has been used. Reference standards used in chromatographic procedures should have suitable documentation of identity and purity. Other components can be accepted on the basis of a manufacturer's certification only.
8. Membrane filters used with parenteral PET drug products should have a manufacturer's certification. Examine the manufacturer's certification for each lot to ensure compliance with written specifications.
9. Media used in the sterility testing of PET drug products may be obtained from commercial sources. If the media is obtained from commercial sources, then growth-promotion testing that uses a suitable single species of organism should be performed on initial qualification of the supplier and periodically (e.g., quarterly) thereafter.

6. PROCESS AND OPERATIONAL CONTROLS

6.1 Process Controls

The following process controls should be established and summarized in a master formula for the PET drug product. A designated person should be responsible for ensuring that these activities are carried out and completed properly.

1. Written acceptance criteria for the identity, strength, quality, and purity of each PET drug product should be established. For PET drug products intended for parenteral administration, specifications should include sterility and bacterial endotoxins. If a *USP* monograph exists or if there are specifications that have been previously accepted by the appropriate regulatory agency (e.g., FDA), then these standards, if applicable, may be applied as the minimum acceptance criteria.
2. Written procedures for the preparation of each PET drug product should provide the following:
   - Incorporate, for each PET drug product intended for parenteral administration, sterile membrane filtration (0.22 µm) or steam sterilization;
   - Incorporate, for each PET drug product intended for inhalation, particulate filtration (0.45 µm);
   - Describe routine cleaning procedures for equipment and facilities;
   - Describe components, materials, and supplies used to make PET drug products, including precursors, standards, reagents, stock solutions, and related items;
   - Describe the process and the steps used to make the PET drug product;
   - Describe the formulation process, including the use of stabilizers, buffers, and other agents;
   - Describe calculations performed for quantitative parameters associated with making and QC testing the PET drug product (e.g., including radiochemical yield, radiochemical purity, specific activity, solvent amounts, etc.);
   - Describe QC tests for the final PET drug product (see *Controls and Acceptance Criteria for Finished PET Drug Products* below), including a schedule that defines whether or not each test should be performed on each batch and that states if the test results should be complete at the time of release.

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3. The quality of each batch of a PET drug product should be verified by full finished product testing prior to use to ensure the product meets all specifications.
4. In cases where testing as described in the previous paragraph is not possible or impractical, the quality of a PET drug product may also be ensured by documented validation studies in lieu of prerelease tests. Such studies should provide the following:
   - Demonstrate a consistent process that is suitable for the intended preparation of the PET drug product;
   - Be completed on three batches made according to the master formula, and all three batches should meet all acceptance criteria;
   - Include evaluation of radiochemical identity and purity, radionuclidic identity and purity, specific activity, sterility (for parenteral PET drug products), bacterial endotoxins (for parenteral PET drug products), pH, appearance, stereochemical purity (for applicable compounds), residual solvents, other toxic chemicals that may have been used during the synthesis or purification procedure, effective concentration of a stabilizer (if any), chemical purity of the PET drug product, and equivalence of initial and final sub-batches (see *Definitions* above);
   - Be repeated if the process and steps described in the master formula have been altered in a way that could change the identity, strength, quality, or purity of the PET drug product;
5. The processes and steps described in the master formula should be updated as needed and should be reviewed annually to ensure they are current. Prior to the implementation of updates, appropriate validation and/or verification should be approved and performed.

Appropriate controls of computer-controlled equipment should ensure that process changes are instituted only by authorized personnel and that such changes are documented and verified. Production, compounding, and test methods should be backed up and controlled to avoid accidental use of outdated methods. In the case of processes or test methods from a vendor that are used without alteration, it is acceptable to rely on vendor certification for software verification and proper operation.

6.2 Operational Controls

The following operational controls should be established and summarized in a batch record that is a subset of the master formula for the PET drug product. The batch record should adequately document the routine process for making the PET drug product. A designated person should be responsible for ensuring that these activities are carried out and completed properly. Completed batch records and associated documentation should be maintained for one year after batch release.

1. Execute suitable line clearance procedures to avoid mix-ups and cross-contamination, including the inspection of areas used to make and test PET drug products, and the inspection of all equipment for cleanliness and suitability before use. Remove extraneous materials and labels from these areas and equipment.
2. Ensure the correct identity, strength, quality, and purity of components, materials, and supplies used in the preparation of the PET drug product. Label components as appropriate for identity and traceability purposes.
3. Execute routine cleaning procedures for equipment and facilities.
4. Prepare the PET drug product according to the current master formula, and for each batch maintain a batch record. Batch records may consist of paper documents, electronic records, or combinations thereof. Spreadsheets and other electronic recordkeeping tools should be verified to ensure traceability, data integrity, accuracy of results for calculations, and so on. The batch record should include the following:
   - Lot numbers or other unique identifiers for all components, materials, and supplies used to make the PET drug product;
   - A description of the individual procedures that were followed;
   - The initials, signature, or other identifier of the responsible individual indicating that critical steps and processes used to make and test the PET drug product were completed;
   - The percent yield calculated on the basis of the known or expected amount of the starting radionuclide that is synthetically incorporated into the PET drug product;
   - Raw analytical data on each batch of the PET drug product;
   - Labeling for the PET drug product (see *Labeling* below);
   - Calculations for key parameters defined in the master formula;
   - Results obtained from QC tests of the PET drug product, including chromatograms, print-outs, and other test data;
   - The initials of the analyst who performed each QC test;
   - A notation of the result for each QC test and whether or not the result meets the acceptance criteria;
   - The date and time of release and the signature of the individual who assumes overall responsibility for, and adherence to, the procedures used to make the batch and authorizes the release of the batch for human administration; and
   - Documentation on the batch record of process deviations, when applicable.

Entries in batch records should be made immediately after the activity is performed and should include the initials, signature, or other identifier for the person making the entry. Corrections to paper entries should be dated and initialed, signed, or noted with an identifier of the person making the corrections but leaving the original entry still readable.

6.3 Aseptic Operations for Parenteral PET Drug Products

Because the sterility test results for parenteral PET drug products are obtained after release for human administration, aseptic operations and procedures should adequately ensure a sterile PET drug product. All aseptically prepared PET drug products for parenteral administration should be filtered through a sterile membrane filter of 0.22-µm or finer pore size into a closed sterile

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vial or container or sterilized by steam sterilization. Although the chemical synthesis of a parenteral PET drug product may take place in an open or closed apparatus, the membrane filtration of the PET drug product should be a closed system downstream of the membrane filter. This system should be aseptically assembled from presterilized, commercially available components.

COMPONENTS

The sterile components used in the aseptically assembled apparatus typically consist of an empty vial, needles, membrane filters, vent needles, syringes, tubing, stopcocks, and perhaps others. All components should be single-use, commercially available, presterilized items. If components in the aseptically assembled apparatus are sterilized by the PET facility, the sterilization processes should be verified. The exact configuration of the PET drug product vial assembly is process dependent. A typical example is a sterile, empty vial with a membrane filter of 0.22-µm pore size attached to a needle that is inserted through the vial septum for filtration, a membrane filter of 0.22-µm pore size attached to a needle that is inserted through the vial septum for venting the vial during filtration, and a syringe with needle inserted through the vial septum for removal of the QC sample after filtration is complete.

PET DRUG PRODUCT VIAL ASSEMBLY

Aseptic techniques should be used in the preparation of the PET drug product vial assembly, especially the assembly of all components downstream from the membrane sterilizing filter. These operations should be performed in an ISO Class 5 environment (see *Facilities and Equipment* above).

Following the creation of the PET drug product vial assembly in the ISO Class 5 environment, the assembly can be removed to another location for filtration. The location can be a noncontrolled environment as long as the integrity of the PET drug product vial assembly is not compromised during the process. Any PET drug product vial assembly that is compromised during this process should be discarded.

ASEPTIC TECHNIQUES

Any sterile component downstream from the membrane filter that contacts the PET drug product should be handled using suitable aseptic techniques inside the aseptic workstation. During aseptic operations, operators should wear proper attire, including a clean laboratory jacket, forearm sleeves, hair cover, sanitized gloves that cover the wrist, and beard/moustache covers (as appropriate). Multiple PET drug product vial assemblies can be prepared in a single aseptic operational cycle. The storage conditions and time for assembled vials should be based on data from aseptic simulations.

STERILITY TEST INOCULATIONS

Sterility tests should be performed to assess the quality of PET drug products intended for parenteral administration. The inoculation of sterility test media should be performed in a manner that is consistent with personnel radiation exposure requirements but that also minimizes the risk of false positives caused by adventitious contamination during the inoculation process. For media tubes with a screw-cap opening, the inoculation should be performed in the aseptic workstation. Media tubes with a septum cap can be inoculated in a shielded area that does not contain a HEPA filter.

7. STABILITY

Written specifications for the expiration time and storage conditions should be established for each PET drug product. The expiration time should be based on the results of stability testing (and specific activity requirements, as appropriate). Stability testing of the PET drug product should be performed at the highest strength of the PET drug product and in the intended final vial or container. At least three batches of the PET drug product should be stored according to proposed conditions and should be examined after a time period equal to the proposed shelf life. In addition, the PET drug product should meet acceptance criteria for radiochemical purity, appearance (color and clarity), pH, and stabilizer effectiveness (as appropriate) and chemical purity at expiry. Analytical methods should be reliable, meaningful, and specific. Stability studies should be repeated if there is a change in strength, stabilizer (or preservative) content that has the potential to affect the stability, the final vial or container, storage conditions, or expiration time. The results of stability testing should be documented.

8. CONTROLS AND ACCEPTANCE CRITERIA FOR FINISHED PET DRUG PRODUCTS

Written specifications for identity, strength, quality, and purity should be established for each PET drug product. For PET drug products intended for parenteral administration, specifications should be included for sterility and bacterial endotoxins.

Written procedures should be developed for QC tests. QC and documentation requirements should be established for each batch or sub-batch of a PET drug product (see *Process and Operational Controls* above). All QC tests should be executed by qualified and trained personnel according to written procedures.

The short half-life of PET radionuclides frequently precludes the completion of all QC tests before shipment of the PET drug product. This effectively creates two levels of release, one for distribution and the other for human administration. This is acceptable as long as the QC tests required for release of the PET drug product for human administration (see below) are completed before administration. The controls used in the release for distribution should be previously established in writing and should be documented in routine practice. It is not necessary to retain reserve samples of PET drug products.

If a *USP* compendial test procedure is used, the procedure should be verified to demonstrate that the test works under the conditions of actual use. Noncompendial test procedures used in the testing of a PET drug product should be reliable and specific. Supporting data for use of all analytical methods should be documented. Data derived from process studies or from

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in-process controls can be used as a basis for the omission of some QC tests. An example of this approach is the chlorodeoxyglucose determination in the testing of [^{18}F]fludeoxyglucose. Supporting data from process studies or in-process controls should be documented.

8.1 Quality Control Tests

The following QC tests should be performed on each batch before release for administration:

1. Appearance by visual inspection for color and clarity (absence of particulate matter) for parenteral dosage forms.
2. Measurement of the pH for parenteral dosage forms.
3. Determination of the radiochemical purity and identity of all dosage forms.
4. Determination of the radionuclidic identity of all dosage forms by half-life measurement.
5. Determination of the strength.
6. Determination of the specific activity of PET drug products that have mass-dependent localization or toxicity concerns.
7. Determination of residual solvents used in the synthesis or purification processes.
8. Determination of the chemical purity and residual compounds used in the synthesis or purification processes (e.g., cryptand [2.2.2]).
9. Determination of preservative or stabilizer, if present.

For PET drug products with very short-lived radionuclides, prepare an initial QC sub-batch that is representative of successive sub-batches prepared in a defined operational cycle. The QC tests described in the previous paragraph should be considered for the QC sub-batch before release of subsequent sub-batches for human administration. For subsequent sub-batches of parenteral and inhaled dosage forms, visual inspection should be performed before human administration. In certain cases, limited testing of each sub-batch before administration may be appropriate (e.g., for pH determination of [^{13}N]ammonia produced by Devarda's alloy).

8.2 Periodic Quality Indicating Tests

For all PET drug products, periodically measure the radionuclidic purity of decayed samples of the PET drug product to assess the presence of long-lived radionuclides that are produced in targetry associated with the particle accelerator. For PET drug products labeled with certain radionuclides (e.g., ^{94m}Tc, ^{124}I, ^{64}Cu, ^{76}Br, and others), consider the measurement of radionuclidic purity by gamma spectrometry. Periodic quality indicating tests for PET drug products also include low-level nontoxic impurities (e.g., Class 3 residual solvents). The periodic testing should be performed at predetermined intervals rather than on a batch-to-batch basis.

8.3 Microbiological Tests for Sterile PET Drug Products

For PET drug products intended for parenteral administration, perform the following QC tests in addition to those described previously:

1. Determine the integrity of the membrane filter. Filter units used to sterilize PET drug products should be subjected to manufacturers' recommended integrity tests such as the bubble point test. Perform the filter integrity test after completion of filtration and before release of the PET drug product for human administration. In the case of PET drug products with $T_{1/2} < 10$ min, the PET drug product can be released for human administration before completion of the filter integrity test. In this case, the test should be completed as soon as possible after release.
2. Perform a test for bacterial endotoxins on each batch or QC sub-batch of a PET drug product. The test can be performed using recognized procedures in *USP* (see *Bacterial Endotoxins Test* ⟨85⟩). Regardless of which test is used, it should be initiated before release of each batch for human administration. For PET drug products with very short-lived radionuclides, complete the test on the QC sub-batch before the release of subsequent sub-batches for human administration. After a record of successful bacterial endotoxin tests is established for a particular PET drug product, it is necessary only to test the first batch prepared each day for that PET drug product.
3. Perform a test for sterility on each batch or QC sub-batch. The sterility test consists of the inoculation and incubation of a sample into each of two media: tryptic soy broth and fluid thioglycollate. The inoculated volume may be adjusted to avoid excessive losses because of sterility testing (e.g., 0.1 mL inoculated into 10 mL of media). The incubation period for sterility tests should begin within 30 hours of the membrane filtration. The samples can be inoculated immediately after completion of the membrane filtration, or they can be allowed to decay in a shielded area for as long as 30 hours before inoculation. It is acceptable to exceed the 30-hour period because of weekends or holidays provided it is shown that the extended period does not significantly reduce the viability of a suitable indicator organism in the sample. The sterility test may be performed using other recognized procedures in *USP* (see *Sterility Tests* ⟨71⟩). Samples should be tested individually and may not be pooled. After a record of successful sterility tests is established for a particular PET drug product, it is only necessary to test the first batch prepared each day for that PET drug product.

8.4 Conditional Final Release Tests

When a required QC test for a PET drug product cannot be completed because of a malfunction of testing equipment, it may be appropriate to conditionally release the batch. PET drug products may not be released without determination of radiochemical identity and purity. The batch may be released if the following conditions are met:

1. Review historical QC data to assess the frequency of out-of-specification (OOS) results or failures associated with the QC test. A conditional release is appropriate only if the historical data reveal a record of successful completion of the QC test.

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2. Confirm that the acceptance criteria are met for all other QC tests for the batch.
3. Retain a sample of the conditionally released batch.
4. Promptly correct the malfunction of the testing equipment.
5. Complete the omitted QC test on the sample as soon as possible after the malfunction has been corrected. This is not necessary if the omitted QC result is meaningless after decay of the PET drug product.
6. If the sample fails the omitted QC test, immediately notify the physician or receiving facility that ordered the PET drug product.
7. Document all actions regarding the conditional release of the PET drug product, including the justification for the release, results of completed testing, and any notifications and corrective and preventive actions resulting from the incident.

In addition to the finished QC testing, other appropriate laboratory determinations could involve in-process testing of an attribute that is equivalent to finished-product testing of that attribute; continuous statistical process monitoring; or some combination of these approaches with finished testing of each PET drug product.

9. IF A PET DRUG PRODUCT DOES NOT CONFORM TO SPECIFICATIONS

When the result of a QC test for a PET drug product does not meet established acceptance criteria, the result is OOS. An OOS result does not necessarily mean that the final PET drug product is a failure and should be rejected. Instead, an OOS investigation should be performed to determine if the OOS result indicates a true failure or an analytical error.

If an OOS investigation concludes that the OOS result was caused by an analytical error, invalidate the original test. If a printout is associated with this test, mark the printout *invalid*, retain it for the batch record, and repeat the test.

If an OOS investigation concludes that the OOS result was a true failure, the batch should be rejected and cannot be released for human administration. Segregate the batch to avoid its potential use. Investigate all failures and document the results according to written procedures. The investigation should include, but is not limited to, the examination of processes, operations, and records from previous batches, as well as complaints and other relevant sources of information. If possible, assign an actual or probable cause to the failure, and document corrective actions undertaken as a result of the investigation. Depending on the nature of the failure, the PET drug product may be reprocessed according to pre-established written procedures (see *Reprocessing* below).

When a sterility test for a PET drug product shows signs of microbial growth, the test result is OOS and should be investigated. Upon completion of the investigation, immediately notify all receiving facilities if the product fails to meet the criterion for sterility, including the microbiological findings from the investigation.

10. REPROCESSING

If a PET drug product is rejected as a true failure, the batch may be reprocessed according to established procedures. It is not possible to describe all possible reprocessing operations, but some examples could include the following:

- pH adjustment;
- A second passage through a membrane filter in the event of a failed filter integrity test; and
- A second passage through a purification column to remove an impurity.

If a PET drug product is reprocessed, the reprocessed batch should be tested to ensure it meets the established acceptance criteria for the PET drug product before release for human administration.

11. LABELING

The following information should appear on the label attached to the final PET drug container:

- The name of the PET drug product, including the dosage form;
- The assigned batch number; and
- Any required warning statements or symbols (e.g., investigational use, radioactive).

The following information should appear on the shielding for the PET drug product:

- The name of the PET drug product, including the dosage form;
- The assigned batch number;
- The date and time of calibration;
- Any required warning statements or symbols (e.g., investigational use, radioactive);
- As appropriate, the total radioactivity in MBq (or mCi) or the strength in MBq/mL (or mCi/mL) at time of calibration;
- Expiration time and date;
- Added substance(s) (e.g., stabilizer inactive ingredients);
- The name of the producer where the PET drug product was made or the name of the distributor;
- Other applicable warning statement(s) (e.g., "Do not use if cloudy or if it contains particulate matter" or investigational use labeling); and
- Other pertinent information (if required), such as storage condition(s), half-life.

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GLOSSARY

Batch: A quantity of PET drug product that is intended to have uniform character and quality, within specified limits, and that is made in a single operational cycle produced according to one or more production order(s).

Conditional final release: A final release for patient administration before completion of required tests because of a malfunction of analytical equipment.

Lot: A quantity of materials (e.g., reagents, solvents, gases, purification columns, and other auxiliary materials) that have uniform character and quality within specified limits and are used to make a PET drug product.

PET drug: A radioactive substance (active pharmaceutical ingredient) that exhibits spontaneous disintegration of unstable nuclei by the emission of positrons and is incorporated into a PET drug product to furnish direct effect in the diagnosis or monitoring of a disease or a manifestation of a disease in humans, or monitoring treatment of disease or therapeutic procedures (e.g., tumor therapy).

PET drug product: A finished dosage form that contains a PET drug, whether or not in association with one or more other ingredients.

Compounding: The practice as described in the Food, Drug and Cosmetic Act (1997) Chapter II, Section 121 (a) (ii) (1) (B) of synthesizing or formulating a PET drug product, by or on the order of a practitioner who is licensed by a State to compound or order compounding for a PET drug product, and is compounded in accordance with that State's law, for a patient or for research, teaching, or quality control.

Line clearance: The segregation and cleaning of different processing and work areas to avoid cross-contamination and mix-ups between the production and/or compounding of different PET drug products.

Manufacturer's certification: Documentation, including, but not limited to, certificates of analysis, certificates of conformance, or certificates of quality obtained from the manufacturer, supplier, or vendor of a material or component that describes critical quality characteristics used to determine acceptability of use.

Out of specification (OOS): A quality control test result for a PET drug product that does not conform to established acceptance criteria.

Production: The process of synthesis or formulation of a PET drug product including processing, packaging, labeling, reprocessing, and testing for investigational or research use.

Quality assurance (QA): A planned system for ensuring that a PET drug product possesses defined identity, strength, quality, and purity required for its intended purpose by procedures, tests, and analytical methods.

Quality control (QC): A system for testing the quality of components, materials, supplies, and PET drug products by procedures, tests, analytical methods, and acceptance criteria.

Specific Activity: The radioactivity of a radionuclide per unit mass of the element or compound. The unit of specific activity is radioactivity per mass expressed on a gram or mole basis (e.g., mCi/μg [MBq/μg], Ci/mmol [GBq/mmol]).

Strength: The radioactivity concentration of the PET drug in the PET drug product on a volume basis at the time of calibration. The unit of strength is the amount of radioactivity per volume at the time of calibration (e.g., mCi/mL [MBq/mL]).

Sub-batch: A quantity of PET drug product having uniform character and quality, within specified limits, that is produced during one succession of multiple irradiations using a given synthesis or purification operation. A group of sub-batches collectively form a batch that is intended to have uniform character and quality, within specified limits. Sub-batches may be required for PET drug products with very short-lived radionuclides (e.g., ^{13}N and ^{15}O) because QC tests cannot be completed before use.

Validation: Establishment of documented evidence that a method, process, or system meets its intended requirements.

Verification: Confirmation that an established method, process, or system meets predetermined acceptance criteria.

Add the following:

▲⟨825⟩ RADIOPHARMACEUTICALS—PREPARATION, COMPOUNDING, DISPENSING, AND REPACKAGING

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APPENDIX

1. INTRODUCTION

Radiopharmaceuticals, as defined in this chapter (see *Glossary*), are a subset of radioactive materials (RAMs) falling under the control of the US Nuclear Regulatory Commission (NRC) or NRC-contracted agreement state agency. Radiopharmaceuticals are also a subset of prescription drugs falling under the control of the US FDA for manufacturing and marketing. Other federal regulatory authorities (e.g., Department of Transportation) have control over certain activities related to radiopharmaceuticals. Compliance with these regulations, as applicable, must be ensured in addition to compliance with the standards described in this chapter. [NOTE—Users outside the US must comply with equivalent regulations, as applicable, pertaining to radiopharmaceuticals.]

This chapter is intended to provide uniform minimum standards for the preparation, compounding, dispensing, and repackaging of sterile and nonsterile radiopharmaceuticals for humans and animals that occur as part of state-licensed activities (e.g., the practice of pharmacy and the practice of medicine). These standards apply to all radiopharmaceutical processing activities, including those with radionuclides that emit a single photon, a positron, or a therapeutic particle. Furthermore, these standards apply to sterile intravascular radioactive devices (e.g., radioactive microspheres for intravascular brachytherapy).

This chapter does not apply to the following activities:

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- Manufacturing of approved radiopharmaceuticals (e.g., NDA, ANDA, BLA) in FDA-registered manufacturing establishments
- Manufacturing of radiopharmaceuticals as investigational agents (e.g., IND, RDRC)
- Compounding of radiopharmaceuticals in a registered FDCA §503B outsourcing facility
- Preparation/compounding of positron emission tomography (PET) drugs that are not manufactured as approved drug products (e.g., NDA, ANDA, BLA) and conforms with *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* ⟨823⟩
- Administration of radiopharmaceuticals to patients

In each of these scenarios except for patient administration, the further processing and manipulation of the drug product after release falls within the scope of this chapter.

This chapter does not apply to the preparation of non-radioactive drugs, including those used as pharmacologic adjuncts for certain nuclear medicine procedures. These drugs must be prepared following standards described in *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩ and *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩.

This chapter applies to all practice settings where radiopharmaceuticals are prepared, compounded, dispensed, or repackaged. Practice settings consist of state-licensed nuclear pharmacies, federal nuclear pharmacy facilities, and other healthcare facilities, including, but not limited to: nuclear medicine departments in hospitals and clinics, nuclear cardiology clinics (fixed site or mobile), and other specialty clinics.

This chapter applies to all individuals who prepare, compound, dispense, or repackage radiopharmaceuticals. Applicable individuals consist of authorized nuclear pharmacists (ANPs) and authorized user (AU) physicians, as well as individuals working under their supervision. This includes, but is not limited to, student pharmacists, nuclear pharmacy technicians, nuclear medicine technologists and students, and physician residents and trainees.

US federal and state radiation regulatory authorities require limiting radiation exposure to personnel who handle radiopharmaceuticals, which necessitates special provisions for radiation protection. The principles of radiation safety involve time, distance, shielding, and radioactive contamination control. Moreover, the use of radiation detection and measuring devices is a necessary component of radiopharmaceutical handling procedures. Strict adherence to all typical aseptic handling practices is not possible in many scenarios where radiopharmaceuticals are handled. Thus, it is necessary to balance aseptic handling practices (patient safety) with radiation protection practices (worker safety). This chapter describes appropriate strategies that provide assurance of maintaining patient safety, while also ensuring the safety of individuals performing these activities. Because radiopharmaceuticals represent a unique class of prescription drugs, the use of technologies, techniques, materials, and procedures other than those described in this chapter are not prohibited so long as they are documented to be equivalent or superior to those described herein.

1.1 Nonsterile Radiopharmaceuticals

Examples of nonsterile radiopharmaceuticals include oral capsules and oral solutions. For conventionally manufactured products or compounded preparations obtained from 503B-registered outsourcing facilities, dispensing can proceed as described in *12. Dispensing*. For prepared or compounded preparations, such preparations must comply with applicable identity, quality, and purity standards, as described in manufacturer labeling, *USP* monographs, or other appropriate sources (e.g., documented, peer-reviewed materials). They can then be dispensed as described in this chapter.

1.2 Sterile Radiopharmaceuticals

Examples of sterile radiopharmaceuticals include injectables (e.g., intravenous, intrathecal, intraperitoneal, subcutaneous, and intradermal), inhalations, ophthalmics, and intra-organ instillations. For conventionally marketed products, see *12. Dispensing*. For prepared or compounded preparations, such preparations must comply with applicable identity, quality, and purity standards. For compounded preparations involving one or more nonsterile components, a sterilization procedure (e.g., filtration with bubble point testing) must be performed prior to dispensing. For injectable compounded preparations involving one or more components that are not certified to be pyrogen-free, bacterial endotoxin testing, as defined in *Bacterial Endotoxins Test* ⟨85⟩, must be performed prior to dispensing.

The most important factor for maintaining sterility is the avoidance of touch contamination. Wipe the vial septum with sterile 70% isopropyl alcohol (IPA) prior to initial needle puncture. If the vial shield top is then closed, the septum must be disinfected again with sterile 70% IPA prior to another needle puncture. Some vial shields are constructed such that the vial septum is recessed and difficult to access. One approach for disinfecting the vial septum in this type of vial shield is to use right-angle forceps to hold a sterile 70% IPA wipe and apply direct contact with the vial septum. It is also acknowledged that such vial shields disrupt first air contacting the vial septum during certain handling conditions. Wipe the septum with sterile 70% IPA frequently whenever multiple punctures are occurring (e.g., removing several individual doses from a multiple-dose container).

2. RADIATION SAFETY CONSIDERATIONS

The handling of radiopharmaceuticals necessitates meeting the radiation regulatory agency requirements for worker safety. This involves licensing commitments to keep all exposure levels for the workers involved as low as reasonably achievable (ALARA) practices. Principles of radiation safety involve time, distance, shielding, and contamination control. Moreover, radiation detection and measuring devices are necessary. Aseptic handling practices must be balanced with radiation safety considerations, based on the following:

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- Knowledge, training, experience, and professional judgment related to the type, abundance, and energy of the radioactive emissions
- The quantity of radioactivity, volume, handling steps, and timing
- Other factors, which can vary on a case-by-case basis

2.1 Time

Radiation exposure to personnel is directly proportional to the quantity of radiation handled and the time handling the RAM; minimizing handling time will minimize radiation exposure. Personnel handling radiopharmaceuticals may work quickly in a controlled and safe manner, including multiple hand movements in and out of the ISO Class 5 primary engineering control (PEC) during aseptic processes.

2.2 Distance

Radiation exposure follows the inverse square law; increasing the distance between the operator and the RAM will decrease radiation exposure to personnel by the square of the distance. Handlers of radiopharmaceuticals may utilize techniques to increase distance, such as using remote handling tools, including within an ISO Class 5 PEC.

2.3 Shielding

Radiation exposure to personnel decreases with the use of shielding materials. Therefore, handlers of radiopharmaceuticals may use various shielding materials (e.g., lead, tungsten) in various configurations. The use of shielding, such as L-block, torso, vial, and syringe shields, is usually required throughout the radiopharmaceutical handling process, including within an ISO Class 5 PEC.

2.4 Radiation Contamination Control

RAM contamination (e.g., spills, drips, sprays, volatility) is an important concern for radiation protection. Therefore, various techniques and materials may be used by handlers of radiopharmaceuticals to minimize radioactive contaminations. For example, container contents are maintained at neutral or negative pressure, because positive pressure in a container is a common cause of radioactive contamination. Disposable absorbent pads are commonly used to contain such radioactive contamination and, when used in an ISO Class 5 PEC, the pads must be clean and low-lint. Vertical air flow, not horizontal, in a PEC is used to control contamination. When exposure to blood and other potentially infectious material is reasonably anticipated, some engineered needlestick prevention devices may pose a radiation hazard to employees. Policies must be implemented for handling biohazardous radioactive sharps while minimizing contamination.

RADIATION DETECTORS AND MEASURING DEVICES

Radiopharmaceuticals require measurement with a suitable radiation measuring device (e.g., dose calibrator). These and other necessary equipment, (e.g., monitors, bar code scanner, label printer) may be placed inside an ISO Class 5 PEC but should be placed in a manner that minimizes disruptions of airflow.

As per RAM license requirements, individuals must wear body and, as required, extremity dosimeters (e.g., a ring worn on a finger) for long-term monitoring of personnel radiation exposure. The body dosimeter should be worn underneath the gown. Any extremity dosimeter must be worn underneath gloves and must not interfere with proper fit of gloves.

3. IMMEDIATE USE OF STERILE RADIOPHARMACEUTICALS

The preparation and dispensing of sterile radiopharmaceuticals in a patient care setting may be handled as an immediate use practice. The information below describes the appropriate handling requirements for immediate use sterile radiopharmaceuticals in an ambient environment that lacks primary and secondary engineering controls (SEC) when intended for a single patient. Strict aseptic technique and limited beyond-use date (BUD) must be adhered to given the lack of engineering controls.

- Appropriate for preparation (including minor deviations) and/or dispensing that is limited to use for a single patient.
- Preparation (including preparations with minor deviations) components must be sterile, conventionally manufactured drug products (e.g., NDA, ANDA).
- Dispensing of drug products produced under an approved IND or RDRC protocol is allowed.
- Manipulations for any unit doses (e.g., decreasing the dosage, needle changes) or dispensing for one patient (e.g., withdrawing a dose) is allowed.
- Must be administered within 1 hour of the first container puncture or exposure of any critical site involved (e.g., syringe tip, needle hub or needle) to ambient air, whichever is first.
- All components involved (e.g., Tc-99m sodium pertechnetate syringe or vial, final prepared radiopharmaceutical kit vial, diluent vial) must be discarded within 1 hour of being punctured or after use for a single patient administration, whichever is first.

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- Dose pooling (combining doses from two or more syringes to meet one patient's need) may be performed as immediate use. Any residual activity that remains must be immediately discarded and not utilized for any other patient.
- Follow hand hygiene and garbing in *4.4 Hand Hygiene and Garbing for Immediate Use Preparations*.
- Follow *10.4 Preparation of Radiolabeled Red Blood Cells for Immediate Use* for red blood cell labeling.
- Follow *12.2 Labeling* for labeling.
- Area for sterile preparation and/or dispensing must be functionally separate from nonsterile compounding area (e.g., radiolabeling food) during the time of use.
- Does not require a segregated radiopharmaceutical processing area (SRPA), classified area, or PEC.
- The number of steps or punctures is not limited.
- Does not require personnel to complete the aseptic qualifications as detailed in *4.1 Aseptic Qualifications* (e.g., aseptic technique training with documented assessment, media fill challenge, gloved fingertip testing).
- While adding a non-radioactive, sterile and commercially manufactured pharmaceutical (e.g., lidocaine) to a unit dose is otherwise considered compounding, it is allowed for immediate use purposes as long as all of the above are adhered to.
- Dose splitting (splitting a unit dose for administration to more than one patient) may not be performed as immediate use; if performed, dose splitting must be done in an ISO class 5 PEC in either an SRPA or in an ISO class 8 or better buffer area.

4. PERSONNEL QUALIFICATIONS, TRAINING, AND HYGIENE

Personnel must be trained to work with radiopharmaceuticals per the policies and standard operating procedures (SOPs) authorized by an ANP or AU physician. These individuals (e.g., nuclear medicine technologists or nuclear pharmacy technicians) must follow these policies and SOPs of the ANP or AU physician and work under their supervision. As appropriate, this should include blood-borne pathogens training.

Individuals entering a compounding area must be properly garbed and must maintain proper personal hygiene to minimize the risk of contamination to the environment and/or radiopharmaceuticals. Individuals who have a condition that may pose a higher potential of contaminating the radiopharmaceutical and the environment with microorganisms (e.g., rashes, sunburn, recent tattoos, oozing sores, conjunctivitis, or active respiratory infection) must report these conditions to their supervisor. The designated person is responsible for evaluating whether these individuals should be excluded from working in sterile processing areas before their conditions are resolved.

4.1 Aseptic Qualifications

Personnel must prove competency, as applicable to their job functions, prior to performing radiopharmaceutical aseptic tasks that are beyond immediate use. These qualifications may be conducted at a different site if all SOPs are identical for the applicable job function. These qualifications must be completed and documented initially, and then successfully repeated at intervals described below in *Timing of Reevaluation and Requalification* under the observation of a designated person and include the following:

- Aseptic technique training with a documented assessment (written or electronic)
- Garbing and hand hygiene, as defined by the policies and SOPs
- PEC cleaning and disinfecting
- Gloved fingertip and thumb sampling
- Media-fill testing

GLOVED FINGERTIP AND THUMB SAMPLING

Appropriate garbing, including sterile gloves, is necessary for personnel who enter and perform tasks in an ISO Class 5 PEC (e.g., aseptic manipulations, cleaning the PEC). Personnel that perform such functions must prove their competency in this process. Gloved fingertip and thumb sampling must be performed initially on both hands, immediately following hand-hygiene and garbing. Successful completion of initial gloved fingertip and thumb sampling is defined as zero colony-forming unis (cfu) and subsequent gloved fingertip and thumb sampling after media-fill testing is defined as ≤3 cfu (total for both hands).

- The gloved fingertip and thumb sampling must be performed with touch plates or other devices (e.g., plates, paddles, or slides) that contain a general microbial growth agar [e.g., trypticase soy agar (TSA) soybean–casein digest media] supplemented with neutralizing additives (e.g., lecithin and polysorbate 80) as this supports both bacterial and fungal growth
- Gloves must not be disinfected immediately before touching the sampling device, as this could cause a false-negative result
- Using a separate sampling device for each hand, a gloved fingertip and thumb sample from both hands must be collected by rolling finger pads and thumb pad over the agar surface
- The plates must be incubated in an incubator at 30°–35° for no less than 48 h, and then at 20°–25° for no less than 5 additional days

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MEDIA-FILL TESTING

Media-fill testing is necessary for all personnel who prepare, compound, dispense, and repackage sterile radiopharmaceuticals. This testing must be reflective of the actual manipulations to be carried out by the individual and must simulate the most challenging and stressful conditions to be encountered in the worker's duties.

- Media-fill tests must be documented as defined by the facility's policies and SOPs.
- Media-fill tests should be performed at the end of a work session in the PEC.
- Media-fill tests must be performed with a commercial source of soybean–casein digest medium. Those performing sterile-to-sterile processing activities must start with sterile media. Those performing nonsterile-to-sterile compounding must use a nonsterile soybean–casein digest powder to make a solution. Dissolve nonsterile commercially available soybean–casein digest medium in nonbacteriostatic water to make a 3% nonsterile solution. Manipulate it in a manner that simulates nonsterile-to-sterile compounding activities. Prepare at least 1 container as the positive control to demonstrate growth promotion, which is indicated by visible turbidity upon incubation.
- The certificate of analysis (CoA) must include documentation of growth promotion testing for each lot of media used.
- Once the media-fill simulation is completed and the final containers are filled with the test medium, incubate media-filled containers in an incubator for 7 days at 20°–25° followed by 7 days at 30°–35° to detect a broad spectrum of microorganisms. Failure is indicated by visible turbidity or other visual manifestations of growth in the medium in 1 or more container–closure unit(s) on or before 14 days.
- In the event of failure, results of the evaluation and corrective actions must be documented and the documentation maintained to provide a record and long-term assessment of personnel competency. Documentation must at a minimum include the name of the person evaluated, evaluation date/time, media and components used including manufacturer, expiration date and lot number, starting temperature for each interval of incubation, dates of incubation, and the results.

4.2 Reevaluation, Retraining, and Requalification

REQUALIFICATION AFTER FAILURE

Personnel who fail visual observation of hand hygiene, garbing, and aseptic technique, gloved fingertip and thumb sampling, or media-fill testing must successfully pass reevaluations in the deficient area(s) before they can resume processing of sterile preparations. All failures, retraining, and reevaluations must be documented.

REQUALIFICATION PROGRAM

Personnel must successfully complete requalification in the core competencies listed in *4.1 Aseptic Qualifications*. Successful completion must be demonstrated through observation, written testing, and hands-on demonstration of skills.

TIMING OF REEVALUATION AND REQUALIFICATION

Visual observation: Personnel must be visually observed while performing hand hygiene, garbing SOPs, and aseptic technique procedures initially, and then at least once every 12 months.
Gloved fingertip and thumb sampling: Personnel must perform fingertip and thumb sampling 3 times initially, and then every 12 months (in conjunction with media-fill testing).
Media-fill testing: After initial qualification, conduct a media-fill test of all personnel engaged in sterile radiopharmaceutical processing at least every 12 months (in conjunction with gloved fingertip and thumb sampling).
Cleaning and disinfecting: Retrain and requalify personnel in the cleaning and disinfecting of sterile processing areas every 12 months or in conjunction with any change(s) in cleaning and disinfecting SOPs, whichever is sooner.
After a pause in sterile radiopharmaceutical processing: Personnel that have not performed radiopharmaceutical processing in more than 6 months must be requalified in all core competencies before resuming duties.
Sterile compounding using a nonsterile drug substance or components: Personnel who perform sterile compounding using a nonsterile drug substance or components (see *11.3 Sterile Compounding Using a Nonsterile Drug Substance or Components*) must be requalified in all core competencies every 6 months.

4.3 Ancillary Personnel

Personnel who are authorized to be within the sterile processing area and do not handle sterile preparations are not required to complete training on media-fill testing but are required to complete all other training and testing. Other personnel or visitors (e.g., auditors, regulators, student observers) must comply with garbing and gloving SOPs but do not need to prove competency.

4.4 Hand Hygiene and Garbing for Immediate Use Preparations

Radiopharmaceuticals may be prepared and dispensed as immediate use, and the precautions related to personal hygiene to be followed must include the following:

- Hand hygiene: Wash hands and arms to the wrists with soap and water or use a suitable alcohol-based hand rub with a time based on institution policies to reduce bioburden on the hands.

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- Garbing: Immediately after hand hygiene, don a clean coat/gown that has not been exposed to a patient or patient care area, and either don sterile gloves or don nonsterile disposable gloves and then disinfect the gloves with sterile 70% IPA. [NOTE—A different lab coat must be worn to care for a patient than the coat/gown used for radiopharmaceutical preparation.]

4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area

In situations involving repackaging, dispensing, preparation, preparation with minor deviations, or compounding of sterile radiopharmaceuticals in an ISO Class 5 PEC, the following precautions related to personal hygiene are to be followed:

- Before entering the SRPA or buffer area, personnel must remove outer garments (e.g., bandanas, coats, hats, jackets, sweaters, vests); all cosmetics; all hand, wrist, and other exposed jewelry including piercings that could interfere with the effectiveness of the garbing (e.g., the fit of gloves, cuffs of sleeves, and eye protection). Nail products (e.g., artificial nails, polish, extenders) are prohibited. Natural nails must be kept neat and trimmed. Remove ear buds and headphones. Radiation dosimetry devices are allowed, as required by the RAM license.
- Do not bring electronic devices that are not necessary for compounding or other required tasks.
- Immediately before entering the SRPA or buffer area, remove visible debris from underneath fingernails under warm running water using a disposable nail cleaner. Personnel must wash hands and arms up the elbows with soap and water for at least 30 s and then dry hands using low-lint towels. Alternatively, hand washing may be performed after donning shoe covers, head/hair covers, and face mask, as described below.
- Personnel must don the following garb—shoe covers, head/hair/facial hair covers, face mask—in an order that eliminates the greatest risk of contamination, as defined in facility SOPs.
- If not already performed, remove visible debris from underneath fingernails under warm running water using a disposable nail cleaner. Personnel must then wash hands and arms up to the elbows with soap and water for at least 30 s and then dry hands using low-lint towels. Electronic hand dryers are not permitted.
- Personnel must then perform hand antisepsis cleansing using a suitable alcohol-based hand rub.
- Personnel must then don a low-lint gown with sleeves that fit snugly around the wrists and enclosed at the neck. Disposable gowns are preferred. If reusable gowns are used, a clean gown must be donned daily.
- Personnel must then aseptically don sterile, powder-free gloves. Gloves must completely and snugly cover the ends of the gown cuffs so that skin on the wrists and upper hands is completely enveloped.
- Because gloves may not remain sterile due to touching or handling potentially nonsterile materials, personnel must periodically apply sterile 70% IPA to gloves while balancing the risk of radioactivity contamination.
- Personnel must also routinely inspect the gloves that they are wearing for holes, punctures, radioactivity contamination, or tears. If a defect, radioactivity contamination, or malfunction is detected, personnel must immediately remove the gloves, repeat antiseptic hand cleansing using an alcohol-based hand rub, and don new sterile gloves.
- Direct personnel touch contamination is the most common source of microorganisms, so personnel must avoid touch contamination of container septa, needles, syringe and needle hubs, and other critical sites.

When personnel exit the buffer area or SRPA, shoe covers, head/hair covers, face masks, and gloves must be properly disposed of and new ones donned for each reentry into the buffer area or SRPA. Gowns may be re-used within the same shift if the gown is maintained in a classified area or in (or immediately outside of) the SRPA that minimizes contamination (e.g., away from sinks).

5. FACILITIES AND ENGINEERING CONTROLS

5.1 Facility Design and Environmental Controls

In addition to minimizing airborne contamination, sterile radiopharmaceutical facilities must be designed and controlled to provide a well-lighted and comfortable working environment (see *Physical Environments That Promote Safe Medication Use* ⟨1066⟩). The classified areas and SRPA must be continuously maintained at a temperature of 25° or cooler and should be continuously maintained at a relative humidity (RH) below 60% to minimize the risk for microbial proliferation and provide comfortable conditions for personnel attired in the required garb. The temperature and humidity must be monitored in the classified areas each day that it is used, either manually or by a continuous recording device. The results of the temperature and humidity readings must be documented at least once daily or stored in the continuous recording device, and must be retrievable. The temperature and humidity readings must be reviewed as described in the facility's SOPs. Free-standing humidifiers/dehumidifiers and air conditioners must not be used within the classified area or SRPA. Temperature and humidity monitoring devices must be verified for accuracy at least every 12 months or as required by the manufacturer.

The designated person is responsible for ensuring that each area related to sterile radiopharmaceutical processes meets the classified air quality standard appropriate for the activities to be conducted in that area. They must also ensure that the ISO Class 5 PECs are located, operated, maintained, monitored, and certified to have appropriate air quality.

TYPES OF SECONDARY ENGINEERING CONTROLS AND DESIGN

Due to the interdependence of the various areas or areas that make up a sterile radiopharmaceutical processing facility, it is essential to define and control the dynamic interactions permitted between areas. When designing doors, consider the

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placement of door closures, door surfaces, and the movement of the door, all of which can affect airflow. Tacky surfaces must not be used in ISO-classified areas.

The PEC must be located in a SEC, which may be either an ISO-classified buffer room with ante-room or an SRPA, in a manner that minimizes conditions that could increase the risk of microbial contamination. For example, strong air currents from opened doors, personnel traffic, or air streams from the HVAC system(s) can disrupt the unidirectional airflow of an open-faced PEC such as a laminar airflow workbench (LAFW) or biological safety cabinet (BSC). The ISO-classified ante-room and buffer area must be separated from the surrounding unclassified areas of the facility with fixed walls and doors. Facility design and controls must be in place to minimize the flow of lower-quality air into the more controlled areas. Air supplied to the classified areas must be introduced through HEPA filters that are located in the ceiling. Returns must be low on the wall unless a visual smoke study demonstrates an absence of stagnant airflow where particulate will accumulate. A smoke study of the PEC must be repeated whenever a change to the placement of the PEC within the area is made. The classified areas must be equipped with a pressure-differential monitoring system. The ante-room must have a line of demarcation to separate the clean side from the less clean side. The ante-room is entered through the less clean side, and the clean side is the area closest to the buffer area. Required garb must be worn prior to crossing the line of demarcation (see *4. Personnel Qualifications, Training, and Hygiene*).

A PEC may be located within an unclassified area, without an ante-room or buffer area. This type of design is called an SRPA. Only sterile radiopharmaceutical preparation, preparation with minor deviations, dispensing, and repackaging may be performed in an SRPA. If the SRPA meets ISO Class 8 total airborne particle count specifications, it can also be used for storage and elution of non-direct infusion radionuclide generators (e.g., Tc-99m). The SRPA must be located away from unsealed windows, doors that connect to the outdoors, and traffic flow which may adversely affect the air quality in the PEC. The impact of activities that will be conducted around or adjacent to the SRPA must be considered carefully when designing such an area. A visible perimeter must establish the boundaries of the SRPA. Access to the SRPA must be restricted to authorized personnel and required materials. An SRPA must not be located adjacent to environmental control challenges.

It is also critical to control materials (e.g., supplies and equipment) as they move from classified areas of lower quality to those of higher quality (e.g., ISO Class 8 ante-room to ISO Class 7 buffer area to ISO Class 5 PEC) to prevent the influx of contaminants. Airlocks and interlocking doors can be used to facilitate better control of air flow between areas of differing ISO classification (e.g., between the buffer area and ante-room), or between a classified area and an unclassified area (e.g., between the ante-room and an unclassified area such as a hallway) See *5.7 Environmental Controls* for a description of air pressure differentials. If a pass-through is used, both doors must never be opened at the same time, which may be achieved using interlocking mechanisms.

THE RADIOPHARMACEUTICAL PROCESSING ENVIRONMENT

The PEC must be certified to meet ISO Class 5 or better conditions (see *Table 1*) and must be designed to minimize microbial contamination during processing of radiopharmaceuticals under dynamic operating conditions.

The airflow in the PEC must be unidirectional (laminar flow), and because of the particle collection efficiency of the filter, the "first air" at the face of the filter is, for the purpose of aseptic processing, free from airborne particulate contamination. HEPA-filtered air must be supplied in the direct processing area (DPA) (ISO Class 5; see *Table 1*) at a velocity sufficient to sweep particles away from aseptic processing areas and maintain unidirectional airflow as much as possible during operations, given the limitations added from the radiation shielding in the DPA. Proper design and control prevents turbulence and stagnant air in the DPA. In situ air pattern analysis via smoke studies must be conducted at the critical area to demonstrate unidirectional airflow and sweeping action under dynamic conditions.

Table 1. ISO Classification of Particulate Matter in Area Air[a]

ISO Class	Particle Count[b] /m³
3	35.2
4	352
5	3520
6	35,200
7	352,000
8	3,520,000

[a] Adapted from ISO 14644-1, Clean areas and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration.
[b] Limits for number of particles ≥0.5 µm measured under dynamic operating conditions.

TYPES OF PECS AND PLACEMENT

Proper placement of the PEC is critical to ensuring an ISO Class 5 environment for preparing radiopharmaceuticals. Placement of the PEC must allow for cleaning around the PEC.

A PEC provides an ISO Class 5 or better environment for sterile radiopharmaceuticals. The unidirectional airflow within the PEC helps protect the DPA from process-generated contamination of an aseptic processing environment. The unidirectional airflow within the PEC helps protect the DPA from process-generated contamination (e.g., opening wrappings of sterile containers, worker movement, etc.) as well as from outside sources.

Laminar airflow workbench (LAFW): An LAFW used for preparing radiopharmaceuticals must provide vertical unidirectional HEPA-filtered airflow. In cases where the LAFW is located within the segregated containment area of a hot-cell, it is acceptable for a horizontal unidirectional HEPA-filtered airflow pattern to be utilized.

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Biological safety cabinet (BSC) Class II: A BSC Class II is a cabinet with an open front, inward airflow, downward unidirectional HEPA-filtered airflow, and HEPA-filtered exhaust. The BSC is designed to provide worker protection from exposure to biohazardous material and to provide an ISO Class 5 or better environment for preparing sterile radiopharmaceuticals.
Placement of PEC: The PEC must be located out of traffic patterns and away from area air currents that could disrupt the intended airflow patterns inside the PEC. If used only to prepare, prepare with minor deviations, dispense, or repackage sterile radiopharmaceuticals the ISO Class 5 PEC may be placed in an unclassified SRPA. If used to compound sterile radiopharmaceuticals, the PEC must be located within an ISO Class 7 or better buffer area with an ISO Class 8 or better ante-room. A dynamic airflow smoke pattern test must be performed initially and at least every 6 months to ensure that the PEC is properly placed into the facility and that workers understand how to utilize the unidirectional airflow to maintain first air as much as possible given the limitations added from the radiation shielding in the DPA.

AIR-EXCHANGE REQUIREMENTS

For classified areas, adequate HEPA-filtered airflow to the buffer area(s) and ante-room(s) is required to maintain the appropriate ISO classification during processing activities. Airflow is measured in terms of the number of HEPA-filtered air changes per hour (ACPH). The ACPH may need to be higher to maintain the required ISO classification and microbial state of control depending on these factors: the number of personnel permitted to work in the area, the number of particulates that may be generated from activities and processes in the area, the equipment located in the area, the area pressure, and the effects of temperature. The summary of ACPH requirements is listed in *Table 2*.

A minimum of 30 total HEPA-filtered ACPH must be supplied to ISO Class 7 areas.

- The total HEPA-filtered air change rate must be adequate to maintain ISO Class 7 under dynamic operating conditions considering factors listed above
- At least 15 ACPH of the total air change rate in a room must come from the HVAC through HEPA filters located in the ceiling
- The HEPA-filtered air from the PEC, when added to the HVAC-supplied HEPA-filtered air, increases the total HEPA-filtered ACPH to at least 30 ACPH
- If the PEC is used to meet the minimum total ACPH requirements, the PEC must not be turned off except for maintenance
- The ACPH from HVAC, ACPH contributed from the PEC, and the total ACPH must be documented on certification reports

A minimum of 20 ACPH of HEPA-filtered air must be supplied to ISO Class 8 areas.

- The total HEPA-filtered air change rate must be adequate to maintain ISO Class 8 under dynamic operating conditions considering factors listed above
- At least 15 ACPH of the total air change rate in a room must come from the HVAC through HEPA filters located in the ceiling
- Ante-rooms where activity levels are high may require more HEPA-filtered ACPH to maintain ISO Class 8 under dynamic operating conditions
- The total ACPH must be documented on certification reports

Table 2. Summary of ACPH Requirements for Sterile Radiopharmaceutical Processing

Processing Area	ACPH Requirement
Unclassified SRPA	No requirement
ISO Class 7 area	≥30 ACPH
ISO Class 8 area	≥20 ACPH

5.2 Creating Areas to Achieve Easily Cleanable Conditions

CLASSIFIED AREAS

The surfaces of ceilings, walls, floors, doors, door frames, fixtures, shelving, work surfaces, counters, and cabinets in the classified area must be smooth, impervious, free from cracks and crevices, and non-shedding, so they can be cleaned and disinfected, and to minimize spaces in which microorganisms and other contaminants can accumulate. Surfaces should be resistant to damage by cleaning agents, disinfectants, and tools used to clean. Junctures between the ceiling and the walls and between the wall and the floor must be sealed to eliminate cracks and crevices where dirt can accumulate. If ceilings consist of inlaid panels, each panel must be caulked or otherwise sealed and secured to seal them to the support frame.

Walls must be constructed of or covered with a durable material (e.g., epoxy-painted walls or heavy-gauge polymer) and the integrity of the surface must be maintained. Panels must be joined together and sealed to each other and the support structure. Floors must include coving to the sidewall or the juncture between the floor and wall must be caulked. Floors must include coving to the sidewall. Classified areas should minimize dust-collecting overhangs such as utility pipes and ledges such as windowsills. If overhangs or ledges are present, they must be easily cleanable. The exterior lens surface of ceiling light fixtures must be smooth, mounted flush, and sealed. Any other penetrations through the ceiling or walls must be sealed.

SRPA

The SRPA and all surfaces (e.g., walls, floors, counters, equipment) within the SRPA must be clean, uncluttered, and dedicated to sterile radiopharmaceutical processing activities. Surfaces in the SRPA should be smooth, impervious, free from cracks and

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crevices, and non-shedding, so they can be easily cleaned and disinfected, and to minimize spaces in which microorganisms and other contaminants can accumulate. Surfaces should be resistant to damage by cleaning agents, disinfectants, and tools used to clean. Dust-collecting overhangs such as utility pipes and ledges such as windowsills should be minimized. If overhangs or ledges are present, they must be easily cleanable.

5.3 Water Sources

The facility where sterile radiopharmaceuticals are prepared must be designed so that activities such as hand hygiene and garbing should not adversely affect the ability of the PEC to function as designed. Sinks should enable hands-free use with a closed system of soap (i.e., non-refillable) to minimize the risk of extrinsic contamination. In facilities with an ante-room and buffer area, the sink used for hand hygiene may be placed either inside or outside of the ante-room. If the sink is located outside of the ante-room, it must be located in a clean space to minimize the risk of bringing in contaminants into the ante-room. If the sink is located inside the ante-room, it may be placed on either the clean side or the less-clean side of the ante-room. [NOTE—The order of hand washing and garbing would depend on the placement of the sink (see *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area*).] The buffer area must not contain plumbed water sources [e.g., sink(s), eyewash(es), shower(s), or floor drain(s)]. The ante-room must not contain floor drain(s). If installed, sprinkler systems in classified areas should be recessed and covered, and should be easily cleanable. In a facility with an SRPA design, the sink must be accessible but located at least 1 m from the PEC and generators, if present. The sink must not be located inside the perimeter of the SRPA.

5.4 Placement and Movement of Materials

Only furniture, equipment, and other materials necessary are permitted in the classified area or SRPA and they should be low-shedding and easily cleaned and disinfected. Their number, design, location, and manner of installation must not adversely impact environmental air quality and must promote effective cleaning and disinfecting. No shipping carton(s) or other corrugated or uncoated cardboard are allowed in the classified area or SRPA.

Carts used to transport components or equipment into classified areas must be constructed from nonporous materials with cleanable casters and wheels. All items must be wiped with low-lint wipers and an appropriate disinfectant by personnel wearing gloves before they are brought into the clean side of ante-room(s), pass-through(s), into an SRPA or into an ISO 5 PEC. However, constraints that would lead to excessive radiation exposure to radiation for workers and thereby be contradictory to following ALARA safety principles (e.g., the wiping of unshielded sources of radioactive material) might preclude this from occurring. In a classified area, carts must not be moved from the dirty side to the clean side of the ante-room unless the entire cart, including casters, is cleaned and disinfected.

5.5 Classified Areas

Activities and tasks carried out within the buffer area must be limited to only those necessary. Food, drinks, and materials exposed in patient care and treatment areas must not enter ante-rooms or buffer areas. When processing activities require the manipulation of blood-derived or other biological material (e.g., radiolabeling patient's or donor's blood cells), the manipulations must be clearly separated from routine material-handling procedures and equipment used in radiopharmaceutical preparation activities, and they must be controlled by specific SOPs to avoid any cross-contamination.

5.6 Remote Aseptic Processing Involving a Hot-Cell

A hot-cell device provides an inherent physical segregation for the ISO Class 5 aseptic processing area. If the hot-cell is located in an ISO-classified space, personnel must garb according to requirements listed in *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area*. In settings where tasks are carried out within the hot-cell enclosure not within an ISO-classified space by remote means (i.e., no direct intervention by personnel into the ISO Class 5 space), it is not necessary for personnel to don the garbing described in *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area* to carry out these aseptic manipulations or to perform other routine tasks in the general area where the hot-cell is located. If hand and arm incursions into the interior of the hot-cell might be necessary for personnel to stage the required materials and supplies, the personnel must garb in relation to the contamination risk associated with the individual hot-cell/ISO Class 5 relationship.

For situations where a PEC device is located within a hot-cell, dynamic airflow smoke pattern tests must show that the staging of supplies and materials in the demarcated PEC area does not allow the influx of unclassified air into the PEC. Personnel may be garbed in nonsterile gloves and a low-particulate lab coat for interventions that are outside of the PEC. A failure of the airflow smoke pattern test requires personnel to garb in accordance with *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area* for all incursions into the hot-cell.

For situations where the hot-cell is an integrated HEPA filtration system with a clear demarcated area that is a PEC, dynamic airflow smoke pattern tests must show that the staging of supplies and materials into the demarcated PEC area does not allow the influx of less than ISO Class 5 quality air into the PEC. Personnel may be garbed in nonsterile gloves and a low-particulate lab coat for interventions that are outside of the PEC. A failure of the airflow smoke pattern test requires personnel to garb in accordance with *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area* for all incursions into the PEC.

Since other hot-cell/PEC configurations and technologies may exist, verification (either by airflow smoke pattern tests or other manufacturer specified methods) must ensure, upon each certification, that the staging of materials and supplies does

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not allow for the intrusion of less than ISO Class 5 air into the designated ISO Class 5 space. A failure of the airflow smoke pattern test requires personnel to garb in accordance with *4.5 Hand Hygiene and Garbing for Buffer Areas and Segregated Radiopharmaceutical Processing Area* for all incursions into the hot-cell.

5.7 Environmental Controls

All RAM users must comply with the conditions specified in their approved RAM license application and regulations, and RAM license conditions may supersede the following requirements for environmental controls described in this section. Pass-through enclosures for transferring radiopharmaceuticals from controlled handling areas (e.g., buffer area) should be designed to provide reasonable balance between maintenance of air quality and other worker safety concerns (e.g., radiation exposure, physical injury from lifting heavy shielded cases). At a minimum, there must be a mechanical system or SOP in place that ensures that both doors cannot be open at the same time. There may be both positive and negative air pressure within the facility; positive pressure to minimize the potential of microbial contamination in sterile drug preparation areas, and negative pressure to minimize potential radioactive contamination from volatile or airborne radiopharmaceuticals. Positive pressure environments must have a minimum differential positive pressure of 0.02-inch water column between each ISO-classified area (e.g., between the buffer area and ante-room). The pressure differential between the ante-room and the unclassified area must be no less than a positive 0.02-inch water column. Refer to the RAM license for negative pressure requirements. For preparation of sterile radiopharmaceuticals, consideration of both concerns could be addressed as follows:

1. Buffer area, if present, must be positive pressure compared to the ante-room
2. Ante-room, if present, must be positive pressure compared to unclassified portions of the restricted area
3. Restricted area, in the presence of volatile or airborne radiopharmaceuticals, must be negative pressure compared to the unrestricted area
4. SRPA must be negative pressure compared to unrestricted areas in the presence of volatile or airborne radiopharmaceuticals (e.g., I-131 sodium iodide and Xenon).

Various environmental controls for various preparation scenarios (see *Table 7* for maximum BUDs for differing environments) are described in the following sections. *Table 1* details the limits for particle counts for each specific ISO classification.

ESTABLISHING AND MAINTAINING PRESSURE DIFFERENTIALS

Any time a pressure differential is required, a pressure monitoring device is required. In a classified area, a pressure differential monitoring system must be used to continuously monitor the pressure differential between the ante-room(s) and buffer area(s) and between the ante-room and the general environment outside the classified area(s) or area(s). The results from the pressure monitoring system must be reviewed and documented at least daily on days the area is used. All pressure monitoring devices must be tested for accuracy and required performance at least every 6 months.

AMBIENT ATMOSPHERE FOR IMMEDIATE USE PREPARATIONS

The following requirements should be met in ambient atmosphere environments:

- Non-patient care space, functionally separate (not necessarily a different area) from the patient care area, such as a radiopharmaceutical handling space, or hot lab, in a hospital, clinic, or mobile coach
- A designated area for medication preparation that is clean and free from clutter
- Low traffic (i.e., limited number of people going in and out or moving around the area during times that radiopharmaceutical processing is being carried out)

SRPA WITH VERTICAL FLOW ISO CLASS 5 PEC(S) FOR RADIOPHARMACEUTICAL PREPARATIONS

An SRPA with vertical ISO Class 5 PECs must meet the following requirements:

- Area surrounding the PEC may be ambient (unclassified) atmosphere
- Area must be clean, uncluttered, and dedicated to the processing of radiopharmaceuticals
- Appropriate for preparation, preparation with minor deviations, repackaging, and dispensing of radiopharmaceuticals

An area that meets ISO Class 8 total airborne particle-count specifications may be used to store and elute non-direct infusion radionuclide generators (e.g., Tc-99m).

AN ISO CLASS 8 BUFFER AREA WITH VERTICAL FLOW ISO CLASS 5 PEC(S) WITH AN ADJACENT ISO CLASS 8 ANTE-ROOM

This environment is appropriate for all activities listed in *SRPA with Vertical Flow ISO Class 5 PEC(s) for Radiopharmaceutical Preparations.*

AN ISO CLASS 7 BUFFER AREA WITH VERTICAL FLOW ISO CLASS 5 PEC(S) WITH AN ADJACENT ISO CLASS 8 OR BETTER ANTE-ROOM

This environment is appropriate for all activities listed in *An ISO Class 8 Buffer Area with Vertical Flow ISO Class 5 PEC(s) with an Adjacent ISO Class 8 Ante-Room* and sterile compounding.

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HOT-CELL

This environment is appropriate for all activities listed in *SRPA with Vertical Flow ISO Class 5 PEC(s) for Radiopharmaceutical Preparations.*

CERTIFICATION OF PECS AND ENVIRONMENT IN WHICH THE PEC IS LOCATED

Certification of the classified areas, including the PEC, must be performed initially and recertification must be performed at least every 6 months using procedures outlined in the current Controlled Environment Testing Association (CETA) certification guide for *Sterile Compounding Facilities*, or an equivalent guideline, and must include the following:

- Airflow testing: To determine acceptability of the air velocity, the air exchange rate, and area pressure cascade to ensure that air consistently flows from most to least clean areas, and that the appropriate quality of air is maintained under dynamic operating conditions.
- HEPA filter integrity testing: HEPA filters must be leak tested after installation and as part of recertification.
- Total particle counts testing: Conducted under dynamic operating conditions using calibrated electronic equipment.
- Smoke visualization studies: Performed under either simulated or dynamic operating conditions to demonstrate unidirectional airflow and sweeping action over and away from the preparation(s).

In cases where technologies exist for hot-cell and PEC configurations that are not consistent for certification by the current CETA standards, other equivalent means for certifying the PEC may be performed and documented per facility SOPs. In this case, the PEC must maintain the environmental equivalent for total particle counts and the protection of the ISO Class 5 area from intrusions of lesser controlled air.

DAILY MONITORING OF ENVIRONMENT

The temperature and humidity must be monitored in the SRPA or area containing a hot-cell, and if in a classified area the pressure must monitored, each day that preparations are made, either manually or by a continuous recording device. These include:

- Relative humidity should be kept at 60% or lower
- Temperature and relative humidity continuous readings must be confirmed daily to have remained within the acceptable range
- Excursions must be documented and, if applicable, appropriate corrective actions taken
- Temperature monitoring devices must be verified for accuracy every 12 months or as required by the manufacturer
- Monitoring of pressure differentials must be performed

See *Packaging and Storage Requirements* ⟨659⟩ for information on controlled area temperature and allowable excursions.

6. MICROBIOLOGICAL AIR AND SURFACE MONITORING

An effective air and surface monitoring program provides information on the environmental quality of the classified areas where sterile radiopharmaceuticals are processed. The program identifies environmental quality trends over time, potential routes of microbiological contamination, and allows for implementation of corrective actions to prevent microbiological contamination of the radiopharmaceuticals. Facilities must develop and implement written air and surface monitoring procedures for all sterile radiopharmaceutical classified areas. Air and surface monitoring results and the corrective actions must be documented, and records must be readily retrievable as required by jurisdictional laws and regulations.

6.1 General Monitoring Requirements

The goals of an air and surface monitoring program are to determine whether microbiological contamination is present at unacceptable levels and to assess whether proper personnel practices are being followed, cleaning and disinfecting agents are effective, and environmental quality is maintained. The microbiological air and surface monitoring program must include viable impact volumetric airborne particulate sampling and surface sampling.

Air and surface sampling must be performed initially for classified areas in a facility to establish a baseline level of environmental quality. After initial sampling, the classified areas must be monitored according to the minimum frequencies described in this section to ensure that the environment remains in a suitable state for aseptic processing tasks.

The air and surface monitoring program involves the collection and evaluation of samples from various air and surface locations to detect viable microbiological contaminants. The data are then used to assess risks for contamination, potential routes of contamination, and the adequacy of cleaning and disinfection techniques and agents specified in the facility SOPs. Regular review of the sampling data must be performed to detect trends such as elevated levels of microbial bioburden, elevated levels of nonviable particulates, or other adverse changes within the environment. Evaluating results collected over a period of time can be useful in identifying trends or determining that a significant change has occurred, even when the results fall within the specified limits.

In addition, results must be reviewed in conjunction with personnel data (i.e., training records, visual observations, competency assessments) to assess the state of control and to identify potential risks of contamination. Prompt corrective action in response to any adverse findings is required to maintain the necessary environmental quality for handling sterile radiopharmaceutical. Data must also be reviewed following corrective actions to confirm that the actions taken have been effective in achieving the required air and surface quality levels (see *Table 3* and *Table 4*).

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Air and surface sampling must be conducted during actual or simulated dynamic operating conditions to confirm that the required environmental quality in classified areas is maintained. Due to radiation exposure concerns for the workers involved, it is permissible for sampling to be carried out at the conclusion of sterile radiopharmaceutical processing but prior to cleaning and disinfecting the surface area. In this case, simulated tasks that are reflective of the routine aseptic activities are performed. In addition to the specific sampling frequencies described in this section, sampling must be performed in any of the following circumstances:

- In conjunction with the certification of new facilities and equipment
- After any modification of facilities or equipment
- In response to identified problems (e.g., positive growth in sterility tests of compounded radiopharmaceuticals)
- In response to identified trends (e.g., repeated positive gloved fingertip sampling results or failed media-fill testing involving more than one operator where a review of the operator technique shows no reasonable flaws in process; repeated observations of air or surface contamination)
- In response to changes that could impact the controlled area environments (e.g., significant change in cleaning process or the agents involved)

To obtain an air and surface sample that is representative of the typical aseptic operating conditions at the facility, air and surface sampling must be conducted under dynamic or simulated dynamic operating conditions in all PECs and classified areas. If conducted during actual sterile processing, the monitoring program must be designed and conducted in a manner that minimizes the chance that the sampling itself will contribute to contamination of the sterile radiopharmaceutical(s) or the environment.

The air and surface monitoring program must be clearly described in the established SOPs of the facility and must include a diagram of the sampling locations, SOPs for collecting samples, frequency of sampling, size of samples (e.g., surface area, volume of air), time of day of sampling in relation to activities in the classified areas, and action levels that will trigger corrective action. The locations of sampling should be carefully selected based on their relationship to the activities performed in the area. It is important to obtain samples from locations that pose the highest possible contamination risk to the sterile radiopharmaceuticals involved with the operation's processes and that are likely to be representative of the conditions throughout the area.

Evaluating results collected over a period of time can be useful in identifying trends or determining that a significant change has occurred, even when the results fall within the specified limits.

It is important that personnel who operate the equipment be trained in the proper operation of the air and surface sampling equipment to ensure accurate and reproducible sampling. All air sampling devices must be serviced and calibrated as recommended by the manufacturer.

6.2 Monitoring Air Quality for Viable Airborne Particles

A monitoring program for viable airborne particles must be developed and implemented to assess microbiological air quality in all classified areas.

VIABLE AIR SAMPLING: TIMING AND LOCATIONS

Volumetric active air sampling of all classified areas (e.g., ISO Class 5 PEC and ISO Class 7 and 8 areas) using an impaction device must be conducted during dynamic operating or simulated operating conditions at least every 6 months.

Air sampling sites must be selected in all classified areas. When conducting sampling of the PEC, care should be taken to avoid disturbing unidirectional airflow if taken during actual sterile processing activities. Viable air sampling must include:

1. Follow the manufacturer's instructions for operation of the air sampling device, including placement of media.
2. Using the sampling device, test at least 1 cubic meter or 1000 liters of air from each location sampled.
3. At the end of the sampling, retrieve the media plates/devices and cover.
4. Invert the media and incubate at 30°–35° for no less than 48 hours. Examine for growth. Record the total number of discrete colonies of microorganisms on each plate as cfu/m^3 of air on an environmental sampling form based on sample type (i.e., viable air). Include sample location and date.
5. Then incubate the inverted media at 20°–25° for no less than 5 additional days. Examine the media plates for growth. Record the total number of discrete colonies of microorganisms on each plate as cfu/m^3 of air on an environmental sampling form based on sample type (i.e., viable air). Include sample location and date.

Alternatively, to shorten the overall incubation period, two samples may be collected for each sample location and incubated concurrently. Both samples could be TSA or one sample could be TSA and the other fungal media [e.g., malt extract agar (MEA) or sabouraud dextrose agar (SDA)]. Incubate each sample in a separate incubator. Incubate one sample at 30°–35° for no less than 48 hours, and incubate the other sample at 20°–25° for no less than 5 days. Fungal media samples must be incubated at 20°–25° for no less than 5 days. Count the total number of discrete colonies of microorganisms on each sample, and record these results as cfu per sample.

Record the results of the sampling on an environmental sampling form based on sample type (i.e., viable air) and include the sample location, and sample date.

A general microbiological growth medium that supports the growth of bacteria and fungi must be used (e.g., TSA medium). CoA(s) from the manufacturer must verify that the medium meets the expected growth promotion, pH, and sterilization requirements. Samples must be incubated in a temperature monitored incubator with a calibrated measuring device. The incubator temperature must be monitored during incubation, either manually or by a continuous recording device, and the results must be reviewed and documented. Incubators used for microbiological testing must be placed in a location outside of

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any classified area or SRPA and kept away from areas where compounding or sterile processing activities are carried out. All sampling activities must be performed by trained individuals.

DATA EVALUATION AND ACTION LEVELS

Evaluate cfu counts against the action levels in *Table 3* and in relation to previous data to identify adverse results and/or trends. If two pieces of media were collected at a single location, all recovered growth on each must be documented and action levels are applied individually to each plate/device (i.e., results from each cubic meter of air sampled must be compared to the action level for that area). If levels measured during the viable air monitoring program exceed the levels in *Table 3* for the ISO classification levels of the area sampled, the cause must be investigated and corrective action must be taken. The corrective action plan must be dependent on the cfu count and the microorganism recovered. Some examples of corrective action include process or facility improvements, personnel training, cleaning and disinfecting, or HEPA filter replacement and/or repair, or reducing the BUD of the radiopharmaceutical during investigation and while carrying out the corrective action plan. The extent of the investigation should be consistent with the deviation and should include an evaluation of trends. The corrective action plan must be documented. If levels measured during viable air sampling exceed the levels in *Table 3*, an attempt must be made to identify any microorganism recovered to the genus level (see *Microbial Characterization, Identification, and Strain Typing* ⟨1113⟩) with the assistance of a qualified individual (e.g., microbiologist or industrial hygienist).

Table 3. Action Levels for Viable Airborne Particle Air Sampling[a]

ISO Class	Air Sampling Action Levels [cfu/m³ (1000 L) of air per plate]
5	≥1
7	>10
8	>100

[a] Adapted from *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice.* US Department of Health and Human Services, Food and Drug Administration (FDA), September 2004.

6.3 Monitoring Surfaces for Viable Particles

Surface sampling is an important component of the maintenance of a suitably controlled environment for sterile radiopharmaceutical processing, especially because transfer of microbial contamination from improperly disinfected work surfaces (e.g., via inadvertent touch contact by personnel) is a potential source of contamination of the radiopharmaceutical(s). Surface sampling is useful for evaluating facility cleaning and material handling procedures, work surface cleaning and disinfecting procedures, and personnel competency in work practices such as proper cleaning and disinfection. All sampling sites and procedures must be described in the facility's SOP.

SURFACE SAMPLING: TIMING AND LOCATIONS

Surface sampling of all classified areas and all PECs must be conducted at least monthly for the detection of microbial contamination. Each classified area must be sampled (see *Microbiological Control and Monitoring of Aseptic Processing Environments* ⟨1116⟩). The DPA of the PEC, and any equipment permanently contained in the PEC, must be sampled. Staging or work surfaces in classified areas near the PEC, frequently touched surfaces in classified areas, and pass-through enclosure(s) for all classified areas are to be evaluated to determine the locations that pose the greatest risk of harboring microbial contamination.

Surface sampling must be performed at the end of the radiopharmaceutical aseptic activities or shift, but before the area has been cleaned and disinfected. However, radiopharmaceutical personnel must also consider the appropriate exposure and contamination prevention measures prior to and while collecting samples. If the worker assesses that the risk for exposure is not in conformance with ALARA safety standards, measures must be taken to eliminate the risk (e.g., implementation of appropriate shielding, performing the sampling at a later time or alternate day).

SAMPLING PROCEDURES

Surface sampling devices (e.g., plates, paddles, or slides) containing microbial growth media must be used for sampling flat surfaces. CoAs from the manufacturer must verify that the media meet the expected growth promotion, pH, and sterilization requirements. Surface sampling devices must contain general microbial growth media (e.g., TSA) supplemented with neutralizing additives (e.g., lecithin and polysorbate 80) to neutralize the effects of any residual disinfecting agents. If used, contact plates must have a raised convex surface. Sterile swabs wetted with sterile water or a sterile neutralizing buffer may be used when sampling irregular surfaces and difficult-to-reach locations, such as crevices, corners, and spaces between surfaces. After sampling, the sampled area must be thoroughly cleaned and disinfected.

Use the following procedures for surface sampling on flat surfaces:

1. Remove the cover from the surface sampling device. Firmly press, using a rolling motion, if possible, the media surface onto the surface to be sampled. The surface sampling device will leave a residue of growth medium on the sample site. After sampling, use sterile 70% IPA to remove residue. Cover each surface sampling device.
2. If using plates, invert the plates.

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3. Incubate the surface sampling devices at 30°–35° for no less than 48 hours. Examine for growth. Record the total number of discrete colonies of microorganisms on each media device as cfu/sample on an environmental sampling form based on sample type (i.e., surface). Include sample location and date.
4. Incubate the device at 20°–25° for no less than 5 additional days. Examine the media plates for growth. Record the total number of discrete colonies of microorganisms (cfu/sample) on the environmental sampling record based on sample type (i.e., surface). Include sample location and date.

Alternatively, to shorten the overall incubation period, two samples may be collected for each sample location.

1. Both samples could be TSA or one sample could be TSA and the other fungal media (e.g., MEA or SDA).
2. Incubate each sample in a separate incubator. Incubate one sample at 30°–35° for no less than 48 hours, and incubate the other sample at 20°–25° for no less than 5 days.
3. If fungal media are used as one of the samples, incubate the fungal media sample at 20°–25° for no less than 5 days.
4. Count the total number of discrete colonies of microorganisms on each sample, and record these results as cfu per sample. Record the results of the sampling.
5. Record the results of the sampling.

DATA EVALUATION AND ACTION LEVELS

Evaluate cfu counts against the action levels in *Table 4* and examine counts in relation to previous data to identify adverse results or trends. If two devices were collected at a single location, all recovered growth on each must be documented and action levels are applied to each piece of media individually (i.e., results from each sampling device must be compared to the action level for the area). If levels measured during surface sampling exceed the levels in *Table 4* for the ISO classification levels of the area sampled, the cause must be investigated and corrective action must be taken. Data collected in response to corrective actions must be reviewed to confirm that the actions taken have been effective. The corrective action plan must be dependent on the cfu count and the microorganism recovered. Examples of corrective action include process or facility improvements, personnel training, cleaning and disinfecting, or HEPA filter replacement and/or repair, or reducing the BUD of the radiopharmaceutical(s) during investigation and while carrying out the corrective action plan. The extent of the investigation should be consistent with the deviation and should include an evaluation of trends. The corrective action plan must be documented. If levels measured during surface sampling exceed the levels in *Table 4*, an attempt must be made to identify any microorganism recovered to the genus level (see ⟨1113⟩) with the assistance of a qualified individual (e.g., microbiologist or industrial hygienist).

Table 4. Action Levels for Surface Sampling

ISO Class	Surface Sampling Action Levels (cfu/device or swab)
5	>3
7	>5
8	>50

7. CLEANING AND DISINFECTING

Cleaning and disinfecting are important because surfaces in classified areas and SRPAs are a potential source of microbial contamination of sterile radiopharmaceuticals. The process of cleaning involves removing organic and inorganic residues from surfaces, usually with a manual or mechanical process and a cleaning agent. The process of disinfecting involves destruction of microorganisms, usually with a chemical or physical agent. Surfaces must be cleaned prior to being disinfected unless an Environmental Protection Agency (EPA)-registered (or equivalent) one-step disinfectant cleaner is used to accomplish both the cleaning and disinfection in one step. After cleaning and disinfecting or the application of a one-step disinfectant cleaner in a PEC, apply sterile 70% IPA to remove any residue.

Cleaning and disinfecting surfaces should occur at the minimum frequencies specified in *Table 5* or if activities are not performed daily, cleaning and disinfecting must be completed before initiating activities. The act of reducing or removing radioactivity (radioactive decontamination) from an object or surface must be balanced with the risk of spreading radioactive contamination. At times the best approach may be to shield the area until the radiation exposure levels are lower. This balance must be specified in SOPs (e.g., trigger levels for safe cleaning). The PEC should be checked for radioactive contamination prior to cleaning and disinfecting to prevent spreading radioactive contamination in the PEC.

All cleaning and disinfecting activities must be performed by trained and appropriately garbed personnel using facility-approved agents and procedures that must be described in written SOPs. Cleaning must be performed in the direction of most to least clean areas. The frequency, method(s), and location(s) of cleaning, disinfecting, and sporicidal agent use must be established in written SOPs, in accordance with the manufacturer's instructions when available, or based on sound microbiological cleaning techniques when unavailable, and must be followed by all cleaning personnel. The manufacturer's direction or published data for the minimum contact time must be followed for the cleaning, disinfecting, and sporicidal agents used. When sterile 70% IPA is used, it must be allowed to dry. All cleaning, disinfecting, and application of sporicidal agents must be documented according to facility SOPs.

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Table 5. Minimum Frequency for Cleaning and Disinfecting Surfaces in Classified Areas and within the Perimeter of the SRPA

Site	Cleaning	Disinfecting[a]	Applying Sporicidal
PEC(s) and equipment inside the PEC(s)	Prior to performing sterile processing of radiopharmaceuticals on each day that activities are carried out, the walls, bars, torso shield and any exposed surface of equipment inside the PEC must be cleaned to the extent possible as specified by the equipment manufacturer or the assessment of a qualified individual (e.g., microbiologist or industrial hygienist). Radioactive contamination may be shielded with appropriate temporary material, providing the material is covered with low-lint absorbent pads or has equivalent low-shedding properties.	Following cleaning on each day that activities are carried out, exposed surfaces of the equipment should be disinfected to the extent possible as specified by the equipment manufacturer or the assessment of a qualified individual (e.g., microbiologist or industrial hygienist). When used, remove low-lint absorbent pads and survey the PEC for radioactive contamination prior to disinfecting. Replace with new pads after disinfecting or as required after spills.	Monthly
Surfaces of sink(s)	Daily	Daily	Monthly
Hot-cells (all interior surfaces, dependent on design, equipment, and shielding present)	Daily	Daily	Monthly
PEC and the equipment inside the PEC(s) located in a hot-cell	Prior to performing sterile processing of radiopharmaceuticals on each day that activities are carried out, the walls, bars, torso shield, and any exposed surface of equipment inside the PEC to the extent possible as specified by the equipment manufacturer or the assessment of a qualified individual (e.g., microbiologist or industrial hygienist). Radioactive contamination may be temporarily shielded with appropriate temporary material providing the material is covered with low-lint absorbent pads or has equivalent low-shedding properties.	Following cleaning on each day that activities are carried out, exposed surfaces of the equipment should be disinfected to the extent possible as specified by the equipment manufacturer or the assessment of a qualified individual (e.g., microbiologist or industrial hygienist) and should be specified by SOPs. Remove low-lint absorbent pads and survey the PEC for radioactive contamination prior to disinfecting. Replace with new pads after disinfecting or as required after spills.	Monthly
Work surface(s) outside the PEC	Daily	Daily	Monthly
Ceiling(s)	Monthly	Monthly	Monthly
Wall(s), door(s), door frame(s), and other fixtures	Monthly	Monthly	Monthly
Floor(s)	Daily	Daily	Monthly
Storage shelving and storage bins	Monthly	Monthly	Monthly

[a] Many disinfectants registered with the EPA are one-step cleaning and disinfecting agents, which means that the disinfectant has been formulated to be effective in the presence of light to moderate soiling without a separate cleaning step. Cleaning and disinfecting must be balanced with the risk of spreading radiation contamination. The best approach may be to shield the area until the radiation exposure levels are lower.

7.1 Cleaning, Disinfecting, and Sporicidal Agents

Cleaning and disinfecting agents must be selected and used with careful consideration of compatibilities, effectiveness, and user safety. Considerations when selecting and using disinfectants include their anti-microbial activity, inactivation by organic matter, residue, shelf life, preparation requirements of the agent, and suitability for surfaces being disinfected (see *Disinfectants and Antiseptics* ⟨1072⟩). After the disinfectant is applied on the surface to be disinfected, the disinfectant must be allowed to dwell for the minimum contact time specified by the manufacturer, during which time the surface cannot be disturbed. Only the 70% IPA used in the ISO Class 5 PEC must be sterile. Sporicidal agents must be used at least monthly on all surfaces in classified areas and SRPAs. Some EPA-registered (or equivalent) one-step disinfectant cleaners may have sporicidal properties. See *Table 6* for a summary of the purpose of the cleaning, disinfecting, and sporicidal agents.

Table 6. Purpose of Cleaning, Disinfecting, and Sporicidal Agents

Type of Agent	Purpose
Cleaning agent	An agent for the removal of residues (e.g., dirt, debris, microbes, and residual drugs or chemicals) from surfaces.
Disinfecting agent	A chemical or physical agent used on inanimate surfaces and objects to destroy fungi, viruses, and bacteria.
Sporicidal agent	A chemical or physical agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

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7.2 Cleaning Supplies

All cleaning supplies (e.g., wipers and mop heads), with the exception of tool handles and holders, must be low-lint and should be disposable. If disposable cleaning supplies are used, they must be discarded after each cleaning activity. Reusable cleaning tools must be made of cleanable materials (e.g., no wooden handles) and must be cleaned and disinfected before and after each use. Reusable cleaning tools must be dedicated for use in the classified areas or SRPAs and must not be removed from these areas except for disposal. They must be discarded after an appropriate amount of time, to be determined based on the condition of the tools. Cleaning supplies and solutions used in the classified areas and SRPAs should be monitored for radioactive contamination after use and prior to disposal, as per facility SOPs. Dispose of cleaning supplies used in the classified areas and SRPAs in a manner that minimizes the potential for dispersing particulates into the air (e.g., with minimal agitation, away from work surfaces).

7.3 Cleaning and Disinfecting the PEC

Clean and disinfect the PEC at the minimum frequencies specified in *Table 5*. If the PEC contains a removable work tray, all sides of the work tray and the area underneath the work tray must be cleaned and disinfected at least monthly.

1. Survey all surfaces of the PEC for radioactive contamination and follow facility SOPs to decontaminate, if necessary.
2. Remove, if necessary, any particles, debris, or residue with an appropriate solution (e.g., *Sterile Water for Injection* or *Sterile Water for Irrigation*) using sterile, low-lint wipers.
3. Apply a cleaning agent followed by a disinfecting agent or apply an EPA-registered (or equivalent) one-step disinfectant cleaner and ensure that the contact time specified per manufacturer instructions is achieved.
4. Apply sterile 70% IPA
5. Allow the surface to dry completely before beginning activities.
6. The PEC must be wiped with a sporicidal agent at least monthly.

7.4 Disinfecting Supplies for Classified Areas and SRPAs

No shipping carton(s) or other corrugated or uncoated cardboard are allowed in the classified area (e.g., clean side of ante-room) or within the perimeter of the SRPA. Before items are introduced into a classified area or SRPA, they must be wiped with a sporicidal agent, EPA-registered (or equivalent) one-step disinfectant cleaner, or sterile 70% IPA using low-lint wipers. After the sporicidal or sterile disinfectant is applied onto the surface, the agent must be allowed to dwell on the surface for the minimum contact time specified by the manufacturer (see *6.1 General Monitoring Requirements*). The agent used for disinfecting the packaging must be compatible with the packaging and must not render the product label unreadable.

Any item to be transferred into the PEC from the classified area or SRPA must be disinfected with a sterile disinfectant (e.g., sterile 70% IPA).

In the case of radiopharmaceuticals being processed by remote means in a hot-cell, the opening of sterile packages (e.g., syringes, luer lock caps) may not be possible by remote means within the ISO Class 5 area. In this case, the syringes may be opened and appropriately labeled outside of the ISO Class 5 environment and placed in disinfected shielding, immediately prior to the forthcoming dispensing cycle.

7.5 Disinfecting Critical Sites

Critical sites (e.g., vial stoppers) must be wiped with sterile 70% IPA. The critical site must be wiped ensuring that both chemical and mechanical actions are used to remove contaminants. The sterile 70% IPA must be allowed to dry before piercing critical sites.

7.6 Cleaning and Disinfecting Items from Patient Care Area

Radiation shielding and equipment used in the classified area/SRPA or PEC that is exposed to patient care areas during the process of administration must be cleaned and disinfected before returning to any classified area (e.g., buffer or ante-room) or SRPA in accordance with the Centers for Disease Control and Prevention guidelines[1] as noncritical equipment requiring low-risk disinfection. Syringes that have been used in a patient care area must not be brought back into the classified area (e.g., buffer or ante-room) or SRPA for re-assaying or disposal unless the syringe is sealed inside an impervious container (e.g., sealed plastic bag) that is disinfected prior to entry into the classified area or SRPA. Equipment that has been exposed to needles and syringes contaminated with blood-borne pathogens and RAMs are considered mixed waste (e.g., syringe shields and syringe carrying containers). This equipment must be cleaned and disinfected through actions regulated by the facilities' SOPs. Equipment that contained or was in contact with mixed waste must be cleaned and disinfected with an appropriate agent(s) for blood.

8. ASSIGNING BUD

BUDs are based on the risk of microbial contamination with the assumption that the radiopharmaceutical(s) should remain chemically and physically stable, and its container–closure system should maintain its integrity for the duration of the BUD

[1] Centers for Disease Control and Prevention. *Guideline for Disinfection and Sterilization in Healthcare Facilities*, 2008.

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(*Table 7*). The time starts at the moment of the first sterile vial puncture or exposure of a critical site (e.g., syringe tip, needle hub, or needle) to ambient air, whichever is first. The BUDs stated in *Table 7* are maximum values in the absence of sterility testing, and the assigned BUD may be shorter for a variety of reasons discussed below. The individual responsible for the manipulation assigns the BUD based on established testing data, either performed in-house or obtained from peer-reviewed literature.

Table 7. Preparation Conditions for Sterile Radiopharmaceuticals

Preparation Conditions			
Manipulation	PEC	SEC	BUD (hours)
Immediate use	—	—	1
Direct infusion system, one puncture only (e.g., PET patient infusion system, Rb-82 generator)	—	—	10
Dispensing, repackaging, preparation, and preparation with minor deviations	ISO Class 5	SRPA	12
Radionuclide generator storage/elution (e.g., non-direct infusion system; Tc-99m or Ga-68)	—	SRPA with ISO Class 8 total airborne particle count	12
Radionuclide generator storage/elution (e.g., non-direct infusion system; Tc-99m or Ga-68)	—	ISO Class 8 or better buffer area with ISO Class 8 or better ante-room	24
Dispensing, repackaging, preparation, and preparation with minor deviations	ISO Class 5	ISO Class 8 or better buffer area with ISO Class 8 or better ante-room	24
Dispensing, repackaging, preparation, preparation with minor deviations, and compounding using sterile components	ISO Class 5	ISO Class 7 or better buffer area with ISO Class 8 or better ante-room	96
Dispensing, repackaging, preparation, preparation with minor deviations, and compounding using a nonsterile component and performing sterilization procedure (e.g., filtration with bubble point testing) but without performing *Sterility Tests* ⟨71⟩ testing	ISO Class 5	ISO Class 7 or better buffer area with ISO Class 8 or better ante-room	24
Radiolabeled blood components for immediate use [e.g., Tc 99m red blood cells (RBC)]	—	—	1
Radiolabeled blood components (e.g., radiolabeled leukocytes)	ISO Class 5 BSC	ISO Class 7 or better buffer area with ISO Class 8 or better ante-room	6 h after the blood sample is obtained

For compounded preparations (sterile and nonsterile), the BUD is also dependent on maintenance of appropriate quality and purity, including radiochemical purity, radionuclidic purity, and other applicable parameters as specified in individual monographs or as clinically appropriate.

For preparations with minor deviations involving conventionally manufactured kits (sterile and nonsterile), the kit may state or suggest a use-by time in the package insert. For certain radiopharmaceuticals transportation time, radionuclide availability, and other factors may necessitate extending manufacturer-stated/suggested use-by time to meet patient needs. Assigning a BUD longer than the manufacturer-stated/suggested use-by time interval must be supported by evidence of the maintenance of appropriate quality and purity, including radiochemical purity and radionuclidic purity as specified in individual monographs, and other applicable parameters as clinically appropriate.

Assignment of a BUD for a radiopharmaceutical(s) must consider several factors, as applicable. Issues of concern include, but are not limited to, the following:

- Sterility: Maintenance of sterility is a major concern for any sterile preparation or product. Good aseptic handling practices in an appropriate environmentally-controlled area are the most critical factors in ensuring sterility. See *Table 7* for maximum BUD. The assigned BUD should not exceed the sterility-related times listed in *Table 7*, unless a longer time is justified by *Sterility Tests* ⟨71⟩.
- Radiochemical purity: Maintenance of radiochemical purity is affected by a variety of factors including, but not limited to, storage temperature, quantity of radioactivity, radioactivity concentration, presence or absence of antioxidants or other stabilizing agents, and container type (e.g., glass vial vs. plastic syringe). The assigned BUD must be based on stability studies in which these variables are controlled and are representative of the conditions of actual use. For factors that allow a range of values (e.g., storage temperature, quantity of radioactivity, radioactivity concentration), studies should be conducted at the extremes of the ranges.
- Radionuclidic purity: Because radionuclidic impurities may decay away more slowly than the primary radionuclide, the radionuclidic purity may decrease over time. For example, the ratio of Mo-99 (half-life of about 66 hours) to Tc-99m

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(half-life of about 6 hours) continuously increases over time. *USP* monographs for Tc-99m radiopharmaceuticals require that the radionuclidic impurity Mo-99 not exceed 0.15 µCi Mo-99 per mCi Tc-99m at the time of administration. Calculation of radionuclidic purity at future times is necessary to ensure compliance throughout the assigned BUD.

- Age of generator eluate: As a generator eluate decays, the desired daughter radionuclide decays to form other nuclides and potential radiolytic products, which may interfere with radiolabeling of kits. For example, Tc-99m undergoes decay to Tc-99. More importantly, increasing amounts of peroxides formed as radiation interacts with water molecules. Increased amounts of Tc-99 and peroxides can interfere with the radiolabeling of many kits. Extension of the BUD for Tc-99m pertechnetate intended for radiolabeling of kits must take into account the build-up of Tc-99 and peroxides over time.
- Number of particles: For radiolabeled particulates, the number of particles per unit radioactivity increases over time as the radionuclide decays. For example, the BUD for Tc-99m albumin aggregated [macroaggregated albumin (MAA)] must take into account the increasing ratio over time of the number of particles per unit radioactivity. For example, if an MAA kit is prepared such that the radioactive patient dose is 200,000 particles at the time of calibration, the same patient dose will contain 700,000 particles at 10.85 hours after calibration. Calculation of the number of MAA particles in the patient dose is necessary to ensure compliance with the prescribed particle range throughout the assigned BUD.
- Specific activity: For some receptor-based radiopharmaceuticals, the mass quantity may influence uptake (i.e., too much mass may result in saturation of receptor sites and reduce target uptake of the radiopharmaceutical). As radioactivity decays over time, specific activity decreases resulting in more mass per unit radioactivity. In such situations, the assigned BUD must ensure that the patient dose contains no more than the specified maximum mass.
- Container type: Because radiochemical stability or other quality attributes of a radiopharmaceutical may be affected by its container characteristics, the BUD for a radiopharmaceutical dose dispensed in a plastic syringe may be different than the BUD of that same radiopharmaceutical maintained in a glass vial. The assigned BUD must be determined in the proper storage container.
- Cell viability: The viability of radiolabeled blood cells (e.g., leukocytes) decreases over time, and may also be affected by other factors such as the suspending medium, temperature, and agitation. The assigned BUD should be as short as circumstances reasonably allow so as to maximize cell viability.
- In the case of manufactured radiopharmaceuticals that are distributed to nuclear pharmacies or other healthcare facilities for terminal distribution/dispensing, the assigned BUD of the dispensed dose cannot exceed the expiration date/time of the manufactured radiopharmaceutical(s).
- In the case of radiopharmaceuticals prepared from kits, the BUD of a dispensed dose cannot exceed the assigned BUD of the finished kit preparation.
- A radiopharmaceutical may not exceed the shortest BUD of any of its components.

The facility must have policies and SOPs appropriate to the assignment of BUD and maintain documentation of applicable study results and calculations. Studies of radiolabeling efficiency and radiochemical stability should employ quality control (QC) testing methods described in the manufacturer's package insert, *USP* monographs and general chapters, or other equivalent testing methods and be sufficiently rigorous to allow statistical confidence in the results.

The facility must have SOPs to collect and evaluate complaints associated with the use of radiopharmaceuticals having assigned BUDs. Policies and SOPs should also be in place to reevaluate the assigned BUD based on complaints, which may include repeating studies and/or performing additional studies on radiolabeling efficiency and/or radiochemical stability.

9. DOCUMENTATION

Applicable records (hard-copy or electronic), including policies and SOPs, must be maintained for all activities involved in repackaging, preparing, preparing with minor deviations, compounding, and dispensing radiopharmaceuticals. Such records include, but are not limited to:

- Personnel training and testing, including visual assessment of aseptic technique competency, validation, garbing, hand hygiene, equipment/environment cleaning and disinfecting, gloved fingertip and thumb sampling, and media fill evaluation initially and follow up testing at specified intervals.
- Testing and monitoring of environmental controls, including ISO classification, ACPH, pressure differentials, temperature, humidity and viable air/surface and total airborne particle test results
- Equipment maintenance and cleaning/disinfecting
- End product radiochemical purity and other testing, as applicable, results of preparations, preparations with minor deviations, and compounded preparations
- Master Formulation Record (MFR) for preparation with minor deviation(s) and compounding
- Validation of stability testing to support the assigned BUD from SOPs by the compounder or derived from accepted literature
- Investigations and corrective actions and tracking of events to closure.

9.1 Master Formulation Record

A MFR is required only for a preparation with minor deviations or compounding, as described in *11. Compounding*. A MFR is not required for a preparation following the manufacturer's instructions.

Data that must be included in the MFR are as follows:

- Name of the radiopharmaceutical

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- Name, identity, strength, purity, quality, and quantity of ingredients with validated documentation (e.g., CoA)
- Detailed procedure (e.g., heating, components, incubation time)
- Range of radioactivity
- Range of volume
- Equipment to be used
- PEC and SEC to be used, if applicable
- Quality control tests to be performed for final release of the radiopharmaceutical (e.g., radiochemical purity, pH)
- Procedures for depyrogenation and sterility procedures and validations, as applicable, including limits
- Trained personnel
- Garbing procedure, if different than standard procedure
- Container(s)
- Reference source of the BUD assignment and storage conditions

9.2 Records for Preparation with Minor Deviations/Compounding

A record for preparation with minor deviation or compounding must include the following:

- Name of the radiopharmaceutical
- Physical form (e.g., capsule or solution)
- Name and quantity of ingredients including calibration time for radioactive ingredients (e.g., 100 mCi Tc 99m sodium pertechnetate @ 1300)
- Total volume
- Reference to the MFR
- Any deviation from the MFR, if applicable
- Name of vendor or manufacturer, lot numbers, and expiration dates of all ingredients and components
- Name of the person who prepared and name of the supervising personnel (e.g., ANP or AU physician)
- Date and time of preparation
- Assigned internal identification number (e.g., lot number)
- Unique reference [e.g., prescription, order number(s)]
- Assigned BUD and storage requirements
- Documentation of QC results

10. PREPARATION

The individual responsible for preparing the radiopharmaceutical(s) must ensure that the final preparation complies with quality and purity specifications throughout the assigned BUD. This includes, as appropriate for the preparation, radionuclidic purity, radiochemical purity, chemical purity, and physical and chemical properties.

10.1 Preparation Following Manufacturer Instructions

NONSTERILE PREPARATIONS

For nonsterile preparations, follow manufacturer preparation instructions (e.g., I-131 NaI capsules or solution), taking into account appropriate radiation safety considerations and environmental controls, if applicable (e.g., negative air pressure area, chemical fume hood, activated charcoal filters when handling a potentially volatile radionuclide). The area should be suitably cleaned and uncluttered to ensure the overall integrity and quality of the prepared radiopharmaceutical(s). There should be a documented process for activities (e.g., cleaning) between the preparation cycles of different nonsterile products, to decrease the likelihood of contamination from other prepared products.

STERILE PREPARATIONS

For sterile preparations (including intravascular devices), follow manufacturer preparation instructions, taking into account appropriate radiation safety considerations, appropriate environmental controls, and aseptic handling practices to maintain sterility. The minimum environmental standard for the preparation of sterile radiopharmaceuticals beyond immediate-use is within an ISO Classified area or device (see *Table 7*). Refer to *5. Facilities and Environmental Controls* and *Table 7* on the location of the PEC and the assignment of the BUD.

10.2 Preparation with Minor Deviations

In some cases, radiopharmaceuticals are prepared with minor deviations from manufacturer instructions that are necessary to accommodate circumstances not contemplated in the FDA-approved labeling. Note that *General Notices, 5.20.20.1 In Compounded Preparations* includes the statement: "Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the

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relevant standards and to preparations produced by following the specified process." However, except for a few receptor-based radiopharmaceuticals where specific activity is an important parameter, there is a very broad range of acceptable values for specific activity and for proportions of ingredients. Deviations from manufacturer preparation instructions for radiopharmaceuticals must maintain the same ingredients but may differ in their proportions.

This requires appropriate in-house QC testing, designed to validate the radiochemical purity of the product for the entirety of the BUD or is supported by appropriate peer-reviewed publications for the minor deviation utilized.

Examples of minor deviations include, but are not limited to, the following:

- Altering the quantity of radioactivity or volume added to the vial
- Changes in step-by-step operations (e.g., dilute Tc-99m sodium pertechnetate after rather than before addition to the vial)
- Using alternative devices or equipment (e.g., a heating block rather than a hot water bath, using a different sized needle, different shielding materials)
- Using QC test methods other than those described in the product labeling (e.g., radiochemical purity)
- Filtering Tc-99m sulfur colloid

10.3 Preparation of Radiolabeled Blood Components

Handling blood and radiolabeling of blood components requires special attention to biological risks and must be handled with standard precautions using aseptic technique to prevent the introduction of new microorganisms into the preparation that will be administered. Due to the potential presence of microorganisms in the original blood sample, the preparation must be administered as soon as possible but no later than 6 hours after the blood sample is obtained from the patient or blood bank.

The presence of microorganisms in a blood sample may present a risk to the individual performing the preparation as well as cross-contamination to other blood samples or other non-blood related radiopharmaceuticals. Equipment and supplies should never be shared with other activities unless they are first thoroughly cleaned and disinfected. Special precautions when radiolabeling of blood components for non-immediate use include:

- There must be complete physical separation (either fixed or non-fixed wall) of areas where blood products are handled from areas where non-blood products are handled. An ISO Class 5 BSC located in an ISO Class 7 buffer area is required for blood-labeling processes. If more than one ISO Class 5 PEC is located within the ISO Class 7 buffer area, policies and SOPs must be in place to include certification that the SEC meets conditions of air quality at maximum occupancy under dynamic operating conditions.
- One radiolabeling procedure per PEC at a time. Blood products from more than one patient must never be manipulated at the same workstation at the same time. Each area should have dedicated supplies, equipment (including dose calibrator), and waste disposal to eliminate sharing of these items or overlap in pathways.
- Thorough cleaning and disinfection of the ISO Class 5 BSC and all reusable equipment within, prior to starting another blood component radiolabeling procedure.
- If a dedicated dose calibrator is not available, then a means of preventing the blood container(s) from contaminating the dose calibrator must be used or the dose calibrator dipper and liner must be cleaned and disinfected following the radioassay.
- Centrifuge should be located within the ISO Class 7 buffer area that is dedicated for blood component radiolabeling processes.
- Dedicated (per each radiolabeling procedure) consumable products (e.g., 0.9% sodium chloride injection, diluent, tubes, syringes, and other supplies) necessary for each individual patient radiolabeling procedure.
- All tubes and syringes in contact with the patient's blood components must be clearly labeled with the patient's name and at least one additional identifier (e.g., date of birth, medical record number, barcode).
- Dedicated syringe shields and vial shields.
- Remove and replace any garb that enters the ISO Class 5 BSC before handling anything else not related to performing this procedure.
- Removal of all disposable items from the ISO Class 5 BSC utilized in each radiolabeling procedure.
- Cleaning and disinfection of all reusable equipment and components (e.g., BSC, centrifuge, dose calibrator, syringe shields, vial shields, syringe transport shields and delivery cases) after each radiolabeling procedure prior to any further use. Policies and SOPs must address cleaning and disinfection processes including the use of an EPA-registered (or equivalent) one-step disinfectant cleaner with activity against blood-borne pathogens followed by sterile 70% IPA. Sterile 70% IPA alone is not sufficient.
- After the completion of blood radiolabeling procedures, follow all requirements in *4.5 Hand Hygiene and Garbing for Buffer Areas and segregated Radiopharmaceutical Processing Area*.

10.4 Preparation of Radiolabeled Red Blood Cells for Immediate Use

In vitro red blood cell labeling must be prepared while following the conditions below:

- A dedicated space for blood handling must be designated through the entirety of the blood radiolabeling process. This area must be free from clutter and not used for any other radiopharmaceutical preparation or handling until the completion of cleaning and disinfection.
- Perform only one radiolabeling procedure at a time or have documented processes that maintain the integrity of samples and environment.

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- Dedicated equipment must be used for blood radiolabeling procedure (e.g., L-block, syringe shield, vial shield, forceps, needle recapper).
- If a dedicated dose calibrator is not available, then a means of preventing the blood container(s) from contaminating the dose calibrator or a cleaning and disinfecting procedure with an appropriate product must be used to decontaminate the dipper and liner of the dose calibrator following the radioassay
- A cleaning and disinfecting procedure with an appropriate agent(s) must be used to decontaminate the area and equipment prior to and after the radiolabeling is complete and all disposable components have been discarded
- Follow all requirements in *4.4 Hand Hygiene and Garbing for Immediate Use Preparations*.
- The start time of the preparation must begin with the initial container puncture or the exposure of a critical site (e.g., syringe tip, needle hub or needle) to ambient air, whichever is first.
- BUD of 1 hour (see *Table 7*).

11. COMPOUNDING

Each compounding activity must be based on a pre-established written procedure and must include maintenance of compounding records. The compounding record must provide traceability (see *9. Documentation*).

All sterile compounding, using aseptic technique, must be performed in an ISO 5 PEC. Refer to *5.7 Environmental Controls* and *Table 7* for further clarification on the location of the PEC and the applicability of the radiopharmaceutical BUD.

Compounding must not be performed for any radiopharmaceutical(s) that has been withdrawn from the market because of safety or lack of effectiveness, unless part of an institutional review board approved investigational study. Radiopharmaceuticals that are essentially copies of marketed FDA-approved radiopharmaceuticals must not be compounded unless there is a change that produces a clinical difference for an identified individual patient, as determined by a prescriber.

11.1 Compounding Nonsterile Radiopharmaceuticals

Compounding nonsterile radiopharmaceuticals is the combining, mixing, diluting, pooling, reconstituting or otherwise altering a drug or bulk drug substance other than as provided by the manufacturer's package insert to create a nonsterile radiopharmaceutical. Examples of compounding nonsterile radiopharmaceuticals include: changing the dosage form of a capsule to a solution, changing an intravenous dosage form to an oral dosage form, and radiolabeling a food for oral administration (e.g., Tc-99m sulfur colloid in eggs). Areas designated for nonsterile compounding must be cleaned and uncluttered and separated from areas designated for sterile radiopharmaceuticals. Compounding should take into account RAM licensing requirements for appropriate radiation safety considerations and utilize appropriate environmental controls, if applicable (e.g., chemical fume hood, activated charcoal filters when handling potentially volatile radionuclides). The placement of equipment and materials must take into account a design that prevents cross-contamination.

When feasible, disposable material should be used to reduce the chance of cross-contamination. Each compound must have a unique MFR (see *9.1 Master Formulation Record*). The preparation information is documented on a compounding record (see *9.2 Records for Preparation with Minor Deviations/Compounding*). The MFR details the selection of all components. The ingredients must be obtained from sources in this preferential order: FDA-approved product; FDA-registered facility; and lastly, if the ingredients for the compound are not available from either of these two sources, the MFR must detail the selection of a material that is suitable for the intended use. The MFR must establish the identity, strength, purity, and quality of the ingredients by validated means (e.g., CoA). Requirements for nonsterile oral meal components are limited to common food grade description and are not required to establish identity by validated means.

A BUD for the compounded radiopharmaceutical must be validated, taking into account the stability of the ingredients, any intermediate containers, the final container, and the storage conditions. A BUD cannot be extended past the labeled expiration date of any component in the compound. If the compounded radiopharmaceutical(s) includes components from other preparations or preparations with minor deviations, the BUD of the final compounded radiopharmaceutical must not exceed the shortest remaining BUD of any of those components.

11.2 Sterile Compounding

Some compounding activities involve only the addition of a conventionally manufactured drug product (e.g., *Ascorbic Acid Injection, Lidocaine Hydrochloride Injection, Sodium Bicarbonate Injection*) approved by the appropriate regulatory agency to a radiopharmaceutical.

Personnel responsible for compounding must consider all possible interactions between the components, such as altered chemical stability, radiochemical stability, solubility, or other parameters (e.g., osmolality) related to changes in pH, excipients, or other factors, in determining an appropriate BUD. In some cases, this may require systematic QC testing over time to validate the appropriateness of a particular BUD.

Another activity that is considered a compounding activity is the splitting of conventionally marketed kits. Kit-splitting (also referred to as "fractionation") may be used to meet patient need. For example, the contents of a kit vial can be reconstituted with 0.9% sodium chloride injection and aliquoted into other containers for storage and subsequent radiolabeling. The individual responsible must consider all possible interactions of kit components with these other containers (e.g., container walls, closures), as well as possible alterations in stability (e.g., physical stability, chemical stability) that may affect radiolabeling yields or performance parameters, when determining an appropriate BUD. Systematic QC testing is required to validate the appropriateness of a particular BUD.

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11.3 Sterile Compounding Using a Nonsterile Drug Substance or Components

Some sterile compounding activities involve the use of materials other than commercially marketed products, such as drug substances and/or radionuclides. If one or more materials or components are not certified to be sterile and pyrogen-free, a sterilization procedure (e.g., filtration with bubble point testing) and testing described in ⟨85⟩ must be performed. The designated person for compounding is responsible for ensuring that the final preparation complies with pre-established standards or acceptance criteria for identity, quality, and purity, and must consider all possible interactions between the components, such as altered chemical stability, radiochemical stability, solubility, or other parameters (e.g., osmolality) related to changes in pH, excipients, or other factors, in determining an appropriate BUD. This may require testing to validate the appropriateness of a particular BUD.

If compounding involves a bulk drug substance, the radiopharmaceutical must comply with standards of an applicable *USP* or *NF* monograph, if one exists, or be a component of an approved drug product. For this chapter, a bulk drug substance includes a radionuclide, a ligand, or other substance, such as a precursor that becomes an active ingredient in the final radiopharmaceutical. Each bulk drug substance should be manufactured by drug establishments registered with FDA and be accompanied by a valid CoA or equivalent testing procedures.

If compounding involves excipients or other inactive ingredients, the excipients or other inactive ingredients must comply with standards of an applicable *USP* or *NF* monograph, if one exists. It is also acceptable that any excipients or other inactive ingredients be approved products, manufactured by a drug establishment registered with the FDA.

12. DISPENSING

12.1 Dispensing and Radioassay

Dispensing refers to the manipulations necessary to transfer the prescribed or ordered amount of radiopharmaceutical into the final container (e.g., syringe or vial). Dispensing can take place from single-dose or multiple-dose containers of prepared, prepared with minor deviations, compounded, or manufactured radiopharmaceuticals, and may involve needle changes, affixing a sterile cap, or dilution (e.g., adding 0.9% sodium chloride injection) in the final container. For nonsterile radiopharmaceuticals, an example is obtaining 1 capsule from a container holding 1 or more capsules. For sterile radiopharmaceuticals, an example is withdrawing a volume of solution from a single-use or multiple-dose container into a syringe. Labeling of the final patient-ready dose or ordered amount of a radiopharmaceutical is also a component of the dispensing process.

Except for an unopened manufacturer container, the final dose or ordered amount must be radioassayed (i.e., in a dose calibrator). The measured activity should be mathematically corrected for radioactive decay to the time of scheduled administration (calibration time) (refer to *14. Quality Assurance and Quality Control*). The activity at calibration time must always be within federal, state, and local variance limits.

12.2 Labeling

The labeling of radiopharmaceuticals can fall under the jurisdiction of numerous regulatory agencies. Individual boards of pharmacy and other regulatory bodies may have very specific statutes and/or regulations concerning this process. The requirements specified in this chapter must be considered the minimum requirements for the labeling of the inner container (e.g., syringe, vial) and the outer shielding (e.g., syringe or vial shielding). Therefore, all personnel distributing and/or dispensing radiopharmaceuticals should verify that any labeling is in compliance with regulatory agencies.

The inner container must be labeled with the following:

- Standard radiation symbol
- The words "Caution—Radioactive Material"
- For all therapeutic and blood-products, the patient name/identifier
- Radionuclide and chemical form (generic name)
- Radioactivity at the date and time of calibration

The outer shielding must be labeled with the following:

- Standard radiation symbol
- The words "Caution—Radioactive Material"
- For all therapeutic and blood-products, the patient name/identifier
- Radionuclide and chemical form (generic name)
- Radioactivity at the date and time of calibration
- Volume or number of units dispensed (e.g., 2 capsules), as applicable
- Product expiration or BUD (see *Table 7*), as applicable, and any special storage and handling instructions for non-immediate use (e.g., refrigeration, resuspension)
- Route of administration

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12.3 Direct Infusion Systems

The information in this section is limited to the sterility and aseptic technique for direct infusion systems. The described infusion systems are FDA-cleared medical devices or FDA-approved direct infusion generators without an ISO-5 environment. The manner in which all necessary solutions (e.g., radiopharmaceutical and eluant/diluent) are used in conjunction with the system was a consideration in the overall approval process for the system. Therefore, all operators of the direct infusion systems must follow the "Instructions for Use" in the device labeling.

- Direct infusion generators (e.g., rubidium chloride Rb 82 injection) may employ a container of eluant (e.g., bag of 0.9% sodium chloride injection) to allow administration of the eluate directly to patient(s).
- Direct infusion devices (e.g., portable PET patient-infusion system) provide a method for dispensing and administration from a multiple-dose container of the radiopharmaceutical (e.g., fludeoxyglucose F 18 injection) and the diluent (e.g., 0.9% sodium chloride injection) directly to patients to reduce the radiation exposure to personnel.

In each of these situations, the radiopharmaceutical container must be attached to or be needle-punctured by the respective direct infusion system. Given that such direct infusion systems are intended for multiple patients over the course of several hours, there could be a sterility concern if not operated properly. Therefore, the following parameters must be considered by the operator of the system:

- Setup attachment or needle-puncture should be performed in a defined environment
- Aseptic handling in ambient air with a maximum BUD of 10 hours is allowed for these direct infusion systems (see *Table 7*)
- The 0.9% sodium chloride bag attached to the device may only be punctured once and may be used for no more than 10 hours. The bag must be labeled with the date and time of puncture and the BUD
- Any nonsterile parts of the device that may encounter the septum of the radiopharmaceutical vial must be disinfected with sterile 70% IPA prior to puncturing the vial with the needle
- The septum of any vial and the ports of any diluent bag must be wiped with sterile 70% IPA prior to puncturing
- When puncturing the vial in ambient air, it must only be punctured once
- If there are problems with the infusion device, no sterile container(s) associated with the system can be repunctured or transferred to a PEC for further manipulations and the container, with contents, must be discarded

12.4 Transporting Generators Between Facilities

The following standards must be followed if transporting generators between facilities:

- The generator needle and/or ports must be capped in ISO Class 8 air or better with sterile protectors
- The generator must be packaged and transported in a manner to maintain the integrity and sterility of the generator system

13. REPACKAGING

Repackaging refers to the act of removing conventionally manufactured radiopharmaceutical(s) from the container in which it was distributed by the original manufacturer and placing it into a different container without further manipulation of the product. Repackaging also includes the act of placing the contents of multiple containers of the same finished drug product into one container, as long as the container does not include other ingredients. Repackaging may be performed for nonsterile radiopharmaceuticals (e.g., I-131 sodium iodide oral capsules) and for sterile radiopharmaceuticals (e.g., thallous chloride Tl 201 injection).

Except for unopened manufacturer dosage units (e.g., capsules, Xe-133 vials), the repackaged radiopharmaceutical must be radioassayed (i.e., in a dose calibrator). The inner container should be labeled with the following:

- Standard radiation symbol
- The words "Caution—Radioactive Material"
- The radionuclide and chemical form (generic name)
- Radioactivity with units at time of calibration and the calibration time

The outer shielding should be labeled with the following:

- Standard radiation symbol
- The words "Caution—Radioactive Material"
- The radionuclide and chemical form (generic name)
- Radioactivity with units at time of calibration and the calibration time
- Volume, or number of units (e.g., capsules), as applicable
- Product expiration or BUD (see *Table 7*), as applicable
- Special storage and handling instructions

14. QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance (QA) is a system of procedures, activities, and oversight that ensures that radiopharmaceutical processing consistently meets quality standards (see *Quality Assurance in Pharmaceutical Compounding* ⟨1163⟩). Quality control (QC) is the

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sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the radiopharmaceutical(s).

A facility's QA and QC programs must be formally established and documented in SOPs that ensure that all aspects of the handling of radiopharmaceuticals are conducted in accordance with this chapter and applicable federal, state, and local laws and regulations. A designated person must ensure that the facility has formal, written QA and QC programs that establish a system of:

1. Adherence to procedures,
2. Prevention and detection of errors and other quality problems,
3. Evaluation of complaints and adverse events, and
4. Appropriate investigations and corrective actions.

The SOPs must describe the roles, duties, and training of the personnel responsible for each aspect of the QA program. The overall QA and QC program must be reviewed at least once every 12 months by the designated person. The results of the review must be documented and appropriate corrective action taken, if needed.

14.1 Notification About and Recall of Out-of-Specification Dispensed Radiopharmaceuticals

If a radiopharmaceutical is dispensed or administered before the results of release testing are known, the facility must have SOPs in place to:

1. Immediately notify the prescriber of a failure of specifications with the potential to cause patient harm (e.g., sterility, strength, purity, bacterial endotoxin, or other quality attributes), and
2. Determine whether a recall is necessary.

The SOP for recall of out-of-specification dispensed radiopharmaceuticals must contain procedures to:

- Determine the severity of the problem and the urgency for the implementation and completion of the recall
- Determine the distribution of any affected radiopharmaceutical, including the date and quantity
- Identify patients who have received the radiopharmaceutical
- Outline the disposition and reconciliation of the recalled radiopharmaceutical

The facility must document the implementation of the recall procedures. The recall must be reported to appropriate regulatory bodies as required by laws and regulations of the applicable regulatory jurisdiction (e.g., state board of pharmacy, state health department).

14.2 Complaint Handling

Radiopharmaceutical facilities must develop and implement SOPs for handling complaints. Complaints may include concerns or reports on the quality and container labeling of, or possible adverse reactions to, a specific radiopharmaceutical.

A designated person must review all complaints to determine if they indicate potential quality problems with the radiopharmaceutical. If a complaint does, an investigation into the potential cause of the problem must be completed. The investigation must consider whether the quality problem could extend to other radiopharmaceuticals. Corrective action, if necessary, must be implemented for all potentially affected radiopharmaceuticals. Consider whether to initiate a recall of potentially affected radiopharmaceuticals and whether to cease sterile compounding until all underlying problems have been identified and corrected.

A readily retrievable record (written or electronic) of each complaint must be kept by the facility, regardless of the source of the complaint (e.g., e-mail, telephone, mail). The record must contain the name of the complainant, the date the complaint was received, the nature of the complaint, the response to the complaint, and, if known, the name and strength of the radiopharmaceutical and the assigned internal identification number (e.g., prescription, order, or lot number).

The record must also include the findings of any investigation and any follow-up. Records of complaints must be easily retrievable for review and evaluation for possible trends and must be retained in accordance with the record keeping requirements in *9. Documentation*. A radiopharmaceutical that is returned in connection with a complaint must be quarantined until it is destroyed after completion of the investigation and in accordance with applicable jurisdictional laws and regulations.

14.3 Adverse Event Reporting

Adverse events potentially associated with the quality of radiopharmaceuticals must be reported in accordance with the facility's SOPs and all applicable jurisdictional laws and regulations. In addition, adverse events potentially associated with the quality of the radiopharmaceutical preparation should be reported to the applicable jurisdictional regulatory body (e.g., state boards of pharmacy, state health departments, FDA's MedWatch program for human drugs).

GLOSSARY

Administration: The direct and immediate application of a radiopharmaceutical to a patient by injecting, infusing, ingesting, or otherwise providing a radiopharmaceutical in its final form.

Airlock: A space with interlocked doors, constructed to maintain air pressure control when items move between two adjoining areas.

Ante-room: An ISO Class 8 or cleaner area with fixed walls and doors where personnel hand hygiene, garbing procedures, and other activities that generate high particulate levels are performed. The ante-room is the transition area between the unclassified area in a facility and the classified buffer area.

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Aseptic processing or preparation: A process by which separate, sterile components (e.g., drugs, containers, or closures) are brought together under conditions that maintain their sterility.

Aseptic technique: Methods utilized during the processing of radiopharmaceuticals to keep objects and areas free of microorganisms and thereby minimize infection risk to the patient. It is accomplished through practices that maintain the microbe count at a nearly irreducible number.

As low as (is) reasonably achievable (ALARA): The effort to maintain exposures to ionizing radiation as far below the dose limits as practical. These efforts should be consistent with the purpose for which the licensed activity is undertaken, in relation to utilization of licensed materials in the public interest. Limiting exposure time, using adequate shielding, and maintaining the most distance possible from all radioactive sources (i.e., time, distance, shielding) are the basic principles for successfully following ALARA guidelines.

Authorized nuclear pharmacist (ANP): A pharmacist recognized by the U.S. Nuclear Regulatory Commission or an Agreement State agency as having met training and experience requirements for the practice of nuclear pharmacy.

Authorized user (AU): A physician recognized by the U.S. Nuclear Regulatory Commission or an Agreement State agency as meeting training and experience requirements for specified medical uses of radioactive material.

Beyond-use date (BUD): The assigned date and time beyond which the radiopharmaceutical must not be administered.

Biological safety cabinet (BSC) Class II: A ventilated cabinet with an open front and inward and downward unidirectional HEPA-filtered airflow and HEPA-filtered exhaust.

Blood components: Any constituent of blood that is separated by physical or mechanical means (e.g., red cells, white cells, platelets)

Buffer area: An ISO Class 8 or cleaner area with fixed walls and doors where PEC(s) that generate and maintain an ISO Class 5 environment are physically located. The buffer area may only be accessed through the ante-room.

Chemical purity: The fraction of the total chemical species present in the radiopharmaceutical as the specified chemical component(s). A chemical impurity is the presence of an unwanted non-radioactive chemical.

Classified area: An area that maintains an air quality classification based on the ISO guidelines (i.e., ante-room, buffer area). See *ISO class*.

Cleaning agent: A material for the removal of residues (e.g., dirt, debris, microbes, and residual drugs or chemicals) from surfaces.

Compounding: The combining, mixing, pooling, or otherwise altering (excluding preparation with minor deviations) of a conventionally manufactured radiopharmaceutical or synthesizing/formulating a radiopharmaceutical from bulk drug substances and radionuclides. See *Preparation with minor deviations*.

Container–closure system: The packaging components that contain or come in contact with the radiopharmaceutical and maintain the integrity of the radiopharmaceutical contained within. Examples include (but are not limited to) vials, tubes and syringes.

Critical site: A location that includes any component or fluid pathway surface (e.g., vial septa, injection ports) or openings (e.g., needle hubs) that, when exposed is at risk for contamination by direct contact with air (e.g., ambient area or HEPA-filtered), moisture (e.g., oral and mucosal secretions), or touch.

Designated person: One or more individuals assigned to be responsible and accountable for the performance and operation of the radiopharmaceutical processing facility and for personnel who prepare, compound, dispense, and repackage radiopharmaceuticals.

Direct infusion system: An FDA-cleared medical device used to dispense and/or administer radiopharmaceuticals to multiple patients. The standards of this chapter pertain to devices with ambient air that lack and ISO Class 5 environment.

Direct processing area (DPA): An area within the ISO Class 5 PEC where critical sites are exposed to unidirectional HEPA-filtered air, also known as first air.

Disinfectant: A chemical or physical agent used on inanimate surfaces and objects to destroy microbiological contamination (e.g., fungi, viruses, and bacteria) when used in the appropriate concentrations and for the appropriate contact times. Sporicidal disinfectant agents are considered a special class of disinfectants that also are effective against bacterial endospores and fungal spores.

Dispensing: The manipulation or labeling of a radiopharmaceutical to render it in its final form for administration, typically obtained from a single-dose or multiple-dose container (e.g., withdrawing a volume of finished product or preparation from a vial into a syringe). Dispensing is performed under the supervision of a physician or pharmacist and for radiopharmaceuticals includes dilution with an appropriate diluent or adjusting the activity in an individual dosage.

Dose pooling: The combining of doses from two or more syringes to meet one patient's need, also see "repackaging".

Dose splitting: The splitting of a patient-ready unit dose for use with more than one patient.

Dynamic operating conditions: Conditions in the SRPA or classified area in which operating personnel are present and performing actual or simulated activities. The PEC should contain equipment and materials regularly used for radiopharmaceutical processing (e.g., low-lint absorbent pads, dose calibrator, syringe shields).

Expiration date: For conventionally manufactured radiopharmaceuticals, the specified date (and time) beyond which the product must not be administered. The expiration date is determined by the manufacturer.

First air: The air exiting the HEPA filter in a unidirectional air stream.

Garb: Gloves, gowns, shoe covers, head (covers ears and all hair) and facial hair covers, masks, and other items designed to reduce particle shedding from personnel and minimize the risk of microbiological contamination to radiopharmaceuticals.

High efficiency particulate air (HEPA) filtration: Using a tested and certified air filter designed to remove 99.97% of airborne particles measuring 0.3-micron or greater in diameter from the air passing through it.

Hot-cell: A device used for the shielding and containment of radioactive materials. The shielding material(s) (e.g., lead) is generally incorporated into the structure of the unit itself. Radiopharmaceutical personnel carry out the majority of the tasks within the hot-cell from the exterior of the unit. This is accomplished by the use of remote manipulation systems (e.g., manipulator arms, automated dispensing system) of various designs. Numerous air quality configurations of the hot-cell may

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exist, including integrated HEPA filtration systems to render all or a specified portion (DPA) of the device capable of certifying to a controlled ISO Class 5 environment. In other situations, the hot-cell offers only radiation protection and a laminar flow PEC, capable of achieving an ISO Class 5 environment, is placed within the enclosure to allow for safe aseptic manipulations. A hot-cell may also be referred to by other designations (e.g., shielded isolator with laminar flow, PET dispensing station, manipulator hot-cell, shielded isolators for dispensing, radiopharmaceutical dispensing isolator).

Hot lab: Unclassified radiopharmaceutical processing area located within a hospital or clinical site that is only appropriate for immediate use radiopharmaceuticals if there is not an ISO 5 PEC within SRPA located within the area.

Immediate use: A preparation (including preparations with minor deviations) and/or dispensing of a sterile radiopharmaceutical that is limited for a single patient. Only sterile conventionally manufactured drug products (e.g., NDA, ANDA) or drugs produced under an approved IND or RDRC protocol may be used. Administration must begin within 1 hour of the first container puncture or exposure of any critical site involved (e.g., syringe tip, needle hub or needle) to ambient air, whichever is first.

Individual dose (unit dose): A radiopharmaceutical in its final form ready for administration (e.g., capsule, sterile solution in a syringe) consisting of the amount (dose) prescribed, ordered, or other intended for an individual patient or research subject.

Inverse square law: The specified physical quantity or intensity of a radiation emission is inversely proportional to the square of the distance from the source of the emission.

ISO class: A quality classification from the International Organization for Standardization based on quantity and size of particles per volume of air.

Kit: Conventionally manufactured package containing all ingredients required to prepare a radiopharmaceutical with the exception of the radionuclide.

Kit-splitting (fractionation): The act of dividing the contents of a kit vial and transferring aliquots into other containers for storage and subsequent radiolabeling.

Ligand: An ion or molecule that incorporates a metal atom to form a coordination complex.

Line of demarcation: A visible line on the floor that separates the clean and less clean sides of the ante-room.

Low-lint wiper: A wiper exhibiting few, if any, fibers or other particulates, visible without magnification, which are separate from, or easily removed from, the wiper material in a dry condition.

Master Formulation Record (MFR): A document or set of documents specifying the starting materials with their quantities and the packaging materials, together with a description of the procedures and precautions required to produce a specified quantity of a finished preparations as well as the processing instructions, including the in-process controls.

Media-fill test: A simulation used to qualify processes and personnel engaged in sterile radiopharmaceutical processing to ensure that the processes and personnel are able to prepare radiopharmaceuticals without microbiological contamination.

Molar mass: The measured mass that is attained from a molar amount of a given substance (e.g., element, compound). It is generally expressed with units such as g/mol and kg/mol.

Multiple-dose container: A container of a radiopharmaceutical for administration that is designed to contain more than one patient dose of the radiopharmaceutical.

Negative-pressure area: An area that is maintained at lower pressure than the adjacent spaces, and therefore the net airflow is into the area. This area is appropriate for volatile or gaseous radionuclides and radiopharmaceuticals (e.g., I-131 NaI, N-13 ammonia) and intended to lend a measure of protection for the radiation workers and the general public.

One-step disinfectant cleaner: A product with an EPA-registered claim (or equivalent) that it can clean and disinfect a nonporous surface in the presence of light to moderate organic soiling without a separate cleaning step.

Pass-through: An enclosure with sealed doors on both sides to ensure that both doors are not opened at the same time. The pass-through is positioned between two spaces creating an airlock for the purpose of minimizing particulate transfer while moving materials from one space to another.

Perimeter: A visible demarcation on the floor that defines the boundaries of the SRPA.

Positive-pressure area: An area that is maintained at higher pressure than the adjacent spaces, and therefore the net airflow is out of the area.

Preparation: The act of combining a conventionally manufactured kit with a conventionally manufactured radionuclide following manufacturer's recommended instructions. Mixing, reconstituting, combining, diluting, or repackaging of a radiopharmaceutical, or other such acts, performed in accordance with directions contained in the FDA-approved labeling.

Preparation with minor deviations: The act of preparing a conventionally manufactured kit with a conventionally manufactured radionuclide with volume, and/or radioactivity, and/or step-by-step deviations from the manufacturers recommended labeling while ensuring that the final preparation maintains appropriate radiochemical and radionuclidic purity for the entirety of the BUD. Examples of minor deviations include, but are not limited to, altering the amount of activity or volume added to the vial, changes in step-by-step operations (e.g., dilute Tc-99m solution after, rather than before, addition to the vial, use of a venting needle or filter), using alternative devices or equipment (e.g., a heating block rather than a hot water bath), and using alternative radiochemical purity testing methods.

Primary engineering control (PEC): A device or zone that provides an ISO Class 5 air quality environment for sterile processing.

Pyrogen: A substance that induces a febrile reaction in a patient.

Quality assurance (QA): The system of procedures, activities, and oversight that ensures that radiopharmaceutical processing consistently meets quality standards.

Quality control (QC): The sampling, testing, and documentation of results that, taken together, ensure that specifications have been met before release of the radiopharmaceutical.

Radioactive materials (RAM) license: A document(s) issued by the US NRC or an Agreement State agency that authorizes various activities involving the use of radioactive materials. These uses can include possession, research and development, distribution, medical use, and other purposes not included in this list. Only those activities specifically authorized are allowed.

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Radioassay: Measurement of the quantity of radioactivity present in a container using a suitable and calibrated instrument, such as a well-type ionization chamber (i.e., dose calibrator).

Radiochemical purity: The ratio, expressed as a percentage, of the radioactivity of the intended active radiopharmaceutical ingredient to the total radioactivity of all radioactive ingredients and impurities present in the radiopharmaceutical preparation (see *Radioactivity* ⟨821⟩).

Radionuclidic purity: The ratio, expressed as a percentage, of the radioactivity of the intended radionuclide to the total radioactivity of all radionuclides in the radiopharmaceutical preparation (see ⟨821⟩).

Radiopharmaceutical (radiopharmaceutical preparation/radioactive drug): (See ⟨821⟩.) A finished dosage form that contains a radioactive substance in association with one or more other ingredients and that is intended to diagnose, stage a disease, monitor treatment, or provide therapy. A radiopharmaceutical includes any non-radioactive reagent kit or radionuclide generator that is intended to be used in the preparation of any such substance. The terms "radiopharmaceutical" and "radioactive drug" are commonly used interchangeably.

Repackaging: The act of removing a conventionally manufactured radiopharmaceutical from the container in which it was distributed by the original manufacturer and placing it into a different container without further manipulation of the product. Repackaging also includes the act of placing the contents of multiple containers (e.g., vials) of the same finished drug product into one container, as long as the container does not include other ingredients. Radiopharmaceutical manipulation in any other way, including reconstitution, dilution, mixing, or combination with another ingredient, is not considered repackaging.

Restricted area: Any area to which access is controlled for the protection of individuals from exposures to radiation and radioactive materials.

Secondary engineering control (SEC): The area where the PEC is placed (e.g., a classified area or an SRPA). It incorporates specific design and operational parameters required to minimize the risk of microbial contamination.

Segregated radiopharmaceutical processing area (SRPA): A designated, unclassified space, area, or room with a defined (by facility procedures) perimeter that contains a PEC. An SRPA is only suitable for radiopharmaceutical preparation (with and without minor deviations), dispensing, and repackaging. If the SRPA is used to elute radionuclide generators it must have ISO Class 8 particle count non-viable particle count air quality.

Shielding: Barriers of appropriate radiation attenuating material, used for radiopharmaceuticals, to protect the personnel. These barriers can be general in nature (e.g., L-block, hot-cell), as to afford protection from a radiation field, or specific to a container used to hold a particular radiopharmaceutical (e.g., syringe shield, vial shields, "pigs").

Single-dose container: A container of a radiopharmaceutical for administration that is designed for use with a single patient as a single administration.

Specific activity: The radioactivity of a radionuclide per unit mass of the compound involved with the radionuclide (see *Radioactivity—Theory and Practice* ⟨1821⟩). The units of specific activity involve those for the activity (e.g., mCi, MBq, Ci, GBq) and those for the unit of mass (e.g., µg, mmol); expressed on an activity per mass basis (e.g., mCi/µg, MBq/µg, Ci/mmol, GBq/mmol).

Sporicidal agent: A chemical or physical agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

Start of preparation: Time at which a vial septum is punctured or a component container is opened (e.g., removal of cap on a pre-filled syringe), whichever comes first.

Sterility: The absence of viable microorganisms.

Strength: The radioactivity concentration of the radiopharmaceutical at the calibration time (see ⟨821⟩). Strength is expressed as the quantity of radioactivity on a volume basis (e.g., mCi/mL or MBq/mL).

Unclassified space: A space not required to meet any ISO air cleanliness classification.

Unrestricted area: An area in which a person should not be exposed to radiation levels in excess of 2 millirems in any 1 h from external sources.

Use-by time: For radiopharmaceuticals prepared from kits, the time period after preparation during which the radiopharmaceutical should be used or administered, as suggested or stated in the manufacturer's prescribing information.

APPENDIX

Abbreviations

ACPH	Air changes per hour
ALARA	As low as reasonably achievable
ANDA	Abbreviated new drug application
ANP	Authorized nuclear pharmacist
AU	Authorized user
BLA	Biologics license application
BSC	Biological safety cabinet
BUD	Beyond-use date
CETA	Controlled Environment Testing Association
cfu	Colony-forming unit
CoA	Certificate of analysis

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Abbreviations *(continued)*

DPA	Direct processing area
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FDCA	Food, Drug, and Cosmetic Act
HEPA	High-efficiency particulate air
HVAC	Heating, ventilation, and air conditioning
IND	Investigational new drug
IPA	Isopropyl alcohol
ISO	International Organization for Standardization
LAFW	Laminar airflow workbench
MAA	Macroaggregated albumin
MEA	Malt extract agar
MFR	Master Formulation Record
NDA	New drug application
NRC	Nuclear Regulatory Commission
PEC	Primary engineering control
PET	Positron emission tomography
RAM	Radioactive material
RDRC	Radioactive drug research committee
RH	Relative humidity
SDA	Sabouraud dextrose agar
SEC	Secondary engineering control
SOP	Standard operating procedure
SRPA	Segregated radiopharmaceutical processing area
TSA	Trypticase soy agar ▲ (USP 1-Dec-2019)

⟨831⟩ REFRACTIVE INDEX

The refractive index (*n*) of a substance is the ratio of the velocity of light in air to the velocity of light in the substance. It is valuable in the identification of substances and the detection of impurities.

Although the standard temperature for Pharmacopeial measurements is 25°, many of the refractive index specifications in the individual monographs call for determining this value at 20°. The temperature should be carefully adjusted and maintained, since the refractive index varies significantly with temperature.

The values for refractive index given in this Pharmacopeia are for the D line of sodium (doublet at 589.0 nm and 589.6 nm). Most instruments available are designed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

The Abbé refractometer measures the range of refractive index for those Pharmacopeial materials for which such values are given. Other refractometers of equal or greater accuracy may be employed.

To achieve the theoretical accuracy of ±0.0001, it is necessary to calibrate the instrument against a standard provided by the manufacturer and to check frequently the temperature control and cleanliness of the instrument by determining the refractive index of distilled water, which is 1.3330 at 20° and 1.3325 at 25°.

⟨841⟩ SPECIFIC GRAVITY

Unless otherwise stated in the individual monograph, the specific gravity determination is applicable only to liquids, and unless otherwise stated, is based on the ratio of the weight of a liquid in air at 25° to that of an equal volume of water at the same temperature. Where a temperature is specified in the individual monograph, the specific gravity is the ratio of the weight of the liquid in air at the specified temperature to that of an equal volume of water at the same temperature. When the substance is a solid at 25°, determine the specific gravity of the melted material at the temperature directed in the individual monograph, and refer to water at 25°.

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Unless otherwise stated in the individual monograph, the density is defined as the mass of a unit volume of the substance at 25°, expressed in kilograms per cubic meter or grams per cubic centimeter (1 kg/m^3 = 10^{-3} g/cm^3). Where the density is known, mass can be converted to volume, or volume converted to mass, by the formula: volume = mass/density.

Unless otherwise directed in the individual monograph, use *Method I*.

METHOD I

Procedure

Select a scrupulously clean, dry pycnometer that previously has been calibrated by determining its weight and the weight of recently boiled water contained in it at 25°. Adjust the temperature of the liquid to about 20°, and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°, remove any excess liquid, and weigh. When the monograph specifies a temperature different from 25°, filled pycnometers must be brought to the temperature of the balance before they are weighed. Subtract the tare weight of the pycnometer from the filled weight.

The specific gravity of the liquid is the quotient obtained by dividing the weight of the liquid contained in the pycnometer by the weight of water contained in it, both determined at 25°, unless otherwise directed in the individual monograph.

METHOD II

The procedure includes the use of the oscillating transducer density meter. The apparatus consists of the following:

- a U-shaped tube, usually of borosilicate glass, which contains the liquid to be examined;
- a magneto-electrical or piezo-electrical excitation system that causes the tube to oscillate as a cantilever oscillator at a characteristic frequency depending on the density of the liquid to be examined;
- a means of measuring the oscillation period (T), which may be converted by the apparatus to give a direct reading of density or used to calculate density by using the constants A and B described below; and
- a means to measure and/or control the temperature of the oscillating transducer containing the liquid to be tested.

The oscillation period is a function of the spring constant (c) and the mass of the system:

$$T^2 = \{(M/c) + [(\rho \times V)/c]\} \times 4\pi^2$$

where ρ is the density of the liquid to be tested, M is the mass of the tube, and V is the volume of the filled tube.

Introduction of two constants $A = c/(4\pi^2 \times V)$ and $B = (M/V)$ leads to the classical equation for the oscillating transducer:

$$\rho = A \times T^2 - B$$

The specific gravity of the liquid is given by the formula:

$$\rho_{(L)}/\rho_{(W)}$$

where $\rho_{(L)}$ and $\rho_{(W)}$ are the densities of the liquid and water, respectively, both determined at 25°, unless otherwise directed in the individual monograph.

Calibration

The constants A and B are determined by operating the instrument with the U-tube filled with two different samples of known density (e.g., degassed water and air). Perform the control measurements daily, using degassed water. The results displayed for the control measurement using degassed water do not deviate from the reference value (ρ_{25} = 0.997043 g/cm^3) by more than its specified error. Precision is a function of the repeatability and stability of the oscillator frequency. Density meters are able to achieve measurements with an error on the order of 1 × 10^{-3} g/cm^3 to 1 × 10^{-5} g/cm^3 and a repeatability of 1 × 10^{-4} g/cm^3 to 1 × 10^{-6} g/cm^3. For example, an instrument specified to ±1 × 10^{-4} g/cm^3 must display 0.9970 ± 0.0001 g/cm^3 in order to be suitable for further measurement, otherwise a readjustment is necessary. Calibration with certified reference materials should be carried out regularly.

Procedure

Using the manufacturer's instructions, perform the measurements using the same procedure as for *Calibration*. If necessary, equilibrate the liquid to be examined at 25° before introduction into the tube to avoid the formation of bubbles and to reduce the time required for measurement. Factors affecting accuracy include the following:

- temperature uniformity throughout the tube,
- nonlinearity over a range of density,
- parasitic resonant effects, and
- viscosity, if the oscillating transducer density meters used do not provide automatic compensation of sample viscosity influence.

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⟨846⟩ SPECIFIC SURFACE AREA

INTRODUCTION

The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

BRUNAUER, EMMETT AND TELLER (BET) THEORY AND SPECIFIC SURFACE AREA DETERMINATION

Multipoint Measurement

The data are treated according to the Brunauer, Emmett and Teller (BET) adsorption isotherm equation:

$$1/[V_a((P_O/P) - 1)] = [(C - 1)/(V_mC)] \times (P/P_O) + (1/V_mC) \quad (1)$$

P	=	partial vapor pressure of adsorbate gas in equilibrium with the surface at 77.4 K (b.p. of liquid nitrogen), in Pa,
P_o	=	saturated pressure of adsorbate gas, in Pa,
V_a	=	volume of gas adsorbed at standard temperature and pressure (STP) [273.15 K and atmospheric pressure (1.013×10^5 Pa)], in mL,
V_m	=	volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in mL,
C	=	dimensionless constant that is related to the enthalpy of adsorption of the adsorbate gas on the powder sample.

A value of V_a is measured at each of not less than three values of P/P_o.
Then the BET value

$$1/[V_a((P_o/P) - 1)]$$

is plotted against P/P_o, according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient, r, of the linear regression is not less than 0.9975; that is, r^2 is not less than 0.995. From the resulting linear plot, the slope, which is equal to $(C - 1)/V_mC$, and the intercept, which is equal to $1/V_mC$, are evaluated by linear regression analysis. From these values, V_m is calculated as $1/(slope + intercept)$, while C is calculated as $(slope/intercept) + 1$. From the value of V_m so determined, the specific surface area, S, in $m^2 \cdot g^{-1}$, is calculated by the equation:

$$S = (V_mNa)/(m \times 22400) \quad (2)$$

N	=	Avogadro constant (6.022×10^{23} mol^{-1}),
a	=	effective cross-sectional area of one adsorbate molecule, in square meters (0.162 nm^2 for nitrogen and 0.195 nm^2 for krypton),
m	=	mass of test powder, in g,
22,400	=	volume, in mL, occupied by one mole of the adsorbate gas at STP allowing for minor departures from the ideal.

A minimum of three data points is required. Additional measurements may be carried out especially when nonlinearity is obtained at a P/P_o value close to 0.3. Because nonlinearity is often obtained at a P/P_o value below 0.05, values in this region are not recommended. The test for linearity, the treatment of the data, and the calculation of the specific surface area of the sample are described above.

Single-Point Measurement

Normally, at least three measurements of V_a, each at different values of P/P_o, are required for the determination of specific surface area by the dynamic flow gas adsorption technique *(Method I)* or by volumetric gas adsorption *(Method II)*. However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of V_a measured at a single value of P/P_o such as 0.300 (corresponding to 0.300 mole of nitrogen or 0.001038 mole fraction of krypton), using the following equation for calculating V_m:

$$V_m = V_a[1 - (P/P_o)] \quad (3)$$

The specific surface area is then calculated from the value of V_m by equation (2) given above.

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The single-point method may be employed directly for a series of powder samples of a given material for which the material constant C is much greater than unity. These circumstances may be verified by comparing values of specific surface area determined by the single-point method with that determined by the multipoint method for the series of powder samples. Close similarity between the single-point values and multipoint values suggests that 1/C approaches zero.

The single-point method may be employed indirectly for a series of very similar powder samples of a given material for which the material constant C is not infinite but may be assumed to be invariant. Under these circumstances, the error associated with the single-point method can be reduced or eliminated by using the multipoint method to evaluate C for one of the samples of the series from the BET plot, from which C is calculated as (1 + *slope/intercept*). Then V_m is calculated from the single value of V_a measured at a single value of P/P_o, by the equation:

$$V_m = V_a[(P_o/P) - 1] [(1/C) + ((C - 1)/C) \times (P/P_o)] \quad (4)$$

The specific surface area is calculated from V_m by equation (2) given above.

EXPERIMENTAL TECHNIQUES

This section describes the methods to be used for the sample preparation, the dynamic flow gas adsorption technique *(Method I)* and the volumetric gas adsorption technique *(Method II)*.

Sample Preparation

OUTGASSING

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface after manufacture and during treatment, handling, and storage. If outgassing is not achieved, the specific surface area may be reduced or may be variable because an intermediate area of the surface is covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials.

The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure, and time are chosen so that the original surface of the solid is reproduced as closely as possible. Outgassing of many substances is often achieved by applying a vacuum by purging the sample in a flowing stream of a nonreactive, dry gas or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

ADSORBATE

The standard technique is the adsorption of nitrogen of analytical quality at liquid nitrogen temperature.

For powders of low specific surface area (< 0.2 m^2g^{-1}), the proportion adsorbed is low. In such cases, the use of krypton at the liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error. The use of larger sample quantities, where feasible (equivalent to 1 m^2 or greater total surface area using nitrogen), may compensate for the errors in determining low surface areas.

All gases used must be free from moisture.

QUANTITY OF SAMPLE

A quantity of the test powder is accurately weighed such that the total surface of the sample is at least 1 m^2 when the adsorbate is nitrogen and 0.5 m^2 when the adsorbate is krypton.

Lower quantities of sample may be used after appropriate validation.

Measurements

Because the amount of gas adsorbed under a given pressure tends to increase when the temperature is decreased, adsorption measurements are usually made at a low temperature. Measurement is performed at 77.4 K, the boiling point of liquid nitrogen.

Method I: The Dynamic Flow Method

PRINCIPLE

In the dynamic flow method (see *Figure 1*), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions.

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A minimum of three mixtures of the appropriate adsorbate gas with helium are required within the P/P_o range 0.05 to 0.30.

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of three data points within the recommended range of 0.05 to 0.30 for P/P_o is determined.

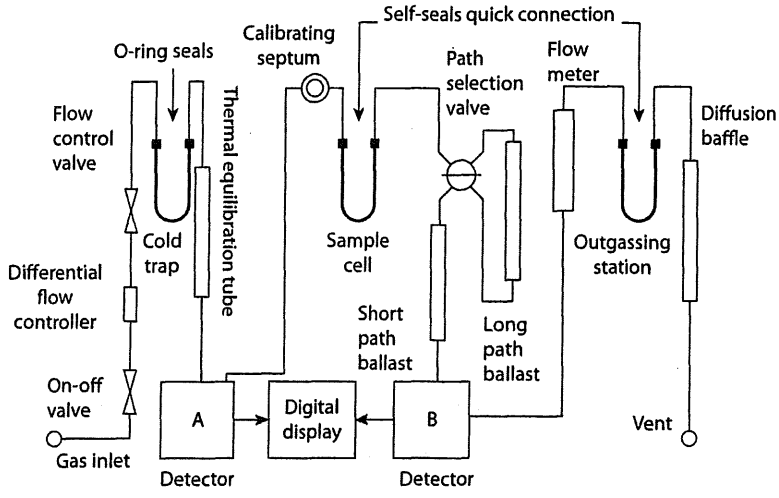


Fig. 1. Schematic diagram of the dynamic flow method apparatus.

PROCEDURE

A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again, through the thermal conductivity cell, and then to a recording potentiometer.

The sample cell is immersed in liquid nitrogen, and the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

The sample is removed from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak. Because this is better defined than the adsorption peak, it is the one used for the determination.

To effect the calibration, a known quantity of adsorbate, sufficient to give a peak of similar magnitude to the desorption peak, is injected into the system, and the proportion of gas volume per unit peak area is obtained.

A mixture of nitrogen and helium is used for a single-point determination; and several such mixtures or premixing two streams of gas are used for a multipoint determination.

The calculation is the same as the volumetric method.

Method II: The Volumetric Method

PRINCIPLE

In the volumetric method (see *Figure 2*), the recommended adsorbate gas is nitrogen, which is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure, P, of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Because only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.

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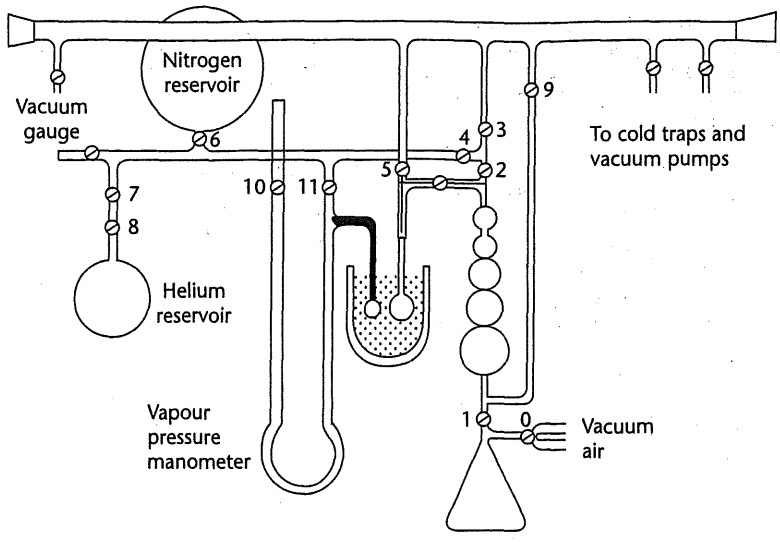


Fig. 2. Schematic diagram of the volumetric method apparatus.

PROCEDURE

A small amount of dry nitrogen is admitted into the sample tube to prevent contamination of the clean surface, the sample tube is removed, a stopper is inserted, the tube is weighed, and the weight of the sample is calculated. Then the sample tube is attached to the volumetric apparatus. The sample is cautiously evacuated down to the specified pressure (e.g., between 2 Pa and 10 Pa). Alternately, some instruments are operated by evacuating to a defined rate of pressure change (e.g., less than 13 Pa/30 s) and by holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a nonadsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements: that is, by means of reference and sample tubes connected by a differential transducer. The adsorption of nitrogen gas is then measured as described below.

Raise a Dewar vessel containing liquid nitrogen at 77.4 K up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed, V_a. For multipoint measurements, repeat the measurement of V_a at successively higher P/P_o values. When nitrogen is used as the adsorbate gas, P/P_o values of 0.10, 0.20, and 0.30 are often suitable.

Reference Materials

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area that have a specific surface area similar to that of the sample to be examined.

⟨852⟩ ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTION

Atomic absorption (AA) spectroscopy is an analytical method that supports qualification and/or quantification of elements. In this use, the AA method supports procedures that measure the absorbance of radiation at a characteristic wavelength by a vapor composed of ground state atoms. A typical instrument consists of a primary energy source that produces the spectrum of the element under examination, a monochromator, and a suitable detector.

For discussion of the theory and principles of measurements, see *Atomic Absorption Spectroscopy—Theory and Practice* ⟨1852⟩, a resource that may be helpful but is not mandatory.

QUALIFICATION OF ATOMIC ABSORPTION SPECTROPHOTOMETERS

Qualification of an AA spectrophotometer can be divided into three elements: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ); see also the general information chapter *Analytical Instrument Qualification* ⟨1058⟩.

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Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location.

Operational Qualification

In OQ, an instrument's performance is characterized using standards of known spectral properties to verify that the system operates within target specifications (see *Table 1* and *Table 2*). The purpose of OQ is to demonstrate that instrument performance is suitable. OQ is a check of the key operational parameters performed following installation and following repairs and/or maintenance.

The OQ tests in the following sections are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package.

Table 1. OQ Test and Acceptance Criteria for Flame AAS

Test	Procedure	Acceptance Criteria
Sensitivity	Aspirate a 0.3-µg/mL Zn standard and record the absorbance.	±20% of AU[a] specified by instrument manufacturer
Linearity	Aspirate blank, 0.05-, 0.075-, 0.10-, 0.25-, and 0.50-µg/mL Zn standards. Generate calibration curve and record correlation coefficient (*R*).	Correlation coefficient NLT 0.995
Precision	Assay 5 separate replicates of the 0.10-µg/mL Zn standard versus the standard curve generated for the *Linearity* test. Calculate the %RSD of the 5 results in µg/mL.	%RSD[b] NMT 3%

[a] AU = absorption unit.
[b] %RSD = % relative standard deviation.

Table 2. OQ Test and Acceptance Criteria for Graphite Furnace AAS

Test	Procedure	Acceptance Criteria
Sensitivity	Prepare a Cu standard and measure the characteristic mass as described by the manufacturer.	±20% of Cu characteristic mass as specified by the manufacturer
Linearity	Generate a calibration curve from a blank, 25-, 50-, 75-, and 100-µg/L Cu standards. Inject each standard in triplicate and record the %RSD.	Correlation coefficient NLT 0.995
		%RSD of triplicate injections of each Cu standard NMT 5% (not including the blank)
Precision	Assay 5 separate replicates of the 50-µg/L Cu standard versus the standard curve generated for the *Linearity* test. Inject each replicate in triplicate.	%RSD of 5 replicates NMT 3%
		%RSD of triplicate injections NMT 5%

Performance Qualification

PQ determines that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement.

Depending on typical use, the specifications for PQ may be different from the manufacturer's specifications. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements.

Specific procedures, acceptance criteria, and time intervals for characterizing AA spectrophotometer performance depend on the instrument and intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from test sample spectra using validated AA procedures.

PROCEDURE

Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in AA analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents. For some analyses, diligence must be exercised to prevent the adsorption of analytes onto the surface of a vessel, particularly in ultra-trace analyses. Contamination of the sample solutions from metal and ions present in the container also can lead to inaccurate results.

For the analysis of a ubiquitous element, it is often necessary to use the purist grade of reagent or solvent available. Check all solutions (diluents, matrix modifier solutions, ionization suppression solutions, reactants, and others) for elemental contamination before they are used in an analysis.

Standard Solution

Prepare as directed in the individual monograph. [NOTE—Commercially available single- or multi-element standard solutions, traceable to the National Institute of Standards and Technology or to an equivalent national metrology organization, can be used in the preparation of standard solutions.] Standard solutions, especially those used for ultra-trace analyses, may have limited shelf life. Standard solutions should be retained for NMT 24 h unless stability is demonstrated experimentally.

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The method of standard additions also can be used. This method involves adding a known concentration of the analyte element to the sample at no fewer than two concentration levels against an unspiked sample preparation. The instrument response is plotted against the concentration of the added analyte element, and a linear regression line is drawn through the data points. The absolute value of the *x*-intercept multiplied by any dilution factor is the concentration of the analyte in the sample.

Sample Solution

Prepare as directed in the individual monograph.

A variety of digestion techniques may be indicated to dissolve the sample. These may include hot-plate and microwave-assisted digestions, including open-vessel and closed-vessel approaches. Note that open-vessel digestion generally is not recommended for the analysis of volatile metals, e.g., selenium and mercury.

Analysis

Follow the procedure as directed in the individual monograph for the instrumental parameters.

The instrument must be standardized for quantification at the time of use. The absorbance of standard solutions that bracket the target concentration is determined against an appropriate blank. The detector response is plotted as a function of the analyte concentration. When an analysis is performed at or near the detection limit, the analyst cannot always use a bracketing standard. This is acceptable for qualitative but not quantitative tests. Regression analysis of the standard plot should be used to evaluate the linearity of detector response, and individual monographs may set criteria for the residual error of the regression line.

To demonstrate the stability of the system's initial standardization, the analyst must reassay a solution used in the initial standard curve as a check standard at appropriate intervals throughout the analysis of the sample set. Unless otherwise indicated in the individual monograph, the reassayed standard should agree with its expected value to within ±3% for an assay or ±20% for an impurity analysis.

Sample concentrations are calculated versus the working curve generated by plotting the detector response versus the concentration of the analyte in the standard solutions.

VALIDATION AND VERIFICATION

Validation

Validation is required when an AA method is intended for use as an alternative to the official procedure for testing an official article.

The objective of an AA procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). [NOTE—For definition of different categories, see *Validation of Compendial Procedures* ⟨1225⟩.] Depending on the category of the test, analytical procedure validation for AA may require the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized methods and to the method of standard additions.

General information chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed to demonstrate suitability for the intended use.

ACCURACY

For Category I assays or Category II tests, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of elements. It is also an acceptable practice to compare assay results obtained using the AA procedure under validation to those of an established analytical procedure. In standard addition methods, accuracy assessments are based on the final intercept concentration, not the recovery calculated from the individual standard additions.
Validation criteria: 95.0%–105.0% mean recovery for the drug substance assay and the drug product assay, and 70.0%–150.0% mean recovery for the impurity analysis. These criteria apply throughout the intended range.

Precision

REPEATABILITY

The analytical procedure should be assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration. Alternatively, three replicates of three separate sample solutions at different concentrations can be used. The three concentrations should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria. If validating a procedure by the method of standard additions, the precision criterion applies to the final experimental result, not the accuracy of the individual standard addition levels.
Validation criteria: The relative standard deviation is NMT 5.0% for the drug substance assay, NMT 5.0% for the drug product assay, and NMT 20% for the impurity analysis.

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INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the procedure should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, the analytical procedure should be assessed by performing the repeatability test in any combination of at least two of the conditions previously mentioned (totaling 12 measurements).
Validation criteria: The relative standard deviation is NMT 8.0% for the drug substance assay, NMT 8.0% for the drug product assay, and NMT 25.0% for the impurity analysis.

SPECIFICITY

The procedure must be able to unequivocally assess each analyte element in the presence of components that may be expected to be present, including any matrix components.
Validation criteria: Demonstrated by meeting the accuracy requirement.

QUANTITATION LIMIT

The limit of quantitation (QL) can be estimated by calculating the standard deviation of NLT six replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used (see ⟨1225⟩).

A measurement of a test solution prepared from a representative sample matrix spiked at the estimated QL concentration must be performed to confirm accuracy. If validating a procedure using the method of standard additions, the validation criterion applies to the final experimental result, not the spike recovery of the individual standard addition levels.
Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

LINEARITY

A response curve between the analyte concentration and absorbance is prepared from NLT five standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods, such as a least-squares regression.

For experiments that do not yield a linear relationship between analyte concentration and AA response, appropriate statistical methods must be applied to describe the analytical response.
Validation criteria: Correlation coefficient (*R*), NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

Range is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. Range is demonstrated by meeting the linearity, precision, and accuracy requirements.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. For AA this can include but is not limited to sample preparation steps and heating programs, including atomization hold time or atomization temperature. Exercise caution when changing fuel and oxidant gas flows and burner hardware, because this could potentially create a flash-back condition.

Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP–NF*, are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of an AA procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, can be executed by the user with suitable accuracy, specificity, linearity, and precision using the instruments, analysts, and sample matrices available. According to *Verification of Compendial Procedures* ⟨1226⟩, if the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Verification of compendial AA methods should, at a minimum, include the execution of the validation parameters for specificity, linearity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in *Validation*.

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⟨853⟩ FLUORESCENCE SPECTROSCOPY

INTRODUCTION

Fluorescence is a two-step process that requires absorption of light at a specific wavelength (excitation) followed by emission of light, usually at a higher wavelength. The emission of light is termed *fluorescence*.

The most common type of fluorescent sample is a submicromolar transparent solution that absorbs light following the Beer–Lambert–Bouguer Law and fluoresces with an intensity that is directly proportional to the concentration, the absorptivity, and the fluorescence quantum yield of the fluorescent species or fluorophore.

Unlike absorption spectroscopy, where deviation from linearity is the exception, fluorescence linearity can be affected by a number of sample-related effects. For additional information, see *Fluorescence Spectroscopy—Theory and Practice* ⟨1853⟩.

Fluorescence methods also are termed *background-free* because little excitation light reaches the detector. This characteristic makes fluorescence detection highly sensitive, down to single-molecule detection in some cases. Fluorescence detection also can be highly specific because a fluorophore emits a characteristic emission pattern. Specificity and sensitivity are two of the more important strengths of fluorescence methods.

QUALIFICATION OF FLUORESCENCE INSTRUMENTS

Analysts ensure the suitability of a specific instrument for a given procedure by using a stepwise evaluation for the desired application from selection to instrument retirement: design qualification (DQ); installation qualification (IQ); an initial performance-to-specification qualification, also known as operational qualification (OQ); and an ongoing performance qualification (PQ). For additional information, see general chapter *Analytical Instrument Qualification* ⟨1058⟩.

DQ and IQ are not further considered in this chapter. The purpose of this section is to provide test methods and acceptance criteria to ensure that the instrument is suitable for its intended use (OQ) and that it will continue to function properly over extended time periods (PQ).

As with any spectrometric device, analysts must qualify a spectrofluorometer for both wavelength (*x*-axis) and relative intensity (*y*-axis or signal axis) accuracy and precision. They also must establish sensitivity. OQ should span the operational ranges required within the laboratory for both intensity and wavelength scales.

Instrument Operational Qualification

The tolerances given in both the instrument OQ and PQ are applicable for general use. Specifications for particular instruments and applications can vary depending on the analytical procedure used and the desired accuracy of the final result. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package.

Wherever possible, analysts should use certified reference materials for purposes of calibration in the steps detailed below in preference to laboratory-prepared solutions. When certified reference materials are obtained from a recognized accredited source, they have independently verified traceable value assignments with associated calculated uncertainties.

Two general types of instrumental measurements are differentiated here: spectral (i.e., those that measure intensity versus wavelength) and fixed (i.e., those that measure intensity at a fixed wavelength and bandwidth).

CONTROL OF WAVELENGTHS

The level of confidence of measured peak positions is defined by wavelength accuracy for spectral measurements. Determination of the accuracy of many wavelengths across the desired wavelength range demonstrates if further calibration beyond a single point is needed. Multipoint calibration involves measuring wavelength biases at multiple wavelengths and correcting for the wavelength dependence of the bias. A single-point calibration often can be applied to the wavelength axis in an instrument's software before data are collected, but a multipoint calibration may require that the correction be applied to spectra after they are collected.

For fixed measurements, the wavelength position and bandwidth must be reproducible. For filter-based wavelength selection, this requires that only the same filter be used when analysts compare data over time. If a different filter must be used (e.g., when data are compared across instruments and laboratories), then the transmission curves of the filters must be compared.

Wavelength precision should be determined over the operational range using at least six replicate measurements. The standard deviation should not exceed ±1 nm.

ATOMIC LINE SPECTRA

This procedure is described as the primary application because the emission lines produced from a discharge lamp are characteristic of the source element, and, as a fundamental physical standard, these wavelengths have been measured with an uncertainty of NMT ± 0.01 nm. In solution spectrofluorometry the wavelength bias required rarely exceeds 1.0 nm. For these reasons, the atomic line standard values are cited without uncertainty. The lamp should be placed at the source position in the spectrofluorometer.

A commonly employed low-pressure mercury lamp has a number of intense lines that cover a large part of the UV and visible range. Manufacturers often use two Xenon lines from the source at 260.5 and 541.9 nm as an internal calibration check, because

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the accuracy of both the excitation and emission monochromators can be verified and can be used for diagnostic purposes (see *Table 1*).[1]

Table 1. Elemental Line Spectra Wavelengths

Element	Wavelength (nm)
Hg	253.7
Xe	260.5
Hg	296.7
Hg	365.0
Hg	404.7
Hg	435.8
Xe	541.9
Hg	546.1
Hg	577.0
Hg	579.1

USE OF RARE EARTH OXIDE SOLUTIONS

This procedure uses a solution of a rare earth oxide prepared by dissolution in acid media. The most frequently used is holmium oxide in perchloric acid in combination with a diffuse reflector located at the sample position. Suitable certified reference materials are available commercially.[2] The wavelength selector not being scanned should be removed; if removal is not practicable, it should be set to zero order (in this position a grating behaves like a mirror reflecting all wavelengths). The diffuse reflector is scanned with and without the rare earth sample in place, and the ratio of the two intensities is calculated to obtain an effective transmittance spectrum. Minima in the intensity ratio correspond to absorption peaks of the sample. For a 4% (w/w) solution of holmium oxide in perchloric acid at 1.0 nm spectral bandwidth and a path length of 1 cm, these minima are in *Table 2*.[3]

Table 2

Wavelength (nm)
241.1
249.9
278.1
287.2
333.5
345.4
361.3
385.6
416.3
451.4
467.8
485.2
536.6
640.5

If the operational range of the spectrophotometer lies outside 240–650 nm, other certified rare earth oxides or other solutions are used.

Didymium (a mixture of neodymium and praseodymium) is available as a traceable standard in both solution and glass presentations. Didymium is similar in preparation to the holmium materials and has useful peak characteristics in the 730–870 nm region. Useful peaks are found in the didymium solution at approximately 731.6, 740.0, 794.1, 799.0, and 864.4 nm.

[1] The rounded values are taken from ASTM Standard E388-04 (2009).

[2] NIST SRM 2034 is no longer available.

[3] The rounded values are taken from the intrinsic wavelength standard absorption band data from Travis et al. *J Phys Chem Ref Data* 2005;34(1):41. The maximum 95% measurement uncertainty is ±0.06 nm.

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USE OF POLYMETHYLMETHACRYLATE-DOPED REFERENCES

This procedure uses solid reference materials manufactured by polymerization of a variety of fluorescent active aromatic ring compounds into an inert polymethylmethacrylate (PMMA) matrix. These materials are supplied as polished blocks for use in a standard cuvette holder (see *Table 3*).

Table 3. Dopant, Excitation, and Emission Data for Selected Reference Materials

Dopant	Excitation λ (nm)	Emission λ (nm)
p-Terphenyl	295	338
Ovalene	342	482
Tetraphenylbutadiene	348	422
Anthracene	360	402

Performance Verification

Results from day-to-day testing of photostable intensity standards are used to verify the performance of an instrument. If the measured intensity does not change from that observed when the instrument was qualified, then instrument performance has not changed and remains qualified. Using such standards to determine an artifact-based or quasi-absolute intensity scale potentially enables measured intensities and instrument sensitivity to be compared over time or between instruments. Intensity measurements should be within the linear range of the instrument's detection system before analysts attempt intensity comparisons, and will be affected by any instrumental effect directly related to the fluorescence signal, e.g., changes in source intensity, detector response, etc.

For instruments with filter-based wavelength selection, analysts use fluorescence standards for spectral correction to determine expected intensity differences caused by filters with different transmission profiles. By compensating for these intensity differences due to spectral mismatch, the analyst can determine a quasi-absolute intensity scale for these instruments. Analysts should approach instrument-to-instrument comparisons with particular caution because of the relatively large and difficult-to-quantify uncertainties involved.

USE OF LOW-CONDUCTIVITY (18-MΩ) WATER

The Raman band of water is used to measure signal-to-noise ratios in fluorescent instruments. The Raman band of water is inherently reproducible and does not degrade with time. Water is convenient to obtain in a pure state and allows interlaboratory comparisons to be made with a high level of confidence. No preparation or dilution is required. The Raman band is a low-level signal that provides a good test for both the optics and the electronics of an instrumental system.

The Raman band of water is not caused by fluorescence but is a result of Raman scattering. For water, the Raman band is always red-shifted 3382 cm^{-1} relative to the excitation.[4] This band usually is measured by excitation at 350 nm, resulting in a Raman peak at 397 nm, but radiation up to 500 nm also can be used as the excitation wavelength, and the corresponding emission peak is 602 nm.

USE OF INTENSITY STANDARDS

Several solid-doped fluorescent materials are available.[5] These polymers or glasses enable the relative spectral correction and day-to-day performance qualification of fluorescence instruments across the UV, visible, and NIR regions from 320 to 830 nm. The high photostability of the materials makes them particularly useful as day-to-day intensity standards, even when spectral correction is not needed or when the excitation wavelength differs from that used for certification. A certified, steady-state emission spectrum is supplied with each certified reference material, along with the estimated total uncertainties. The reference is available in the form of a solid glass, standard-sized cuvette (12.5 mm × 12.5 mm × 45 mm) with three polished long faces for 90° detection and one frosted long face for front-face or epifluorescence detection.

Alternatively, analysts can use fluorophores in solutions that have been shown to be stable.[6,7]

QUALITATIVE AND QUANTITATIVE FLUORESCENCE MEASUREMENTS

Two general classes of procedural measurements commonly are performed by fluorescence spectrometry: qualitative and quantitative.

[4] The red-shift value is taken from Parker CA. Raman spectra in spectrofluorimetry. *Analyst.* 1959;84:446–453.

[5] Available from commercial vendors and from NIST as SRMs 2940 (orange emission), 2941 (green emission), 2942 (UV emission), 2943 (blue emission), and 2944 (red/NIR emission).

[6] Commercial vendors provide a 1-mg/L solution of quinine sulfate dehydrate in 0.105 M perchloric acid that has been fully characterized by NIST as SRM 936a.

[7] A series of day-to-day Intensity Standards is available from the German Federal Institute for Materials Research and Testing (BAM).

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Qualitative Fluorescence Measurements

Qualitative fluorescence measurements are used to detect the presence of particular analytes and yield a positive or negative answer. The excitation and emission wavelengths often are selected at the peak maximum of the fluorophore to be detected. The minimum amount of analyte needed for a positive result should be considered by the analyst to ensure that the method is appropriate for the particular application. The observation of fluorescence at the peak position above the limit of detection (usually 3 times the noise level) indicates a positive result.

Quantitative Fluorescence Measurements

Quantitative fluorescence measurements are used to determine amounts or concentrations of analytes in unknown samples. These quantities may be determined in absolute units, such as moles or moles per L, or in relative units, such as the ratio of the concentrations of two fluorescent analytes contained in a single unknown solution. These determinations use the following proportionality relating fluorescence signal (S) at a given pair of excitation and emission wavelengths (λ_{ex}, λ_{em}) to fluorescent analyte concentration (c):

$$S \propto I_0 \times \Omega \times R_d \times \alpha \times \Phi \times c$$

I_0 = intensity of the excitation beam
Ω = fraction of the fluorescence collected by the detection system
R_d = responsivity of the detection system
α = absorption coefficient
Φ = fluorescence quantum yield
c = concentration of the fluorescent analyte

This linear proportionality with concentration applies to optically dilute samples (e.g., solutions with an absorbance of less than 0.05 at a path length of 1 cm).

GOOD SPECTROSCOPIC PRACTICE

Comparisons of a test specimen with a Reference Standard are best made at a peak of spectral emission for the compound of interest. Assays based on spectrofluorometry give the commonly accepted wavelengths for excitation and peak spectral emission of the substance in question. Different spectrofluorometers may show minor variation in the apparent wavelength of this peak. Comparisons should be made at the wavelength at which peak emission occurs. If this differs from the wavelength specified in the monograph by more than ±1 nm in the range of 200–400 nm or by more than ±2 nm in the range of 400–800 nm, recalibration of the instrument may be indicated.

Use of Reference Standards

With few exceptions, pharmacopeial spectrofluorometric procedures provide results by comparison against a USP Reference Standard. This ensures measurement under identical conditions for the test specimen and the Reference Standard. These conditions could include wavelength setting, spectral bandwidth selection, cell placement and correction, and intensity levels. Cells that exhibit identical optical fluorescence characteristics at a specific wavelength may differ considerably at other wavelengths. Analysts should establish and use appropriate cell corrections where required.

The terms *similar preparation* and *similar solution* in tests and assays that involve spectrofluorometry indicate that the Reference Standard should be prepared and observed in a manner that is identical to that used for the sample under test. Usually when a solution of the specified Reference Standard is prepared at (i.e., within 10% of) the desired concentration, the fluorescence intensity is calculated on the basis of the exact amount weighed out. If analysts have not used a previously dried specimen of the Reference Standard, they should correct this intensity on the anhydrous basis.

The expressions *concomitantly determine* and *concomitantly measured* as used in procedures that involve spectrofluorometry indicate that the fluorescence of both the sample solution and the standard solution, relative to the specified test blank, are to be measured in immediate succession.

Sample Solution Preparation

For determinations using UV or visible spectrofluorometry, the specimen generally is dissolved in a solvent. Unless otherwise directed in the monograph, determinations are made at room temperature by using a path length of 1 cm. Many solvents are suitable for these ranges, including water, alcohols, chloroform, lower hydrocarbons, ethers, and dilute solutions of strong acids and alkalis. Solvents should be free from contaminants that fluoresce in the spectral region under examination. For the solvent, water-free methanol or alcohol or alcohol that has been denatured by the addition of methanol but does not contain benzene or other interfering impurities should be used. Spectrophotometric-quality solvents that are guaranteed to be free from contaminants are available commercially from several sources, but some analytical reagent-grade organic solvents may contain traces of impurities that fluoresce strongly in the UV region. New lots of these solvents should be checked for their transparency, and analysts should take care to use the same lot of solvent for the preparation of the sample solution, the standard solution, and the blank. Solvents that do not have an interfering fluorescence signature at the wavelength(s) of interest should be used. In normal usage, the fluorescence baseline intensity should not be more than 2% of the expected measurement signal unless a larger value previously has been justified.

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Assays in the visible region usually call for comparing concomitantly the fluorescence intensities produced by the sample solution with that produced by a standard solution that contains approximately an equal quantity of a USP Reference Standard. In some situations, it may be permissible to omit the use of a Reference Standard. This is true when spectrofluorometric assays are made with routine frequency, when a suitable standard curve is available and is prepared with the appropriate USP Reference Standard, and when the substance assayed conforms to Beer–Lambert–Bouguer Law within the range of about 75%–125% of the final concentration used in the assay. Under these circumstances, the fluorescence intensity found in the assay can be interpolated on the standard curve, and the assay result can be calculated. Such standard curves should be confirmed frequently and always when a new spectrofluorometer or new lots of reagents are put into use.

VALIDATION AND VERIFICATION

Validation

Validation is required when a procedure based on fluorescence spectroscopy is intended for use as an alternative to the official procedure. The objective of validation is to demonstrate that the measurement is suitable for its intended purpose, including the following: quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). Depending on the category of the test (for additional information, see *Table 2* in *Validation of Compendial Procedures* ⟨1225⟩), the process for analytical procedure validation for fluorescence requires testing for linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. These analytical performance characteristics apply to externally standardized procedures and those that use standard additions. Chapter ⟨1225⟩ provides definitions and general guidance about analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for fluorescence technology. For each particular application tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I, Category II, and Category III procedures, accuracy is determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. Analysts also can compare assay results obtained using the fluorescence procedure under validation to those from an established analytical procedure.
Validation criteria: 98.0%–102.0% mean recovery for a drug substance, 95.0%–105.0% mean recovery for a drug product assay, and 80.0%–120.0% mean recovery for impurity analysis. These criteria must be met throughout the intended range.

Precision

REPEATABILITY

Repeatability of the analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration. Alternatively, repeatability is assessed by measuring concentrations of three replicates of three separate sample solutions at different concentrations. The three concentrations should be sufficiently similar so that the repeatability is similar across the concentration range. If this is done, the repeatability at the three concentrations can be pooled for comparison to the acceptance criteria.
Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance, NMT 2.0% for a drug product assay, and NMT 20.0% for impurity analysis.

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the procedure should be evaluated. Typical variables include performing the analysis on different days, using different instrumentation, and having the method performed by two or more analysts. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.
Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance, NMT 3.0% for a drug product assay, and NMT 25.0% for impurity analysis.

SPECIFICITY

In fluorescence measurements, specificity is ensured by the use of a Reference Standard wherever possible and is demonstrated by the lack of interference from other components present in the matrix.
Validation criteria: Demonstrated by meeting the accuracy requirement

DETECTION LIMIT

Analysts can estimate the detection limit (DL) by calculating the standard deviation of NLT six replicate measurements of a blank solution and multiplying by 3.3. Alternatively, the standard deviation can be determined from the error of the intercept from a calibration curve or by demonstration that the signal-to-noise ratio is >3.3. Analysts must confirm the estimated DL by analyzing samples at the calculated concentration.

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QUANTITATION LIMIT

Analysts can estimate the quantitation limit (QL) by calculating the standard deviation of NLT six replicate measurements of a blank solution and multiplying by 10. Alternatively, the standard deviation can be determined from the error at the intercept from a calibration curve or by demonstration that the signal-to-noise ratio is >10.

A sample solution prepared from a representative sample matrix spiked at the required QL concentration is measured to confirm sufficient sensitivity and adequate precision. The observed signal-to-noise ratio at the required QL should be >10.

Validation criteria: For the estimated limit of quantitation to be considered valid, the measured concentration must be accurate and precise at a level equal to or less than 50% of the specification.

LINEARITY

A response curve between the analyte concentration and the fluorescence signal is prepared from NLT five Standard solutions at concentrations that encompass the anticipated concentration of the sample solution. Analysts then should evaluate the standard curve for linearity using appropriate statistical methods such as a least-squares regression. Deviation from linearity can result from either instrumental or sample factors, or both, and can be reduced to acceptable levels by reduction of the analyte concentration and thereby the associated absorbance values.

Validation criteria: The correlation coefficient (R) must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

The operational range of an analytical instrument (and the analytical procedure as a whole) is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the instrumental response function has a suitable level of precision, accuracy, and linearity.

Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

Analysts should demonstrate the reliability of an analytical measurement by deliberate changes to experimental parameters. For fluorescence these changes can include measuring the stability of the analyte under specified storage conditions, varying pH, removal of oxygen, and adding possible interfering species, to list a few examples. Analysts should determine robustness concurrently using a suitable design-of-experiments procedure.

Verification

Analytical procedures described in *USP–NF* do not require validation. Instead, a verification is used to determine a procedure's suitability under actual conditions of use.

Thus the objective of fluorescence procedure verification is to demonstrate the suitability of a test procedure under actual conditions of use. Performance characteristics that verify the suitability of a fluorescence procedure are similar to those required for any analytical procedure. For additional information, see *Verification of Compendial Procedures* ⟨1226⟩ for a discussion of the applicable general principles. Verification should be performed using a reference material and a well-defined matrix. Verification of compendial fluorescence procedures should at a minimum include the execution of the validation parameters for specificity, accuracy, precision, and quantitation limit, when appropriate, as indicated in *Validation*.

Indirect Measurement Requirements

Some fluorescence procedures employ chromogenic reactions. Generally the requirements for the analytical performance characteristics should be used. In some instances the required accuracy and precision for the direct measurements may not be achievable. Under these circumstances, the accuracy and precision requirements may be widened by as much as 50%. However, any such widening must be justified on scientific grounds and with documented evidence. Under these circumstances, the amount of replication required to produce a scientifically sound reportable value may be increased.

⟨854⟩ MID-INFRARED SPECTROSCOPY

INTRODUCTION

Mid-infrared (mid-IR) spectroscopy is an instrumental method used to measure the absorption of electromagnetic radiation over the wavenumber range between 4000 and 400 cm^{-1} (corresponding to wavelengths between 2.5 and 25 µm). Unless otherwise specified in a monograph or other validated procedure, the region from 3800 to 650 cm^{-1} (corresponding to wavelengths from 2.6 to 15 µm) should be used to ensure compliance with monograph specifications for IR absorption. The absorption of photons causes the promotion of molecules from a ground state of their vibrational mode to an excited vibrational state.

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Vibrational modes are defined by the motion of all atoms in a molecule. When molecules contain certain functional groups, IR absorption often occurs in specific narrow spectral ranges. In these cases, the wavenumbers at which these transitions occur are known as group frequencies. When a vibrational mode involves atomic motions of more than just a few atoms, the frequencies occur over wider spectral ranges and are not characteristic of a particular functional group but are more characteristic of the molecules as a whole. Such bands are known as fingerprint bands. All strong bands that absorb at wavenumbers above 1500 cm^{-1} are group frequencies. Strong bands that absorb below 1500 cm^{-1} can be either group frequencies or fingerprint bands.

For discussion of the theory and principles of measurements, see *Mid-Infrared Spectroscopy—Theory and Practice* ⟨1854⟩, which may be a helpful, but not mandatory, resource.

QUALIFICATION OF IR SPECTROPHOTOMETERS

Qualification of mid-IR spectrophotometers is divided into three components: *Installation Qualification* (IQ), *Operational Qualification* (OQ), and *Performance Qualification* (PQ). For further information, see *Analytical Instrument Qualification* ⟨1058⟩.

Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location.

Operational Qualification

Because the majority of mid-IR spectra are measured with Fourier-transform IR (FTIR) spectrophotometers, only these instruments will be discussed. [NOTE—No recommended values for signal-to-noise ratio or 100% line stability are included in this chapter because these vary with manufacturer, model, and age of the instrument.]

WAVENUMBER ACCURACY

The most commonly used wavenumber standard for IR spectrophotometry is an approximately 35 µm thick, matte polystyrene film. The spectrum of such a film has several sharp bands at 3060.0, 2849.5, 1942.9, 1601.2, 1583.0, 1154.5, and 1028.3 cm^{-1}. The most frequently chosen band for wavenumber accuracy determination is located at 1601.2 cm^{-1}. Using a suitable polystyrene film or other well-characterized wavenumber standard, scan from 3800 to 650 cm^{-1} wavenumbers, and compare the wavenumber of maximum response of the chosen band using the center-of-gravity, polynomial spline procedure, or other peak-picking algorithms to the known absorption wavenumber of the standard. The acceptable tolerance for the measured wavenumber is ±1.0 cm^{-1}.

Performance Qualification

The purpose of performance qualification (PQ) is to determine that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement.

PROCEDURE

Mid-IR spectra can be measured by transmission, external reflection, internal reflection (often called attenuated total reflection), diffuse reflection, and photoacoustic spectroscopy. Different sample preparation techniques are available for these options. The most common sample preparation techniques are presented below.

Potassium Bromide (KBr) Disks

Certain powdered alkali halides such as potassium bromide, potassium chloride, and caesium iodide coalesce under high pressure and can be formed into self-supporting disks that are transparent to mid-IR radiation. The alkali halide most commonly used is powdered, dry, highly pure potassium bromide, which is transparent to mid-IR radiation above 400 cm^{-1}.

Commercial presses and dies in a range of diameters are available for the preparation of alkali halide and similar disks.

Mineral Oil Mulls

A typical procedure to prepare a mull is to place 10–20 mg of the sample into an agate mortar, and then grind the sample to a fine particle-size powder using a vigorous rotary motion of the pestle. A small drop of the mulling agent is added to the mortar. Rotary motion of the pestle is used to mix the components into a uniform paste, which is transferred to the center of a clean IR-transparent window (e.g., potassium bromide, sodium chloride, silver bromide, or caesium iodide). A second matching window is placed on top of the mull, and the mull is squeezed to form a thin, translucent film that is free from bubbles.

The most widely used mulling agent for the mid-IR region is a saturated hydrocarbon mineral oil (liquid paraffin, Nujol).

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Self-Supported Polymer Films

The mid-IR transmission spectrum of many polymers used as packaging materials is at times recorded from samples prepared as thin self-supporting films using hot compression molding or microtoming.

Capillary Films

Nonvolatile liquids can be examined neat in the form of a thin layer sandwiched between two matching windows that are transparent to mid-IR radiation. The liquid layer must be free of bubbles and must completely cover the diameter of the IR beam focused on the sample.

Liquids and Solutions in Transmission Cells

For the examination of liquid and solution samples, transmission cell assemblies that comprise a window pair, spacer, filling ports, and a holder are available commercially in both macro- and micro-sample configurations.

For laboratory applications, spacers typically are formed from lead, poly(tetrafluoroethylene), or poly(ethylene terephthalate) and can be supplied, depending on spacer materials, in standard thickness path lengths from approximately 6 µm to 1 mm or larger.

Gases

Mid-IR transmission cells for static or flow-through gas and vapor sampling are available in a wide range of materials to suit the application, from laboratory to process scale. In the laboratory, the traditional gas cell has been a 10 cm long cylinder made from borosilicate glass or stainless steel with an approximately 40-mm aperture at each end. Each open end is covered with an end cap that contains one of a pair of mid-IR–transparent windows constructed from, e.g., potassium bromide, zinc selenium, or calcium fluoride.

Attenuated Total Reflection

Attenuated total reflectance spectroscopy relies on the optical phenomenon of radiation passing through a medium of high refractive index at a certain angle of incidence entirely reflected internally at a boundary in contact with a material of lower refractive index. The medium of high refractive index is also known as the internal reflection element (IRE).

The sample under examination should be placed in close contact with the IRE such as diamond, germanium, zinc selenide, or another suitable material of high refractive index. Ensure close and uniform contact between the substance and the whole crystal surface by applying pressure in the case of solid samples or by dissolving the substance in an appropriate solvent and then covering the IRE with the solution and evaporating to dryness.

Diffuse Reflection

The most important and commonly used form of sample preparation for diffuse reflection is to dilute the sample by intimately mixing it with 90%–99% of nonabsorbing diluents such as finely powdered potassium bromide or potassium chloride. The sample dilution has the added benefit of reducing absorption band intensities to an appropriate level.

Microscope Sampling

Coupling a light microscope with a mid-IR spectrophotometer allows spectra to be obtained from very small samples. Generally applied in transmittance or reflectance modes, it provides, for example, a powerful tool for obtaining spectroscopic data of contaminants in pharmaceutical samples.

Change to read:

VALIDATION AND VERIFICATION

Validation

Validation is required when an IR method is intended for use as an alternative to the official procedure for testing an official article.

The objective of an IR method validation is to demonstrate that the measurement is suitable for its intended purpose including: quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV, see *Table 2* in *Validation of Compendial Procedures* ⟨1225⟩). Depending on the category of the test, the validation process for IR may require the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. If the IR procedure employs a chemometrics model calculated against the response of another analytical technology (e.g., HPLC), then the principles of ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), specifically the *Method Validation* section, is to be applied.

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Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation for each characteristic. The following sections are intended to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I and II procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. It is also an acceptable practice to compare assay results obtained using the IR procedure under validation with those obtained from an established alternative analytical method.
Validation criteria: 98.0%–102.0% mean recovery for drug substances, 95.0%–105.0% mean recovery for drug product assay, and 70.0%–150.0% mean recovery for impurity analysis. These criteria are met throughout the intended range.

PRECISION

Repeatability: The analytical procedure is assessed by measuring the concentrations of six independently prepared sample preparations at 100% of the assay test concentration. Alternatively it can be based on measurements of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.
Validation criteria: The relative standard deviation is NMT 1.0% for drug substance, NMT 2.0% for drug product, and NMT 20.0% for impurity analysis.
Intermediate precision: The effect on analytical precision caused by changes in variables such as performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts needs to be assessed. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.
Validation criteria: The relative standard deviation is NMT 1.0% for drug substance, NMT 3.0% for drug product assay, and NMT 25.0% for impurity analysis.

SPECIFICITY

For Category IV tests, the identity of the analyte should be ensured. Regarding Category I and II procedures, the accuracy requirement also demonstrates specificity for the targeted analytes.
In the case of identification tests, the ability to select between compounds of closely related structure that are likely to be present should be demonstrated. This should be confirmed by obtaining positive results (perhaps by comparison to a known reference material) from samples containing the analyte, coupled with negative results from samples that do not contain the analyte and by confirming that a positive response is not obtained from materials structurally similar to or closely related to the analyte.

QUANTITATION LIMIT

The quantitation limit can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank preparation divided by the slope of the calibration line and multiplying by 10. Other suitable approaches can be used (see ⟨1225⟩). A measurement of a representative sample matrix spiked at the estimated quantitation limit concentration must be performed to confirm accuracy.
Validation criteria: For the estimated quantitation limit to be considered valid, the measured concentration must be accurate and precise at a level equal to or less than 50% of the specification.

LINEARITY

A linear relationship between the analyte concentration and the IR spectral response is demonstrated by preparing NLT 5 standard preparations at concentrations encompassing the anticipated concentration of the test preparation. The standard curve should then be evaluated using appropriate statistical methods such as a least squares regression. For experiments that do not have a linear relationship between analyte concentration and IR spectral response, appropriate statistical methods should be applied to describe the analytical response.
Validation criteria: Correlation coefficient (R), NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

This parameter is demonstrated by meeting linearity, precision, and accuracy requirements.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For non-centered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters. For mid-IR this can include but is not limited to changes in sample preparation procedure or changes in hardware settings.

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Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of an IR procedure verification is to demonstrate that the method, as prescribed in specific monographs, is being executed with suitable accuracy, sensitivity, and precision. *Verification of Compendial Procedures* ⟨1226⟩ notes that if the verification of the compendial procedure, according to the monograph, is not successful, the procedure is not suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*.

Although complete revalidation of a compendial procedure is not required, verification of the compendial Mid-IR procedure includes the execution of certain critical parameters. When the method being verified is for identification purposes, specificity is the only parameter required. For quantitative applications, additional validation parameters are studied. Typically these include accuracy, precision, and quantitation limit, as indicated in *Validation*.

Change to read:

⟨855⟩ NEPHELOMETRY ▲AND▲ (USP 1-May-2019) TURBIDIMETRY ▲▲ (USP 1-May-2019)

Add the following:

▲1. INTRODUCTION
2. TERMS AND DEFINITIONS
3. APPLICATIONS
4. INSTRUMENTATION
5. FORMAZIN TURBIDITY STANDARDS
    5.1 Preparation of the Formazin Standards
6. QUALIFICATION OF TURBIDIMETERS AND NEPHELOMETERS
    6.1 Calibration
    6.2 Stray Light
    6.3 Range of Measuring Capability
    6.4 Resolution
    6.5 Accuracy
    6.6 Performance Qualification
7. PROCEDURES
    7.1 Turbidimetric Procedures
    7.2 Nephelometric Procedures
8. VALIDATION AND VERIFICATION
    8.1 Validation
    8.2 Verification ▲ (USP 1-May-2019)

Change to read:

▲1. INTRODUCTION

Nephelometry and turbidimetry are analytical techniques that are based on the principles of light-scattering phenomena. Light scattering is the physical phenomenon in which a beam of light changes its direction of propagation (known as deflection) as a result of interaction with sufficiently small matter particles. It has been established from the Maxwell electromagnetic theory that a prerequisite for scattering to occur is that the refractive indexes of the suspended particles must be different from those of the suspending liquid. The larger the difference, the more intense the scattering becomes. There are two types of light scattering: 1) elastic scattering, in which the wavelength of the scattered light and incident light are the same; and 2) inelastic light scattering, in which the wavelength of the scattered light and incident light are different. Only the first type of light scattering (elastic) is relevant to nephelometry and turbidimetry.

In turbidimetry, the intensity of the transmitted light is measured and the attenuation of the intensity of incident light as a result of scattering is measured at the direction of incident light (i.e., 0°) and compared to the intensity of incident light (blank measurement). The measured property is an indirect measurement of the scattering effect of the suspended particles and is referred to as turbidance. Any absorbance of light by the suspended sample will result in additional attenuation of light intensity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩ and *Ultraviolet-Visible Spectroscopy—Theory and Practice* ⟨1857⟩). Hence, it is important to ensure that the material being measured does not absorb light at the measurement wavelength. Indeed the equations governing absorption and turbidimetry are the same (albeit with different values for the attenuation constants). In

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nephelometric techniques, the intensity of the scattered light at a 90° angle from the propagation direction of the incident light is measured. Therefore, a nephelometric measurement is a direct measurement of the scattering effect of suspended matter.

2. TERMS AND DEFINITIONS

Terms commonly used in describing turbidimetric and nephelometric techniques are:

- Turbidance (symbol, S): A measure of the decrease of the transmitted incident light beam intensity as a result of the light-scattering effect of suspended particles. The amount of suspended matter may be measured by observation of either the transmitted light (turbidimetry) or the scattered light (nephelometry).

$$\log I_0/I_t = kbc = T$$

I_0 = intensity of incident light
I_t = intensity of transmitted light
k = molar turbidity coefficient
b = cell path length
c = concentration
T = turbidance

- Turbidity (symbol, τ): In turbidimetric measurements, the turbidity is the measure of the decrease in incident beam intensity/unit length of a given suspension. The International Organization for Standardization defines turbidity as "the reduction of transparency of a liquid caused by the presence of undissolved matter".
- Turbidity Measurement Units: The turbidity units are stated using a descriptor which indicates the method of measurement.
- Nephelometric Turbidity Units (NTUs): When the turbidity is measured using a nephelometer, which measures the scattered light at a 90° angle from the direction of propagation of incident light, the units of turbidity are called nephelometric turbidity units (NTUs). The magnitude of NTU is defined based on the turbidity generated by primary formazin standard (a suspension made by mixing solutions of hydrazine sulfate and hexamethylenetetramine in water). Safer polymer-bead suspensions are now commercially available and are recognized as an acceptable alternative. However, all those standards are traced to formazin. The primary formazin standard solution has been assigned a turbidity of 4000 NTUs.

Other recognized units for turbidity include the formazin turbidity unit (FTU) and the formazin nephelometric unit (FNU). These units are equivalent to NTU for the range from 0–40 NTUs.

3. APPLICATIONS

Turbidimetric and nephelometric techniques have applications that include 1) concentration determination of solutions and/or suspensions (determination of several cations and anions by precipitating and suspending the resulting precipitate at well-controlled reaction parameters); 2) measurement of the degree of turbidity of turbid solutions or suspensions; 3) determination of weight-average molecular weights and dimensions of polydisperse systems in the molecular weight range from 1000 to several hundred million; 4) measurement of immunoassays' reaction kinetics or kinetics of immunoprecipitations (rate nephelometry); 5) monitoring of cell and bacteria growth; and 6) particle size distribution determination of suspended material, particle counting, etc.

Rate nephelometry is widely used for vaccine components assays and/or quantitation of components in blood serum. It is also used for host cell protein qualification in recombinant biopharmaceuticals. When using the technique, the measurement of the change in the light-scattering response by antigen–antiserum or antigen-purified antibody complexes is used to calculate the amount of antigen (Ag) or antibody (Ab) responsible for the immunological Ab-Ag precipitation reaction or agglutination reaction. Often the antigens under consideration are linked covalently or adsorbed to polymeric microspheres to increase the scattering efficiency; the resulting technique is known as "particle-enhanced immunoassay". Although the technique is described as nephelometry, usually both scattered and transmitted light are measured using the ratio instruments.

Nephelometric measurements are more reliable in low turbidity ranges (relatively low concentration of the scattering medium). In this range, a linear relationship is observed between the sample concentration and the detector's signal intensity expressed as NTU. As the concentration increases, so does the incidence of multiple scattering that deviates the response from the linearity. The maximum NTU value, which supports a reliable linearity relationship, is in the range of 1750–2000 NTUs. Turbidimetry is preferred for higher turbidity ranges (concentrations of the scattering media). To achieve consistent results, all measurement variables must be carefully controlled. Where such control is possible, extremely dilute suspensions may be measured.

Change to read:

4. INSTRUMENTATION

Instruments used for turbidimetric and nephelometric measurements are called turbidimeters and nephelometers, respectively. Generally, these instruments consist of a mercury lamp with filters for the strong green or blue lines, a shutter, a set of neutral filters with known transmittance, and a sensitive photomultiplier, which can be mounted fixed at 0° or at a 90° angle from the incident light propagation direction, or on an arm that can be rotated around the solution cell and set at any

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angle from –135° to 0° to +135° by a dial outside of the light-tight housing. Solution cells are of various shapes, such as square for measuring 90° scattering; semioctagonal for 45°, 90°, and 135° scattering; and cylindrical for scattering at all angles (see *Figure 1*).

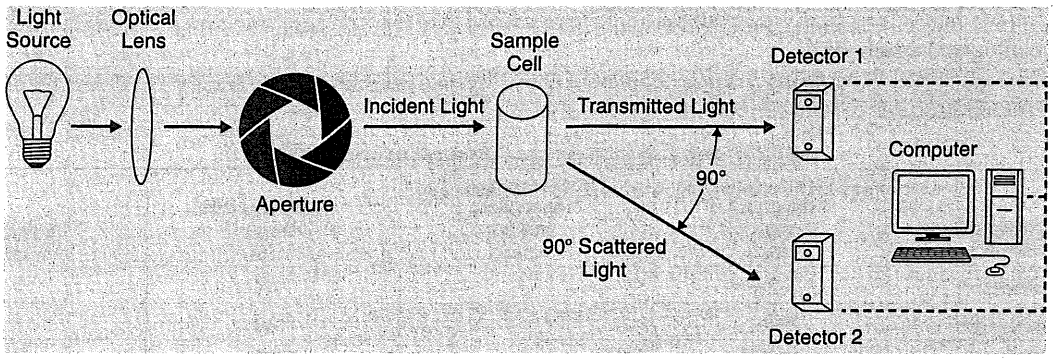


Figure 1. Representative nephelometric (turbidimetric) instrument. Note that Detector 2 may be mounted on a movable arm.

Turbidity also can be measured with a standard photoelectric filter photometer or spectrophotometer, preferably with illumination in the blue portion of the spectrum. Nephelometric measurements require an instrument with a photocell placed so as to receive scattered, rather than transmitted, light. Because this is the same geometry used in fluorometers, they can be used as nephelometers by proper selection of filters. A ratio turbidimeter combines the technology of 90° nephelometry and turbidimetry. It contains photocells that receive and measure scattered light at a 90° angle from the sample as well as receiving and measuring the forward scatter in front of the sample. It also measures light transmitted directly through the sample. Linearity is attained by calculating the ratio of the 90° angle scattered light measurement to the sum of the forward scattered light measurement and the transmitted light measurement. The benefit of using a ratio turbidimetric system is that the measurement of stray light becomes negligible. In addition, the determination of turbidity of colored suspensions is done exclusively using turbidimetric or nephelometric instruments with ratio mode because this procedure compensates for the attenuation of light as the result of the suspension color. Typically, the light source in these instruments is a tungsten lamp with most of the light intensity at about 550 nm operating at the filament temperature of 2700 K. Other suitable light sources are also available. Typically, the detectors are ▲silicon diodes▲ (ERR 1-May-2019) and photomultipliers. An alternative for eliminating the color effect involves using an infrared light-emitting diode as a light source, which yields an emission maximum centered at about 860 nm and a spectral bandwidth of 60 nm. When laser light sources are also used, especially in nephelometric instruments, the technique is commonly known as "laser nephelometry". The advantage of using laser nephelometers is the significant improvement in signal-to-noise ratio at very low detection levels. Usually the light source is a laser diode with a working wavelength at 660 nm. The high-power density of the laser beam gives rise to higher scattered intensity from smaller particles. Combined with a light trap, which absorbs the unscattered light, the system lowers the stray light significantly. When the use of a nephelometer or turbidimeter is indicated for a procedure in a monograph, instruments working in ratio mode may be used instead.

Change to read:

5. FORMAZIN TURBIDITY STANDARDS

Formazin is the only known primary turbidity standard. All other standards are secondary and must be traced to formazin. The primary standard is defined in the ▲*IUPAC Compendium of Chemical Terminology*,▲ (ERR 1-May-2019) 2nd ed. (the Gold Book) as one that is prepared by the user from traceable materials using well-defined methodologies and conditions.

Formazin suspension has many features that ensure its suitability as a primary standard. It can be consistently and accurately prepared from reagent-grade starting materials. The suspension consists of random polymers with different lengths and of random configurations, which result in moieties of varying shapes and sizes ranging from less than 0.1 µm to more than 10 µm. Although the polymer chain length distribution has been shown to vary from preparation to preparation, the overall resulting turbidity has been very reproducible.

5.1 Preparation of the Formazin Standards

[NOTE—All procedures described below must be performed at 20 ± 2° (see *Volumetric Apparatus* ⟨31⟩.]

- **Hydrazine sulfate solution:** Dissolve 1.000 g of ACS grade hydrazine sulfate ($N_2H_4 \cdot H_2SO_4$) in particle-free water in a 100-mL Class A volumetric flask and dilute with particle-free water to volume. Allow this solution to stand for 4–6 h.
- **Primary formazin standard:** Dissolve 2.50 g of analytical grade hexamethylenetetramine $[(CH_2)_6N_4]$ in 25.0 mL of particle-free water in a 100-mL flask. Add 25.0 mL of hydrazine sulfate solution using a Class A 25-mL "to deliver" pipette and mix thoroughly. Allow the preparation to stand for 48 h at 25 ± 1° before using. The resulting suspension is stable for 2 months.

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- **Formazin stock standard suspension 1:** Using a 15-mL Class A "to deliver" pipette, transfer 15 mL of the *Primary formazin standard* to a 1-L volumetric flask, and dilute with particle-free water to volume and mix. The resulting suspension has a turbidity of 60 NTU.
- **Formazin stock standard suspension 2:** Using a 50-mL Class A "to deliver" pipette, transfer 50 mL of *Primary formazin standard* to a 200-mL volumetric flask, and dilute with particle-free water to volume and mix. The resulting suspension has a turbidity of 1000 NTUs.
- **Formazin reference suspensions:** Prepare by mixing in a 100-mL volumetric flask, portions of the respective *Formazin stock standard suspension* and particle-free water according to *Table 1*.

Table 1. Formazin Reference Suspensions Preparations

Formazin Reference Suspensions	Formazin Stock Standard Suspension 1 Volume (mL)	Formazin Stock Standard Suspension 2 Volume (mL)	Particle-Free Water Volume (mL)	Turbidity Value (NTU)
Reference suspension I	5.0	—	95.0	3
Reference suspension II	10.0	—	90.0	6
Reference suspension III	30.0	—	70.0	18
Reference suspension IV	50.0	—	50.0	30
Stock standard suspension 1	100	—	0	60
Reference suspension VI	—	10.0	90.0	100
Reference suspension VII	—	20.0	80.0	200
Reference suspension VIII	—	40.0	60.0	400
Reference suspension IX	—	70.0	30.0	700
Stock standard suspension 2	—	100	0	1000

6. QUALIFICATION OF TURBIDIMETERS AND NEPHELOMETERS

The suitability of a specific instrument for a given procedure is ensured by a stepwise life cycle evaluation for the desired application from selection to instrument retirement. The qualification comprises three components: 1) installation qualification (IQ), 2) operational qualification (OQ), and 3) performance qualification (PQ) (see *Analytical Instrument Qualification* ⟨1058⟩).

The purpose of this section is to provide test methods and acceptance criteria to ensure that the instrument is suitable for its intended use (OQ), and that it will continue to function properly over extended time periods (PQ). As with any spectrometric device, a turbidimetric and nephelometric spectrometer must be qualified for both wavelength (*x*-axis, if not fixed) and photometric (*y*-axis, or signal axis) accuracy and precision, and meet the requirements for the stray light. OQ is carried out across the operational ranges required within the laboratory for both the absorbance and wavelength scales.

Acceptance criteria for critical instrument parameters that establish "fitness for purpose" are verified during IQ and OQ. Specifications for particular instruments and applications can vary depending on the analytical procedure used and the desired accuracy of the final result. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package.

Wherever possible in the procedures detailed as follows, primary reference standards or certified reference materials (CRMs) are to be used. Formazin is the only primary reference standard used in turbidimetry and nephelometry. All the other standards, including the CRMs, must be correlated to formazin. The CRMs should be obtained from a recognized accredited source and include independently verified traceable value assignments with associated calculated uncertainty. CRMs must be kept clean and free from dust. Recertification should be performed periodically to maintain the validity of the certification.

6.1 Calibration

All of the turbidimetric and nephelometric instruments are calibrated against standards of known turbidity. The instrument must be calibrated using formazin turbidity standards prior to its first time use and at least every 3 months or as specified by the vendor. Calibration is performed using at least four formazin turbidity standards whose turbidity proportionally covers the range of interest. Many instrument manufactures provide calibration verification standards. They usually consist of sealed sample cells filled with a latex suspension or with metal oxide particles in polymer gel. These standards must be used only for checking the calibration in the time intervals between the instrument recommended calibrations.

6.2 Stray Light

Stray light (stray radiant energy) is a very significant error source, especially for measurements in the range of the lower turbidity readings. It is defined as external light that reaches the detector without being scattered from the sample. There are several sources of stray light including the inherent cell surface imperfections, reflections from within the cell that are unaccounted for, optical system parts, light sources, and, to a smaller degree, the electronics fluctuations. Although there are many design features that instrument vendors use to minimize the stray light, a complete mitigation of the stray light cannot be achieved. Unlike spectrophotometric measurements, the stray light cannot be compensated for in turbidimetry. The stray

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light must be measured and the values should be within the specification range set by the vendor of the particular instrument or <0.15 NTUs for the measurement in the range of 0–10 NTUs and 0.5 NTUs for the measurements in the range of 10–1100 NTUs, whichever is smaller.

6.3 Range of Measuring Capability

The instrument must be able to measure the turbidity in the range of 0.01–1100 NTUs or from 50%–200% of the target turbidity. To demonstrate the linearity for the intended measurements range, choose at least four appropriate reference suspensions from *Table 1*.

6.4 Resolution

Instrument resolution must be 0.01 NTU or less for the measurements range of 0–9.99 NTUs; 0.1 NTU or less for the measurements range of 10–99.9 NTUs; and 1 NTU for the measurements above 100 NTUs.

6.5 Accuracy

The instrument reading accuracy must be ±10% of the reading + 0.01 NTU for the measurement range from 0–19.9 NTUs, and ±7.5% of the reading for the measurement range from 20–1100 NTUs.

6.6 Performance Qualification

The instrument PQ is accomplished periodically or as needed between the calibrations. Primary turbidity standards (formazin) or secondary calibration verification standards (latex suspensions or metal oxide particles in polymer gels contained in sealed sample cells) supplied by instrument manufacturers may be used.

7. PROCEDURES

7.1 Turbidimetric Procedures

SAMPLE CELL PREPARATION

The sample cells for sample measurements must be clean. Follow the sample cell or instrument manufacturer recommendations for cleaning the sample cells appropriately. For low turbidity measurements it is a good practice to use a single-indexed sample cell or a flow cell, which help ensure adequate precision and repeatability of the measurements. Using particle-free water, find the sample cell orientation in the sample cell holder that gives the lowest reading. For higher values of turbidity, different sample cells may be used. However, the sample cells must be matched (the difference in readings for a standard prepared at nominal sample concentration from two different sample cells must be within ±0.005 NTU or below the measurement precision requirement, whichever is lower).

SAMPLE PREPARATION

Prepare the samples as prescribed in the individual monograph. Carefully mix the samples thoroughly by swirling or inverting the volumetric flask slowly several times. Avoid shaking or stirring since it may introduce bubbles. Degassing the samples helps to improve the measurements. For degassing, the samples could stand for several minutes or a vacuum could be applied, or they could be gently sonicated using an ultrasonic bath. After degassing, let the samples stand for several minutes and mix again by carefully inverting two to three times. Transfer the sample to the sample cell and take the readings.

USE OF FLOW CELLS

Flow cells are mainly used for low turbidity measurements for samples with small particles. When such cells are used, the sample is introduced by carefully pouring it down the interior edge of the inlet reservoir.

In practice, it is advisable to ensure that settling of the particles being measured is negligible. This is usually accomplished by including a protective colloid in the liquid-suspending medium. It is important that results be interpreted by a comparison of readings with those representing known concentrations of suspended matter, produced under precisely the same conditions.

7.2 Nephelometric Procedures

Nephelometric procedures are performed similarly to turbidimetric procedures for both direct measurements and measurements in the ratio mode as described above.

RATE NEPHELOMETRIC PROCEDURES

The overall procedure for monitoring the progress of the reaction consists of three well-defined steps: 1) record a baseline reading of the turbidity of the medium (blank); 2) record the turbidity after the first reagent (antigen) is added, which results

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in an increase of the turbidity until a plateau is reached; and 3) add the second reagent (antibody), which results in another turbidity increase and a second plateau followed by a final turbidity increase that continues until a third plateau is reached. The measurement zone is selected from the addition of the antibody until the third plateau, depending on the purpose of the assay and the respective component concentrations. *Kinetic nephelometry* and *Endpoint nephelometry* are two general procedures that are used for quantifying the immune complexes formed in the immunoassay methods (also known as immunonephelometry because the measured turbidity is due to immunocomplexes that are formed). For each procedure, there are several parameters that need to be optimized in each individual application. The main parameters are 1) with or without particle enhancement; 2) particle types, sizes, and respective optimum wavelength, if applicable; 3) monitoring reaction kinetic or endpoint; 4) antibody/antigen under consideration and, related to that, the optimum level of antigen loading; 5) buffers and other ionic species and respective optimal pH; 6) type and concentration of polymers used to modify the solubility of proteins; and 7) temperature and other environmental factors. Generally these parameters are optimized during the method development and the values are given in specific monograph(s) and/or chapter(s) as applicable.

Kinetic nephelometry: The kinetic nephelometry is advantageous compared to the endpoint nephelometry mainly because of the capability to take a sample blank reading in addition to a reagent blank reading. This procedure assesses the rate of the immunocomplex formation based on the increased intensity response of the scattered light of the chosen wavelength. The reaction kinetic may be monitored continuously or a certain number of data points may be taken, depending on the time response of the instrument used and the type of application. At times it may involve only two data points; however, proper care must be exercised because the choice of point selection can influence the overall accuracy in cases where differences in reaction kinetics exist between samples and calibrating standards. Careful consideration should be given to the appropriate choice of specificity control strategy.

Endpoint nephelometry: In this method, an initial measurement is performed before adding the reagent, which represents the blank reading. A second measurement is performed after the immune complex is formed after approximately 60 min. The difference between these two measurements is proportional to the content of the component being assayed.

8. VALIDATION AND VERIFICATION

8.1 Validation

Validation is required when a nephelometric/turbidimetric method is intended for use as an alternative to the official procedure for testing an official article. The objective of nephelometric/turbidimetric method validation is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). Depending on the category of the test (see *Validation of Compendial Procedures* ⟨1225⟩, *Table 2*), the analytical method validation process for nephelometry/turbidimetry requires testing for accuracy, precision, specificity, detection limit (DL), quantitation limit (QL), linearity, range, and robustness. These analytical performance characteristics apply to externally standardized procedures and those that use standard additions.

Validation of Compendial Procedures ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I, II, and III procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. Analysts can also compare the assay results obtained using the nephelometric/turbidimetric procedure under validation to those from an established analytical procedure.

Validation criteria: 98.0%–102.0% mean recovery for the drug substances, 95.0%–105.0% mean recovery for the drug product assay, and 80.0%–120.0% mean recovery for the impurity analysis. These criteria are met throughout the specified range.

PRECISION

Repeatability: The repeatability of the analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration. Alternatively, it can be assessed by measuring the concentrations of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for the drug substance, NMT 2.0% for the drug product assay, and NMT 20.0% for the impurity analysis.

Intermediate precision: The effect of random events on the analytical precision of the method must be established. Typical variables include performing the analysis on different days, using different instrumentation, and/or having the method performed by two or more analysts. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.5% for the drug substance, NMT 3.0% for the drug product assay, and NMT 25.0% for the impurity analysis.

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SPECIFICITY

In nephelometric/turbidimetric measurements, specificity is demonstrated by the lack of interference from other components present in the matrix (other components of the matrix produce a true solution).

DETECTION LIMIT

The DL can be estimated by calculating the concentration of a solution that would give the signal-to-noise ratio of ≥3.3. The estimated DL must be confirmed by analyzing samples at the calculated concentration.

QUANTITATION LIMIT

The QL can be estimated by calculating the concentration of a solution that would give the signal-to-noise ratio of ≥10.0. The estimated QL must be confirmed by analyzing samples at the calculated concentration. Measurement of a test solution prepared from a representative sample matrix spiked at the required QL concentration must be performed to confirm sufficient sensitivity and adequate precision. The observed signal-to-noise ratio at the required QL should be >10.
Validation criteria: For the estimated limit of quantitation to be considered valid, the measured concentration must be accurate and precise at a level ≤50% of the specification.

LINEARITY

A linear relationship between the analyte concentration and measured turbidity response must be demonstrated by preparation of at least four standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods such as a least-squares regression. Deviation from linearity results from instrumental or sample factors, or both, can be reduced to acceptable levels by reducing or increasing the analyte concentration, thereby respectively decreasing or increasing the turbidity readings to within the nephelometer/turbidimeter instrument linearity range.
Validation criteria: The correlation coefficient (R) must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

The operational range of an analytical instrument (and the analytical procedure as a whole) is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the instrumental response function has a suitable level of precision, accuracy, and linearity.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For non-centered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. For nephelometry/turbidimetry this can include, for example, measuring the stability of the analyte under specified storage conditions, varying pH, and adding possible interfering species. Robustness is determined concurrently using a suitable design for the experimental procedure.

8.2 Verification

Current U.S. Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in the *U.S. Pharmacopeia* and *National Formulary* are not required to validate these procedures if provided in a monograph. Instead, they simply must verify their suitability under actual conditions of use.

The objective of nephelometric/turbidimetric procedure verification is to demonstrate the suitability of a test procedure under actual conditions of use. Performance characteristics that verify the suitability of a nephelometric/turbidimetric procedure are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in *Verification of Compendial Procedures* ⟨1226⟩. Verification is usually performed using a reference material and a well-defined matrix. Verification of compendial nephelometric/turbidimetric procedures includes, at minimum, the execution of the validation parameters for specificity, accuracy, precision, and QL, when appropriate, as indicated in *8.1 Validation*.▲ (USP 1-May-2019)

Add the following:

▲⟨856⟩ NEAR-INFRARED SPECTROSCOPY

1. INTRODUCTION
2. QUALIFICATION OF NIR SPECTROMETERS
    2.1 Installation Qualification
    2.2 Operational Qualification

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2.3 Performance Qualification
3. PROCEDURE
4. VALIDATION AND VERIFICATION
4.1 Validation
4.2 Verification
USP Reference Standards

1. INTRODUCTION

Near-infrared (NIR) spectroscopy covers the electromagnetic field wavelength range from 780 to 2500 nm, with the frequencies expressed in wavenumbers (about 12,821–4000 cm^{-1}). NIR spectroscopy is a vibrational spectroscopic technique that measures the absorption of radiation in this wavelength range that is resonant with frequencies from molecular dipole changes in the sample; thus, NIR spectroscopy is related to infrared (IR) spectroscopy. The NIR spectroscopic region is dominated by overtone and combination frequencies of the molecular fundamental harmonic frequencies in the mid-IR region. The molar absorptivities in the NIR region tend to be much lower than those in the IR region, and the radiation can penetrate several millimeters into the material, giving rise to a wavelength-dependent scattering signal overlapping the molecular absorbance signals.

Not all materials absorb NIR radiation, and materials such as glass are relatively transparent. These attributes of NIR spectroscopy have been shown to be useful in the area of pharmaceutical analysis for both qualitative and quantitative applications. For further discussion of the theory and applications, see *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩, which may be a helpful, but not mandatory, resource.

The instrument qualification tests and acceptance criteria provided in this chapter may not be appropriate for some instrument configurations. In such cases, alternative instrument qualification and performance checks should be scientifically justified and documented before use. In addition, validation parameters discussed in this chapter may not be appropriate for all applications of NIR spectroscopy. Validation parameters characterized for a specific NIR-spectroscopic application should demonstrate the suitability of the application for its intended use. For further discussion of the theory and applications of chemometrics, see *Chemometrics* ⟨1039⟩.

2. QUALIFICATION OF NIR SPECTROMETERS

Qualification of NIR spectrometers is divided into three components: 1) installation qualification (IQ), 2) operational qualification (OQ), and 3) performance qualification (PQ). For further discussion on qualification, see *Analytical Instrument Qualification* ⟨1058⟩.

2.1 Installation Qualification

The IQ requirements elicit evidence that the hardware and software are properly installed in the desired location.

2.2 Operational Qualification

In OQ, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. Because there are so many different approaches for measuring NIR spectra, OQ using standards with known spectral properties is recommended. This may be achieved by using the instrument performance checks, and external, traceable certified reference materials (CRMs) as appropriate. As is the case with any spectroscopic device, wavelength accuracy, photometric linearity, and noise characteristics of NIR instruments must be qualified against target specifications for the intended application.

The OQ tests described in the sections that follow are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance specifications given in this section are applicable for general use specifically, whereas specifications for particular instruments and applications can vary, depending on the analytical method used and the desired accuracy of the final result.

2.2.1 CHARACTERIZING INSTRUMENT PERFORMANCE

Use the apparatus according to the manufacturer's instructions, and carry out the prescribed verification at regular intervals. For in-line and on-line applications, the use of alternative means of control of instrument performance must be scientifically justified. For example, use the manufacturer's internal standards or use separate channels/probes, with scientific justification, to demonstrate instrument performance. System suitability tests may be required prior to sample scanning, and the instrument attributes with the most variation (typically photometric noise and wavelength accuracy) must be tested. The frequency at which each performance test is conducted must be assessed for risk, depending on the instrument type, application, and its environment. For example, instruments placed in harsh environments, such as environments with changing temperature and humidity, will need frequent performance testing.

Wherever possible, in the procedures for determining wavelength accuracy and linearity, analysts must use CRMs rather than laboratory-prepared solutions. These CRMs should be obtained from a recognized, accredited source and should include

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independently verified, traceable value assignments with associated calculated uncertainty. CRMs are to be kept clean and free from dust. Recertification is to be performed periodically to maintain the validity of the certification.

2.2.2 WAVELENGTH ACCURACY

NIR spectra from sample and/or reference standard materials can be used to demonstrate an instrument's suitable wavelength-dispersion performance against target specifications. Typically, wavelength accuracy is characterized from a single spectrum (collected with the same spectral resolution used to obtain the standard value) using a minimum of three peaks that cover a suitable spectral range of the instrument.

Verification of wavelength scale (except for filter apparatus): Verify the wavelength scale utilized, which is generally in the region between about 780 and about 2500 nm (about 12,800 to about 4000 cm^{-1}) or in the intended spectral range using one or more suitable wavelength standards that have characteristic maxima or minima within the range of methodology wavelengths to be used. Fourier transform (FT) instruments have a linear frequency range; therefore, wavelength certification at a single frequency is sufficient. The USP Near IR System Suitability RS can be used for wavelength verification. Suitable materials for demonstrating wavelength dispersion performance must be used. With appropriate justification, alternative standards may be used. Additional information on calibration considerations may be found in ⟨1856⟩.

Typical tolerances for agreement with standard values are:

- ±1.0 nm at 780 nm (±16 cm^{-1} at 12,800 cm^{-1})
- ±1.0 nm at 1200 nm (±8 cm^{-1} at 8300 cm^{-1})
- ±1.0 nm at 1600 nm (±6 cm^{-1} at 6250 cm^{-1})
- ±1.5 nm at 2000 nm (±4 cm^{-1} at 5000 cm^{-1})
- ±3.0 nm at 2500 nm (±2 cm^{-1} at 4000 cm^{-1})

For the reference material used, apply the tolerance for the nearest wavelength (or wavenumber) from the above information for each peak used. For diode array instruments, the pixel resolution (wavelength between pixels) can be as large as 10 nm. The pixel resolution must be consistent with the required spectral resolution. The peak-finding algorithms are critical to wavelength accuracy. From a practical standpoint, ±2 nm is appropriate for peak wavelength accuracy when using such instrumentation. Alternatively, refer to the manufacturer's specifications for acceptance.

2.2.3 PHOTOMETRIC LINEARITY AND RESPONSE STABILITY

Photometric linearity is typically characterized in the range from 10% to 90% reflectance (or transmittance). NIR applications based on measuring an absorbance larger than 1.0 may require standards with reflectivity properties between 2% and 5% reflectance (or transmittance) for characterizing instrument performance at low reflectance. The purpose is to demonstrate a linear relationship between NIR reflectance and/or transmittance and instrument response across the scanning range of the instrument. Subsequent verifications of the photometric model can use the initial observed absorbance values as the established values.

Measure four photometric standards across the working method absorbance range. Typical tolerances for a linear relationship are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept of a plot of the measured photometric response versus the standard photometric response. Alternative tolerances may be used when justified for specific applications. A tolerance of ±2% is acceptable for long-term stability.

2.2.4 PHOTOMETRIC NOISE

NIR instrument software may include built-in procedures to automatically determine system noise and to provide a statistical report of noise or signal-to-noise ratio (S/N) over the instrument's operating range. In addition, it may be desirable to supplement such checks with measurements that do not rely directly on manufacturer-supplied procedures. Typical procedures involve measuring spectra of traceable reference materials with both high and low reflectance. Determine the photometric noise using suitable reflectance standards. Tolerances for these procedures should demonstrate suitable S/N for the intended application. Follow the manufacturer's methodology and specifications.

High-flux noise: Instrument noise is evaluated at high-light flux by measuring reflectance or transmittance of the reference standard, with the reference material (e.g., 99% reflectance standard) acting as both the sample and the background reference.

Low-flux noise: The same procedure may be used with a lower-reflectivity reference material (e.g., 10% reflectance standard) to determine system noise at reduced-light flux. The source, optics, detector, and electronics make significant contributions to the noise under these conditions.

2.3 Performance Qualification

The objective of PQ is to ensure that the instrument is performing within specified limits with respect to critical operational parameters. In certain cases, when the instrument has been set up for a specific measurement, it might no longer be possible or desirable to measure the wavelength and photometric (signal) qualification reference standards used in the OQ. Provided that the instrument OQ has shown that the equipment is fit for use, a single external performance verification standard can be used on a continuing basis (e.g., daily or before use). The performance verification standard must match the format of the samples in the current analysis as closely as possible and must use similar spectral acquisition parameters. Quantitative measurements of an external performance verification standard can be used to check both the wavelength accuracy and the photometric precision. Favorable comparison of a series of performance verification spectra demonstrates proper continued operation of the instrument.

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Specific procedures, acceptance criteria, and time intervals for characterizing and monitoring NIR instrument performance are selected based on the instrument and intended application. Many NIR applications use previously validated models that relate NIR spectral response to a physical or chemical property of interest. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from sample spectra using previously validated chemometric models.

3. PROCEDURE

NIR spectra can be obtained from several sample presentations, including solids, powders, slurries, gels, liquids, films, and gases. In addition, measurement can be performed through glass or plastic films that are normally used for containment. Typically, NIR spectroscopy does not require any sample preparation because the detectors have a wide dynamic range, and the collection time can be adjusted by the user. Acceptable acquisition parameters or ranges hereof, must be included as a part of the analytical test procedure. Equally important, data pretreatment must be properly implemented such that the intended measurement sensitivity is not subsequently degraded. Specific values for tests such as spectrometer noise and bandwidth will be dependent on the instrument chosen and the purpose required. For this reason, specific instrument tests for these parameters are not indicated here. Additional details can be found in ⟨1856⟩.

4. VALIDATION AND VERIFICATION

The objective of NIR procedure validation, as is the case with validation of any analytical procedure, is to demonstrate that it is suitable for its intended purpose. NIR spectroscopy is somewhat different from conventional vibrational spectroscopic techniques, because it is not a primary technique. Validation can be achieved by the assessment of variability (precision) and comparison to a reference standard or the result of a validated primary method (accuracy). The procedure for validation is related to the fundamental validation characteristics required for any analytical procedure. Data pretreatment is often a vital step in the chemometric analysis of NIR spectral data. Many suitable data pretreatments exist; the selection should be based on sound scientific judgment and suitability for the intended application.

4.1 Validation

The validation criteria described below are only required when an NIR spectroscopic procedure is intended for use as an alternative to the monograph procedure for testing an official article. The objective of procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including: quantitative determination of the main component in a drug substance or drug product (Category I assays); quantitative determination of impurities or limit tests (Category II); and identification tests (Category IV; see *Validation of Compendial Procedures* ⟨1225⟩, *Table 2*). Depending on the category of the test, the analytical procedure validation process for an NIR spectroscopic method requires the testing of accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness as appropriate. Note that validated NIR spectroscopy procedures may not be transferrable to all configurations of NIR spectrometers because of differences in their inherent performance characteristics.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The following sections are intended to provide the user with specific validation criteria that represent the minimum expectations for this technology, assuming use of the typical Category I USP specifications of 98.0%–102.0% for a drug substance and 90.0%–110.0% for a drug product. The actual validation performance characteristics will depend on the specifications in place and must provide adequate evidence that the measurement capability is sufficient for those specifications. For certain particular applications, tighter criteria may be needed in order to demonstrate suitability for the intended use.

4.1.1 ACCURACY

Accuracy is demonstrated by showing the closeness of agreement between the value that is obtained and either a conventional true value or an accepted reference value from a reference procedure. Accuracy can be determined by direct comparison between validation results and actual or accepted reference values. Suitable agreement with reference values is based on required measurement capability for a specific application. The purpose is to demonstrate a linear relationship between the NIR spectroscopic results and actual values. If a chemometric model is used, accuracy can be determined by using the methodologies in ⟨1039⟩. The error of the reference procedure may be known 1) on the basis of historical data, 2) through validation results specific to the reference procedure, or 3) by calculating the standard error of the laboratory (SEL).

For Category I and Category II procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. It is also an acceptable practice to compare assay results obtained using the NIR spectroscopy procedure under validation with those obtained from an established alternative analytical method.

Validation criteria

Criteria 1: Suitable agreement between SEP and SEL is based on required measurement capability for a specific application. For more information, see ⟨1039⟩.

Criteria 2: In addition to Category I and Category II procedures, 98.0%–102.0% recovery for a drug substance, 95.0%–105.0% recovery for a drug product assay, and 70.0%–150.0% recovery for an impurity analysis. These criteria must be met throughout the intended range.

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4.1.2 PRECISION

The precision of an NIR spectroscopic procedure expresses the closeness of agreement among a series of measurements under prescribed conditions. Precision measurements are typically expressed as the relative standard deviation of a series of results and should be suitable for the intended application. Two levels of precision should be considered for NIR spectroscopic procedures: repeatability and intermediate precision.

Repeatability: Repeatability can be demonstrated by statistical evaluation of multiple NIR procedure results, with each result derived from a replicate analysis of a sample after repositioning between spectral acquisitions. System precision may be obtained directly from the analysis of variance (ANOVA) table. The analytical procedure can be assessed by measuring the concentrations of six independently prepared sample preparations at 100% of the assay test concentration. Alternatively, it can be based on measurements of three replicates of three separate samples at different analyte levels. The three levels should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations may be pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance assay, NMT 2.0% for a drug product assay, and NMT 20.0% for an impurity analysis.

Intermediate precision: Analysts must test the effects of changes in variables, such as performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts in the repeatability study. At a minimum, any scientifically justified combination of at least two of these factors totaling (based upon intended application) six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance assay, NMT 3.0% for a drug product assay, and NMT 25.0% for an impurity analysis.

4.1.3 SPECIFICITY

The extent of specificity testing depends on the intended application. Specificity is typically demonstrated by using the following approaches:

Qualitative: Identification testing is a common application of qualitative NIR spectroscopy. Identification is achieved by comparing a sample spectrum to a reference spectrum or spectra, for instance, by establishing a mathematical relationship (chemometric model). The specificity of the NIR identification procedure is demonstrated by obtaining positive identification from samples, coupled with negative results from materials that should not meet the criteria for positive identification. Materials to demonstrate specificity should be selected on the basis of sound scientific judgment and risk specific to the application (e.g., materials handled in the same area).

Quantitative: Quantitative applications of NIR spectroscopy typically first establish a mathematical relationship (chemometric model) between NIR spectral response and a physical or chemical property of interest. Demonstration of specificity against a physical or chemical property of interest is based on interpreting both NIR spectral attributes and chemometric parameters in terms of the intended application. The demonstration of specificity may include the following:

- Spectral regions can be correlated to the property of interest
- Wavelengths used by regression analysis for the calibration (e.g., for multiple linear regression models) or the loading vector for each factor (e.g., for partial least squares or principal component regression models) can be examined to verify relevant spectroscopic information that is used for the mathematical model
- Variation in spectra from samples for calibration should be examined and interpreted
- Variation in material composition and sample matrix may be shown to have no significant effect on quantification of the property of interest within the specified procedure range

For Category I and Category II procedures, the specificity is demonstrated by meeting the accuracy requirements.

For Category IV tests, the identity of the analyte must be confirmed by comparison with appropriate reference substances.

4.1.4 QUANTITATION LIMIT

Given that NIR methods are usually macro techniques, there is normally no need for determining the quantitation limit. However, fitness for purpose must be demonstrated over the operational range of the analyte.

4.1.5 LINEARITY

Quantitative procedures generally attempt to demonstrate a linear relationship between NIR spectral response function and the property of interest. Although demonstrating a linear response is not required for all NIR applications, the model chosen should properly represent the relationship.

Linearity of NIR spectroscopic procedures depends on variables such as matrix effects and data pretreatment. Validation of linearity in NIR procedures may be accomplished by examining NLT 5 samples that span the operational range and by plotting either the NIR spectral response functions versus actual or accepted values for the property of interest. Many applications may require models of higher order, and various statistical procedures are available for evaluation of the goodness of fit. Applicable statistics and graphical procedures may be used as appropriate.

The Pearson correlation coefficient, r, measures the strength and direction of the association between two variables (x and y), in this instance, concentration and absorbance. The coefficient of determination, r^2, is a measure of the fraction of the data's variation that is adequately modelled and not a measure of linearity. Linearity depends on the standard error of the calibration function (and hence the reference procedure) and on the range of the calibration data. Thus, although values very near 1.00, such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability.

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Validation criteria: The coefficient of determination, r^2, must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests. Visual inspection of the residual plots should reveal no significant pattern. For further guidance on multivariate procedures, see ⟨1039⟩.

4.1.6 RANGE

The specified range of an NIR spectroscopic procedure depends on the specific application. The range typically is established by confirming suitable measurement capability (accuracy and precision) over the proposed operational range. Controls must be used to ensure that results outside of the validated range are not accepted. In certain circumstances, it may not be possible or desirable to extend the validated range to include sample variability outside of the validated range. Extending the range of an NIR spectroscopic procedure requires demonstration of suitable measurement capability within the limits of the expanded range. Examples of situations in which only a limited sample range may be available are samples from a controlled manufacturing process and in-process samples. A limited procedure range does not preclude the use of an NIR spectroscopic procedure.

Validation criteria: For Category I procedures, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the range is 70.0%–130.0%. For Category II procedures, the validation range covers typically 50.0%–120.0% of the acceptance criteria.

4.1.7 ROBUSTNESS

The robustness of an analytical measurement must be demonstrated during development by making deliberate changes to experimental parameters. NIR spectroscopic measurement parameters selected to demonstrate robustness will vary depending on the application and the sample's interface with the instrument. Critical measurement parameters associated with robustness often are identified and characterized during method development. Typical measurement parameters include the following:

- Effect of environmental conditions (e.g., temperature, humidity, and vibration)
- Effect of sample temperature
- Sample handling (e.g., fiber-optic probe depth, compression of material, sample depth/thickness)
- Influence of instrument changes (e.g., lamp change, warm-up time)

4.2 Verification

US Current Good Manufacturing Practices regulations [21 Code of Federal Regulations (CFR) 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of method verification is to demonstrate that the procedure, as prescribed in specific monographs, is being executed with suitable accuracy, sensitivity, and precision. *Verification of Compendial Procedures* ⟨1226⟩ notes that if the verification of the compendial procedure, according to the monograph, is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices*.

Although complete revalidation of a compendial procedure is not required, verification of the compendial NIR spectroscopic procedure includes the determination of certain validation parameters. When the method being verified is for identification purposes, specificity is the only parameter required. For quantitative applications, additional validation parameters, such as accuracy and precision, must be studied as indicated in *Validation of Compendial Procedures* ⟨1225⟩, *Validation* as appropriate.

USP Reference Standards ⟨11⟩

USP Near IR System Suitability RS▲ (USP 1-May-2020)

⟨857⟩ ULTRAVIOLET-VISIBLE SPECTROSCOPY

Change to read:

INTRODUCTION

▲For the purposes of this chapter, an ultraviolet-visible (UV-Vis) spectrometer is defined as an optical system capable of producing monochromatic radiation in the range of 200–780 nm and as a device capable of detecting the optical transmittance, usually expressed in absorbance (*A*), whose primary function is to measure the stated absorbance/transmittance at defined wavelength(s). [NOTE—A UV-Vis spectrometer may also be referred to as a spectrophotometer or absorption spectrometer.]

This functionality may be extended to incorporate multiple channel measurements, either by instrumental design or with time, e.g., in dissolution or kinetic studies.

Chromatographic detectors are specifically excluded from this chapter.

For essential supporting information on the best practices and principles of measurements, see *Ultraviolet-Visible Spectroscopy —Theory and Practice* ⟨1857⟩.

The suitability of a specific instrument for a given procedure is ensured by a stepwise life cycle evaluation for the desired application from selection to instrument retirement: design qualification (DQ); installation qualification (IQ); an initial

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performance-to-specification qualification, also known as operational qualification (OQ); and an ongoing performance qualification (PQ). For more details, see *Analytical Instrument Qualification* ⟨1058⟩. *Acceptance criteria* for "fitness for purpose" are based on the uncertainty of the reference material and the performance specification of the instrument.

Principles of Measurement ▲(USP 1-Dec-2019)

UV-Vis spectra are derived when the interaction between incident radiation and the electron cloud in a chromophore results in an electronic transition involving the promotion of one or more of the outer shell or the bonding electrons from a ground state into a state of higher energy. The UV and visible spectral bands of substances generally are broad and do not possess a high degree of specificity for compound identification. Nevertheless, they are suitable for quantitative assays and, for many substances, are useful as an additional means of identification.

In the Beer–Lambert law the absorbance (A_λ) of a solution at given wavelength, λ, is defined as the logarithm to base 10 of the reciprocal of the transmittance (T_λ):

$$A_\lambda = \log_{10}\left(\frac{1}{T_\lambda}\right) \text{ and } T_\lambda = \frac{I_\lambda}{I_{\lambda 0}}$$

T_λ = transmittance
I_λ = intensity of the transmitted radiation at the same wavelength λ
$I_{\lambda 0}$ = intensity of the incident radiation at wavelength λ

In the absence of any other physical or chemical factors, A_λ is proportional to path length, b, through which the radiation passes, and to the concentration, c, of the substance in the solution in accordance with the following:

$$A_\lambda = \varepsilon_\lambda c b$$

ε_λ = molar absorptivity
c = solute concentration (mol/L)
b = path length (cm)

If the concentration, c, is expressed in g/L, the constant ε_λ becomes a_λ, which is called the absorptivity.

The expression $A_{1\text{ cm}}^{1\%}$, which represents the specific absorbance of a dissolved substance, refers to the absorbance of a 10-g/L solution (1%, m/v) in a 1-cm cell measured at a defined wavelength so that:

$$A_{1\text{ cm}}^{1\%} = 10a_\lambda = 10\varepsilon_\lambda / M$$

a_λ = absorptivity
ε_λ = molar absorptivity
M = molar concentration of the solution

When solutions are observed in 1-cm cells, concentrations of about 10 ▲µg/mL▲ (USP 1-Dec-2019) often will produce absorbances of 0.2–0.8 in the UV or visible region.

▲▲ (USP 1-Dec-2019)

Change to read:

QUALIFICATION OF UV-VIS SPECTROMETERS

▲▲ (USP 1-Dec-2019)

The purpose of this section of the chapter is to provide test methodologies and acceptance criteria to ensure that the instrument is suitable for its intended use (OQ), and that it will continue to function properly over extended time periods as part of PQ. As with any spectrometric device, a UV-Vis ▲spectrometer▲ (USP 1-Dec-2019) must be qualified for both wavelength (x-axis) and photometric (y-axis, or signal axis) accuracy and precision, and the fundamental parameters of stray light and resolution must be established. OQ is carried out across the operational ranges required within the laboratory for both the absorbance and wavelength scales.

Installation Qualification

▲Documented proof of the▲ (USP 1-Dec-2019) IQ requirements provide evidence that the hardware and software are properly installed in the desired location.

Operational Qualification

Acceptance criteria for critical instrument parameters that establish "fitness for purpose" are verified during IQ and OQ. Specifications for particular instruments and applications can vary, depending on the analytical procedure used and the desired

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accuracy of the final result. ▲OQ should establish suitable control over the operational range of wavelength, absorbance, and the evaluation of stray light and resolution, i.e., spectral bandwidth (SBW).▲ (USP 1-Dec-2019) Instrument vendors often have ▲reference materials and protocols▲ (USP 1-Dec-2019) available as part of the IQ/OQ package.

Wherever possible in the procedures detailed as follows, certified reference materials (CRMs) are to be used in preference to laboratory-prepared solutions. These CRMs should be obtained from a recognized accredited source and include independently verified, traceable value assignments with associated calculated uncertainty. CRMs must be kept clean and free from dust. Recertification should be performed periodically to maintain the validity of the certification.

Control of Wavelengths

Ensure that the accuracy of the wavelength axis (*x*-axis) over the intended operational range is correct within acceptable limits. ▲The control of wavelength OQ must include at least one method in each operational range where it is intended for use. The options and acceptance criteria are summarized in *Table 1*.

Table 1. Wavelength Ranges and Procedures for Control of Wavelengths

Method	UV (200–400 nm)	Vis (400–780 nm)	Vis/NIR (400–900 nm)
Mercury (Hg) emission lines	Accuracy ±1 nm Precision ≤0.5 nm	Accuracy ±2 nm Precision ≤0.5 nm	—
Deuterium (D_2) emission lines	—	Accuracy ±2 nm Precision ≤0.5 nm	—
Cerium oxide solutions	Accuracy ±1 nm Precision ≤0.5 nm	—	—
Holmium oxide solutions or glasses	Accuracy ±1 nm Precision ≤0.5 nm	Accuracy ±2 nm Precision ≤0.5 nm	—
Didymium solutions or glasses	—	—	Accuracy ±2 nm Precision ≤0.5 nm

[NOTE—Certified reference standards, traceable to the National Institute of Standards and Technology (NIST) (www.nist.gov) or to other recognized standards-setting organizations, are commercially available, and should be used where possible.▲ (USP 1-Dec-2019)]

For non-diode array instruments, wavelength accuracy and precision are determined over the operational range using at least six replicate measurements. For wavelength accuracy, the difference of the mean measured value to the certified value of the CRM must be within ±1 nm in the UV region (200–400 nm), and in the visible region ▲(400–780 nm) and visible/NIR (near-infrared) region (400–900 nm)▲ (USP 1-Dec-2019) must be within ±2 nm.

For wavelength precision, ▲the standard deviation of the mean value of the six wavelength measurements▲ (USP 1-Dec-2019) must not exceed 0.5 nm. For diode array instruments, only one wavelength accuracy measurement is required, and no precision determination needs to be performed, ▲due to the non-mechanical design of the monochromator.

Establishment of Acceptance Limits (Wavelength)—Choice of Standards

For all *Control of Wavelengths* accuracy procedures, acceptance limits for adequate calibration are established by adding the expanded uncertainty of the CRM to the instrument vendor specification at the wavelength(s) required in a linear manner, and these values must lie within the values specified in *Table 1*. In the case where atomic line spectra are used, this expanded uncertainty of the CRM is deemed to be zero for this process, i.e., the limit simply becomes the instrument vendor specification.[1] ▲ (USP 1-Dec-2019)

ATOMIC LINE SPECTRA

This procedure is described as the primary application because the emission lines produced from a discharge lamp are characteristic of the source element and, as a fundamental physical standard, these wavelengths have been measured with an uncertainty of NMT ±0.01 nm. In solution spectrometry, the wavelength accuracy required rarely exceeds ▲1.0▲ (USP 1-Dec-2019) nm. For these reasons, the atomic line standard values are cited without uncertainty.

▲The arc of the atomic emission source, or its image, needs to be located on the same optical path as the image of the primary light source of the spectrometer;▲ (USP 1-Dec-2019) thus, it can be used only in ▲spectrometers▲ (USP 1-Dec-2019) that can be operated in a single-beam intensity mode and practically should be implemented only on a system designed to accommodate these sources, e.g., as an accessory.

A commonly employed low-pressure mercury lamp has a number of intense lines that cover a large part of the UV and visible spectra. Two deuterium lines from the source at 486.0 and 656.1 nm often are used by manufacturers as an internal calibration check and can be used for diagnostic purposes, ▲as can the Xe line at 260.6 nm, if appropriate▲ (USP 1-Dec-2019) (see ▲*Table 2*).▲ (USP 1-Dec-2019)[2]

[1] NIST Special Publication 829—Use of NIST Standard Reference Materials for Decisions on Performance of Analytical Chemical Methods and Laboratories. www.nist.gov/sites/default/files/documents/mml/csd/inorganic/NIST_SpecialPub829.pdf.

[2] The rounded values are taken from ASTM Standard E275.

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▲Table 2.▲ (USP 1-Dec-2019) Recommended Atomic Lines from Low-Pressure Mercury (Hg) and Deuterium (D_2) Lamps for Wavelength Calibration Purposes

Element	nm
Hg	253.7
▲Xe	260.6▲ (USP 1-Dec-2019)
Hg	296.7
▲Hg	313.2▲ (USP 1-Dec-2019)
Hg	365.0
Hg	404.7
Hg	435.8
D_2	486.0
Hg	546.1
Hg	577.0
Hg	579.1
D_2	656.1

▲[NOTE—Select the nearest wavelength(s) spanning the operational range required.]▲ (USP 1-Dec-2019)

RARE EARTH OXIDE SOLUTIONS

This procedure uses solutions of rare earth oxides prepared by dissolution in acid media. The most frequently used is holmium oxide in perchloric acid.▲[3]▲ (USP 1-Dec-2019) Holmium oxide solution has been internationally accepted as an intrinsic wavelength standard, and suitable CRMs are available commercially.[4]

The observed peak maxima are determined using the normal scan mode on the ▲spectrometer.▲ (USP 1-Dec-2019) The peak maxima for a 4% (m/v) solution of holmium oxide in perchloric acid at 1.0-nm SBW and a path length of 1 cm are shown in ▲*Table 3*▲ (USP 1-Dec-2019).

▲Table 3.▲ (USP 1-Dec-2019) Recommended Peak Maxima from a 4% Solution of Holmium Oxide in Perchloric Acid for Wavelength Calibration Purposes

nm
241.1
249.9
278.1
287.2
333.5
345.4
361.3
385.6
416.3
451.4
467.8
485.2
536.6
640.5

▲[NOTE—Select the nearest wavelength(s) spanning the operational range required.]▲ (USP 1-Dec-2019)

If the operational range of the ▲spectrometer▲ (USP 1-Dec-2019) lies outside the range 240–650 nm, other certified rare earth oxides or other solutions can be used if they are traceable to a national or international standard.

[3] ▲▲ (USP 1-Dec-2019) Travis JC, Acosta JC, Andor G, Bastie J, Blattner P, Chunnilall CJ, et al. Intrinsic wavelength standard absorption bands in holmium oxide solution for UV/visible molecular absorption spectrophotometry. *J Phys Chem Ref Data.* 2005;34(1):41–57. ▲▲ (USP 1-Dec-2019)

▲[4] CRMs produced under appropriate control, which may be demonstrated by accreditation to ISO/EC 17025 and/or ISO Guide 34 (ISO 17034).▲ (USP 1-Dec-2019)

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▲Cerium is available as a traceable solution CRM in acidic media, e.g., in sulfuric acid. It has useful peak characteristics in the 200–300 nm region at approximately 201.1, 211.4, 222.6, 240.4, and 253.7 nm.▲ (USP 1-Dec-2019)

Didymium (a mixture of neodymium and praseodymium) is available as a traceable ▲CRM▲ (USP 1-Dec-2019) both in solution and as a glass. Didymium is similar in preparation to the holmium materials and has useful peak characteristics in the 730–870-nm region, ▲which may vary from melt to melt of the glass.▲ (USP 1-Dec-2019) Useful peaks are found in the didymium solution at approximately 731.6, 740.0, 794.1, ▲800.0,▲ (USP 1-Dec-2019) and 864.4 nm.

RARE EARTH GLASSES

This procedure uses glasses manufactured by fusing the appropriate rare earth oxide in a base glass matrix. The most frequently used is holmium, for which the reference wavelengths have been well defined. Although manufacturing can cause batch variation in these glasses, traceable CRMs are commercially available and can be used. Typical values for a holmium glass using a 1.0-nm SBW are the following: 241.5, 279.2, 287.5, 333.8, 360.9, 418.8, 445.8, 453.7, 460.2, 536.5, and 637.7 nm. ▲▲ (USP 1-Dec-2019)

Control of Absorbance

To establish the transmittance accuracy, precision, and linearity of a given system, it is necessary to verify the absorbance accuracy of a system over its intended operational range by ▲selection and use of▲ (USP 1-Dec-2019) the following procedures as appropriate for the wavelength and absorbance ranges required.

▲The control of absorbance OQ must include at least one assessment at each wavelength range in the 0–2.00 *A* region. If absorbance >2.00 *A* is to be used for quantitation, then control of absorbance must also be evaluated in the >2.00 *A* range.

Establishment of Acceptance Limits (Absorbance)—Choice of Standards

For all *Control of Absorbance* procedures, acceptance limits for adequate calibration are established by adding the expanded uncertainty of the CRM to the instrument vendor specification at the wavelength(s) and absorbance levels required in a linear manner, and these values must lie within the values specified in *Table 4*.

[NOTE—CRMs, traceable to NIST Standard Reference Materials (SRMs), or other international or national standards, are commercially available and should be used where possible.]

Control of Photometric Response (Linearity)

Verification of photometric response (linearity) is required, and it should be evaluated using a standard type appropriate for the wavelength(s) required, where at least three different absorbance levels appropriate to and spanning the required operational range are measured. The options and acceptance criteria are summarized in *Table 4*.

[NOTE—Given the above requirement, it is not necessary to calculate the correlation coefficient of the standard response to demonstrate linearity; just demonstrate that at the different absorbance levels selected the acceptance limits have been met.]

Table 4. Available CRMs for the Control of Absorbance

Absorbance Range	Standard	UV (200–400 nm)	Vis (400–780 nm)
0–1 *A*	Certified nicotinic acid solution	6–24 mg/L Evaluate at 213 and 261 nm. Accuracy ±0.010 A_i Precision <0.005 A_i	—
0–1 *A*	Certified potassium dichromate ($K_2Cr_2O_7$) solution	20–60 mg/L Evaluate at 235, 257, 313, and 350 nm. Accuracy ±0.010 A_i Precision <0.005 A_i	600 mg/L Evaluate at 430 nm. Accuracy ±0.010 A_i Precision <0.005 A_i
0–1 *A*	Certified neutral density glass filters	—	Evaluate at 440, 465, 546.1, 590, and 635 nm. Accuracy ±0.008 A_i Precision <0.005 A_i
0–1 *A*	Certified metal-on-fused-silica filters	Evaluate at 250, 280, 340, 360, and 400 nm. Accuracy ±0.010 A_i Precision <0.005 A_i	Evaluate at 465, 500, 546.1, 590, and 635 nm. Accuracy ±0.008 A_i Precision <0.005 A_i
1–3 *A*	Certified potassium dichromate ($K_2Cr_2O_7$) solution	80–200 mg/L Evaluate at 235, 257, 313, and 350 nm. Accuracy ±1% Precision <0.5%	—

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Table 4. Available CRMs for the Control of Absorbance *(continued)*

Absorbance Range	Standard	UV (200–400 nm)	Vis (400–780 nm)
1–3 *A*	Certified neutral density glass filters	—	Evaluate at 440, 465, 546.1, 590, and 635 nm. Accuracy ±0.8% Precision <0.5%
UV Photometric linearity	Certified nicotinic acid solutions	All concentrations meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified potassium dichromate ($K_2Cr_2O_7$) solutions	All concentrations meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified metal-on-fused-silica filters	At least three certified filters evaluated at 250, 280, 340, 360, or 400 nm. All filters meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified neutral-density glass filters	—	At least three certified filters evaluated. All filters meet accuracy of absorbance acceptance criteria.

[NOTE—Select the appropriate absorbance CRMs spanning the operational range(s) required.]

ACIDIC NICOTINIC ACID SOLUTIONS IN 0.1 N HYDROCHLORIC ACID

In the 0–24 mg/L range, nicotinic acid solutions provide reference values of up to 1.0 *A* at one of the certified values of 213 or 261 nm. These solutions are available as CRMs, in the 0–60 mg/L range, providing reference values up to 2.5 *A*. Using nicotinic acid solutions, the absorbance accuracy must be ± 0.01 A_λ.

The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. These sets of measurements should be performed, i.e., repeated at both the upper and lower absorbances values of the operational range. In each case, absorbance deviations from the mean must not exceed ± 0.005 A_λ (for values below 1.0 A_λ). ▲(USP 1-Dec-2019)

ACIDIC POTASSIUM DICHROMATE SOLUTIONS IN 0.001 M PERCHLORIC ACID

In the 0–200 mg/L range, potassium dichromate solutions provide reference values of up to ▲3.0▲(USP 1-Dec-2019) absorbance units at one of the certified values of 235, 257, 313, or 350 nm. These solutions are available as CRMs or can be prepared according to NIST from ▲appropriate available SRMs, e.g., SRM 935*x*, or SRM 136*x* (where *x* can be any letter from "a" to "z").▲(USP 1-Dec-2019) Using potassium dichromate solutions, the absorbance accuracy must be ±1% ▲A_λ▲(USP 1-Dec-2019) (for values above ▲1.00 A_λ)▲(USP 1-Dec-2019) or ±▲0.010 A_λ▲(USP 1-Dec-2019) (for values below 1.00 ▲A_λ).

The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. These sets of measurements should be performed, i.e., repeated at both the upper and lower absorbance values of the operational range. In each case, absorbance deviations from the mean must not exceed ±0.5% A_λ (for values above 1.0 A_λ) or ±0.005 A_λ (for values below 1.0 A_λ).▲(USP 1-Dec-2019)

NEUTRAL-DENSITY GLASS FILTERS

These gray glass filters are manufactured from doped glass and have a nominally flat spectrum in the region of the calibration wavelengths. They provide reference values of up to ▲3.5▲(USP 1-Dec-2019) absorbance units at the certified values of 440, 465, 546.1, 590, and 635 nm. These filters are available as CRMs that are traceable to NIST SRM 930e, 1930, and 2930. Other certified standard solutions or optical filters can be used if they are traceable to a national or international standard. Using gray glass filters, the absorbance accuracy must be ±0.8% ▲A_λ (for values above 1.00 A_λ) or ±0.008 A_λ (for values below 1.00 A_λ). The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. The absorbance deviation from the mean must not exceed ±0.5% *A* (for values above 1.0 *A*) or ±0.005 A_λ (for values below 1.0 *A*).

Metal-on-Fused-Silica Filters

These neutral density filters are manufactured by deposition of a metallic film on a silica substrate and have a substantially flat spectrum in the region of the calibration wavelengths. They provide reference values of up to 2 *A* at the usually certified values of 250, 280, 340, 360, and 400 nm in the UV range, and reference values of 465, 500, 546.1, 590, and 635 nm in the visible range. These filters are available as CRMs that are traceable to NIST SRM 2031*x* (where *x* = "a" to "z"), or to other national or international standards. In the UV range, the absorbance accuracy must be ±1% A_λ (for values above 1.00 A_λ) or ±0.01 A_λ (for values below 1.00 A_λ). In the visible range, the absorbance accuracy must be ±0.8% A_λ (for values above 1.00 A_λ) or ±0.008 A_λ (for values below 1.00 A_λ). The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements at the upper and lower absorbance values of the operational range. The standard deviation from the mean must not exceed ±0.5% A_λ (for values above 1.00 A_λ) or ±0.005 A_λ (for values below 1.00 A_λ) in both the UV and visible ranges.

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Estimation of the ▲(USP 1-Dec-2019) Limit of Stray Light (Stray Radiant Energy)

Although the measurement of absorbance or transmittance is a ratio measurement of intensities and therefore theoretically is independent of monochromatic source intensity, practical measurements are affected by the presence of unwanted radiation called "stray radiant energy" or "stray light". In addition, the adverse effect of stray light increases with aging of optical components and lamps in a ▲spectrometer.▲(USP 1-Dec-2019) The effects are greater at the extremes of detector and lamp operational ranges. ▲The limit of stray light OQ must include evaluation at one or more UV wavelengths, by selection of appropriate material(s) shown in *Table 5* to span the UV operational range required. In the visible region, i.e., above 400 nm, stray light does not need to be evaluated.

[NOTE—Published stray light specifications for a given spectrometer must be verified in the OQ.]

Analysts must choose and use the appropriate reference(s) to monitor the level of stray light as part of PQ. Stray light can be detected at a given wavelength with a suitable liquid filter by either of the two procedures (*A* or *B*) detailed below.

[NOTE—If measurements are being performed in the 250–330 nm region, on a spectrometer using individual sources for the UV and visible regions of the spectrum, then an additional PQ verification using acetone should be performed.▲(USP 1-Dec-2019)]

These solutions are available as CRMs or can be prepared at the concentrations shown in ▲*Table 5*▲(USP 1-Dec-2019) by using reagent-grade materials.

▲Table 5.▲(USP 1-Dec-2019) Spectral Ranges of Selected Materials for Monitoring Stray Light

▲Recommended Wavelength (nm)▲(USP 1-Dec-2019)	Spectral Range (nm)	Liquid or Solution
▲198▲(USP 1-Dec-2019)	190–▲210▲(USP 1-Dec-2019)	Aqueous potassium chloride (12 g/L)
▲220▲(USP 1-Dec-2019)	210–▲270▲(USP 1-Dec-2019)	Aqueous sodium iodide or potassium iodide (10 g/L)
▲300▲(USP 1-Dec-2019)	250–▲330▲(USP 1-Dec-2019)	Acetone
▲340▲(USP 1-Dec-2019)	300–▲400▲(USP 1-Dec-2019)	Aqueous sodium nitrite (50 g/L)

▲Procedure A

The aim of this procedure is to produce the differential spectrum resulting from the subtraction of a spectrum produced by a 5-mm path length cell from that of a 10-mm cell, both filled with the same filter solution. This spectrum will contain a peak maximum absorbance value, and analysts can calculate the stray light value from the observed maximum absorbance using the formula:

$$s_\lambda = 0.25 \times 10^{-2A_\lambda}$$

s_λ = stray light value, in transmittance (T)
A_λ = observed maximum absorbance

This procedure requires the 10-mm cell measurement to be subtracted from the 5-mm cell, both filled with the same cut-off solution filter. This measurement can be achieved by either using the physical capabilities of the spectrometer, i.e., using the double-beam capability, with the 5-mm cell as the reference, or by mathematically subtracting the spectra of the 10-mm cell from the 5-mm cell, chronologically produced by sequential measurement of both the 5- and 10-mm cells, using the single-sample beam, with the spectrometer referenced to air (blank holder).

ACCEPTANCE CRITERIA: $s_\lambda \le 0.01$ or $A_\lambda \ge 0.7\ A$

Procedure B

Analysts can measure the absorbance of the cut-off solution filters specified in *Table 5* against a 10-mm cell filled with an appropriate reference, and record the maximum absorbance value (A_{max}) or the minimum % Transmittance ($\%T_{min}$) at the recommended wavelength listed in *Table 5*.

ACCEPTANCE CRITERIA: $A_{max} \ge 2.0\ A$ or $\%T_{min} < 1\%T$

Control of ▲(USP 1-Dec-2019) Resolution

If accurate absorbance measurements must be made on benzenoid compounds or other compounds with sharp absorption bands (natural half-bandwidths of <15 nm), the SBW of the ▲spectrometer▲(USP 1-Dec-2019) used should not be greater than 1/8th the natural half-bandwidth of the compound's absorption; ▲i.e., this equates to a spectrometer with a SBW of 2 nm or less.

Determine the resolution of the spectrometer in the UV region by using the following procedure. Using UV-grade *n*-hexane as the reference, measure the absorbance of a 0.020% (v/v) solution of toluene in UV-grade *n*-hexane at the maximum, about 269 nm, and minimum, about 266 nm. The ratio of the absorbance at the maximum to the absorbance at the minimum will typically fall in the 1.0–2.6 range.

For most compendial quantitative purposes, a SBW of 2 nm or less is sufficient, and the *Acceptance criteria* for the ratio is NLT 1.3.▲(USP 1-Dec-2019)

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Performance Qualification

The purpose of PQ is to determine that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement and to ensure that it will function properly over extended periods of time.

Change to read:

PROCEDURE

With few exceptions, compendial ▲spectrometric▲ (USP 1-Dec-2019) tests and assays call for comparison against a USP Reference Standard. This helps ensure measurement under identical conditions for the test specimen and the reference substance. These conditions could include wavelength setting, SBW selection, cell placement and correction, and transmittance levels. Cells that exhibit identical transmittance at a given wavelength may differ considerably in transmittance at other wavelengths. Appropriate cell corrections should be established and used where required.

Comparisons of a test specimen with a reference standard are best made at a peak of spectral absorption for the compound concerned. Assays that prescribe ▲spectrometry▲ (USP 1-Dec-2019) give the commonly accepted wavelength for peak spectral absorption of the substance in question. Different ▲spectrometers▲ (USP 1-Dec-2019) may show minor variation in the apparent wavelength of this peak. Good practice demands that comparisons be made at the wavelength at which peak absorption occurs. Should this differ by >±1 nm (in the range 200–400 nm) or ±2 nm (in the range 400–800 nm) from the wavelength specified in the individual monograph, recalibration of the instrument may be indicated.

The expressions "similar preparation" and "similar solution" as used in tests and assays involving ▲spectrometry▲ (USP 1-Dec-2019) indicate that the reference comparator, generally a USP Reference Standard, should be prepared and observed in an identical manner for all practical purposes to that used for the test specimen. Usually when analysts make up the solution of the specified reference standard, they prepare a solution of about (i.e., within 10%) of the desired concentration, and they calculate the absorptivity on the basis of the exact amount weighed out. If a previously dried specimen of the reference standard has not been used, the absorptivity is calculated on the anhydrous basis. The expressions "concomitantly determine" and "concomitantly measure" as used in tests and assays involving ▲spectrometry▲ (USP 1-Dec-2019) indicate that the absorbances of both the solution containing the test specimen and the solution containing the reference specimen, relative to the specified test blank, must be measured in immediate succession.

Sample Solution Preparation

For determinations using UV or visible ▲spectrometry,▲ (USP 1-Dec-2019) the specimen generally is dissolved in a solvent. Unless otherwise directed in the monograph, analysts make determinations at room temperature using a path length of 1 cm. Many solvents are suitable for these ranges, including water, alcohols, lower hydrocarbons, ethers, and dilute solutions of strong acids and alkalis. Precautions should be taken to use solvents that are free from contaminants that absorb in the spectral region under examination. For the solvent, analysts typically should use water-free methanol or alcohol or alcohol denatured by the addition of methanol but without benzene or other interfering impurities. Solvents of special ▲spectrometric▲ (USP 1-Dec-2019) quality, guaranteed to be free from contaminants, are available commercially from several sources. Some other analytical reagent-grade organic solvents may contain traces of impurities that absorb strongly in the UV region. New lots of these solvents should be checked for their transparency, and analysts should take care to use the same lot of solvent for preparation of the test solution, the standard solution, and the blank. The best practice is to use solvents that have NLT 40% transmittance (39.9% $T = 0.399$ A) at the wavelength of interest.

Assays in the visible region usually call for concomitantly comparing the absorbance produced by the assay preparation with that produced by a standard preparation containing approximately an equal quantity of a USP Reference Standard. In some situations, analysts can omit the use of a reference standard (e.g., when ▲spectrometric▲ (USP 1-Dec-2019) assays are made with routine frequency) when a suitable standard curve is available and is prepared with the appropriate USP Reference Standard, and when the substance assayed conforms to the Beer–Lambert law within the range of about 75%–125% of the final concentration used in the assay. Under these circumstances, the absorbance found in the assay may be interpolated on the standard curve, and the assay result can be calculated. Such standard curves should be confirmed frequently and always when a new ▲spectrometer▲ (USP 1-Dec-2019) or new lots of reagents are put into use.

Change to read:

▲VERIFICATION AND VALIDATION

Verification

Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* are not required to validate these procedures if provided in a monograph. Instead, they simply must verify their suitability under actual conditions of use.

The objective of a UV-Vis procedure verification is to demonstrate the suitability of a test procedure under actual conditions of use. Performance characteristics that verify the suitability of a UV-Vis procedure are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in *Verification of Compendial Procedures* ⟨1226⟩. Verification is usually performed using a reference material and a well-defined matrix. Verification of compendial UV-Vis procedures includes

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at minimum the execution of the validation parameters for specificity, accuracy, precision, and quantitation limit, when appropriate, as indicated in *Validation*. ▲ (USP 1-Dec-2019)

Validation

Validation is required when a UV-Vis method is intended for use as an alternative to the official procedure for testing an official article ▲or when no official procedure exists in the current *USP–NF*. ▲ (USP 1-Dec-2019)

The objective of UV-Vis method validation is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). ▲For dissolution procedures, see *The Dissolution Procedure: Development and Validation* ⟨1092⟩. ▲ (USP 1-Dec-2019)Depending on the category of the test (see *Validation of Compendial Procedures* ⟨1225⟩, *Table 2*), the analytical method validation process for UV-Vis requires testing for linearity, range, accuracy, specificity, precision, detection limit, ▲or▲ (USP 1-Dec-2019) quantitation limit. ▲▲ (USP 1-Dec-2019) These analytical performance characteristics apply to externally standardized procedures and those that use standard additions.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I, II, and III procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. Analysts also can compare assay results obtained using the UV-Vis procedure under validation to those from an established analytical procedure.

Validation criteria: 98.0%–102.0% mean recovery for the drug substances, 95.0%–105.0% mean recovery for the drug product assay, and 80.0%–120.0% mean recovery for the impurity analysis. These criteria are met throughout the intended range.

Precision

REPEATABILITY

The repeatability of the analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration. Alternatively, it can be assessed by measuring the concentrations of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for the drug substance ▲assay,▲ (USP 1-Dec-2019) NMT 2.0% for the drug product assay, and NMT ▲15.0%–▲ (USP 1-Dec-2019)20.0% for the impurity analysis.

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the method must be established. Typical variables include performing the analysis on different days, using different instrumentation, and/or having the method performed by two or more analysts. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.5% for the drug substance ▲assay,▲ (USP 1-Dec-2019) NMT 3.0% for the drug product assay, and NMT ▲15.0%–▲ (USP 1-Dec-2019)25.0% for the impurity analysis.

SPECIFICITY

In UV-Vis measurements, specificity is ensured by the use of a reference standard wherever possible and is demonstrated by the lack of interference from other components present in the matrix.

DETECTION LIMIT

The detection limit (DL) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank solution and multiplying by 3.3. Alternatively, the standard deviation can be determined from the error of the intercept from a calibration curve or by determining that the signal-to-noise ratio is >3.3. The estimated DL must be confirmed by analyzing samples at the calculated concentration.

QUANTITATION LIMIT

The quantitation limit (QL) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank solution and multiplying by 10. Alternatively, the standard deviation can be determined from the error of the intercept from a calibration curve or by determining that the signal-to-noise ratio is >10.

Measurement of a test solution prepared from a representative sample matrix spiked at the required QL concentration must be performed to confirm sufficient sensitivity and adequate precision. The observed signal-to-noise ratio at the required QL

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should be >10. [NOTE—A suitable procedure for measuring the signal-to-noise ratio is given in ASTM 1657-98 ▲(2011)▲ (USP 1-Dec-2019) *Standard Practice for the Testing of Variable-Wavelength Photometric Detectors Used in Liquid Chromatography*.]
Validation criteria: For the estimated limit of quantitation to be considered valid, the measured concentration must be accurate and precise at a level ≤50% of the specification.

LINEARITY

A linear relationship between the analyte concentration and UV-Vis response must be demonstrated by preparation of NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods such as a least-squares regression. Deviation from linearity results from either instrumental or sample factors, or both, and can be reduced to acceptable levels by reducing the analyte concentration and thereby the associated absorbance values.

▲The (Pearson) correlation coefficient, r, measures the strength and direction of the association between two variables (x and y), in this instance, concentration and absorbance.

The coefficient of determination, r^2, is a measure of the fraction of the data's variation that is adequately modeled and not a measure of linearity. Linearity depends on the standard error of the calibration equation (and hence the reference procedure) and on the range of the calibration data. Thus, although values very near 1.00, such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability.▲ (USP 1-Dec-2019)
Validation criteria: The ▲coefficient of determination, r^2,▲ (USP 1-Dec-2019) must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests. ▲Visual inspection of the residual plots should not reveal any significant pattern.▲ (USP 1-Dec-2019)

RANGE

The operational range of an analytical instrument (and the analytical procedure as a whole) is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the instrumental response function has a suitable level of precision, accuracy, and linearity.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For non-centered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

▲This parameter is evaluated during method development.▲ (USP 1-Dec-2019)
The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. For UV-Vis, this can include measuring the stability of the analyte under specified storage conditions, varying pH, and adding possible interfering species, to list a few examples. Robustness is determined concurrently using a suitable design for the experimental procedure.

INDIRECT MEASUREMENT REQUIREMENTS

For certain UV-Vis procedures, chromogenic reactions are employed. Generally, the requirements for the analytical performance characteristics are used. In some instances, the required accuracy and precision criteria for the direct measurements may not be achievable. Under these circumstances, the accuracy and precision requirements can be widened by as much as 50%. Any such widening must be justified on scientific grounds and with documented evidence. It may be necessary to increase the amount of replication required to produce a scientifically sound reportable value.
▲▲ (USP 1-Dec-2019)

⟨861⟩ SUTURES—DIAMETER

Change to read:
▲Diameters of surgical sutures can be determined by either contact or noncontact methods.

CONTACT METHODS

The gauge for determining suture diameter shall be mechanical or electrical, equipped with a direct-reading dial, a digital readout, or a printed readout. The gauge shall have a minimum accuracy of 0.002 mm, an anvil 50 ± 5 mm in diameter, and a flat contact point (presser foot) 12.70 ± 0.02 mm in diameter. The flat contact point and anvil surfaces shall be plane to within 0.005 mm and parallel to each other to within 0.005 mm. For suture sizes 7-0 and larger, the presser foot and moving parts attached to it shall apply a force of 210 ± 3 g to the specimen. For sutures of size 8-0 and smaller, the presser foot and moving parts attached to it shall apply a force of 60 ± 3 g to the specimen.
Measure sutures, whether packaged in dry or fluid form, immediately after removal from the container and without prior drying or conditioning. Do not stretch the suture before measuring.

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MONOFILAMENT SUTURES (ABSORBABLE AND NONABSORBABLE)

For monofilament sutures, lay the strand across the center of the anvil (see *Figure 1*). Apply tension to the suture NMT required to straighten the suture. Measure the diameter of the suture by slowly lowering the presser foot to avoid crushing the suture. Measure the suture diameter at 3 locations corresponding to approximately one-fourth, one-half, and three-fourths of its labeled length. Each location is considered an individual measurement.

Repeat the test on a total of 10 suture strands. Compare each individual measurement to the individual specifications in the appropriate monograph. Compare the average of the 30 individual measurements to the average specification in the appropriate monograph.

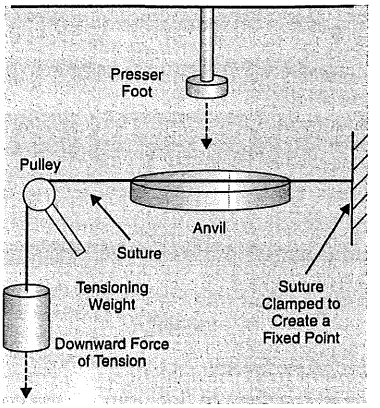


Figure 1. Equipment Setup.

MULTIFILAMENT SUTURES (ABSORBABLE AND NONABSORBABLE)

Diameter Measurement Strategy

Refer to *Table 1* for the diameter measurement strategy for various suture lengths.

Table 1. Diameter Measurement Strategy

Condition	Suture Length as Noted on Package (inches)	Number of Data Points/Strand	Number of Suture Strands	Sample Type
1	≤4	1	30	Use a noncontact method
2	4 to ≤12	1	30	Strand
3	>12 to ≤48	3	10	Strand
4	>48	3	10	Random subsection

For Condition 1, where the suture length is less than 4 inches during diameter testing, use a noncontact method. For Condition 2, measure the suture diameter at a single location on 30 strands for a total of 30 individual diameter measurements. For Condition 3, measure the suture diameter at 3 locations corresponding to approximately one-fourth, one-half, and three-fourths of the labeled length. Measure 10 strands for a total of 30 individual diameter measurements. For Condition 4, measure the suture diameter at 3 locations corresponding to approximately one-fourth, one-half, and three-fourths on a random subsection NLT 48 inches in length from the total suture product. Measure 10 strands for a total of 30 individual diameter measurements.

Preparation

Mount the suture on a tensioning board by clamping one end of the suture so that the strand lies across the center of the anvil in the same plane as the surface of the anvil and routing the free end of the suture to be tensioned, such as by passing the suture around a cylinder or a pulley and attaching a weight to the free end, ensuring the strand does not twist or untwist. Apply a load of about one-half of the knotted tensile strength for the nonsterilized Class I suture as given in *Table 2*. Class III sutures do not require tension.

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Measurement

Measure the diameter of the suture by slowly lowering the presser foot to avoid crushing the suture. Measure the suture diameter as specified in *Diameter Measurement Strategy*.

For suture sizes 2-0 and larger: Take 2 measurements at right angles to each other at each location. Average the 2 measurements taken at each location and record as the observed diameter at that location for comparison to the individual specifications. For suture sizes 3-0 and smaller, take a single measurement at each location for comparison to the individual specifications. Each location is considered an individual measurement. The average diameter of the samples are within the limits on average diameter (for all 30 measurements) per the monograph for the size stated on the label. None of the observed individual measurements should be less than or greater than the limits on individual diameter in the monograph. None of the individual observed measurements should be less or greater than the tolerances in the monograph.

Data Analysis

Compare each individual measurement to the individual specifications in the appropriate monograph. Compare the average of the 30 individual measurements to the average specification in the appropriate monograph.

NONCONTACT METHODS

The laser micrometer (optical micrometer) for determining suture diameter shall be equipped with a digital or printed readout. The laser micrometer shall have a minimum accuracy of 0.002 mm, and be capable of dual axis measurements. The test method should be correlated with the contact method and the appropriate specifications should be established based on the correlation.

Place the suture without any preconditioning into the path of the laser beam, ensuring the suture is appropriately aligned. For a monofilament suture, hold the suture with minimal applied tension. For a multifilament suture, apply a tensile load of about one-half of the knotted tensile strength (see *Tensile Strength* ⟨881⟩) for the nonsterilized Class I suture of the given size. Measure each suture at 3 points corresponding roughly to one-fourth, one-half, and three-fourths of its length.

TENSIONS

Tensions for both contact and noncontact methods are calculated to represent approximately one-half of the knot-pull limit for nonsterile Class I sutures of the designated size in *Table 2*.

Table 2. Tensioning Weights (in grams) for Measuring Multifilament Strand Diameters

Size	Nonabsorbable	Absorbable
12-0	0.6	N/A
11-0	3.8	N/A
10-0	12	12
9-0	27	25
8-0	38	35
7-0	69	70
6-0	125	125
5-0	250	340
4-0	375	475
3-0	600	887
2-0	899	1341
0	1351	1948
1	1702	2539
2	2199	3176
3 and 4	3046	3645
5	3849	N/A
6	4550	N/A
7	5647	N/A▲ (USP 1-Dec-2019)

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⟨871⟩ SUTURES—NEEDLE ATTACHMENT

Change to read:

Absorbable ▲▲ (USP 1-Dec-2019) and nonabsorbable surgical sutures with standard needle attachment are such that the needles are firmly attached and are not intended to be separated. Sutures supplied with eyeless needles attached fall into either the category of standard needle attachment or the category of removable needle attachment. Removable needle attachment of both absorbable and nonabsorbable surgical sutures is such that the needle may be deliberately separated from the suture by means of a quick tug. Both types of attachments are tested on equipment as specified in *Tensile Strength* ⟨881⟩.

Change to read:

PROCEDURE

Clamp each of 5 sutures in the ▲tensile testing device▲ (USP 1-Dec-2019) so that the needle is in the fixed clamp with all of the swaged portion exposed and in line with the direction of force applied to the suture by the moving clamp. Determine the force required to detach the suture from the needle. In the case of standard needle attachment, the suture may break without needle detachment.

Standard Needle Attachment

The requirements are met if neither the average of the 5 values nor any individual value is less than the ▲limits▲ (USP 1-Dec-2019) given for the designated size in *Table 1*.

Table 1. Standard Needle Attachment for Absorbable and Nonabsorbable Sutures

Metric Size (gauge no.)		Limits on Needle Attachment				
Absorbable Collagen Suture	Nonabsorbable and Synthetic Absorbable Sutures	USP Size	Average (in kgf) (min.)	Individual (in kgf) (min.)	Average (in N) (min.)	Individual (in N) (min.)
—	0.1	11-0 ▲and smaller▲ (USP 1-Dec-2019)	0.007	0.005	0.069	0.049
—	0.2	10-0	0.014	0.010	0.137	0.098
0.4	0.3	9-0	0.021	0.015	0.206	0.147
0.5	0.4	8-0	0.050	0.025	0.490	0.245
0.7	0.5	7-0	0.080	0.040	▲0.785▲ (USP 1-Dec-2019)	0.392
1	0.7	6-0	0.17	0.08	1.67	0.784
1.5	1	5-0	0.23	0.11	▲2.26▲ (USP 1-Dec-2019)	1.08
2	1.5	4-0	0.45	0.23	4.41	▲2.26▲ (USP 1-Dec-2019)
3	2	3-0	0.68	0.34	6.67	3.33
3.5	3	2-0	1.10	0.45	10.8	4.41
4	3.5	0	1.50	0.45	14.7	4.41
5	4	1	1.80	0.60	▲17.7▲ (USP 1-Dec-2019)	5.88
6 and larger	5 and larger	2 and larger	1.80	0.70	▲17.7▲ (USP 1-Dec-2019)	6.86

Removable Needle Attachment

▲For USP sizes 5-0 through 2-0,▲ (USP 1-Dec-2019) the requirements are met if the individual values of the 5 sutures are ▲NLT 0.028 kgf (0.275 N) and NMT 1.59 kgf (15.6 N).▲ (USP 1-Dec-2019)

[NOTE—For either type of attachment, if NMT one of the individual values falls outside the prescribed limits, repeat the test on an additional 10 sutures. The requirements of the test are met if none of the 10 additional values falls outside the individual limit requirements.]

▲▲ (USP 1-Dec-2019)

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⟨881⟩ TENSILE STRENGTH

Change to read:

▲Testing devices▲ (USP 1-Dec-2019) for measurement of tensile strength used in the United States may be calibrated in the English units of measure. The following directions are given in metric units with the understanding that the corresponding English equivalents may be used.

Change to read:

SURGICAL SUTURE

Determine the tensile strength of surgical suture on a motor-driven tensile strength testing machine having suitable clamps for holding the specimen firmly and using ▲▲ (USP 1-Dec-2019) the principle of constant rate of elongation of specimen, as described below. The apparatus has two clamps for holding the strand. One of these clamps is mobile. The clamps are designed so that the strand being tested can be attached without any possibility of slipping. Gauge length is defined as the interior distance between the two clamps. For gauge lengths of 125–200 mm, the mobile clamp is driven at a constant rate of elongation of 30 ± 5 cm/min. For gauge lengths of less than 125 mm, the rate of elongation per minute is adjusted to equal 2 times the gauge length per minute. For example, a 5-cm gauge length has a rate of elongation of 10 cm/min.

Determine the tensile strength of the suture, whether packaged in dry form or in fluid, promptly after removal from the container, without prior drying or conditioning. ▲Except where straight pull (no knot required) is indicated in the suture monographs, tie the test suture into a simple knot formed by placing one end of a strand held in the right hand over the other end held in the left hand. Then pass one end over the strand and through the loop that is formed (see *Figure 1*), and pull the knot tight so as not to affect the integrity of the suture.

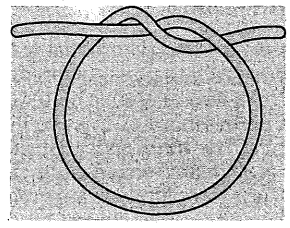


Figure 1. Knot for test suture.

Place the specimen in the testing device with the knot approximately midway between the clamps.▲ (USP 1-Dec-2019) Attach one ▲free▲ (USP 1-Dec-2019) end of the suture to the clamp at the load end of the machine, pass the other ▲free▲ (USP 1-Dec-2019) end through the opposite clamp, applying sufficient tension so that the specimen is taut between the clamps, and engage the second clamp. Perform as many breaks as are specified in the individual monograph. If the break occurs at ▲or in▲ (USP 1-Dec-2019) the clamp, discard ▲and replace with an additional pull with a new test suture. The discarded result should be noted and recorded, but not included in the averaging of the results.

For suture devices that are not knotted, a straight pull should be used for testing. Strength should be compared to the knot tie values for labeled sizes in the monograph table.▲ (USP 1-Dec-2019)

Add the following:

▲BARBED SUTURES

Barbed suture type devices that do not require the use of knots in their application should not be tested for USP knot-pull tensile strength by the use of knots. The alterations to the surface (i.e., barbing) of these devices would lead to testing bias were a USP knot pull be applied to the device. For these types of devices, refer to the manufacturer's specifications with respect to the USP size designation identified and perform straight pull testing accordingly. Compare results generated to the identified USP size knot-pull specifications.▲ (USP 1-Dec-2019)

Change to read:

TEXTILE FABRICS AND FILMS

Determine the tensile strength of textile fabrics, including adhesive tape, on a constant-speed or pendulum type of testing ▲device▲ (USP 1-Dec-2019) of the following general description.

The clamps for holding the specimen are smooth, flat, parallel jaws that are NLT 25 mm in length in the dimension parallel to the direction of application of the load. When the width of the strip being tested does not exceed 19 mm, the jaws of the clamp should be at least 25 mm wide. If the width of the strip is greater than 19 mm and NMT 44 mm, the width of the jaws of the clamp should be at least 50 mm. If the width of the specimen is greater than 44 mm, cut a 25-mm strip, and use a clamp

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with jaws NLT 50 mm wide. Round all edges that might have a cutting action on the specimen to a radius of 0.4 mm. The jaws are 76.2 mm apart at the beginning of the test, and they separate at the rate of 30.5 cm ± 13 mm/min. The machine is of such capacity that when the break occurs, the deviation of the pendulum from the vertical is 9°–45°.

⟨891⟩ THERMAL ANALYSIS

INTRODUCTION

Precisely determined thermodynamic events, such as a change of state, can indicate the identity and purity of drugs. Compendial standards have long been established for the melting or boiling temperatures of substances. These transitions occur at characteristic temperatures, and the compendial standards therefore contribute to the identification of the substances. Because impurities affect these changes in predictable ways, the same compendial standards contribute to the control of the purity of the substances.

Thermal analysis in the broadest sense is the measurement of physical–chemical properties of materials as a function of temperature. Instrumental methods have largely supplanted older methods dependent on visual inspection and measurements under fixed or arbitrary conditions, because they are objective, provide more information, afford permanent records, and are generally more sensitive, precise, and accurate. Furthermore, they may provide information on desolvation, dehydration, decomposition, crystal perfection, polymorphism, melting temperature, sublimation, glass transitions, evaporation, pyrolysis, solid-solid interactions, and purity. Such data are useful in the characterization of substances with respect to compatibility, stability, packaging, and quality control. The measurements used most often in thermal analysis, i.e., transition and melting point temperatures by differential scanning calorimetry (DSC), thermogravimetric analysis, hot-stage microscopy, and eutectic impurity analysis, are described here.

TRANSITION AND MELTING POINT TEMPERATURES

As a specimen is heated, transitions can be observed using differential scanning calorimetry (DSC), differential thermal analysis (DTA), or hot-stage microscopy. In heat-flux DSC, the heat differential between the sample and reference material is determined. In power compensation DSC, the sample and reference materials are maintained at the same temperature, using individual heating elements, and the difference in power input to the two heaters is recorded. DTA monitors the difference in temperatures between the sample and the reference. The transitions that may be observed include those shown in *Table 1* below. In the case of melting, both an "onset" and a "peak" temperature can be determined objectively and reproducibly, often to within a few tenths of a degree. Although these temperatures are useful for characterizing substances, and the difference between the two temperatures is indicative of purity, the values cannot be directly compared to visual "melting-range" or "melting-point" values or with constants such as the triple point of the pure material.

Furthermore, caution should be used when comparing results obtained by different methods of analysis. Optical methods may measure the melting point as the temperature where the last trace of solid coalesces. In contrast, melting points measured by DSC may refer to the onset temperature or the temperature where the maximum melting rate (vertex) was observed. However, the vertex is sensitive to sample weight, heating rate, and other factors, whereas the onset temperature is less affected by these factors. With thermal techniques, it is necessary to consider the limitations of solid solution formation, insolubility in the melt, polymorphism, and decomposition during the analysis.

Table 1

Solid to liquid	Melting	Endothermic
Liquid to gas	Vaporization	Endothermic
Liquid to solid	Freezing	Exothermic
	Crystallization	Exothermic
Solid to gas	Sublimation	Endothermic
Solid to solid	Glass transition	Second order event
	Desolvation	Endothermic
	Amorphous to crystalline	Exothermic
	Polymorphic	Endothermic or Exothermic

Reporting Results of Instrumental Methods

A complete description of the conditions employed should accompany each thermogram, including make and model of instrument; record of last calibration; specimen size and identification (including previous thermal history); container; identity, flow rate, and pressure of gaseous atmosphere; direction and rate of temperature change; and instrument and recorder sensitivity.

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DETERMINATION OF TRANSITION TEMPERATURE (MELT ONSET TEMPERATURE) AND MELTING POINT TEMPERATURE

Apparatus

Use DTA or DSC instrumentation equipped with a temperature-programming device, thermal detector(s), and a recording system that can be connected to a computer, unless otherwise prescribed by the specific monograph for which this chapter is being used.

Calibration

Calibrate instrumentation for temperature and enthalpy changes, using indium or another suitable certified material. Temperature calibration is performed by heating a standard through the melting transition and comparing the extrapolated onset of melting point of the standard to the certified onset of melting point. The temperature calibration should be conducted at the same heating rate as the experiment. Enthalpy calibration is performed by heating a standard through the melting transition and comparing the calculated heat of fusion to the theoretical value.

Procedure

Accurately weigh an appropriate quantity of the substance to be examined in the sample pan, as described in the specific monograph. Set initial temperature, heating rate, direction of temperature change, and final temperature as specified in the monograph. If not specified in the monograph, these parameters are determined as follows: make a preliminary examination over a wide range of temperatures (typically, room temperature to decomposition temperature, or about 10° to 20° above the melting point) and over a wide range of heating rates (1° to 20° per minute) to reveal any unexpected effects. Then determine a lower heating rate such that decomposition is minimized and the transition temperature is not compromised. Determine a temperature range bracketing the transition of interest such that the baseline can be extended to intersect with the tangent of the melt (see *Figure 1*).

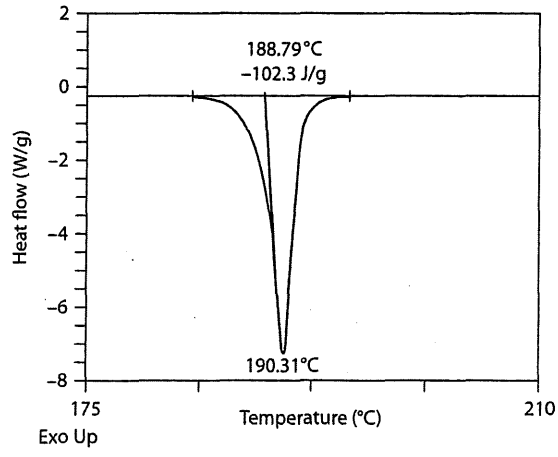


Figure 1. Thermogram.

In examining pure crystalline materials, rates as low as 1° per minute may be appropriate, whereas rates of up to 20° per minute are more appropriate for polymeric and other semicrystalline materials. Begin the analysis, and record the differential thermal analysis curve with the temperature on the *x*-axis and the energy change on the *y*-axis. The melting temperature (melt onset temperature) is the intersection (188.79°) of the extension of the baseline with the tangent at the point of greatest slope (inflexion point) of the curve (see *Figure 1*). The vertex is the temperature at the peak of the curve (190.31°). The enthalpy of the event is proportional to the area under the curve after application of a baseline correction.

THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis involves the determination of the mass of a specimen as a function of temperature, or time of heating, or both. It is often used to investigate dehydration/desolvation processes and compound decomposition. When thermogravimetry is properly applied, it provides more useful information than loss on drying at fixed temperature, often for a fixed time and in what is usually an ill-defined atmosphere. Usually, loss of surface-absorbed solvent can be distinguished from solvent in the crystal lattice and from degradation losses. The measurements can be carried out in atmospheres having controlled humidity and oxygen concentration to reveal interactions with the drug substance, between drug substances, and between active substances and excipients or packaging materials.

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Apparatus

While the details depend on the manufacturer, the essential features of the equipment are a recording balance and a programmable heat source. Equipment differs in the ability to handle specimens of various sizes, the means of sensing specimen temperature, and the range of atmosphere control.

Calibration

Calibration is required with all systems: that is, the mass scale is calibrated by the use of standard weights, and calibration of the temperature scale involves the use of standard materials, because it is assumed that the specimen temperature is the furnace temperature. Weight calibration is conducted by measuring the mass of a certified or standard weight and comparing the measured mass with the certified value. Temperature calibration is performed by analyzing a high-purity magnetic standard such as nickel for its curie temperature and comparing the measured value to the theoretical value.

Procedure

Apply the method to the sample, using the conditions described in the monograph, and calculate the mass gain or loss, expressing the change in mass as percentage. Alternatively, place a suitable quantity of material in the sample holder, and record the mass. Because the test atmosphere is critical, the pressure or flow rate and the composition of the gas are specified. Set the initial temperature, heating rate, and final temperature according to the manufacturer's instructions, and initiate the temperature increase. Alternatively, conduct an examination of the thermogram over a wide range of temperatures (typically, from room temperature to the decomposition temperature, or 10° to 20° above the melting point at a heating rate of 1° to 20° per minute). Calculate the mass gain or loss, expressing the change in mass as percentage.

HOT-STAGE MICROSCOPY

Hot-stage microscopy is an analytical technique that involves monitoring the optical properties of the sample using a microscope as a function of temperature. Hot-stage microscopy may be used as a complementary technique to other thermal analysis techniques such as DSC, DTA, and variable temperature X-ray powder diffraction for the solid-state characterization of pharmaceutical compounds. It is useful to confirm transitions such as melts, recrystallizations, and solid-state transformations using a visual technique. The hot-stage microscope must be calibrated for temperature.

EUTECTIC IMPURITY ANALYSIS

The basis of any calorimetric purity method is the relationship between the melting and freezing point depression and the level of impurity. The melting of a compound is characterized by the absorption of latent heat of fusion, ΔH_f, at a specific temperature, T_o. In theory, a melting transition for an absolutely pure crystalline compound should occur within an infinitely narrow range. A broadening of the melting range, due to impurities, provides a sensitive criterion of purity. The effect is apparent visually by examination of thermograms of specimens differing by a few tenths percent in impurity content. A material that is 99% pure is about 20% molten at 3° below the melting point of the pure material (see *Figure 2*).

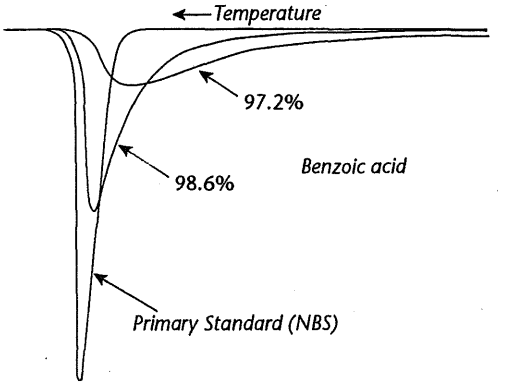


Figure 2. Superimposed thermograms illustrating the effect of impurities on DSC melting peak shape.

The parameters of melting (melting range, ΔH_f, and calculated eutectic purity) are readily obtained from the thermogram of a single melting event using a small test specimen, and the method does not require multiple, precise actual temperature measurements. Thermogram units are directly convertible to heat transfer, millicalories per second.

The lowering of the freezing point in *dilute solutions* by molecules of nearly equal size is expressed by a modified van't Hoff equation:

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$$\frac{dT}{dX_2} = \frac{RT^2}{\Delta H_f} \cdot (K_D - 1) \qquad (1)$$

in which T = absolute temperature in kelvins; X_2 = mole fraction of minor component (solute, impurity); ΔH_f = molar heat of fusion of the major component in Joules per mol: R = gas constant in Joules per mol × kelvins; and K_D = distribution ratio of solute between the solid and liquid phases.

Assuming that the temperature range is small and that no solid solutions are formed ($K_D = 0$).

Integration of the van't Hoff equation yields the following relationship between the mole fraction of impurity and the melting-point depression:

$$X_2 = \frac{(T_o - T_m)\Delta H_f}{RT_o^2} \qquad (2)$$

in which T_o = melting point of the pure compound, in kelvins, and T_m = melting point of the test specimen, in kelvins.

With no solid solution formation, the concentration of impurity in the liquid phase at any temperature during the melting is inversely proportional to the fraction melted at that temperature, and the melting-point depression is directly proportional to the mole fraction of impurity. A plot of the observed test specimen temperature, T_s, versus the reciprocal of the fraction melted, 1/F, at temperature T_s, should yield a straight line with the slope equal to the melting-point depression ($T_o - T_m$). The theoretical melting point of the pure compound is obtained by extrapolation to 1/F = 0:

$$T_s = T_o - \frac{RT_o^2 X_2 (1/F)}{\Delta H_f} \qquad (3)$$

Substituting the experimentally obtained values for $T_o - T_m$, ΔH_f, and T_o in equation (2) yields the mole fraction of the total eutectic impurity, which, when multiplied by 100, gives the mole percentage of total eutectic impurities.

Deviations from the theoretical linear plot also may be due to solid solution formation ($K_D \neq 0$), so that care must be taken in interpreting the data.

To observe the linear effect of the impurity concentration on the melting-point depression, the impurity must be *soluble* in the liquid phase or melt of the compound, but *insoluble* in the solid phase, i.e., no solid solutions are formed. Some chemical similarities are necessary for solubility in the melt. For example, the presence of ionic compounds in neutral organic compounds and the occurrence of thermal decomposition may not be reflected in purity estimates. The extent of these theoretical limitations has been only partially explored.

Impurities present from the synthetic route often are similar to the end product, hence there usually is no problem of solubility in the melt. Impurities consisting of molecules of the same shape, size, and character as those of the major component can fit into the matrix of the major component without disruption of the lattice, forming solid solutions or inclusions; such impurities are not detectable by DSC. Purity estimates are too high in such cases. This is more common with less-ordered crystals as indicated by low heats of fusion.

In addition, the method is reliable when the purity of the major component is greater than 98.5 mol% and the materials are not decomposed during the melting phase.

Impurity levels calculated from thermograms are reproducible and generally reliable within 0.1% for ideal compounds.

Compounds that exist in polymorphic form cannot be used in purity determination unless the compound is completely converted to one form. On the other hand, DSC and DTA are inherently useful for detecting, and therefore monitoring, polymorphism.

Procedure

The actual procedure and the calculations to be employed for eutectic impurity analysis are dependent on the particular instrument used. Consult the manufacturer's literature and/or the thermal analysis literature for the most appropriate technique for a given instrument. In any event, it is imperative to keep in mind the limitations of solid solution formation, insolubility in the melt, polymorphism, and decomposition during the analysis.

⟨905⟩ UNIFORMITY OF DOSAGE UNITS

This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and the *Japanese Pharmacopoeia*. Portions of the general chapter text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•) to specify this fact.

[NOTE—In this chapter, *unit* and *dosage unit* are synonymous.•]

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit. The uniformity of dosage units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The term "uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified elsewhere in this Pharmacopeia.

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The uniformity of dosage units can be demonstrated by either of two methods, *Content Uniformity* or •*Weight*•*Variation* (see *Table 1*). The test for *Content Uniformity* of preparations presented in dosage units is based on the assay of the individual content of drug substance(s) in a number of dosage units to determine whether the individual content is within the limits set. The *Content Uniformity* method may be applied in all cases.

The test for •*Weight*•*Variation* is applicable for the following dosage forms:

(W1)	Solutions enclosed in unit-dose containers and into soft capsules;
(W2)	Solids (including powders, granules, and sterile solids) that are packaged in single-unit containers and contain no active or inactive added substances;
(W3)	Solids (including sterile solids) that are packaged in single-unit containers, with or without active or inactive added substances, that have been prepared from true solutions and freeze-dried in the final containers and are labeled to indicate this method of preparation; and
(W4)	Hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting the requirements for *Content Uniformity*.

The test for *Content Uniformity* is required for all dosage forms not meeting the above conditions for the •*Weight*•*Variation* test.[1]

Table 1. Application of Content Uniformity (CU) and Weight Variation (WV) Tests for Dosage Forms

Dosage Form	Type	Subtype	Dose & Ratio of Drug Substance	
			≥25 mg and ≥25%	<25 mg or <25%
Tablets	Uncoated		WV	CU
	Coated	Film	WV	CU
		Others	CU	CU
Capsules	Hard		WV	CU
	Soft	Suspension, emulsion, or gel	CU	CU
		Solutions	WV	WV
Solids in single-unit containers	Single component		WV	WV
	Multiple components	Solution freeze-dried in final container	WV	WV
		Others	CU	CU
Solutions in unit-dose containers •and into soft capsules•			WV	WV
Others			CU	CU

CONTENT UNIFORMITY

Select not fewer than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the preparation and for the *Content Uniformity* test, it may be necessary to establish a correction factor to be applied to the results of the latter.

Solid Dosage Forms

Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see *Table 2*).

Liquid or Semi-Solid Dosage Forms

Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use, and express the results as delivered dose. Calculate the acceptance value (see *Table 2*).

[1] •European Pharmacopoeia and Japanese Pharmacopoeia text not accepted by the United States Pharmacopeia: Alternatively, products listed in item (4) above that do not meet the 25 mg/25% threshold limit may be tested for uniformity of dosage units by Mass Variation instead of the Content Uniformity test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in *Table 2*.•

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Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks$$

in which the terms are as defined in *Table 2*.

Table 2

Variable	Definition	Conditions	Value
$\bar{X}$	Mean of individual contents (χ_1, χ_2, …, χ_n), expressed as a percentage of the label claim		
χ_1, χ_2, …, χ_n	Individual contents of the units tested, expressed as a percentage of the label claim		
n	Sample size (number of units in a sample)		
k	Acceptability constant	If n = 10, then k =	2.4
		If n = 30, then k =	2.0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}(\chi_i - \bar{X})^2}{n-1}\right]^{\frac{1}{2}}$
RSD	Relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$100s/\bar{X}$
M (case 1) to be applied when T ≤101.5	Reference value	If 98.5% ≤$\bar{X}$ ≤101.5%, then	M = $\bar{X}$ (AV = ks)
		If $\bar{X}$ <98.5%, then	M = 98.5% (AV = 98.5 − $\bar{X}$ + ks)
		If $\bar{X}$ >101.5%, then	M = 101.5% (AV = $\bar{X}$ − 101.5 + ks)
M (case 2) to be applied when T >101.5	Reference value	If 98.5 ≤$\bar{X}$ ≤T, then	M = $\bar{X}$ (AV = ks)
		If $\bar{X}$ <98.5%, then	M = 98.5% (AV = 98.5 − $\bar{X}$ + ks)
		If $\bar{X}$ >T, then	M = T% (AV = $\bar{X}$ − T + ks)
Acceptance value (AV)			General formula: $\|M - \bar{X}\| + ks$ (Calculations are specified above for the different cases.)
L1	Maximum allowed acceptance value		L1 = 15.0 unless otherwise specified
L2	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than [1−(0.01)(L2)]M, while on the high side, no dosage unit result can be greater than [1 + (0.01)(L2)]M. (This is based on an L2 value of 25.0.)	L2 = 25.0 unless otherwise specified
T	Target content per dosage unit at the time of manufacture, expressed as a percentage of the label claim. Unless otherwise stated, T is 100.0%, or T is the manufacturer's approved target content per dosage unit.		

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·WEIGHT, VARIATION

Carry out an assay for the drug substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as percentage of label claim (see *Calculation of Acceptance Value*). Assume that the concentration (weight of drug substance per weight of dosage unit) is uniform. Select not fewer than 30 dosage units, and proceed as follows for the dosage form designated.

Uncoated or Film-Coated Tablets

Accurately weigh 10 tablets individually. Calculate the content, expressed as percentage of label claim, of each tablet from the ·weight, of the individual tablet and the result of the *Assay*. Calculate the acceptance value.

Hard Capsules

Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by a suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net ·weight, of its contents by subtracting the ·weight, of the shell from the respective gross ·weight,. Calculate the drug substance content of each capsule from the ·net weight, of the individual capsule ·content, and the result of the *Assay*. Calculate the acceptance value.

Soft Capsules

Accurately weigh 10 intact capsules individually to obtain their gross ·weights,, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 minutes, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the ·weight, of product removed from the individual capsules and the result of the *Assay*. Calculate the acceptance value.

Solid Dosage Forms Other Than Tablets and Capsules

Proceed as directed for *Hard Capsules*, treating each unit as described therein. Calculate the acceptance value.

Liquid Dosage Forms

Accurately weigh the amount of liquid that is removed from each of 10 individual containers in conditions of normal use. If necessary, compute the equivalent volume after determining the density. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the *Assay*. Calculate the acceptance value.

Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that the individual contents of the units are replaced with the individual estimated contents defined below.

$\chi_1, \chi_2, \ldots, \chi_n$	=	individual estimated contents of the units tested, where $\chi_i = w_i \times A/\overline{W}$
$w_1, w_2, \ldots, w_n$	=	individual ·weights, of the units tested
A	=	content of drug substance (% of label claim) obtained using an appropriate analytical method
$\overline{W}$	=	mean of individual ·weights, $(w_1, w_2, \ldots, w_n)$

CRITERIA

Apply the following criteria, unless otherwise specified.

Solid, Semi-Solid, and Liquid Dosage Forms

The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to L1%. If the acceptance value is > L1%, test the next 20 units, and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is ≤ L1%, and no individual content of ·any, dosage unit is less than [1 – (0.01)(L2)]M nor more than [1 + (0.01)(L2)]M ·as specified, in the *Calculation of Acceptance Value* under *Content Uniformity* or under ·*Weight, Variation*. Unless otherwise specified, L1 is 15.0 and L2 is 25.0.

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⟨911⟩ VISCOSITY—CAPILLARY METHODS

The following procedures are used to determine the viscosity of a Newtonian fluid, i.e., a fluid having a viscosity that is independent of the rate of shear. [NOTE—For additional information, see *Rheometry* ⟨1911⟩.]

• **METHOD I. SUSPENDED-LEVEL (OR UBBELOHDE-TYPE) CAPILLARY VISCOMETER**

Apparatus: The determination may be carried out with a suspended-level capillary viscometer (*Figure 1*).

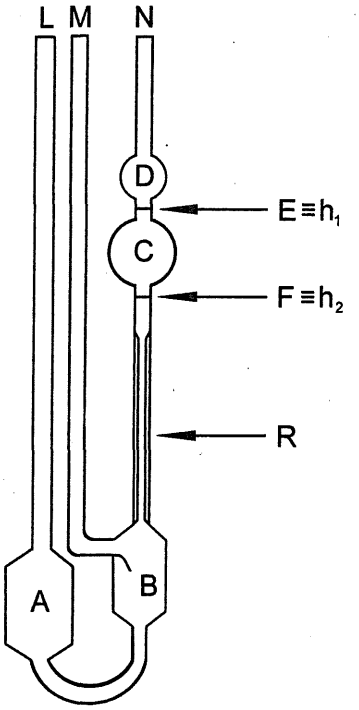


Figure 1. Suspended-level (or Ubbelohde-type) capillary viscometer.

Other viscometers may be used provided that the accuracy and precision is NLT that obtained with the viscometers described in this chapter.

Procedure: Fill the viscometer through tube (L) with a sufficient quantity of the sample liquid that is appropriate for the viscometer being used or by following the manufacturer's instructions. Carry out the experiment with the tube in a vertical position. Fill bulb (A) with the liquid, and also ensure that the level of liquid in bulb (B) is below the exit to the ventilation tube (M). Immerse the viscometer in a water or oil bath stabilized at the temperature specified in the individual monograph, and control the temperature to ±0.1°, unless otherwise specified in the individual monograph. Maintain the viscometer in a vertical position for a time period of NLT 30 min to allow the sample temperature to reach equilibrium. Close tube (M), and raise the level of the liquid in tube (N) to a level about 8 mm above mark ($E \equiv h_1$). Keep the liquid at this level by closing tube (N) and opening tube (M). Open tube (N), and measure the time required for the level of the liquid to drop from mark ($E \equiv h_1$) to mark ($F \equiv h_2$), using an appropriate accurate timing device. [NOTE—The minimum flow time should be 200 s.]

Calibration: Calibrate each viscometer at the test temperature by using fluids of known viscosities of appropriate viscosity standards to determine the viscometer constant, k. The viscosity values of the calibration standards should bracket the expected viscosity value of the sample liquid.

Calculate the viscometer constant, k, in mm^2/s^2:

$$k = \eta/(\rho \times t)$$

η = known viscosity of the liquid (mPa · s)
ρ = density of the liquid (g/mL)
t = flow time for the liquid to pass from the upper mark to the lower mark (s)

Calculation of kinematic and Newtonian viscosities of sample fluid: A capillary viscometer is chosen so that the flow time, t, is NLT 200 s, and the kinematic energy correction is typically less than 1%. If the viscosity constant, k, is known, use the following equation to calculate the kinematic viscosity, v, in mm^2/s, from the flow time, t, in s.

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$$v = k \times t$$

If the density of the fluid is known at the temperature of the viscosity measurement, then the Newtonian viscosity, η, in mPa · s, is calculated:

$$\eta = v \times \rho$$

ρ = density of the fluid (g/mL)

The flow time of the fluid under examination is the mean of NLT three consecutive determinations. The result is valid if the percentage of the relative standard deviation (%RSD) for the three readings is NMT 2.0%.

• **METHOD II. SIMPLE U-TUBE (OR OSTWALD-TYPE) CAPILLARY VISCOMETER**

Apparatus: The determination may be carried out with a simple U-tube capillary viscometer (*Figure 2*).

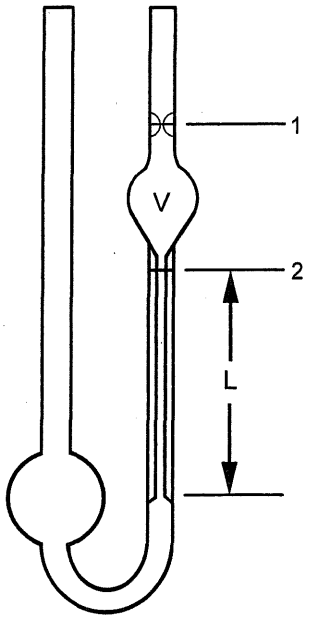


Figure 2. Simple U-tube (or Ostwald-type) capillary viscometer.

Variables and line numbers in *Figure 2* are defined as:

Line 1	= upper mark in the tube
V	= given volume of fluid (m^3)
Line 2	= lower mark in the tube
L	= capillary tube length (m)

Other viscometers, such as modified Ostwald-type capillary viscometers,[1] may be used provided that the accuracy and precision is NLT that obtained with the viscometers described in this chapter.

Procedure: Fill the tube with an amount of the sample that is appropriate for the viscometer being used or by following the manufacturer's instructions. The volume of fluid used should be such that the lower bulb is not entirely emptied when the fluid is drawn up through the capillary tube to the uppermost graduation mark. Carry out the experiment with the tube in a vertical position. Immerse the viscometer in a water or oil bath stabilized at the temperature specified in the individual monograph, and control the temperature to ±0.1°, unless otherwise specified in the individual monograph. Maintain the viscometer in a vertical position for a time period of NLT 30 min to allow the sample temperature to reach equilibrium. Using suction, draw the fluid up through the capillary tube until the meniscus is at the level of the uppermost graduation. With both the filling and capillary tubes open to atmospheric pressure, record the time, in s, required for the liquid to flow from the upper mark to the lower mark in the capillary tube. [NOTE—The minimum flow time should be 200 s.]

[1] For example, the Cannon-Fenske capillary viscometer is one of the simple U-tube capillary viscometers and is also called a modified Ostwald-type capillary viscometer.

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Calibration and **Calculation of kinematic and Newtonian viscosities of sample fluid:** Proceed as directed in *Method I.* For certain simple U-tube capillary viscometers, determine the viscometer constant at the same temperature as the sample liquid under test.

⟨912⟩ VISCOSITY—ROTATIONAL METHODS

The principle of the method is to measure the force (torque) acting on a rotor when it rotates at a constant angular velocity or rotational speed in a liquid. Rotational rheometers/viscometers are used for measuring the viscosity of fluids, both Newtonian and non-Newtonian. The following procedures are used to determine the viscosity of Newtonian fluids or the apparent viscosity of non-Newtonian fluids. The calculated viscosity of Newtonian fluids should be the same (within experimental error), regardless of the rate of shear (or rotational speed). Given the dependence of viscosity on temperature, the temperature of the substance being measured should be controlled to within ±0.1°, unless otherwise specified in the individual monograph. [NOTE—For additional information, see *Rheometry* ⟨1911⟩.]

• **METHOD I. SPINDLE VISCOMETERS**

Apparatus: In the spindle viscometer, the apparent viscosity is determined by rotating a cylinder- or disk (or disc)-shaped spindle, as shown in *Figure 1* and *Figure 2*, respectively, immersed in a large volume of liquid.

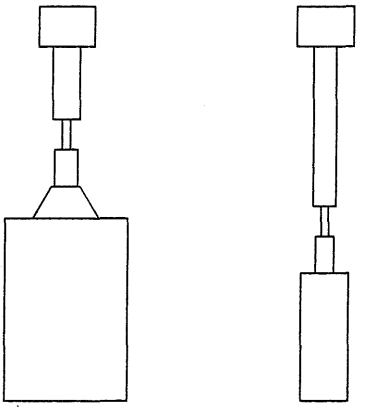


Figure 1. Cylinder-shaped spindles.

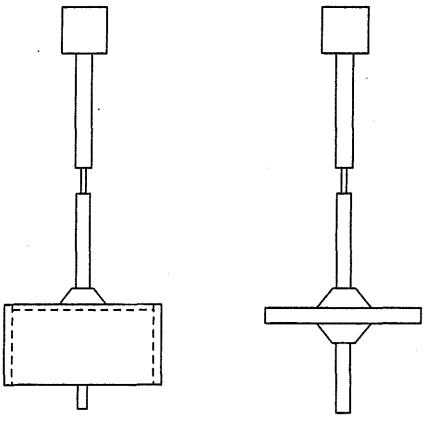


Figure 2. Disk-shaped spindles.

An absolute viscosity cannot be calculated due to the large gap between the spindle and the container wall, or the geometry of the spindle. The torque to maintain a given angular velocity does give a measure of the liquid resistance to flow but is often described as an apparent viscosity.

Other spindle type viscometers may be used provided that the accuracy and precision is NLT that obtained with the viscometers described in this chapter.

Procedure: When viscosity measurement is performed in a beaker or cup, as the shear rate is unknown, in order to enable reproducibility among labs that measure viscosity using different instrumentation, these parameters must be reported along with the measured viscosity:

1. Size and geometry of spindle
2. Angular velocity or rotational speed of the spindle
3. Temperature of the test substance

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The spindle should be immersed to the recommended depth maintaining at least 1 cm clearance from the bottom and side of the container.

The preparation of the test specimen, including its temperature equilibration, is specified in each individual monograph. Follow the instrument manufacturer's recommendations regarding sample loading, spindle selection, and viscometer operation.

Calibration check: Check the calibration of a particular viscometer configuration at the test temperature using one or more fluids of known viscosities (Newtonian viscosity standards). The viscosity values of the calibration standards should bracket the expected viscosity value of the sample liquid. [NOTE—To help verify the linearity of the apparatus, it is suggested to perform measurements of a Newtonian viscosity standard at multiple rotational speeds at the test temperature.]

A viscometer is deemed to be calibrated if the measured apparent viscosities are within ±5% of the stated values.

Generally, calibration, operation, and cleaning of the viscometer should be performed according to the recommendations of the instrument manufacturer.

• **METHOD II. CONCENTRIC CYLINDER RHEOMETERS**

Apparatus: In the concentric cylinder rheometer, the apparent viscosity is determined by placing the liquid in the gap between the inner cylinder and the outer cylinder. Both controlled-stress and controlled-rate rotational rheometers are available commercially in configurations with absolute geometries (e.g., very small annular gaps between concentric cylinders) that allow calculation of apparent viscosities for non-Newtonian fluids. Controlled shear stress rheometers measure the shear rates resulting from the application of a given force or torque (the stress). Controlled shear rate rheometers measure the shear stress (from the torque on the rotor axis) resulting from a given shear rate (or rotational speed). Concentric cylinder rotational rheometers are sometimes referred to as cup-and-bob rheometers. These rheometers involve an additional design consideration depending on whether the outer cylinder (the cup) or the inner cylinder (the bob) rotates. Rotating-cup rheometers are called Couette systems, while rotating-bob rheometers are called Searle systems, as shown in *Figure 3* and *Figure 4*, respectively.

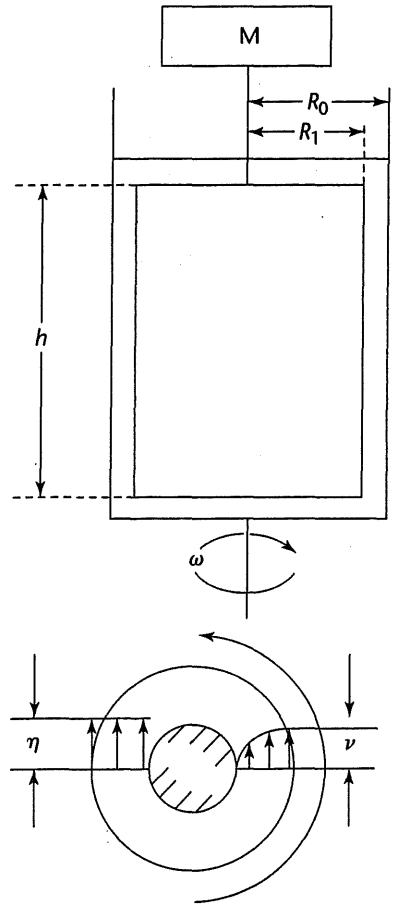


Figure 3. Couette concentric cylinder system for rotational rheometry.

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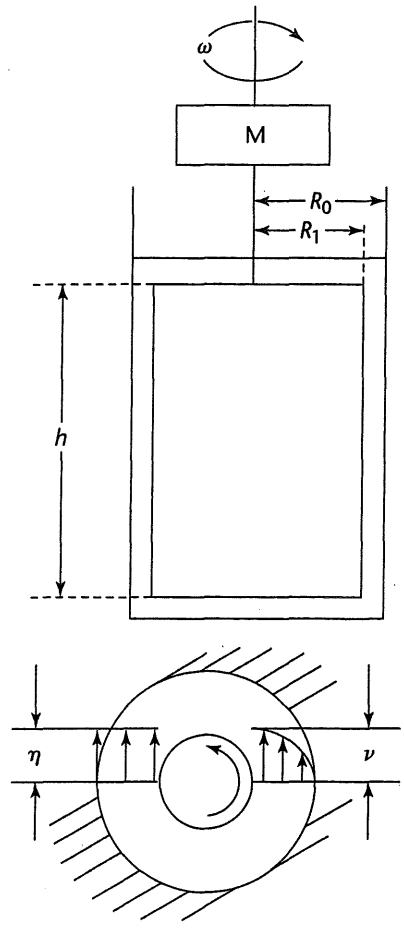


Figure 4. Searle concentric cylinder system for rotational rheometry.

Variables in *Figure 3* and *Figure 4* are defined as:

M = torque acting on the cylinder surface (N · m)
R_0 = radius of the outer cylinder (m)
R_1 = radius of the inner cylinder (m)
h = height of immersion of the inner cylinder in the liquid medium (m)
ω = angular velocity (radians/s)
η = viscosity (Pa · s)
v = velocity (m/s)

Procedure: Place a sufficient quantity of test fluid in the rheometer, and allow the sample to reach thermal equilibrium, as indicated in the individual monograph. Operate the rheometer following the procedure recommended by the instrument manufacturer. For non-Newtonian systems, the monograph indicates the type of rheometer that should be used and the shear rate(s) at which the measurements should be made. Determine apparent viscosities by changing the shear rate (or shear stress, if using a controlled shear stress rheometer) over a range appropriate to the use of the material under test. From a series of such viscosity measurements, the relationship between the shear rate and the shear stress of a non-Newtonian liquid can be obtained.

• **Method III. Cone-and-Plate Rheometers**

Apparatus: In the cone-and-plate rheometer, the liquid is introduced into the fixed gap between a flat disk or plate and a cone forming a defined angle. The angle of the cone ensures a constant shear rate due to the increase in both the gap and the linear speed as the distance increases from the origin. Viscosity measurement can be performed by rotating the cone or the plate, as shown in *Figure 5* and *Figure 6*, respectively. [Note—Because the volume of sample is small, even a small absolute loss of solvents can cause a large percentage change in viscosity. Such a loss is particularly relevant for volatile solvents but could be significant even for nonvolatile solvents such as water.]

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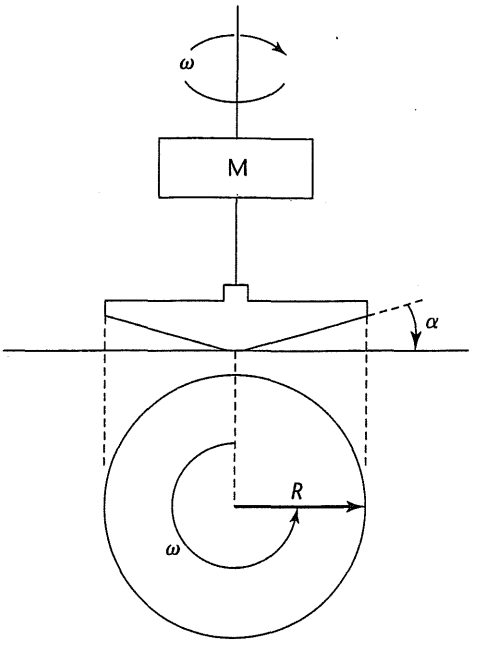


Figure 5. Cone-and-plate rotational rheometer with rotating cone.

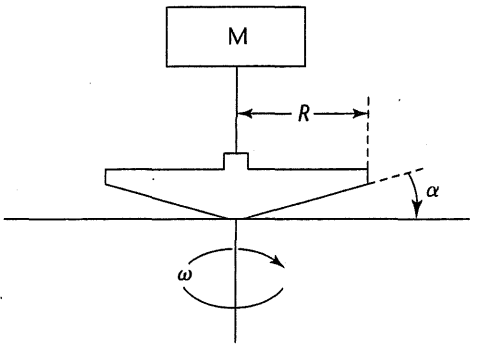


Figure 6. Cone-and-plate rotational rheometer with rotating plate.

Variables in *Figure 5* and *Figure 6* are defined as:

ω = angular velocity (radians/s)
M = torque acting on the flat plate or cone surface (N · m)
α = angle between the flat plate and cone (radians)
R = radius of the cone (m)

Procedure: Proceed as directed for *Method II. Concentric Cylinder Rheometers.*

• **METHOD IV. PARALLEL PLATE (OR PARALLEL DISK) RHEOMETERS**

Apparatus: Parallel plate rheometers are similar to cone-and-plate rheometers except that the sample to be measured is introduced into the gap between a flat plate or disk (or disc) and another parallel flat plate or disk. Measurements are made typically with the lower plate or disk remaining stationary as the upper plate or disk is rotated at a constant angular velocity, ω (*Figure 7*).

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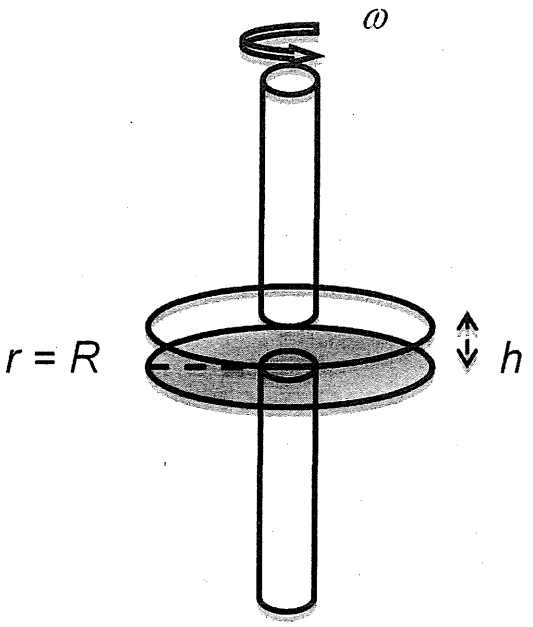


Figure 7. Parallel plate rotational rheometer.

Variables in *Figure 7* are defined as:

ω = angular velocity (radians/s)
R = radius of the plate (m)
h = distance between the two parallel plates (m)

In contrast to the cone-and-plate rheometer, the shear rate between parallel plates increases with distance from the origin of the axis of rotation due to the increasing linear speeds for a given angular velocity with a constant gap. Thus, an average shear rate is obtained. Nonetheless, some advantages of the parallel plate rheometer include ease of sample loading (especially for very viscous liquids and soft semisolids), and suitability for suspensions of particulates. For suspensions, the gap should be set high enough to avoid grinding particles between the plates. Parallel plates have a user-definable gap (within practical limits) and therefore, in the absence of large particles, may be used at narrower gaps. As with cone-and-plate rheometers, evaporative loss of solvent can markedly affect the viscosity of the sample, so adequate precautions need to be taken to minimize solvent loss.

Procedure: Proceed as directed for *Method II. Concentric Cylinder Rheometers.*

⟨913⟩ VISCOSITY—ROLLING BALL METHOD

The following procedure is used to determine the viscosity of a Newtonian fluid, i.e., a fluid having a viscosity that is independent of the shearing stress or rate of shear.

[NOTE—For additional information, see *Rheometry* ⟨1911⟩.]

APPARATUS

See *Figure 1*.

The basic design of a rolling ball viscometer consists of a tube (or capillary) that contains the sample liquid under test and a ball chosen so that it will require a minimum rolling time of 20 s at the measuring angle in the sample liquid.

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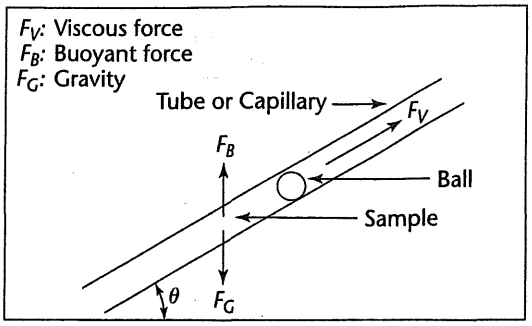


Figure 1. Basic design for rolling ball viscometer.

MEASURING PRINCIPLE

The rolling ball measurement is based on Stokes's Law, as influenced by the angle of inclination of the tube (or capillary). Calculate the Newtonian viscosity, η, in mPa · s:

$$\eta = [(\rho_1 - \rho_2) \times g \times r^2 \times \sin\theta]/v_\infty$$

ρ_1 = density of the ball used (g/mL)
ρ_2 = density of the sample liquid (g/mL)
g = gravitational constant (mm/s^2)
r = radius of the ball (mm)
θ = angle of inclination of the tube (or capillary)
v_∞ = terminal velocity of the ball (mm/s)

Determine the viscosity of a liquid by observing the rolling time of a solid sphere (ball) under the influence of gravity in an inclined cylindrical tube filled with the sample liquid. Measure the time taken by the ball to travel the fixed distance between two ring marks or measuring sensors. For each single measured rolling time, the resulting viscosity can be expressed as dynamic viscosity (mPa · s) as well as kinematic viscosity (mm^2/s) for a sample of known density.

PROCEDURE

Select a measuring system [tube (or capillary) and ball combination] within the anticipated range of viscosity of the sample liquid. As necessary, heat the clean and dry tube and ball of the viscometer to the temperature specified in the individual monograph, and control the temperature to ±0.1°, unless otherwise specified in the individual monograph. Select a measuring angle to obtain a minimum rolling time of 20 s. Fill the tube with the sample liquid, being careful to avoid bubble formation. Close the tube, and insert it in the instrument. Allow to equilibrate at the specified temperature for NLT 15 min for a rolling ball viscometer with a tube of large diameter. For a micro rolling ball viscometer, follow the instrument manufacturer's instructions regarding temperature equilibration. Release the ball, and record the time required for the ball to roll from the upper to the lower ring mark (or measuring sensor). Repeat the test run at least four times.

The rolling time in the fluid under examination is the mean of NLT four consecutive determinations. The result is valid if the percent relative standard deviation (%RSD) for the four readings is NMT 2.0%.

CALCULATION AND CALIBRATION

Calculate the Newtonian viscosity, η, in mPa · s:

$$\eta = k \times (\rho_1 - \rho_2) \times t$$

k = calibration constant of the instrument (mm^2/s^2) at a specified measuring angle and temperature
ρ_1 = density of the ball used (g/mL)
ρ_2 = density of the sample liquid (g/mL)
t = rolling time of the ball (s)

Calibrate each tube (or capillary) and ball combination at the test temperature and test angle using fluids of known viscosities and densities (viscosity standards) to determine the measuring system constant, k.

[NOTE—Some automated viscometers use a polynomial function to determine the calibration for different angles and temperatures.]

The viscosity values of the calibration standards should bracket the expected viscosity value of the sample liquid.

Calibrations are specific to the ball radius, ball density, temperature, and test angle. Recalibration is necessary when any of these parameters is changed.

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Where no reference values at the required test temperature are available, follow the manufacturer's instructions for mathematical corrections to the calibration function. When the materials of the ball and tube are dissimilar, apply corrections calculated using the linear thermal expansion coefficients of the materials.

⟨914⟩ VISCOSITY—PRESSURE DRIVEN METHODS

INTRODUCTION

The viscometers/rheometers that are included in this chapter can be used to measure the viscosity of both Newtonian and non-Newtonian fluids. The devices include slit viscometers/rheometers and capillary-extrusion viscometers.

Capillary viscometers that are described in *Viscosity—Capillary Methods* ⟨911⟩ are based on the principle that the flow is driven by gravity, and these viscometers can be classified under one of the pressure driven methods. However, limitations of glass capillary viscometers render them inherently unsuitable for use in characterizing non-Newtonian fluids, and their use in measurements of high-viscosity fluids is impractical as well. Chapter ⟨914⟩ does not address such viscometers.

[NOTE—For additional information, see *Rheometry* ⟨1911⟩.]

• METHOD I. SLIT VISCOMETERS/RHEOMETERS

Apparatus: See *Figure 1*.

The basic design of a slit viscometer/rheometer consists of a rectangular, slit channel with a uniform cross-sectional area. A test liquid is pumped at a constant flow rate through this channel. Multiple pressure sensors that are flush mounted at linear distances along the streamwise direction measure the pressure drop, as depicted in *Figure 1*.

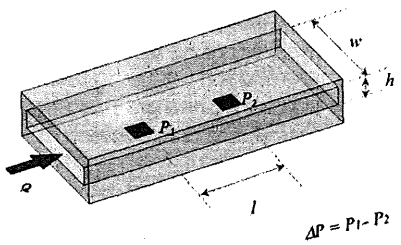


Figure 1. Basic design of the slit viscometer/rheometer.

Measuring principle: The slit viscometer/rheometer is based on the fundamental principle that a viscous liquid resists flow, exhibiting a decreasing pressure along the length of the slit. The pressure decrease or drop (ΔP) is correlated with the shear stress at the wall boundary. The apparent shear rate is directly related to the flow rate and the dimension of the slit. The apparent shear rate ($\dot{\gamma}_a$), the shear stress (σ), and the apparent viscosity (η_a), respectively, are calculated as follows:

$$\dot{\gamma}_a = \frac{6 \times Q}{w \times h^2}$$

$$\sigma = \frac{h \times w}{2 \times (w + h)} \times \frac{\Delta P}{l}$$

$$\eta_a = \frac{\sigma}{\dot{\gamma}_a}$$

$\dot{\gamma}_a$ = apparent shear rate (s^{-1})
Q = flow rate (μL/s)
w = width of the flow channel (mm)
h = depth of the flow channel (mm)
σ = shear stress (Pa)
ΔP = pressure difference between the leading pressure sensor and the last pressure sensor (Pa)
l = distance between the leading pressure sensor and the last pressure sensor (mm)
η_a = apparent viscosity (Pa · s)

To determine the viscosity of a liquid, pump the liquid sample through the slit channel at a constant flow rate and measure the pressure drop. Using the equations shown above, calculate the apparent viscosity for the apparent shear rate. For a Newtonian liquid, the apparent viscosity is the same as the true viscosity, and the single shear-rate measurement is sufficient. For non-Newtonian liquids, the apparent viscosity is not true viscosity. To obtain true viscosity, measure the apparent viscosities at multiple apparent shear rates. Calculate true viscosities, η, at various shear rates using the Weissenberg–Rabinowitsch–Mooney correction factor:

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$$\frac{1}{\eta}=\frac{1}{2\times\eta_a}\times\left(2+\frac{d\ln\dot{\gamma}_a}{d\ln\sigma}\right)$$

The calculated true viscosity should be the same as the value that is obtained using a cone-and-plate rheometer at the same shear rate.

Procedure: Select slit dimensions and full-scale pressure of pressure sensors to attain the desired range of viscosity and shear rates. With a syringe pump to control the flow rate—or interchangeably, the shear rate—load a test sample into a glass syringe. As necessary, control the temperature of the sample with the syringe and the slit viscometer specified in the individual procedure to ±0.1°, unless otherwise specified. Allow to equilibrate at the specified temperature for about 3–5 min. Set each pressure sensor to zero. Select the first flow rate or shear rate. Pump the sample at the flow rate, and measure the pressure readings once the steady-state values are reached. Increase the flow rate (or shear rate) until the pressure reading of the leading sensor is >5% of the full-scale value. Take the average of each pressure sensor over time, and calculate the pressure drops. Calculate the apparent viscosity. If required for the procedure, repeat the measurements four times. If the test sample is non-Newtonian, vary the shear rate and calculate the corresponding apparent viscosities.

Calculation and calibration: Calculate the apparent viscosity by dividing the shear stress by the apparent shear rate. For non-Newtonian liquids, apply the Weissenberg–Rabinowitsch–Mooney corrections to obtain the true viscosity curve. Commercial viscometers are provided with a built-in correction.

Calibrate individual pressure sensors by following the recommendations from the manufacturer. The slit viscometer, however, can be calibrated with a Newtonian standard of known viscosity. Choose a standard that covers the viscosity range of interest for the selected slit and pressure sensors. Measure the viscosities at shear rates for 10%, 50%, and 90% of full scale of the leading pressure sensor, and take the average. Calculate a constant (k) for the ratio of the reference value (η_{ref}) to the measured value (η_{meas}) and record.

$$k=\frac{\eta_{ref}}{\eta_{meas}}$$

η_{ref} = reference viscosity (Pa · s)
η_{meas} = measured viscosity (Pa · s)

Multiply new viscosity data by k, because the value of k will correct for the uncertainty and variation of the slit geometry. Ideally, the value of k should remain constant within a temperature change of 100°. If the k value varies by >1%, consult with the manufacturer regarding the temperature compensation of the pressure sensors.

⟨921⟩ WATER DETERMINATION

Many Pharmacopeial articles either are hydrates or contain water in adsorbed form. As a result, the determination of the water content is important in demonstrating compliance with the Pharmacopeial standards. Generally one of the methods given below is called for in the individual monograph, depending upon the nature of the article. In rare cases, a choice is allowed between two methods. When the article contains water of hydration, *Method I (Titrimetric)*, *Method II (Azeotropic)*, or *Method III (Gravimetric)* is employed, as directed in the individual monograph, and the requirement is given under the heading *Water.*

The heading *Loss on Drying* (see *Loss on Drying* ⟨731⟩) is used in those cases where the loss sustained on heating may be not entirely water.

METHOD I (TITRIMETRIC)

Determine the water by *Method Ia,* unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

PRINCIPLE

The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the *Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the *Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen. In some cases, other suitable solvents may be used for special or unusual test specimens. In these cases, the addition of at least 20% of methanol or other primary alcohol is recommended.

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APPARATUS

Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 s to 30 min, depending upon the solution being titrated. The time is shortest for substances that dissolve in the reagent. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. Commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant, and the titration vessel may be purged by means of a stream of dry nitrogen or current of dry air.

REAGENT

Prepare the Karl Fischer Reagent as follows. Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and, keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 h before use, or daily if in continuous use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light, and under refrigeration. For determination of trace amounts of water (less than 1%), it is preferable to use a *Reagent* with a water equivalency factor of not more than 2.0, which will lead to the consumption of a more significant volume of titrant.

A commercially available, stabilized solution of Karl Fischer type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine or alcohols other than methanol may be used also. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol or other suitable solvent, such as ethylene glycol monomethyl ether, may be used as the diluent.

TEST PREPARATION

Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 2–250 mg of water. The amount of water depends on the water equivalency factor of the *Reagent* and on the method of endpoint determination. In most cases, the minimum amount of specimen, in mg, can be estimated using the formula:

$$FCV/KF$$

in which F is the water equivalency factor of the *Reagent,* in mg per mL; C is the used volume, in percent, of the capacity of the buret; V is the buret volume, in mL; and KF is the limit or reasonable expected water content in the sample, in percent. C is generally between 30% and 100% for manual titration, and between 10% and 100% for the instrumental method endpoint determination.

[NOTE—It is recommended that the product of FCV be greater than or equal to 200 for the calculation to ensure that the minimum amount of water titrated is greater than or equal to 2 mg.]

Where the specimen under test is an aerosol with propellant, store it in a freezer for not less than 2 h, open the container, and test 10.0 mL of the well-mixed specimen. In titrating the specimen, determine the endpoint at a temperature of 10° or higher.

Where the specimen under test is capsules, use a portion of the mixed contents of not fewer than four capsules.

Where the specimen under test is tablets, use powder from not fewer than four tablets ground to a fine powder in an atmosphere of temperature and relative humidity known not to influence the results.

Where the monograph specifies that the specimen under test is hygroscopic, take an accurately weighed portion of the solid into the titration vessel, proceeding as soon as possible and taking care to avoid moisture uptake from the atmosphere. If the sample is constituted by a finite amount of solid as a lyophilized product or a powder inside a vial, use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into a tared container, and shake to dissolve the specimen. Using the same syringe, remove the solution from the container and transfer it to a titration vessel prepared as directed for *Procedure.* Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured, add this washing to the titration vessel, and immediately titrate. Determine the water content, in mg, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed for *Standardization of Water Solution for Residual Titration,* and subtract this value from the water content, in mg, obtained in the titration of the specimen under test. Dry the container and its closure at 100° for 3 h, allow to cool in a desiccator, and weigh. Determine the weight of specimen tested from the difference in weight from the initial weight of the container.

When appropriate, the water may be desorbed or released from the sample by heat in an external oven connected with the vessel, to where it is transferred with the aid of an inert and dried gas such as pure nitrogen. Any drift due to the transport gas should be considered and corrected. Care should be taken in the selection of the heating conditions to avoid the formation of water coming from dehydration due to decomposition of the sample constituents, which may invalidate this approach.

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STANDARDIZATION OF THE REAGENT

Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Reagent* to give the characteristic endpoint color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential.

Purified Water, sodium tartrate dihydrate, a USP Reference Standard, or commercial standards with a certificate of analysis traceable to a national standard may be used to standardize the *Reagent.* The reagent equivalency factor, the recommended titration volume, buret size, and amount of standard to measure are factors to consider when deciding which standard and how much to use.[1] For *Purified Water* or water standards, quickly add the equivalent of between 2 and 250 mg of water. Calculate the water equivalency factor, *F,* in mg of water per mL of reagent:

$$W/V$$

in which *W* is the weight, in mg, of the water contained in the aliquot of standard used; and *V* is the volume, in mL, of the *Reagent* used in the titration. For sodium tartrate dihydrate, quickly add 20–125 mg of sodium tartrate dihydrate ($C_4H_4Na_2O_6 \cdot 2H_2O$), accurately weighed by difference, and titrate to the endpoint. The water equivalence factor *F,* in mg of water per mL of reagent, is given by the formula:

$$W/V\,(36.04/230.08)$$

in which 36.04 is two times the molecular weight of water and 230.08 is the molecular weight of sodium tartrate dihydrate; *W* is the weight, in mg, of sodium tartrate dihydrate; and *V* is the volume, in mL, of the *Reagent* consumed in the second titration. Note that the solubility of sodium tartrate dihydrate in methanol is such that fresh methanol may be needed for additional titrations of the sodium tartrate dihydrate standard.

PROCEDURE

Unless otherwise specified, transfer enough methanol or other suitable solvent to the titration vessel, ensuring that the volume is sufficient to cover the electrodes (approximately 30–40 mL), and titrate with the *Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed, because it does not enter into the calculations.) Quickly add the *Test Preparation,* mix, and again titrate with the *Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen taken, in mg:

$$SF$$

in which *S* is the volume, in mL, of the *Reagent* consumed in the second titration; and *F* is the water equivalence factor of the *Reagent.*

Method Ib (Residual Titration)

PRINCIPLE

See the information given in the section *Principle* under *Method Ia.* In the residual titration, excess *Reagent* is added to the test specimen, sufficient time is allowed for the reaction to reach completion, and the unconsumed *Reagent* is titrated with a standard solution of water in a solvent such as methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

APPARATUS, REAGENT, AND TEST PREPARATION

Use *Method Ia.*

STANDARDIZATION OF WATER SOLUTION FOR RESIDUAL TITRATION

Prepare a *Water Solution* by diluting 2 mL of water with methanol or other suitable solvent to 1000 mL. Standardize this solution by titrating 25.0 mL with the *Reagent,* previously standardized as directed under *Standardization of the Reagent.* Calculate the water content, in mg per mL, of the *Water Solution* taken:

$$V'F/25$$

in which *V'* is the volume of the *Reagent* consumed, and *F* is the water equivalence factor of the *Reagent.* Determine the water content of the *Water Solution* weekly, and standardize the *Reagent* against it periodically as needed.

PROCEDURE

Where the individual monograph specifies that the water content is to be determined by *Method Ib,* transfer enough methanol or other suitable solvent to the titration vessel, ensuring that the volume is sufficient to cover the electrodes (approximately

[1] Consider a setup in which the reagent equivalency factor is 5 mg/mL, and the buret volume is 5 mL and an instrumental endpoint. Standard amounts equivalent to between 2.5 mg and 22.5 mg of water (10%–90% of buret capacity) could be used based on the buret and the reagent equivalency factor. The upper end of this range would involve an excessive amount of sodium tartrate dihydrate. If *Purified Water* or a standard is weighed, an analytical balance appropriate to the amount weighed is required.

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30–40 mL), and titrate with the *Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation,* mix, and add an accurately measured excess of the *Reagent.* Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken:

$$F(X' - XR)$$

in which F is the water equivalence factor of the *Reagent*; X' is the volume, in mL, of the *Reagent* added after introduction of the specimen; X is the volume, in mL, of standardized *Water Solution* required to neutralize the unconsumed *Reagent;* and R is the ratio, $V'/25$ (mL *Reagent*/mL *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration.*

Method Ic (Coulometric Titration)

PRINCIPLE

The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the endpoint. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer solution after each determination is not necessary because individual determinations can be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen is compatible with the other components, and no side reactions take place. Samples are usually transferred into the vessel as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system; thus, the introduction of solids into the cell may require precautions, such as working in a glove-box in an atmosphere of dry inert gas. Control of the system may be monitored by measuring the amount of baseline drift, which does not preclude the need of any blank correction when used as a vehicle for sample introduction. This method is particularly suited to chemically inert substances like hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micro-method.

When appropriate, the water may be desorbed or released from the sample by heat in an external oven connected with the vessel, to where it is transferred with the aid of an inert and dried gas such as pure nitrogen. Any drift due to the transport gas should be considered and corrected. Care should be taken in the selection of the heating conditions to avoid the formation of water coming from dehydration due to decomposition of the sample constituents, which may invalidate this approach.

APPARATUS

Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary, as the current consumed can be measured absolutely.

REAGENT

See the manufacturer's recommendations.

TEST PREPARATION

Where the specimen is a soluble solid, an appropriate quantity, accurately weighed, may be dissolved in anhydrous methanol or other suitable solvents.

Where the specimen is an insoluble solid, an appropriate quantity, accurately weighed, may be extracted using a suitable anhydrous solvent, and may be injected into the anolyte solution. Alternatively, an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas. The gas is then passed into the cell.

Where the specimen is to be used directly without dissolving in a suitable anhydrous solvent, an appropriate quantity, accurately weighed, may be introduced into the chamber directly.

Where the specimen is a liquid, and is miscible with anhydrous methanol or other suitable solvents, an appropriate quantity, accurately weighed, may be added to anhydrous methanol or other suitable solvents.

PROCEDURE

Using a dry device, inject or add directly an accurately measured amount of the sample or sample preparation estimated to contain between 0.5 and 5 mg of water, or an amount recommended by the instrument manufacturer into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the liquid *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, as needed, and make any necessary corrections.

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METHOD II (AZEOTROPIC—TOLUENE DISTILLATION)

Apparatus

Use a 500-mL glass flask A connected by means of a trap B to a reflux condenser C by ground glass joints (see *Figure 1*).

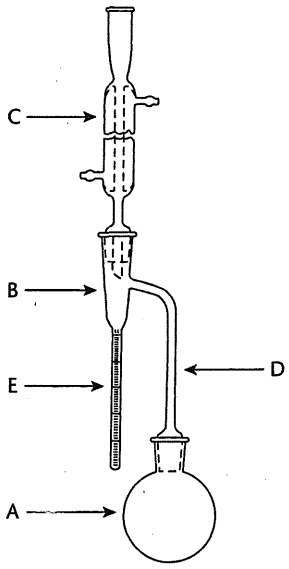


Figure 1. Toluene moisture apparatus.

The critical dimensions of the parts of the apparatus are as follows. The connecting tube D is 9–11 mm in internal diameter. The trap is 235–240 mm in length. The condenser, if of the straight-tube type, is approximately 400 mm in length and not less than 8 mm in bore diameter. The receiving tube E has a 5-mL capacity, and its cylindrical portion, 146–156 mm in length, is graduated in 0.1-mL subdivisions, so that the error of reading is not greater than 0.05 mL for any indicated volume. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Clean the receiving tube and the condenser with a suitable cleanser, thoroughly rinse with water, and dry in an oven. Prepare the toluene to be used by first shaking with a small quantity of water, separating the excess water, and distilling the toluene.

Procedure

Place in the dry flask a quantity of the substance, weighed accurately to the nearest centigram, which is expected to yield 2–4 mL of water. If the substance is of a pasty character, weigh it in a boat of metal foil of a size that will just pass through the neck of the flask. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask, or a number of capillary melting-point tubes, about 100 mm in length, sealed at the upper end. Place about 200 mL of toluene in the flask, connect the apparatus, and fill the receiving tube E with toluene poured through the top of the condenser. Heat the flask gently for 15 min and, when the toluene begins to boil, distill at the rate of about two drops per s until most of the water has passed over, then increase the rate of distillation to about four drops per s. When the water has apparently all distilled over, rinse the inside of the condenser tube with toluene while brushing down the tube with a tube brush attached to a copper wire and saturated with toluene. Continue the distillation for five min, then remove the heat, and allow the receiving tube to cool to room temperature. If any droplets of water adhere to the walls of the receiving tube, scrub them down with a brush consisting of a rubber band wrapped around a copper wire and wetted with toluene. When the water and toluene have separated completely, read the volume of water, and calculate the percentage that was present in the substance.

METHOD III (GRAVIMETRIC)

Procedure for Chemicals

Proceed as directed in the individual monograph preparing the chemical as directed under *Loss on Drying* ⟨731⟩.

Procedure for Biologics

Proceed as directed in the individual monograph.

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Procedure for Articles of Botanical Origin

Place about 10 g of the drug, prepared as directed (see *Methods of Analysis* under *Articles of Botanical Origin* ⟨561⟩) and accurately weighed, in a tared evaporating dish. Dry at 105° for 5 h, and weigh. Continue the drying and weighing at 1-h intervals until the difference between two successive weighings corresponds to not more than 0.25%.

⟨941⟩ CHARACTERIZATION OF CRYSTALLINE AND PARTIALLY CRYSTALLINE SOLIDS BY X-RAY POWDER DIFFRACTION (XRPD)

INTRODUCTION

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially three types of information can be derived from a powder diffraction pattern: the angular position of diffraction lines (depending on geometry and size of the unit cell), the intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample), and diffraction line profiles (depending on instrumental resolution, crystallite size, strain, and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (e.g., identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions[1] can also be made.

The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually nondestructive in nature (to ensure a randomly oriented sample, specimen preparation is usually limited to grinding). XRPD investigations can also be carried out under *in situ* conditions on specimens exposed to nonambient conditions such as low or high temperature and humidity.

PRINCIPLES

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive when the path difference between two diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see *Figure 1*).

$$2d_{hkl}\sin\theta_{hkl} = n\lambda$$

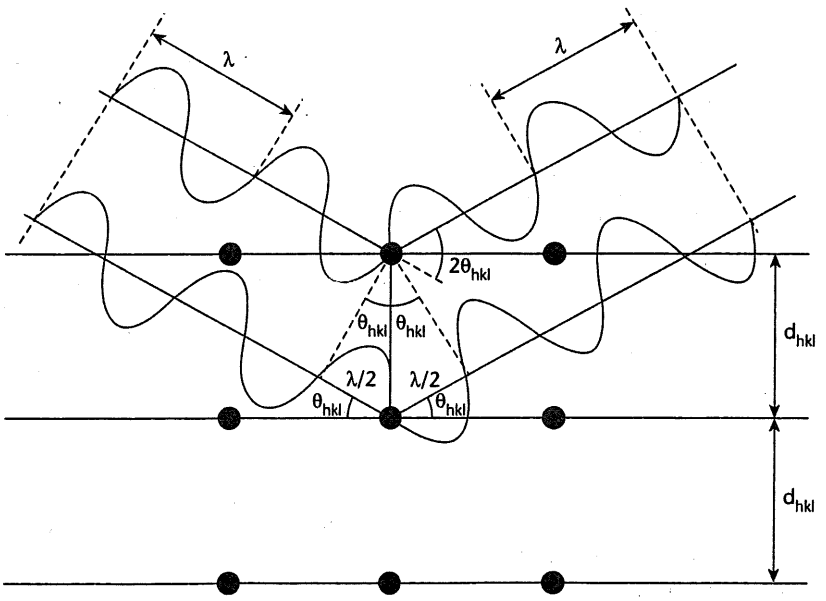


Figure 1. Diffraction of X-rays by a crystal according to Bragg's Law.

[1] There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances, such as determination of crystal structures, refinement of crystal structures, determination of the crystallographic purity of crystalline phases, and characterization of crystallographic texture. These applications are not described in this chapter.

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The wavelength, λ, of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or d_{hkl} (also called d-spacings). θ_{hkl} is the angle between the incident ray and the family of lattice planes, and sin θ_{hkl} is inversely proportional to the distance between successive crystal planes or d-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices {hkl}. These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings a, b, and c, and the angles between them α, β, and γ.

The interplanar spacing for a specified set of parallel hkl planes is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the d values for the related families of planes nh, nk, nl are diminished by the factor 1/n (n being an integer: 2, 3, 4, etc.).

Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with it (for a specific λ).

A powder specimen is assumed to be polycrystalline so that at any angle θ_{hkl} there are always crystallites in an orientation allowing diffraction according to Bragg's law.[2] For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as "lines", "reflections", or "Bragg reflections") are characteristic of the crystal lattice (d-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles depend on the perfection and extent of the crystal lattice. Under these conditions, the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion, and structural imperfections, as well as from instrument characteristics.

The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity, and Lorentz factor.

The main characteristics of diffraction line profiles are 2θ position, peak height, peak area, and shape (characterized by, e.g., peak width, or asymmetry, analytical function, and empirical representation). An example of the type of powder patterns obtained for five different solid phases of a substance are shown in *Figure 2*.

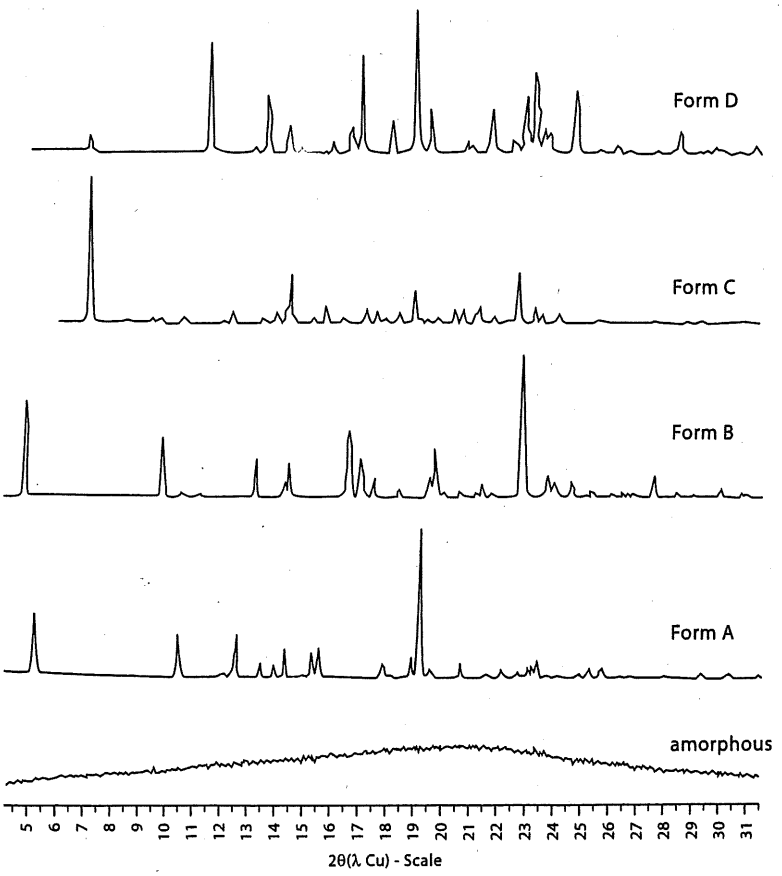


Figure 2. X-ray powder diffraction patterns collected for five different solid phases of a substance (the intensities are normalized).

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more or less uniform background, upon which the peaks are superimposed. Besides specimen preparation, other factors contribute to the background—for example, sample holder, diffuse scattering from air and equipment, and other instrumental parameters such as detector noise and general radiation from the X-ray tube. The peak-to-background ratio can be increased by minimizing background and by choosing prolonged exposure times.

[2] An ideal powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.

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INSTRUMENT

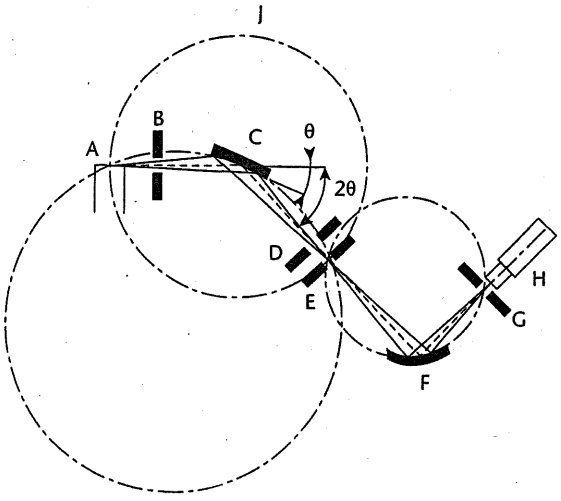
Instrument Setup

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras.

A powder diffractometer generally comprises five main parts: an X-ray source; the incident beam optics, which may perform monochromatization, filtering, collimation, and/or focusing of the beam; a goniometer; the diffraction beam optics, which may include monochromatization, filtering, collimation, and focusing or parallelizing of beam; and a detector. Data collection and data processing systems are also required and are generally included in current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure powder patterns are powder cameras. Replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing, but parafocusing, such as in Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical $\theta/2\theta$ geometry or a vertical θ/θ geometry. For both geometries, the incident X-ray beam forms an angle θ with the specimen surface plane, and the diffracted X-ray beam forms an angle 2θ with the direction of the incident X-ray beam (an angle θ with the specimen surface plane). The basic geometric arrangement is represented in *Figure 3*. The divergent beam of radiation from the X-ray tube (the so-called primary beam) passes through the parallel plate collimators and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle 2θ converge to a line at the receiving slit. A second set of parallel plate collimators and a scatter slit may be placed either behind or before the receiving slit. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-ray quanta are counted by a radiation detector, usually a scintillation counter, a sealed-gas proportional counter, or a position-sensitive solid-state detector such as an imaging plate or CCD detector. The receiving slit assembly and the detector are coupled together and move tangentially to the focusing circle. For $\theta/2\theta$ scans, the goniometer rotates the specimen around the same axis as that of the detector, but at half the rotational speed, in a $\theta/2\theta$ motion. The surface of the specimen thus remains tangential to the focusing circle. The parallel plate collimator limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.



A. X-ray tube
B. Divergence slit
C. Sample
D. Anti-diffusion slit
E. Receiving slit
F. Monochromator
G. Detector receiving slit
H. Detector
J. Focusing circle

Figure 3. Geometric arrangement of the Bragg-Brentano parafocusing geometry.

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5- to 2-mm thickness can also be used for small sample amounts.

X-Ray Radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is converted to heat, which limits the power of the tubes and requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained by using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

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The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation and additional characteristic radiation that depends on the type of anode. Only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources used for X-ray diffraction are vacuum tubes using copper, molybdenum, iron, cobalt, or chromium as anodes; copper, molybdenum, or cobalt X-rays are employed most commonly for organic substances (the use of a cobalt anode can especially be preferred to separate distinct X-ray lines). The choice of radiation to be used depends on the absorption characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the K_α radiation from the anode. Consequently, it is advantageous to make the X-ray beam "monochromatic" by eliminating all the other components of the emission spectrum. This can be partly achieved using K_β filters—that is, metal filters selected as having an absorption edge between the K_α and K_β wavelengths emitted by the tube. Such a filter is usually inserted between the X-ray tube and the specimen. Another more commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as a "monochromator"). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e., K_α and K_β) at different angles so that only one of them may be selected to enter into the detector. It is even possible to separate $K_{\alpha1}$ and $K_{\alpha2}$ radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating K_α and K_β wavelengths is by using curved X-ray mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

RADIATION PROTECTION

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions be taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

SPECIMEN PREPARATION AND MOUNTING

The preparation of the powdered material and the mounting of the specimen in a suitable holder are critical steps in many analytical methods, particularly for X-ray powder diffraction analysis, since they can greatly affect the quality of the data to be collected.[3] The main sources of errors due to specimen preparation and mounting are briefly discussed in the following section for instruments in Bragg-Brentano parafocusing geometry.

Specimen Preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is particularly evident for needle-like or platelike crystals when size reduction yields finer needles or platelets. Preferred orientation in the specimen influences the intensities of various reflections so that some are more intense and others less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution, and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 µm will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately 0.5 µm) may cause line broadening and significant changes to the sample itself, such as

- specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.),
- reduced degree of crystallinity,
- solid-state transition to another polymorph,
- chemical decomposition,
- introduction of internal stress, and
- solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the nonground specimen with that corresponding to a specimen of smaller particle size (e.g., a milled specimen). If the X-ray powder diffraction pattern obtained is of adequate quality considering its intended use, then grinding may not be required.

It should be noted that if a sample contains more than one phase and if sieving is used to isolate particles to a specific size, the initial composition may be altered.

[3] Similarly, changes in the specimen can occur during data collection in the case of a nonequilibrium specimen (temperature, humidity).

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Specimen Mounting

EFFECT OF SPECIMEN DISPLACEMENT

A specimen surface that is offset by D with reference to the diffractometer rotation axis causes systematic errors that are very difficult to avoid entirely; for the reflection mode, this results in absolute $D \cdot \cos\theta$ shifts[4] in 2θ positions (typically of the order of 0.01° in 2θ at low angles

$$[\cos\theta \approx 1]$$

for a displacement D = 15 µm) and asymmetric broadening of the profile toward low 2θ values. Use of an appropriate internal standard allows the detection and correction of this effect simultaneously with that arising from specimen transparency. This effect is by far the largest source of errors in data collected on well-aligned diffractometers.

EFFECT OF SPECIMEN THICKNESS AND TRANSPARENCY

When the XRPD method in reflection mode is applied, it is often preferable to work with specimens of "infinite thickness". To minimize the transparency effect, it is advisable to use a nondiffracting substrate (zero background holder)—for example, a plate of single crystalline silicon cut parallel to the 510 lattice planes.[5] One advantage of the transmission mode is that problems with sample height and specimen transparency are less important.

The use of an appropriate internal standard allows the detection and correction of this effect simultaneously with that arising from specimen displacement.

CONTROL OF THE INSTRUMENT PERFORMANCE

The goniometer and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to adequately minimize systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought while performing the alignment procedure. There are many different configurations, and each supplier's equipment requires specific alignment procedures. The overall diffractometer performance must be tested and monitored periodically, using suitable certified reference materials. Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

QUALITATIVE PHASE ANALYSIS (IDENTIFICATION OF PHASES)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its X-ray powder pattern to the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ-diffraction angles or d-spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the comparison data can be based on either a more or less extended 2θ range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list of d-spacings and normalized intensities, I_{norm}, a so-called (d, I_{norm}) list extracted from the pattern, is the crystallographic fingerprint of the material and can be compared to (d, I_{norm}) lists of single-phase samples compiled in databases.

For most organic crystals, when using Cu K_α radiation, it is appropriate to record the diffraction pattern in a 2θ-range from as near 0° as possible to at least 40°. The agreement in the 2θ-diffraction angles between specimen and reference is within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions, and as such, shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2-θ positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g., inorganic salts), it may be necessary to extend the 2θ region scanned to well beyond 40°. It is generally sufficient to scan past the 10 strongest reflections identified in single-phase X-ray powder diffraction database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

- noncrystallized or amorphous substances,
- the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10% m/m),
- pronounced preferred orientation effects,
- the phase has not been filed in the database used,
- the formation of solid solutions,
- the presence of disordered structures that alter the unit cell,

[4] Note that a goniometer zero alignment shift would result in a constant shift on all observed 2θ-line positions; in other words, the whole diffraction pattern is, in this case, translated by an offset of Z° in 2θ.

[5] In the case of a thin specimen with low attenuation, accurate measurements of line positions can be made with focusing diffractometer configurations in either transmission or reflection geometry. Accurate measurements of line positions on specimens with low attenuation are preferably made using diffractometers with parallel beam optics. This helps to reduce the effects of specimen thickness.

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- the specimen comprises too many phases,
- the presence of lattice deformations,
- the structural similarity of different phases.

QUANTITATIVE PHASE ANALYSIS

If the sample under investigation is a mixture of two or more known phases, of which not more than one is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can in many cases be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines,[6] or on the full pattern. These integrated intensities, peak heights, or full-pattern data points are compared to the corresponding values of reference materials. These reference materials must be single phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require, in particular, homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects.

In favorable cases, amounts of crystalline phases as small as 10% may be determined in solid matrices.

Polymorphic Samples

For a sample composed of two polymorphic phases *a* and *b*, the following expression may be used to quantify the fraction F_α of phase *a*:

$$F_\alpha = 1/[1 + K(I_b/I_a)]$$

The fraction is derived by measuring the intensity ratio between the two phases, knowing the value of the constant K. K is the ratio of the absolute intensities of the two pure polymorphic phases I_{oa}/I_{ob}. Its value can be determined by measuring standard samples.

Methods Using a Standard

The most commonly used methods for quantitative analysis are

- the external standard method,
- the internal standard method, and
- the spiking method (also often called the standard addition method).

The external standard method is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material can be used that has a crystallite size and X-ray absorption coefficient comparable to those of the components of the sample and with a diffraction pattern that does not overlap at all that of the sample to be analyzed. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the internal standard method, requires precise measurement of diffraction intensities.

In the spiking method (or standard addition method), some of the pure phase a is added to the mixture containing the unknown concentration of a. Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative x-intercept is the concentration of the phase a in the original sample.

ESTIMATE OF THE AMORPHOUS AND CRYSTALLINE FRACTIONS

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

- If the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances, as described above. The amorphous fraction is then deduced indirectly by subtraction.
- If the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase (the "degree of crystallinity") can be estimated by measuring three areas of the diffractogram:

  A = total area of the peaks arising from diffraction from the crystalline fraction of the sample,

  B = total area below area A,

  C = background area (due to air scattering, fluorescence, equipment, etc).

When these areas have been measured, the degree of crystallinity can be roughly estimated as:

$$\% \text{ crystallinity} = 100A/(A + B - C)$$

[6] If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components are not known, the Pawley method or the partial least-squares (PLS) method can be used.

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It is noteworthy that this method does not yield an absolute degree of crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

SINGLE CRYSTAL STRUCTURE

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low, and the scattering properties are normally very low. For any given crystalline form of a substance, the knowledge of the crystal structure allows for calculating the corresponding XRPD pattern, thereby providing a preferred orientation-free reference XRPD pattern, which may be used for phase identification.

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