2006

USP 29 THE UNITED STATES PHARMACOPEIA

NF 24 THE NATIONAL FORMULARY

By authority of The United States Pharmacopeial Convention, meeting at Washington, D.C., March 9–13, 2005. Prepared by the Council of Experts and published by the Board of Trustees

Official from January 1, 2006

The designation on the cover of this publication, "USP NF 2006," is for ease of identification only. The publication contains two separate compendia: *The United States Pharmacopeia*, Twenty-Ninth Revision, and the *National Formulary*, Twenty-Fourth Edition.

THE UNITED STATES PHARMACOPEIAL CONVENTION
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12601 Twinbrook Parkway, Rockville, MD 20852
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ISSN 0195-7996
ISBN 1-889788-39-2
Printed in Canada by Webcom Limited, Toronto, Ontario

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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.

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.

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*.

⟨197⟩ SPECTROPHOTOMETRIC IDENTIFICATION TESTS

Spectrophotometric tests contribute meaningfully toward th identification of many compendial chemical substances. The te procedures that follow are applicable to substances that absorb I and/or UV radiation (see *Spectrophotometry and Light-Scatterin* ⟨851⟩).

The IR absorption spectrum of a substance, compared with th obtained concomitantly for the corresponding USP Referen Standard, provides perhaps the most conclusive evidence of th identity of the substance that can be realized from any single test. Th UV absorption spectrum, on the other hand, does not exhibit a hig degree of specificity. Conformance with both IR absorption and U absorption test specifications, as called for in a large proportion compendial monographs, leaves little doubt, if any, regarding th identity of the specimen under examination.

INFRARED ABSORPTION

Six methods are indicated for the preparation of previously drie test specimens and Reference Standards for analysis. The referenc ⟨197K⟩ in a monograph signifies that the substance unde examination is mixed intimately with potassium bromide. Th reference ⟨197M⟩ in a monograph signifies that the substance und examination is finely ground and dispersed in mineral oil. Th reference ⟨197F⟩ in a monograph signifies that the substance und examination is suspended neat between suitable (for example, sodiu chloride or potassium bromide) plates. The reference ⟨197S⟩ signifi that a solution of designated concentration is prepared in the solve specified in the individual monograph, and the solution is examine in 0.1-mm cells unless a different cell path length is specified in th individual monograph. The reference ⟨197A⟩ signifies that th substance under examination is intimately in contact with an intern reflection element for attenuated total reflectance (ATR) analysis. Th reference ⟨197E⟩ signifies that the substance under examination pressed as a thin sample against a suitable plate for IR microscop analysis. The ATR ⟨197A⟩ and the ⟨197E⟩ techniques can be used alternative methods for ⟨197K⟩, ⟨197M⟩, ⟨197F⟩, and ⟨197S⟩ whe testing is performed qualitatively and the Reference Standard spect are similarly obtained.

Record the spectra of the test specimen and the corresponding US Reference Standard over the range from about 2.6 µm to 15 µm (380 cm^{-1} to 650 cm^{-1}) unless otherwise specified in the individu monograph. The IR absorption spectrum of the preparation of the te specimen, previously dried under conditions specified for th corresponding Reference Standard unless otherwise specified, unless the Reference Standard is to be used without drying, exhibi maxima only at the same wavelengths as that of a similar preparatio of the corresponding USP Reference Standard.

Differences that may be observed in the spectra so obtaine sometimes are attributed to the presence of polymorphs, which a not always acceptable (see *Procedure* under *Spectrophotometry an Light-Scattering* ⟨851⟩). Unless otherwise directed in the individu monograph, therefore, continue as follows. If a difference appears i the IR spectra of the analyte and the standard, dissolve equal portion of the test specimen and the Reference Standard in equal volumes of suitable solvent, evaporate the solution to dryness in simila containers under identical conditions, and repeat the test on th residues.

ULTRAVIOLET ABSORPTION

The reference ⟨197U⟩ in a monograph signifies that a test solutio and a Standard solution are examined spectrophotometrically, in 1 cm cells, over the spectral range from 200 to 400 nm unless otherwis specified in the individual monograph.

Dissolve a portion of the substance under examination in th

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Record and compare the spectra concomitantly obtained for the test solution and the Standard solution. Calculate absorptivities and/or absorbance ratios where these criteria are included in an individual monograph. Unless otherwise specified, absorbances indicated for these calculations are those measured at the maximum absorbance at about the wavelength 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 (min) and (sh) are used to indicate a minimum and shoulder, respectively, in an absorption spectrum. The requirements are met if the UV absorption spectra of the test solution and the Standard solution exhibit maxima and minima at the same wavelengths and absorptivities and/or absorbance ratios are within specified limits.

⟨201⟩ THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

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 $500J$ 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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