

**Tartrate**—Dissolve a few mg of a tartrate salt in 2 drops of sodium metaperiodate solution (1 in 20). Add a drop of 1 N sulfuric acid, and after 5 minutes add a few drops of sulfuric acid followed by a few drops of fuchsin–sulfurous acid TS: a reddish-pink color is produced within 15 minutes.

**Thiocyanate**—With ferric chloride TS, solutions of thiocyanates yield a red color that is not destroyed by moderately concentrated mineral acids.

**Thiosulfate**—With hydrochloric acid, solutions of thiosulfates yield a white precipitate that soon turns yellow, and sulfur dioxide, which blackens filter paper moistened with mercurous nitrate TS. The addition of ferric chloride TS to solutions of thiosulfates produces a dark violet color that quickly disappears.

**Zinc**—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. Ammonium sulfide TS produces a similar precipitate in neutral and in alkaline solutions. With potassium ferrocyanide TS, zinc salts in solution yield a white precipitate that is insoluble in 3 N hydrochloric acid.

graphic 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  $\mu$ L 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*.

## 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.

**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  $\times$  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  $\mu$ L 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 chromato-

## 197 SPECTROPHOTOMETRIC IDENTIFICATION TESTS

Spectrophotometric tests contribute meaningfully toward the identification of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb IR and/or UV radiation (see *Spectrophotometry and Light-Scattering* (851)).

The IR absorption spectrum of a substance, compared with that obtained concomitantly for 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 absorption spectrum, on the other hand, does not exhibit a high degree of specificity. Conformance with both IR absorption and UV absorption test specifications, as called for in a large proportion of compendial monographs, leaves little doubt, if any, regarding the identity of the specimen under examination.

## INFRARED ABSORPTION

Seven methods are indicated for the preparation of previously dried test specimens and Reference Standards for anal-

ysis. The reference <197K> in a monograph signifies that the substance under examination is mixed intimately with potassium bromide. The reference <197M> in a monograph signifies that the substance under examination is finely ground and dispersed in mineral oil. The reference <197F> in a monograph signifies that the substance under examination is suspended neat between suitable (for example, sodium chloride or potassium bromide) plates. The reference <197S> signifies that a solution of designated concentration is prepared in the solvent specified in the individual monograph, and the solution is examined in 0.1-mm cells unless a different cell path length is specified in the individual monograph. The reference <197A> signifies that the substance under examination is intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis. The reference <197E> signifies that the substance under examination is pressed as a thin sample against a suitable plate for IR microscopic analysis. The reference <197D> in a monograph signifies that the substance under examination is mixed intimately with an IR-transparent material and transferred to a sample container for diffuse reflection (DR) analysis. The ATR <197A> and the <197E> techniques can be used as alternative methods for <197K>, <197M>, <197F>, and <197S> where testing is performed qualitatively and the Reference Standard spectra are similarly obtained.

Record the spectra of the test specimen and the corresponding USP Reference Standard over the range from about 2.6  $\mu\text{m}$  to 15  $\mu\text{m}$  (3800  $\text{cm}^{-1}$  to 650  $\text{cm}^{-1}$ ) unless otherwise specified in the individual monograph. The IR absorption spectrum of the preparation of the test specimen, previously dried under conditions specified for the corresponding Reference Standard unless otherwise specified, or 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.

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

### ULTRAVIOLET ABSORPTION

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

Dissolve a portion of the substance under examination in the designated *Medium* to obtain a test solution having the concentration specified in the monograph for *Solution*. Similarly prepare a Standard solution containing the corresponding USP Reference Standard.

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 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* <621>) apply 10  $\mu\text{L}$  of this solution and 10  $\mu\text{L}$  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