

〈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 μ L of this solution and 10 μ 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 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 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 μ L 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.

USP Reference Standards (11)

USP Bacitracin Zinc RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Official