

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 × 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 RF 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 °C 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 RF value, intensity, and appearance to that obtained from the Standard Solution.