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Tobramycin and Dexamethasone Ophthalmic Ointment

DEFINITION

Tobramycin and Dexamethasone Ophthalmic Ointment contains NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$), and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Solution A: 100 mg/mL of [sodium sulfate](#) in [water](#)

Diluent: [Butyl alcohol](#) and [pyridine](#) (100:1)

Standard solution: 6 mg/mL of [USP Tobramycin RS](#) in [water](#)

Sample solution: To 1 g of Ophthalmic Ointment in a test tube add 2 mL of [chloroform](#), and shake to dissolve. Add 0.5 mL of *Solution A*, shake vigorously, and centrifuge. Use the clear supernatant aqueous liquid. If, after centrifuging, an oily film remains on top of the supernatant aqueous liquid, transfer the supernatant aqueous liquid to a second test tube, and wash it with 2 mL of [chloroform](#).

Solution B: *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See [Chromatography \(621\)](#), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of [chromatographic silica gel mixture](#)

Application volume: 3 µL

Developing solvent system: [Methanol](#), [chloroform](#), and [ammonium hydroxide](#) (60:25:30)

Spray reagent: 10 mg/mL of [ninhydrin](#) in *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Solution B*

Apply the *Standard solution*, the *Sample solution*, and *Solution B* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in 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, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.

Acceptance criteria: Tobramycin appears as a pink spot, and the R_f values of the spots of the *Sample solution* and of *Solution B*, respectively, correspond to those of the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Dexamethasone.

ASSAY

• TOBRAMYCIN

Mobile phase: Dissolve 2.0 g of [tris\(hydroxymethyl\)aminomethane](#) in 800 mL of [water](#). Add 20 mL of [1 N sulfuric acid](#), and dilute with [acetonitrile](#) to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.

Solution A: 10 mg/mL of [2,4-dinitrofluorobenzene](#) in [alcohol](#). This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of [tris\(hydroxymethyl\)aminomethane](#) in [water](#). This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of [tris\(hydroxymethyl\)aminomethane](#) prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add [dimethyl sulfoxide](#) while mixing, and dilute with [dimethyl sulfoxide](#) to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg of [USP Tobramycin RS](#) prepared as follows. Transfer 55 mg of [USP Tobramycin RS](#) into a 50-mL volumetric flask. Add 1 mL of [1 N sulfuric acid](#) and enough [water](#) to dissolve it, and dilute with [water](#) to volume.

Standard solution: 0.22 mg/mL of [USP Tobramycin RS](#) from *Standard stock solution* in [water](#)

Sample solution: Nominally 0.045 mg/mL of tobramycin from Ophthalmic Ointment in [water](#) prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 4.5 mg of tobramycin to a separator. Add 50 mL of [ether](#), and extract with four 20- to 25-mL portions of [water](#). Combine the water extracts in a 100-mL volumetric flask, and dilute with [water](#) to volume.

Derivatized standard solution, Derivatized sample solution, and Blank solution: Proceed as follows. Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 15.0 mL of the *Sample solution*, and 4.0 mL of [water](#). To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at $60 \pm 2^\circ$, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add [acetonitrile](#) to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with [acetonitrile](#) to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in [acetonitrile](#). Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing [L1](#)

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of tobramycin from the *Derivatized sample solution*

r_S = peak area of tobramycin from the *Derivatized standard solution*

C_S = concentration of [USP Tobramycin RS](#) in the *Standard solution* (mg/mL)

C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)

P = potency of tobramycin in [USP Tobramycin RS](#) (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

• DEXAMETHASONE

Mobile phase: [Methanol](#) and [water](#) (55:45)

Diluent: [Methanol](#) and [water](#) (75:25)

System suitability stock solution: 1 mg/mL of anhydrous chlorobutanol and 0.2 mg/mL of [USP Dexamethasone RS](#) in *Diluent*

System suitability solution: 0.3 mg/mL of anhydrous chlorobutanol and 0.06 mg/mL of [USP Dexamethasone RS](#) in *Diluent* prepared as follows. Transfer 15.0 mL of the *System suitability stock solution* to a separator containing about 50 mL of [n-hexane](#), and shake. Allow the layers to separate, and drain the lower phase into a 50-mL volumetric flask. Repeat the extraction with two 15-mL portions of *Diluent*, combining the lower phase from each extraction in the same 50-mL volumetric flask. Dilute with *Diluent* to volume.

Standard stock solution: 0.2 mg/mL of [USP Dexamethasone RS](#) in *Diluent*

Standard solution: 0.06 mg/mL of [USP Dexamethasone RS](#) in *Diluent* prepared as follows. Transfer 15.0 mL of the *Standard stock solution* to a separator containing about 50 mL of [n-hexane](#), and shake. Allow the layers to separate, and drain the lower phase into a 50-mL volumetric flask. Repeat the extraction with two 15-mL portions of *Diluent*, combining the lower phase from each extraction in the same 50-mL volumetric flask. Dilute with *Diluent* to volume.