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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 ± 2°, 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.

Sample solution: Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone to a separator containing 50 mL of *n*-hexane, and shake. Add 15 mL of *Diluent*, 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, mix, and centrifuge. Use the clear solution.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 8.0-mm × 10-cm; packing L1

Flow rate: 3 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for chlorobutanol and dexamethasone are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between chlorobutanol and dexamethasone, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Column efficiency: NLT 350 theoretical plates, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Ointment taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)

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

P = potency of dexamethasone in USP Dexamethasone RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS ⟨71⟩:** Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests ⟨771⟩, Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Dexamethasone RS

USP Tobramycin RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
TOBRAMYCIN AND DEXAMETHASONE OPHTHALMIC OINTMENT	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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