Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-Aug-2017
Document Type: USP Monographs
DocId: GUID-09279FE0-36AA-4C6D-9B8E-89B0E4940CBB_2_en-US
DOI: https://doi.org/10.31003/USPNF_M34913_02_01
DOI Ref: 8ova8

Gentamicin and Prednisolone Acetate Ophthalmic Ointment

DEFINITION

Gentamicin and Prednisolone Acetate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of gentamicin, and NLT 90.0% and NMT 110.0% of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 1 mg/mL of USP Gentamicin Sulfate RS in water

Sample solution: Nominally 1 mg/mL of gentamicin from Ophthalmic Ointment prepared as follows. Shake a quantity of Ophthalmic Ointment, containing nominally 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer.

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Mix chloroform, methanol, and ammonium hydroxide (20:13:10), allow to separate, and use the lower layer.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and *Sample solution* to the plate. Place the plate in a chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals.

Acceptance criteria: The intensities and R_F values of the three principal spots from the *Sample solution* correspond to those from the *Standard solution*.

• **B.** The retention time of the prednisolone acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Prednisolone Acetate*.

ASSAY

• **Gentamicin**

(See *Antibiotics—Microbial Assays ⟨81⟩*.)

Sample solution: Shake a portion of Ophthalmic Ointment containing nominally 1 mg of gentamicin with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.3* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.3* to a suitable volume to obtain a *Test Dilution* having a gentamicin concentration that is nominally equivalent to the median level of the standard.

Analysis: Proceed as directed in the chapter.

Acceptance criteria: 90.0%–120.0%

• **Prednisolone Acetate**

Mobile phase: Acetonitrile and water (40:60)

Internal standard solution: 2.7 mg/mL of fluorometholone acetate in methanol

Standard stock solution: 0.38 mg/mL of USP Prednisolone Acetate RS in methanol

Standard solution: 0.06 mg/mL of USP Prednisolone Acetate RS in methanol prepared as follows. Transfer 8.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution,* dilute with methanol to volume, and shake vigorously for 30 s. Allow the layers to separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume. Centrifuge a portion of this solution, and use the clear supernatant.

Sample solution: Nominally 0.06 mg/mL of prednisolone acetate from Ophthalmic Ointment in methanol prepared as follows. Transfer a portion of Ophthalmic Ointment, containing nominally 3 mg of prednisolone acetate, to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution*, dilute with methanol to volume, and shake vigorously for 30 s. Allow the layers to

separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume. Centrifuge a portion of this solution, and use the clear supernatant.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the prednisolone acetate and fluorometholone acetate peaks

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area response ratio of prednisolone acetate to fluorometholone acetate from the *Sample solution*

R_S = peak area response ratio of prednisolone acetate to fluorometholone acetate from the *Standard solution*

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

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

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. Store at controlled room temperature.

• **USP Reference Standards ⟨11⟩**

USP Gentamicin Sulfate RS

USP Prednisolone Acetate RS

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

Topic/Question	Contact	Expert Committee
GENTAMICIN AND PREDNISOLONE ACETATE OPHTHALMIC OINTMENT	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 39(5)

Current DocID: GUID-09279FE0-36AA-4C6D-9B8E-89B0E4940CBB_2_en-US
Previous DocID: GUID-09279FE0-36AA-4C6D-9B8E-89B0E4940CBB_1_en-US
DOI: https://doi.org/10.31003/USPNF_M34913_02_01

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