Status: Currently Official on 15-Feb-2025
Official Date: Official Prior to 2013
Document Type: USP Monographs
DocId: GUID-C234E5D3-C294-45A1-ABC0-9712774AE619_2_en-US
DOI: https://doi.org/10.31003/USPNF_M34909_02_01
DOI Ref: 9m8f4

Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution

» Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone acetate ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Betamethasone Acetate RS
USP Gentamicin Sulfate RS

Identification—

A: Apply 10 µL of Ophthalmic Solution and 10 µL of a Standard solution containing 5 mg per mL of USP Gentamicin Sulfate RS in water to a thin-layer chromatographic plate (see *Chromatography ⟨621⟩*) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and in a paper-lined tank develop the chromatogram in a solvent system consisting of the lower phase mixture of dichloromethane, methanol, and ammonium hydroxide (1:1:1) 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 plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone acetate*.

pH ⟨791⟩: between 5.5 and 7.0.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* in *Test for Sterility of the Product To Be Examined*.

Other requirements—It meets the requirements under *Antimicrobial Effectiveness Tests ⟨51⟩*.

Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays ⟨81⟩*, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for betamethasone acetate—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (8:7). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Internal standard solution—Dissolve a quantity of *o*-phenylphenol in methanol to obtain a solution containing about 0.55 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.45 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.09 mg of USP Betamethasone Acetate RS per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 2 mg of betamethasone acetate, to a 10-mL volumetric flask. Dilute with methanol to volume, and mix. Transfer a portion of this solution to a centrifuge tube, and centrifuge. Transfer 4.0 mL of the clear supernatant to a 10-mL volumetric flask. Add 1.0 mL of *Internal standard solution*, dilute with a mixture of methanol and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 254-mm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative retention times are about 1.3 for *o*-phenylphenol and 1.0 for betamethasone acetate; the resolution, *R,* between the betamethasone acetate and *o*-phenylphenol peaks is not less than 3.9; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone acetate

($C_{24}H_{31}FO_6$) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Acetate RS, calculated on the anhydrous basis, in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation*; and R_U and R_S are the ratios of the betamethasone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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

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

Chromatographic Database Information: Chromatographic Database

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Current DocID: GUID-C234E5D3-C294-45A1-ABC0-9712774AE619_2_en-US
Previous DocID: GUID-C234E5D3-C294-45A1-ABC0-9712774AE619_1_en-US
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