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Gentamicin Sulfate and Betamethasone Valerate Otic Solution

» Gentamicin Sulfate and Betamethasone Valerate Otic Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of beta methasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in tight containers.

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

USP Reference standards ⟨11⟩—

USP Beclomethasone Dipropionate RS
USP Betamethasone Valerate RS
USP Gentamicin Sulfate RS

Identification—

A: Transfer an amount of Otic Solution, equivalent to about 3 mg of gentamicin, to a centrifuge tube. Dissolve an accurately weighed quantity of USP Gentamicin Sulfate RS quantitatively in water to obtain a solution having a concentration of about 5 mg per mL. Transfer 1.0 mL of this solution to a centrifuge tube. To each centrifuge tube add 3 mL of water and 4 g of potassium carbonate, and mix. Add 1 mL of isopropyl alcohol to each tube, mix, and centrifuge. Use the upper phases as the test solution and Standard solution, respectively. Separately apply 20 µL of each of these solutions 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 develop the chromatogram in a solvent system consisting of the lower phase of a mixture of methanol, dichloromethane, 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*.

pH ⟨791⟩: between 3.0 and 5.0.

Microbial enumeration tests ⟨61⟩ and Tests for specified microorganisms ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.

Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays ⟨81⟩*, using an accurately measured volume of Otic 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—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Diluent—Prepare a mixture of methanol and glacial acetic acid (1000:1).

Internal standard solution—Dissolve a quantity of USP Beclomethasone Dipropionate RS in methanol to obtain a solution containing about 0.8 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.2 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 5.0 mL of *Internal standard solution,* dilute with *Diluent* to volume, and mix to obtain a solution having a known concentration of about 0.24 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Add 5.0 mL of *Internal standard solution* to a 10-mL volumetric flask. Transfer to the flask an accurately measured volume of Otic Solution, equivalent to about 2 mg of betamethasone, dilute with methanol to volume, and mix. Centrifuge a portion of this solution, and use the clear supernatant as the *Assay preparation*.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 254-nm 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 1.6 for beclomethasone dipropionate and 1.0 for

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betamethasone valerate; the resolution, *R,* between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 3.5; 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 ($C_{22}H_{29}FO_5$) in each mL of the Otic Solution taken by the formula:

$$(392.47/476.58)(10C/V)(R_U/R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the betamethasone valerate 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 VALERATE OTIC SOLUTION	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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