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Gentamicin Sulfate and Betamethasone Valerate Ointment

» Gentamicin Sulfate and Betamethasone Valerate Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone 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 collapsible tubes or other tight containers.

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

USP Reference standards ⟨11⟩—

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

Identification—

A: Transfer an amount of Ointment, equivalent to about 15 mg of gentamicin, to a centrifuge tube, and add 10 mL of a mixture of methanol and 0.1 N hydrochloric acid (4:1) and 25 mL of solvent hexane. Rotate for 30 minutes, and centrifuge. Discard the upper phase. Apply 25 µL of the lower phase and 25 µL of a Standard solution containing 3 mg per mL of USP Gentamicin Sulfate RS in a mixture of methanol and 0.1 N hydrochloric acid (4:1) to a suitable 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 chloroform, 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 spots 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*.

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*.

Minimum fill ⟨755⟩: meets the requirements.

Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays ⟨81⟩*, using an accurately weighed quantity of Ointment, equivalent to about 3 mg of gentamicin, shaken with about 50 mL of ether in a separator and extracted with three 25-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute 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 methanol and water (475:300). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Diluent—Transfer 25 mL of water to a 500-mL volumetric flask. Add 2.5 mL of glacial acetic acid, dilute with methanol to volume, and mix.

Internal standard solution—Dissolve a quantity of USP Beclomethasone Dipropionate RS in *Diluent* to obtain a solution containing about 0.4 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 0.45 mg per mL. Transfer 5.0 mL of this solution to a stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.15 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 2 mg of beta methasone, to a 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution* and 5.0 mL of *Diluent*, and shake vigorously for 10 minutes. Place the tube in an ice–methanol bath for 15 minutes, then centrifuge to separate the phases. Transfer the clear supernatant to a stoppered flask, and allow to warm to room temperature (*Assay preparation*).

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak

responses as directed for *Procedure:* the relative retention times are about 1.5 for beclomethasone dipropionate and 1.0 for 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 20 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 the portion of Ointment taken by the formula:

$$(392.47/476.58)(15C)(R_U/R_S)$$

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;* 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 OINTMENT	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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