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 (C₂₄H₃₁FO₆).

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 Ri 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 (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 Antibacterial 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 No. 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. 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-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 1.3 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 (C₂₄H₃₁FO₆) in each mL of the Ophthalmic Solution taken by the formula:

\[
25(C / V)(R_s / R_i)
\]

in which C is the concentration, in mg per mL, of USP Betamethasone Acetate RS, calculated on anhydrous basis, in the Standard preparation; V is the volume, in mL, of Ophthalmic Solution taken to prepare the Assay preparation; and Rs and Ri 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.

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 (C₂₂H₂₉FO₃).

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 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 Ri 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 (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 Anti-microbial 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 No. 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. 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.
minutes: the $R_t$ 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**.

**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 No. 3**. Combine the aqueous extracts, and dilute quantitatively and stepwise with **Buffer No. 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 Betamethasone V alerate RS in **Diluent** to obtain a solution containing about 0.4 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone V alerate 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 stopped 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 betamethasone, 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 supernatant to a stopped 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, $R_t$, 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:

\[
(392.47 \div 476.58)(150)(R_t / R_i)
\]

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 V alerate RS in the **Standard preparation**; and $R_t$ and $R_i$ 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.

**Gentamicin Sulfate and Betamethasone Valerate Otic Solution**

- Gentamicin Sulfate and Betamethasone V alerate Otic Solution contains not less than 90.0 per cent and not more than 125.0 per cent 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 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 $R_t$ 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 No. 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).