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Delete the following:

▲Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension

» Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension is a suspension of Penicillin G Procaine, Neomycin Sulfate, Polymyxin B Sulfate, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate in a suitable vehicle. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of neomycin, penicillin G, and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocortisone acetate ($C_{23}H_{32}O_6$) and hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$).

Packaging and storage—Preserve in well-closed containers.

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

USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
USP Hydrocortisone Hemisuccinate RS
USP Neomycin Sulfate RS
USP Penicillin G Potassium RS
USP Polymyxin B Sulfate RS

Identification—

• *A:* Shake a quantity of Topical Suspension, equivalent to about 50,000 USP Polymyxin B Units, with 20 mL of chloroform, add 5 mL of 0.1 N hydrochloric acid, shake vigorously, centrifuge, and use the clear supernatant liquid as the *Sample solution.* Apply separately 10 µL of the *Sample solution* and 10 µL of a *Standard solution* of USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid containing 10,000 USP Polymyxin B Units per mL to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture (see *Chromatography* ⟨621⟩). Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol, water, and ammonium hydroxide (24:17:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 105° for 5 minutes. Spray the plate with a 1 in 200 solution of ninhydrin in butyl alcohol, and heat the plate at 105° for 15 minutes: the R_f value of the principal spot in the chromatogram obtained from the *Sample solution* corresponds to that of the principal spot in the chromatogram obtained from the *Standard solution*.

• *B:* It responds to the *Identification* test under *Penicillin G Procaine Intramammary Infusion*.

• *C:* The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate* exhibits major peaks for hydrocortisone acetate and hydrocortisone sodium succinate, the retention times of which, relative to that of the internal standard, correspond to those for hydrocortisone acetate and hydrocortisone hemisuccinate exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate*.

Water Determination, ⟨921⟩*Method I***:** not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for penicillin G—Proceed with the Topical Suspension as directed in the *Assay for penicillin G* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for neomycin—Proceed with the Topical Suspension as directed in the *Assay for neomycin* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for polymyxin B—Proceed with the Topical Suspension as directed in the *Assay for polymyxin B* under *Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for hydrocortisone acetate and hydrocortisone sodium succinate—

Mobile phase—Prepare a filtered and degassed mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (544:544:58:29:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Extraction solution—Prepare a mixture of chloroform and glacial acetic acid (1000:30).

Internal standard solution—Prepare a solution in tetrahydrofuran containing about 1.4 mg of methylprednisolone per mL.

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Standard preparation—Transfer about 7.5 mg of USP Hydrocortisone Acetate RS, accurately weighed, and 7.5*J* mg of USP Hydrocortisone Hemisuccinate RS, accurately weighed, to a conical flask, *J* being the ratio of the labeled amount, in mg, of hydrocortisone sodium succinate to the labeled amount, in mg, of hydrocortisone acetate in the Topical Suspension. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution*, and mix.

Resolution solution—Dissolve about 3.7 mg of penicillin G procaine in 10 mL of *Standard preparation*.

Assay preparation—Transfer an accurately measured portion of well-mixed Topical Suspension, equivalent to about 7.5 mg of hydrocortisone acetate, to a conical flask. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution*, and shake by mechanical means for about 15 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation*.

Chromatographic system

(see *Chromatography, ⟨621⟩*)—The liquid chromatograph is equipped with a 254-nm detector, a guard column containing packing L3, and a 3.9-mm × 30-cm analytical column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and measure the peak responses as directed for *Procedure:* the relative retention times for penicillin G procaine, hydrocortisone acetate, hydrocortisone hemisuccinate, and methylprednisone are about 0.3, 0.4, 0.7, and 1.0, respectively, and the resolution, *R,* between penicillin G procaine and hydrocortisone acetate is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation of the ratios of the hydrocortisone acetate peak to the internal standard peak and the hydrocortisone sodium succinate peak to the internal standard peak is not more than 2%.

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 hydrocortisone acetate in the portion of Topical Suspension taken by the formula:

$$W(R_U/R_S)$$

in which *W* is the quantity, in mg, of USP Hydrocortisone Acetate RS taken to prepare the *Standard preparation*, and R_U and R_S are the ratios of the hydrocortisone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of hydrocortisone sodium succinate in the portion of Topical Suspension taken by the formula:

$$(484.52/462.54)(W)(R_U/R_S)$$

in which 484.52 and 462.54 are the molecular weights of hydrocortisone sodium succinate and anhydrous hydrocortisone hemisuccinate, respectively, *W* is the quantity, in mg, of USP Hydrocortisone Hemisuccinate RS taken to prepare the *Standard preparation*, and R_U and R_S are the ratios of the hydrocortisone hemisuccinate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.▲ (USP 1-Dec-2024)

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PENICILLIN G, NEOMYCIN, POLYMYXIN B, HYDROCORTISONE ACETATE, AND HYDROCORTISONE SODIUM SUCCINATE TOPICAL SUSPENSION	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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