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Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment

» Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of neomycin, and not less than 92.5 percent and not more than 117.5 percent of the labeled amounts of isoflupredone acetate ($C_{23}H_{29}FO_6$) and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or well-closed containers.

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

USP Reference standards ⟨11⟩—

USP Isoflupredone Acetate RS
USP Neomycin Sulfate RS
USP Tetracaine Hydrochloride RS

Identification—

A: *Thin-Layer Chromatographic Identification Test ⟨201⟩*—

Test solution—To 2 g of Ointment in a centrifuge tube add 25 mL of chloroform, and heat at 60° for 5 minutes, with occasional shaking. Centrifuge, discard the chloroform layer, add 5 mL of water, shake, and filter. Use the filtrate.

Standard solution—Prepare a solution containing 2 mg of USP Neomycin Sulfate RS per mL.

Application volume: 1 µL.

Developing solvent system: a mixture of water, butyl alcohol, glacial acetic acid, and pyridine (35:30:22:6).

Spray reagent: a solution of triketohydrindene hydrate in butyl alcohol (2 in 1000).

Procedure—Proceed as directed in the chapter, except to locate the spots by spraying with *Spray reagent* and heating at 100° for 5 minutes.

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

Minimum fill ⟨755⟩: meets the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, a mixture of methanol and chloroform (3:2) being used instead of methanol in the titration vessel and the titration vessel being heated to between 45° and 55°.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays ⟨81⟩*. Place an accurately weighed portion of Ointment in a centrifuge tube with 25 mL of chloroform. Heat at 60° for 3 minutes, shake until the Ointment is dissolved, centrifuge, and remove and discard the chloroform. Add 15 mL of chloroform, shake, centrifuge, and remove and discard the chloroform. Add 5.0 mL of water and 15 mL of chromatographic *n*-heptane, shake, centrifuge, and remove and discard the *n*-heptane layer. Dilute an accurately measured volume of the water layer quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.

Assay for isoflupredone acetate—

Mobile phase, *Diluent*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Isoflupredone Acetate.*

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution*, 32.0 mL of *Diluent*, and about 10 glass beads. Shake for about 15 minutes, centrifuge, and use the clear chloroform portion.

Procedure—Proceed as directed in the *Assay* under *Isoflupredone Acetate.* Calculate the quantity, in mg, of isoflupredone acetate ($C_{23}H_{29}FO_6$) in the portion of Ointment taken by the formula:

$$W_S(R_U/R_S)$$

in which the terms are as defined therein.

Assay for tetracaine hydrochloride—

Standard preparation—Prepare a solution in chloroform having a known concentration of about 5.0 µg of USP Tetracaine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1.25 mg of tetracaine hydrochloride, to a 250-mL volumetric flask, add about 100 mL of chloroform, and warm on a steam bath for about 3 minutes to dissolve the Ointment. Cool to room temperature, dilute with chloroform to volume, and mix.

Blank solution—Transfer an accurately weighed portion of the ointment base, equivalent to the weight used in the *Assay preparation*, to a 250-mL volumetric flask. Add 100 mL of chloroform, warm on a steam bath for about 3 minutes to dissolve, and allow to stand until the solution has equilibrated to room temperature. Dilute with chloroform to volume, and mix well.

Procedure—Concomitantly determine the absorbances of the *Standard preparation,* the *Blank solution*, and the *Assay preparation* with a suitable spectrophotometer at the wavelength of maximum absorbance at about 310 nm, using chloroform to zero the instrument. Calculate the absorbance of the *Blank solution*, A_B, adjusted for weight difference between the *Assay preparation* and the *Blank solution*, by the formula:

$$A(W_T/W_B)$$

in which A is the absorbance of the *Blank solution;* W_T is the weight, in mg, of Ointment taken to prepare the *Assay preparation;* and W_B is the weight, in mg, of the ointment base taken to prepare the *Blank solution.* Calculate the quantity, in mg, of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Ointment taken by the formula:

$$250C\,[(A_U - A_B)/A_S]$$

in which C is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation;* A_B is as obtained above; and A_U and A_S are the absorbances of 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
NEOMYCIN SULFATE, ISOFLUPREDONE ACETATE, AND TETRACAINE HYDROCHLORIDE OINTMENT	Julie Zhang Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO42020 Biologics Monographs 4 - Antibiotics

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