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Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream

» Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS
USP Pramoxine Hydrochloride RS

Identification—

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

Test solution—Disperse a quantity of Cream, equivalent to about 25 mg of neomycin, with 20 mL of chloroform in a 60-mL separator. Add 0.2 mL of 2.5 N hydrochloric acid, and shake. Allow the layers to separate for about 30 minutes. Discard the lower chloroform layer, and centrifuge the upper aqueous layer. Use a portion of the centrifuged aqueous layer.

Standard solution—Dissolve suitable quantities of USP Neomycin Sulfate RS and USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing the equivalent of about 3.5 mg of neomycin and 10,000 USP Polymyxin B Units per mL.

Developing solvent system—Dissolve 0.1 g of benzalkonium chloride in a mixture of isopropyl alcohol, water, and ammonium hydroxide (60:40:10).

Procedure—Proceed as directed in the chapter. Place the plate in a chromatographic chamber saturated with *Developing solvent system*, and develop the chromatogram. Dry the plate at 105° for about 10 minutes, spray with a solution of ninhydrin in butyl alcohol (1 in 200), and heat the plate at 105° for about 15 minutes. The R_F values of the two principal spots in the chromatogram obtained from the *Test solution* correspond to those of the two principal spots in the chromatogram obtained from the *Standard solution*.

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

pH ⟨791⟩—Transfer 1 g of Cream to a small beaker, add 10 mL of carbon dioxide-free water, and mix: the pH is between 3.3 and 6.0.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays ⟨81⟩*, using an accurately weighed portion of Cream, equivalent to about 3.5 mg of neomycin, blended for 3 to 5 minutes in a high-speed blender with 249 mL of *Buffer B.3* and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with *Buffer B.3* to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median level of the Standard (1 µg of neomycin per mL).

Assay for polymyxin—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays ⟨81⟩*, using an accurately weighed portion of Cream, equivalent to about 10,000 USP Polymyxin B Units, blended for 3 to 5 minutes in a high-speed blender with 199 mL of *Buffer B.6* and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with*Buffer B.6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard (10 USP Polymyxin B Units per mL).

Assay for pramoxine hydrochloride—

Mobile phase—Dissolve 3.5 g of dibasic potassium phosphate in 1000 mL of water. Prepare a mixture of this solution, acetonitrile, and triethylamine (700:300:2), and adjust with phosphoric acid to a pH of 4.0 ± 0.1. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Standard preparation—Prepare a solution of USP Pramoxine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 10 mg of pramoxine hydrochloride, to a 50-mL volumetric flask, add about 5 mL of chloroform, and sonicate at about 40° to disperse the Cream. Allow to cool to room temperature, dilute

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with methanol to volume, and mix. Pass a portion of this solution through a glass fiber filter and a PTFE filter having a 0.45-µm porosity, discarding the first few mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector, a guard column that contains packing L7, and a 4.6-mm × 25-cm analytical column that contains packing L7. The column is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* 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 areas for the major peaks. Calculate the quantity, in mg, of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in each g of Cream taken by the formula:

$$50(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pramoxine Hydrochloride RS in the *Standard preparation; W* is the weight, in g, of Cream taken to prepare the *Assay preparation;* and r_U and r_S are the peak areas for pramoxine 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
NEOMYCIN AND POLYMYXIN B SULFATES AND PRAMOXINE HYDROCHLORIDE CREAM	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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