Add 120 mL of acetonitrile, mix, and degas. [NOTE—Protect the *Mobile phase* from air to prevent absorption of carbon dioxide.] Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Increasing the proportion of buffer solution decreases the retention times of the analytes.

Internal standard solution—Dissolve a suitable quantity of ammonium bromide in water to obtain a solution having a concentration of about 2.2 mg per mL.

Standard preparation—To a 100-mL volumetric flask transfer about 246 mg of sodium chloride (4.2 mEq), previously dried at 105° for 2 hours and accurately weighed, and about 682 mg of anhydrous sodium sulfate (9.6 mEq), previously dried at 105° for 2 hours and accurately weighed, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix. Filter this solution through a 0.5-µm or finer porosity filter, and store the filtrate in a suitable glass container. This *Standard preparation* contains about 24.6 µg of sodium chloride (0.00042 mEq of chloride) and about 68.2 µg of sodium sulfate (0.00096 mEq of sulfate) per mL.

Assay preparation—Use the *Assay preparation* prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.042 mEq of chloride and 0.096 mEq of sulfate per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a conductivity detector, a 4-mm × 5-cm guard column containing packing L23, and a 4-mm × 30-cm analytical column maintained at 35 ± 1° containing packing L23. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative retention times are about 0.25 for chloride, 0.4 for bromide, and 1.0 for sulfate, the resolution, *R,* between the chloride and bromide peaks is not less than 1.5 and between the bromide and sulfate peaks is not less than 4.5. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by changing the in-line filters and frits in the columns. Column efficiency may be improved by backflushing the analytical column with 50 mL of the buffer solution used to prepare the *Mobile phase*.]

Procedure—[NOTE—Use peak heights where peak responses are indicated.] 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 mEq of chloride per L of constituted Oral Solution taken by the formula:

$$(500 / 58.44)(C / 6)(R_U / R_S)$$

in which 58.44 is the molecular weight of sodium chloride, *C* is the concentration, in µg per mL, of sodium chloride in the *Standard preparation*, and R_U and R_S are the peak response ratios of chloride to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the mEq of sulfate per L of constituted Oral Solution taken by the formula:

$$(500 / 71.02)(C / 6)(R_U / R_S)$$

in which 71.02 is one-half of the molecular weight of sodium sulfate, *C* is the concentration, in µg per mL, of anhydrous sodium sulfate in the *Standard preparation*, and R_U and R_S are the peak response ratios of sulfate to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for polyethylene glycol 3350—

Salt solution—Prepare a solution in water containing 0.35 mg of sodium chloride, 0.18 mg of potassium chloride, 0.40 mg of sodium bicarbonate, 1.37 mg of anhydrous sodium sulfate, and 0.88 mg of ammonium bromide per mL.

Mobile phase—Dilute 40.0 mL of *Salt solution* with water to 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 360 mg of USP Polyethylene Glycol 3350 RS, accurately weighed, to a 500-mL volumetric flask, add 20.0 mL of *Salt solution* and about 250 mL of water, dissolve by swirling, dilute with water to volume, and mix. This *Standard preparation* contains about 0.72 mg of polyethylene glycol 3350 per mL.

Assay preparation—Use the *Assay preparation,* prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.72 mg of polyethylene glycol 3350 per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—[NOTE—Use peak heights where peak responses are indicated.] The liquid chromatograph is equipped with a refractive index detector maintained at 34 ± 0.5°, a 7.8-mm × 4.5-cm guard column containing packing L25, and a 7.8-mm × 30-cm analytical column containing packing L25 and maintained at ambient temperature. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by cleaning the frits in the guard column or by replacing the guard column. Baseline drift may be reduced by maintaining strict control of ambient temperature, by insulating the lines, the *Mobile phase* reservoir, and the columns, and by increasing the time of equilibration.]

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 content, in g, of polyethylene glycol 3350 per L of constituted Oral Solution taken by the formula:

$$500(C / 6)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of polyethylene glycol 3350 in the *Standard preparation,* and r_U and r_S are the polyethylene glycol 3350 peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Polymyxin B Sulfate

Polymyxin B, sulfate.
Polymyxin B sulfate [1405-20-5].

» Polymyxin B Sulfate is the sulfate salt of a kind of polymyxin, a substance produced by the growth of *Bacillus polymyxa* (Prazmowski) Migula (Fam. Bacillaceae), or a mixture of two or more such salts. It has a potency of not less than 6000 Polymyxin B Units per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Where packaged for prescription compounding, the label states the number of Polymyxin B Units in the container and per milligram, that it is not intended for manufacturing use, that it is not sterile, and that its potency cannot be assured for longer than 60 days after opening. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

USP Reference standards ⟨11⟩**—**
USP Polymyxin B Sulfate RS

Identification—

A: *Liquid Chromatographic Identification Test—*

Mobile phase—Prepare a mixture of 0.1 M tribasic sodium phosphate and acetonitrile (77:23), and adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩.

Standard solution—Prepare a solution of USP Polymyxin B Sulfate RS in *Mobile phase* having a concentration of about 3.5 mg per mL. Protect this solution from light.

Test solution—Prepare a solution of Polymyxin B Sulfate in *Mobile phase* having a concentration of about 3.5 mg per mL. Protect this solution from light.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 212-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. The chromatogram obtained from the *Test solution* corresponds qualitatively to that obtained from the *Standard solution*, exhibiting a major peak corresponding to polymyxin B1 and peaks at relative retention times of about 0.5 (polymyxin B2) and 0.6 (polymyxin B3).

B: Dissolve 2 mg in 5 mL of water, add 5 mL of 2.5 N sodium hydroxide, mix, and add 5 drops of cupric sulfate solution (1 in 100), shaking after the addition of each drop: a reddish violet color is produced.

C: A solution (1 in 20) meets the requirements of the tests for *Sulfate* ⟨191⟩.

pH ⟨791⟩: between 5.0 and 7.5, in a solution containing 5 mg per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 7.0% of its weight.

Content of phenylalanine—Transfer about 0.375 g of Polymyxin B Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with 0.1 N hydrochloric acid to volume, and mix. Measure the absorbances of this solution at the maxima at about 264 nm (A_{264}), 258 nm (A_{258}), and 252 nm (A_{252}), and the absorbances at 280 nm (A_{280}) and 300 nm (A_{300}). Calculate the percentage of phenylalanine in the portion of Polymyxin B Sulfate taken by the formula:

$$(9.4787/W)(A_{258} - 0.5A_{252} + 0.5A_{264} - 1.84A_{280} + 0.8A_{300})$$

in which W is the weight, in g, of Polymyxin B Sulfate taken: it contains between 9% and 12% of phenylalanine, calculated on the dried basis.

Other requirements—If for prescription compounding, it meets the requirements for *Residue on ignition* under *Polymyxin B for Injection*. Where the label states that Polymyxin B Sulfate is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and, where intended for injectable dosage forms, for *Pyrogen* under *Polymyxin B for Injection.* Where the label states that Polymyxin B Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Pyrogen* under *Polymyxin B for Injection.*

Assay—Proceed with Polymyxin B Sulfate as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Polymyxin B for Injection

» Polymyxin B for Injection contains an amount of Polymyxin B Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of polymyxin B.

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩, protected from light.

Labeling—Label it to indicate that where it is administered intramuscularly and/or intrathecally, it is to be given only to patients hospitalized so as to provide constant supervision by a physician.

USP Reference standards ⟨11⟩**—**
USP Polymyxin B Sulfate RS

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Pyrogen—It meets the requirements of the *Pyrogen Test* ⟨151⟩, the test dose being 1.0 mL per kg of a solution in pyrogen-free saline TS containing 20,000 Polymyxin B Units per mL.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Residue on ignition ⟨281⟩: not more than 5.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Heavy metals, *Method II* ⟨231⟩: not more than 0.01%.

Other requirements—It meets the requirements for *pH* and *Loss on drying* under *Polymyxin B Sulfate*. It also meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* under *Injections* ⟨1⟩.

Assay—

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer No. 6* to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Assay preparation 2 (where the label states the quantity of polymyxin B in a given volume of constituted solution)—Constitute 1 container of Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer No. 6* to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol

» Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of polymyxin B and bacitracin.

Packaging and storage—Preserve in pressurized containers, and avoid exposure to excessive heat.