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 x 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 (A264), 258 nm (A258), and 252 nm (A252), and the absorbances at 280 nm (A280) and 300 nm (A300). Calculate the percentage of phenylalanine in the portion of Polymyxin B Sulfate taken by the formula:

\[(9.4787/W)A_{258} - 0.5A_{252} + 0.5A_{264} - 1.8A_{280} + 0.8A_{300}\]

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 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 (201BPN): 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.

Constituted solution—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.
USP Reference standards (11)—
USP Bacitracin Zinc RS
USP Polymyxin B Sulfate RS

Identification—Collect in a suitable container the expelled contents of 1 Aerosol container, shake with a volume of 0.1 N hydrochloric acid sufficient to obtain a solution containing about 500 USP Bacitracin Units per mL, centrifuge, and use the clear supernatant as the test solution. Proceed as directed under Thin-Layer Chromatographic Identification Test (2018BNP). The specified result is observed.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Collect aseptically in a suitable container half the contents expelled from 5 containers, dissolve in 500 mL of Fluid A containing 0.25 g of sodium thioglycollate and adjusted with sodium hydroxide to a pH of 6.6 ± 0.6, pass through a membrane filter as directed for Membrane Filtration under Test for Sterility of the Product to be Examined under Sterility Tests (71), except to place the filter on the surface of Soybean–Casein Digest Agar Medium in a Petri dish, incubate for 7 days at 30° to 35°, and count the number of colonies on the filter. Similarly prepare a second specimen, except to incubate at 20° to 25°. Not more than 20 colonies are observed from the two specimens. It meets also the requirements of the tests for absence of Staphylococcus aureus and Pseudomonas aeruginosa under Microbial Enumeration Tests (61) and Tests for Specified Microorganisms (62).

Water, Method I (921):—Store 1 container of Topical Aerosol in a freezer for not less than 2 hours, open the container, and transfer 10.0 mL of the freshly mixed specimen to a titration vessel containing 20 mL of a mixture of toluene and methanol (7:3) instead of methanol. In titrating the specimen, determine the endpoint at a temperature of 10° or higher; not more than 0.5% of water is found.

Other requirements—It meets the requirements for Pressure Test, Minimum Fill, and Leakage Test under Aerosols, Metered-Dose Inhalers, and Dry Powder Inhalers (601).

Assay for polymyxin B—Proceed as directed for polymyxin B under Antibiotics—Microbial Assays (81), using an accurately weighed portion of Topical Powder, equivalent to about 5000 USP Polymyxin B Units, shaken with 20 mL of water in a suitable volumetric flask. Dilute with Buffer No. 6 to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively with suitable buffers and preservatives. Proceed as directed for assay for polymyxin B under Antibiotics—Microbial Assays (81), using an accurately weighed portion of Topical Powder, equivalent to about 800 USP Bacitracin Units, added to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively and stepwise with Buffer No. 1 to obtain a Test Dilution having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard. Prepare each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the Test Dilution.

Polymyxin B Sulfate and Hydrocortisone Otic Solution

Polymyxin B Sulfate and Bacitracin Zinc Topical Powder

Polymyxin B Sulfate and Hydrocortisone Otic Solution is a sterile solution containing not less than 90.0 percent and not more than 130.0 percent of the labeled amount of polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone (C21H30O5). It may contain one or more suitable buffers and preservatives.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—
USP Hydrocortisone RS
USP Polymyxin B Sulfate RS