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Bleomycin Sulfate

Bleomycin sulfate (salt).

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CAS RN®: 9041-93-4; UNII: 7DP3NTV15T.

» Bleomycin Sulfate is the sulfate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus*, or produced by other means. It has a potency of not less than 1.5 Bleomycin Units and not more than 2.0 Bleomycin Units per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—

USP Bleomycin Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

B: It responds to the tests for *Sulfate ⟨191⟩*.

pH ⟨791⟩: between 4.5 and 6.0, in a solution containing 10 Bleomycin Units per mL.

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

Copper—

Dilute nitric acid—Dilute 20 mL of nitric acid to 2000 mL with water.

Copper stock solution—Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with *Dilute nitric acid* to volume, and mix. Store in a polyethylene bottle. This solution contains 1000 µg of copper per mL.

Standard preparations—Transfer 5.0 mL of *Copper stock solution* to a 100-mL volumetric flask, dilute with *Dilute nitric acid* to volume, and mix. Transfer 3.0, 9.0, and 15.0 mL, respectively, of this solution to separate 100-mL volumetric flasks, dilute the contents of each flask with *Dilute nitric acid* to volume, and mix. These *Standard preparations* contain, respectively, 1.5, 4.5, and 7.5 µg of copper per mL.

Test preparation—Dissolve about 75 mg of Bleomycin Sulfate, accurately weighed, in 10.0 mL of *Dilute nitric acid*.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the copper emission line at 324.8 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy ⟨852⟩*) equipped with a copper hollow-cathode lamp and an air–acetylene flame, using *Dilute nitric acid* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of copper, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of copper in the *Test preparation*. Calculate the percentage of copper in the portion of Bleomycin Sulfate taken by the formula:

$$C/W$$

in which *W* is the weight, in mg, of Bleomycin Sulfate taken to prepare the *Test preparation:* not more than 0.1% is found.

Content of bleomycins—

Mobile phase—Dissolve 960 mg of sodium 1-pentane sulfonate in 1000 mL of deaerated 0.08 N acetic acid, adjust with ammonium hydroxide to a pH of 4.3, filter, and degas. [Note—1.86 g of edetate disodium may be included if needed to obtain satisfactory chromatography.] Use a linear gradient of 10% to 40% methanol mixed with this solution, with a gradient mixing time of 60 minutes, and allow chromatography to proceed with the final gradient mixture for a further 20 minutes or until demethylbleomycin A_2 has been eluted.

Test preparation—Dissolve Bleomycin Sulfate in deaerated water to obtain a solution having a concentration of about 2.5 Bleomycin Units per mL. Store this solution in a refrigerator until just prior to use.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 250-mm stainless steel column containing packing L1. The flow rate is about 1.2 mL per minute.

Procedure—Inject about 10 µL of the *Test preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatogram, and measure the peak responses for all peaks. The elution order is bleomycinic acid, bleomycin A_2 (major peak), bleomycin A_5, bleomycin B_2 (major peak), bleomycin B_4, and demethylbleomycin A_2. Calculate the percentage contents of bleomycin A_2,

bleomycin B_2, and bleomycin B_4 taken by the formula:

$$100r_f/r_t$$

in which r_f is the peak response corresponding to the particular bleomycin and r_t is the total of the responses of all peaks: the content of bleomycin A_2 is between 55% and 70%; the content of bleomycin B_2 is between 25% and 32%; the content of bleomycin B_4 is not more than 1%; and the combined percentage of bleomycin A_2 and bleomycin B_2 is not less than 90%.

Other requirements—Where the label states that Bleomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Bleomycin for Injection*. Where the label states that Bleomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Bleomycin for Injection*.

Assay—

Assay preparation—Dissolve a suitable quantity of Bleomycin Sulfate, accurately weighed, in *Buffer B.16*, and quantitatively dilute with *Buffer B.16* to obtain a solution having a convenient concentration.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.16* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
BLEOMYCIN SULFATE	Jennifer Tong Sun Senior Scientist II	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. Information currently unavailable

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