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Bleomycin for Injection

General Notices

Bleomycin Sulphate for Injection

Action and use

Antineoplastic; treatment of a range of cancers.

DEFINITION

Bleomycin for Injection is a sterile material consisting of Bleomycin Sulfate with or without excipients. It is supplied in a sealed container.

The injection complies with the requirements stated under Parenteral Preparations.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum of bleomycin sulfate *(RS 367)*.
B. In the test for Composition, the retention time and size of the two principal peaks in the chromatogram obtained with solution (1) are approximately the same as those of the two principal peaks in the chromatogram obtained with solution (2).
C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 10,000 IU of bleomycin per mL. The pH of the resulting solution is 4.5 to 6.0, Appendix V L.

Colour of solution

Dissolve a quantity in sufficient *water* to produce a solution containing 40,000 IU of bleomycin per mL. The *absorbance* of the solution at 430 nm is not greater than 0.15, Appendix II B.

Loss on drying

Combine the contents of two containers. When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 6.0% of their weight.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 1000 IU of bleomycin per mL.
(2) 0.05% w/v of *bleomycin sulfate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 254 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *methanol* and 90 volumes of buffer prepared in the following manner. Dissolve 0.960 g of *sodium pentanesulfonate* in 900 mL of 0.08M *acetic acid*, add 1.86 g of *disodium edetate*, dilute to 1000 mL with 0.08M *acetic acid* and adjust to pH 4.3 with 18M *ammonia*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	100	0	isocratic
15-75	100→70	0→30	linear gradient
75-95	70	30	isocratic
95-100	70→100	30→0	linear gradient
100-110	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the resolution between the bleomycin A_2 and bleomycin B_2 peaks is at least 5.

LIMITS

Using the chromatogram obtained with solution (1), calculate the percentage content of bleomycin components by *normalisation*. The proportions are within the following limits:

- bleomycin A_2, between 55% and 70%;

- bleomycin B_2, between 25% and 32%;

- the sum of the contents of bleomycin A_2 and bleomycin B_2 is not less than 85%;

- demethylbleomycin A_2 (retention time 1.5 to 2.5, relative to bleomycin A_2), not greater than 8%;

- the total content of other related substances is not greater than 9.5%.

Disregard any impurity present at less than 0.1%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to produce a solution containing 15,000 IU of bleomycin per mL (solution A). The endotoxin limit concentration of solution A is 50 IU of endotoxin per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Mix the contents of the containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A, Method A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU.

STORAGE

Bleomycin for Injection should be used immediately after opening and should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (Units) contained in it.