Bleomycin sulfate (Bleomycini sulfas)

Bleomycin sulfate (non-injectable)

Bleomycin sulfate, sterile

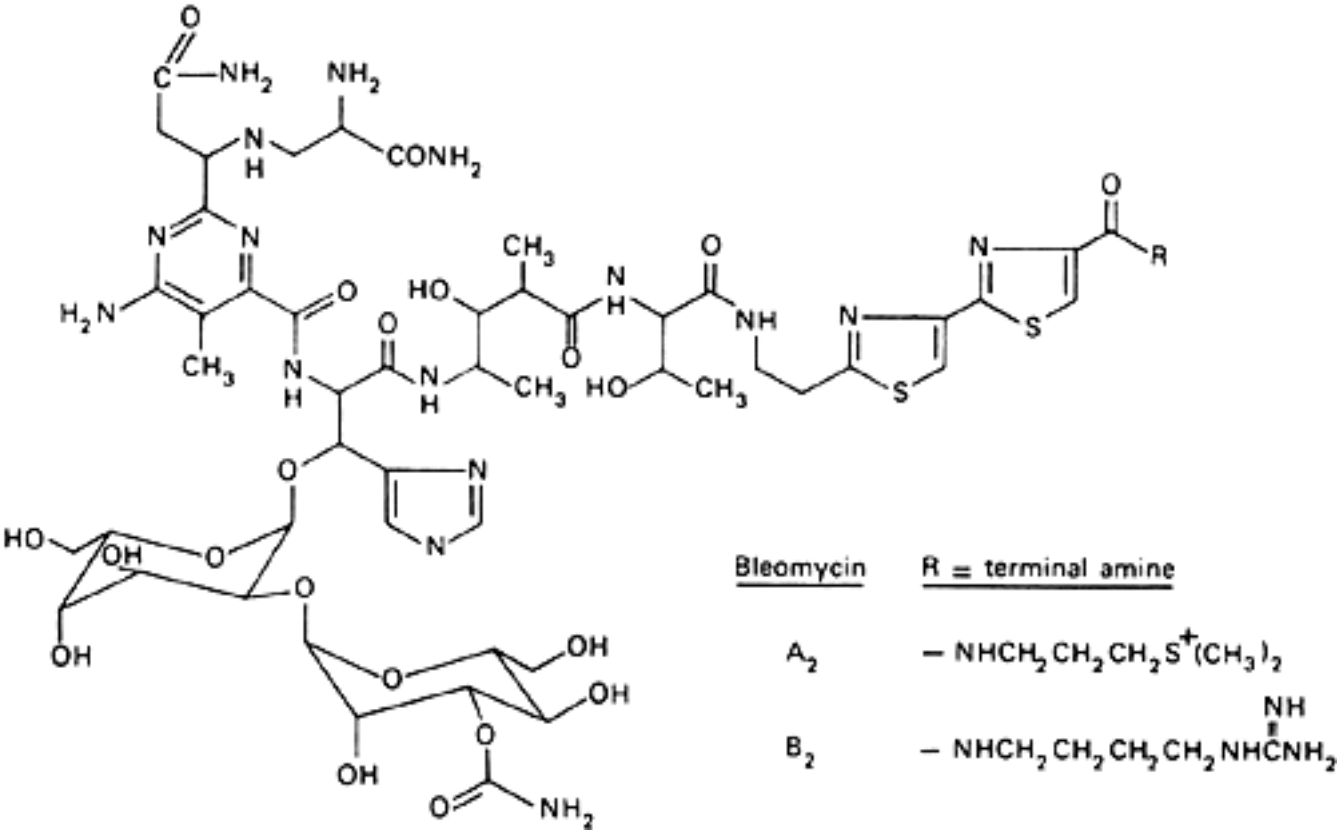
2022-01

CAS Reg. No. 9041-93-4

Molecular formula. Bleomycin A_2 sulfate: $C_{55}H_{84}N_{17}O_{21}S_3,H_2SO_4$; Bleomycin B_2 sulfate: $C_{55}H_{84}N_{20}O_{21}S_2,H_2SO_4$

Relative molecular mass. Bleomycin A_2 sulfate: 1514; Bleomycin B_2 sulfate: 1524.

Graphic formulas for the bleomycin A_2/B_2 bases.



Chemical names for the bleomycin A_2/B_2 bases. Bleomycin A_2 sulfate: N^1 -[3-(Dimethylsulfonio)propyl]bleomycinamide hydrogen sulfate; [3- [2'-[2-[(2*S*,3*R*)-2-[(2*S*,3*S*,4*R*)-4-[(2*S*,3*R*)-2-[6-amino-2-[(1*S*)-1-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-2-carbamoylethyl]-5-methyl-4-pyrimidinecarboxamido]-3-[[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-3-imidazol-4-ylpropionamido]-3-hydroxy-2-methylvaleramido]-3-hydroxybutyramido]ethyl][2,4'-bithiazole]-4-carboxamido]propyl]dimethylsulfonium hydrogen sulfate.

Bleomycin B_2 sulfate: N^1 -(Guanidinobutyl)bleomycinamide; (β*S*)-4-amino-β-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-6-[[(1*S*,2*R*)-2-[[2- *O*-(3-*O* -carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-1-[[(1*R*,2 *S*,3*S*)-3-[[(1*S*,2*R*)-1-[[2-[4-[(4-guanidinobutyl)carbamoyl][2,4'-bithiazol]-2'-yl]ethyl]carbamoyl]-2-hydroxypropyl]carbamoyl]-2-hydroxy-1-methylbutyl]carbamoyl]-2-imidazol-4-ylethyl]carbamoyl]-5-methyl-2-pyrimidinepropionamide sulfate (salt); N^1 -[4-[(aminoiminomethyl)amino]butyl]bleomycinamide sulfate (salt).

Description. A white or cream-coloured, amorphous powder.

Solubility. Very soluble in water.

Category. Cytotoxic drug.

Storage. Bleomycin sulfate should be kept in a tightly closed container.

Labelling. The designation sterile Bleomycin sulfate indicates that the substance complies with the additional requirements for sterile Bleomycin sulfate and may be used for parenteral administration or for other sterile applications. CAUTION: Bleomycin sulfate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Manufacture. The substance is produced by methods of manufacture designed to eliminate or minimize substances lowering blood pressure.

Requirements

Definition. Bleomycin sulfate is the sulfate salt of a mixture of substances produced by the growth of *Streptomyces verticillus.* The main components of the mixture are bleomycin A_2 and bleomycin B_2.

Bleomycin sulfate contains, when tested according to assay A, not less than 1500 and not more than 2000 International Units of bleomycin A_2 /B_2 per mg, calculated with reference to the dried substance.

Further, Bleomycin sulfate contains, when tested according to assay B, not less than 55.0% and not more than 70.0% of bleomycin A_2 and not less than 25.0% and not more than 32.0% of bleomycin B_2; the total of bleomycin A_2 and bleomycin B_2 is not less than 85%. The content of bleomycin A_5 is not more than 7.0%, of bleomycin B_4 not more than 1.0%, and of demethylbleomycin A_2 not more than 3.0%.

Identity tests

A. Dissolve about 5 mg in 10 mL of water, add 5 µl of copper(II) sulfate (160 g/L) TS, and dilute with water to 100 mL; the absorption spectrum exhibits maxima at about 242 nm and 290 nm, and a minimum at about 268 nm.

B. A 10 mg/mL solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 60 mg/g.

pH value. pH of a 5.0 mg/mL solution, 4.5-6.0.

Copper content. Transfer 75 mg, accurately weighed, to a 60-mL separating funnel and dissolve in 10 mL of hydrochloric acid (0.1 mol/L) VS. Transfer 10 mL of copper standard TS2 to an additional separating funnel. To both funnels, add 10 mL of zinc bis(dibenzyldithiocarbamate) TS and shake vigorously for 1 minute. Allow the layers to separate. Filter the lower layer through 1 g of anhydrous sodium sulfate R to remove excess water. Measure the absorbances of a 1-cm layer at the maximum at about 435 nm, using a solvent cell containing carbon tetrachloride R.

Calculate the content of copper in mg/g from the formula $(A_0 \times 15)/(A_s \times W)$ where A_0 is the absorbance of the substance to be examined, A_s is the absorbance of copper standard TS2, and W is the weight in mg of the substance to be examined; the copper content is not more than 0.2 mg/g.

Assay

Microbiological assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Mycobacterium smegmatis* (ATCC 607) as the test organism. Prepare the inoculum as follows: the test organism is grown for 40-48 hours at a temperature of 27 °C on the surface of culture medium Cm8. Using 3 mL of saline TS wash the growth into a flask containing 100 mL of culture medium Cm9 and 50 g of glass beads, and incubate at 25-27 °C for 5 days with constant mechanical agitation using an orbital shaker. The resulting suspension should be used for no longer than 14 days, and kept at a temperature below 5 °C. For the preparation of inoculated plates , use 0.5 mL of the suspension or a suitable volume previously determined using test plates with culture medium Cm8 at a temperature of 27 °C. Prepare the reference solution in phosphate buffer, pH 7.0, TS, diluting the International Reference Preparation of bleomycin A_2/B_2 to an appropriate concentration (usually between 10 and 200 µg per mL). The precision of the assay is such that the fiducial limits of error of the estimated potency (P = 0.95) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency (P = 0.95) is not less than 1500 IU and the lower fiducial limit is not more than 2000 IU of bleomycin A_2/B_2 per mg, calculated with reference to the dried substance.

Content of the bleomycin components. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a column 25 cm long and 4.6 mm in internal diameter packed with particles of silica gel, 5-10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups. As the mobile phase for a linear gradient development, start with a mixture of 9 volumes of 1-pentanesulfonic acid TS and 1 volume of methanol R, both previously filtered and deaerated, and end with a composition of 6 volumes of 1-pentanesulfonic acid TS and 4 volumes of methanol R, using a suitable linear rate of change of mobile phase so as to reach the final composition in 60 minutes. (If needed, add the following to the mobile phase to obtain satisfactory chromatography: 1.86 g of disodium edetate R per litre.) As detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm, fitted with a low-volume flow cell (8-20 µl is suitable). Inject 5 µl of a solution of the test substance in water containing the equivalent of 5 IU of bleomycin per mL. Proceed with the gradient elution, pumping the mobile phase mixture at the condition mentioned above for about 80 minutes or until the demethylbleomycin A_2 is eluted.

The elution order of the bleomycin components is the following: void volume, bleomycin acid, bleomycin A_2, bleomycin B_2, bleomycin A_5, bleomycin B_4, and demethylbleomycin A_2.

Calculate in % the content of each bleomycin component, comparing the ratios of the individual areas of the peaks with that of the total area of all the bleomycins.

Additional Requirements for Bleomycin Sulfate for sterile use

Storage. Sterile Bleomycin sulfate should be kept in a hermetically closed container.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 10.0 IU of endotoxin RS per mg of bleomycin.

Sterility. Complies with 3.2 Test for sterility.