

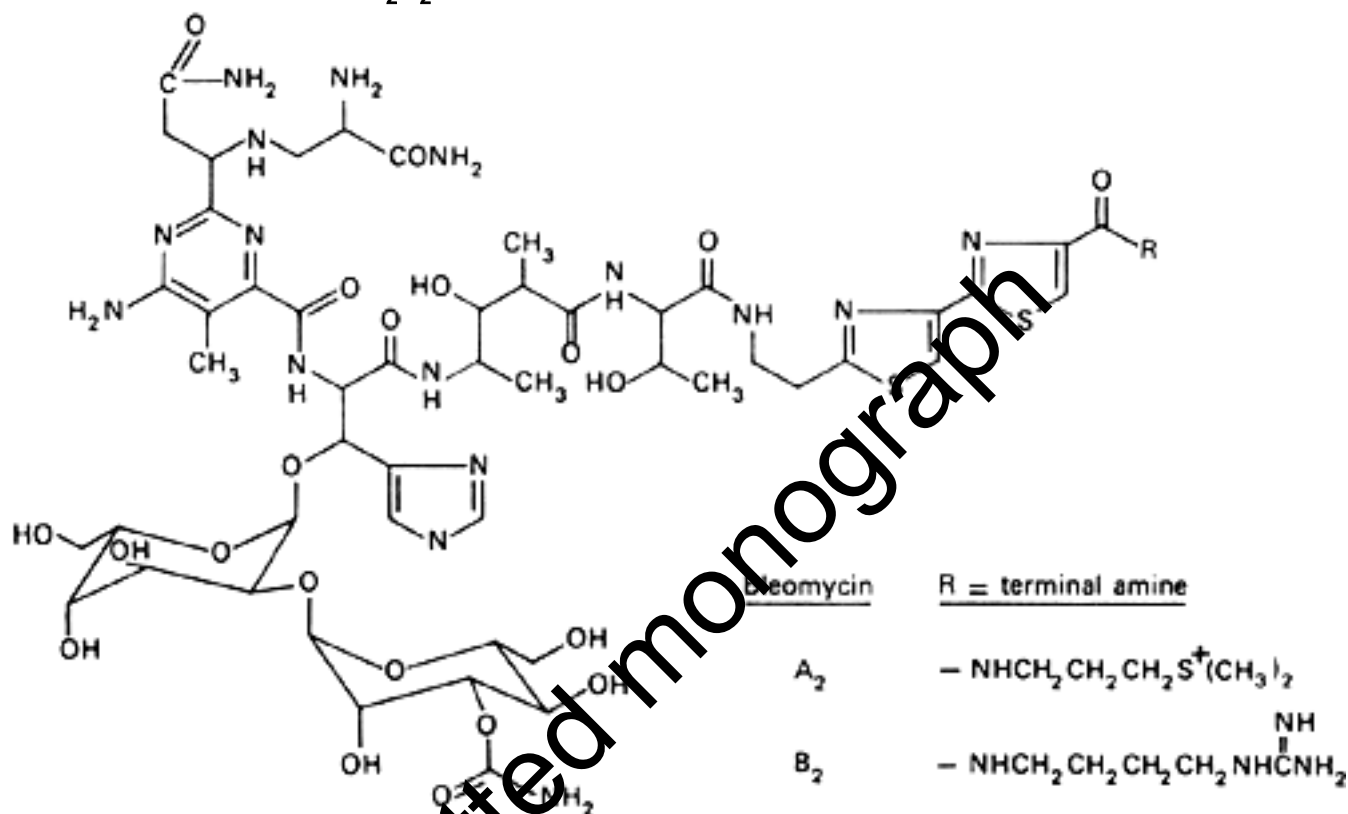
**Bleomycin hydrochloride (Bleomycini hydrochloridum)****Bleomycin hydrochloride (non-injectable)****Bleomycin hydrochloride, sterile**

**Chemical name.** Bleomycin hydrochloride; CAS Reg. No. 67763-87-5.

**Molecular formula.** Bleomycin A<sub>2</sub> hydrochloride: C<sub>55</sub>H<sub>84</sub>N<sub>17</sub>O<sub>21</sub>S<sub>3</sub>.Cl. Bleomycin B<sub>2</sub> hydrochloride: C<sub>55</sub>H<sub>84</sub>N<sub>20</sub>O<sub>21</sub>S<sub>2</sub>.HCl.

**Relative molecular mass.** Bleomycin A<sub>2</sub> hydrochloride: 1452; Bleomycin B<sub>2</sub> hydrochloride: 1461.

**Graphic formulas for the bleomycin A<sub>2</sub>/B<sub>2</sub> bases.**



**Chemical names for the bleomycin A<sub>2</sub>/B<sub>2</sub> bases.** Bleomycin A<sub>2</sub> hydrochloride: N<sup>1</sup>-[3-(Dimethylsulfonio)-propyl]bleomycinamide chloride; [3-[2'-[2-[(2S,3R)-2-[(2S,3S,4R)-4-[(2S,3R)-2-[6-amino-2-[(1S)-1-[(2S)-2-amino-2-carbamoylethyl]amino]-2-carbamoylethyl]-5-methyl-4-pyrimidin-5-carboxamido]-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 chloride; CAS Reg. No. 49830-49-1.

Bleomycin B<sub>2</sub> hydrochloride: N<sup>1</sup>-(Guanidinobutyl)bleomycinamide hydrochloride; (βS)-4-amino-β-[[[(2S)-2-amino-2-carbamoylethyl]amino]-6-[[[(1S,2R)-2-[[2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-1-[[[(1R,2S,3S)-3-[[[(1S,2R)-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 hydrochloride; N<sup>1</sup>-[4-[(aminoiminomethyl)amino]butyl]bleomycinamide hydrochloride; CAS Reg. No. 55658-44-1.

**Description.** A white to yellowish white powder.

**Solubility.** Freely soluble in water and in methanol R; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

**Category.** Cytotoxic drug.

**Storage.** Bleomycin hydrochloride should be kept in a tightly closed container.

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

**Requirements**

**Definition.** Bleomycin hydrochloride is the hydrochloride salt of a mixture of substances produced by the growth of *Streptomyces*

*verticillus*. The main components of the mixture are bleomycin A<sub>2</sub> and bleomycin B<sub>2</sub>.

Bleomycin hydrochloride contains, when tested according to assay A, not less than 1500 and not more than 2000 International Units of bleomycin A<sub>2</sub>/B<sub>2</sub> per mg, calculated with reference to the dried substance.

Further, Bleomycin hydrochloride contains, when tested according to assay B, not less than 55.0% and not more than 70.0% of bleomycin A<sub>2</sub> and not less than 25.0% and not more than 32.0% of bleomycin B<sub>2</sub>; the total of bleomycin A<sub>2</sub> and bleomycin B<sub>2</sub> is not less than 85%. The content of bleomycin A<sub>5</sub> is not more than 7.0%, of bleomycin B<sub>4</sub> not more than 1.0%, and of demethylbleomycin A<sub>2</sub> not more than 3.0%.

**Manufacture.** The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

**Histamine-like substances.** Carry out the test as described under [3.6 Test for histamine-like substances \(vasodepressor substances\)](#) using 1 mL per kg of body mass of a solution in saline TS containing a quantity equivalent to 500 IU per mL.

#### 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 B described under [2.1 General identification tests](#) as characteristic of chlorides.

**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(dibenzylthiocarbamate) 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

**A. 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<sub>2</sub>/B<sub>2</sub> 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<sub>2</sub>/B<sub>2</sub> per mg, calculated with reference to the dried substance.

**B. Content of the bleomycin components.** Carry out the test as described under [1.14.4 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<sub>2</sub> is eluted.

The elution order of the bleomycin components is the following: void volume, bleomycin acid, bleomycin A<sub>2</sub>, bleomycin B<sub>2</sub>,

bleomycin A<sub>5</sub>, bleomycin B<sub>4</sub>, and demethylbleomycin A<sub>2</sub>.

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 Hydrochloride for sterile use**

**Storage.** Sterile Bleomycin hydrochloride 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](#).

Omitted monograph