Bacitracin zinc (Bacitracinum zincum)

**Bacitracin zinc (non-injectable)**

**Bacitracin zinc, sterile**

**Chemical name.** Bacitracin zinc; CAS Reg. No. 1405-89-6.

**Description.** A white or pale brownish yellow powder; odourless or with a faint characteristic odour.

**Solubility.** Soluble in 900 parts of water and in 500 parts of ethanol (~750 g/l) TS; very slightly soluble in ether R.

**Category.** Antiinfective drug.

**Storage.** Bacitracin zinc should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 25 °C. If it is intended for parenteral administration, the container should be sterile and sealed so as to exclude microorganisms.

**Labelling.** The designation sterile Bacitracin zinc indicates that the substance complies with the additional requirements for sterile Bacitracin zinc and may be used for other sterile applications.

**Additional information.** Bacitracin zinc is hygroscopic.

**Requirements**

**Definition.** Bacitracin zinc is a zinc complex of bacitracin, a polypeptide produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis*. The main components are Bacitracin A, B₁ and B₂.

Bacitracin zinc contains not less than 55 International Units of bacitracin per mg, calculated with reference to the dried substance.

**Identity tests**

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 60 volumes of 1-butanol R, 10 volumes of water, 6 volumes of pyridine R, 15 volumes of glacial acetic acid R, and 5 volumes of ethanol (~750 g/l) TS as the mobile phase. Apply separately to the plate 1 μl of each of 2 solutions in disodium edetate (10 g/l) TS containing (A) 6.0 mg of the test substance per mL and (B) 6.0 mg of bacitracin zinc RS per mL. A third spot (C) is made by applying 1 μl of each of solutions A and B at the same point of application, allowing the plate to dry between the two loadings. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with triketohydrindene/pyridine/butanol TS, and heat it at 110°C for 10 minutes. Allow to cool, and examine the chromatogram in daylight. The spots obtained with solution A correspond in position, appearance and intensity with that obtained with solution B. A single spot is obtained with solution C.

B. Ignite 30 mg; dissolve half of the residue in 1.0 mL of hydrochloric acid (~70 g/l) TS and add 1.0 mL of potassium ferrocyanide (45 g/l) TS; a white precipitate is produced. Dissolve the remaining residue in 1.0 mL of sulfuric acid (~100 g/l) TS, add 0.05 mL of copper(II) sulfate (1 g/l) TS and 2.0 mL of ammonium mercurithiocyanate TS; a violet precipitate is produced.

**Loss on drying.** Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

**pH value.** Shake 1.0 g with 10 mL of carbon-dioxide-free water R and filter; pH of the filtrate, 6.0-7.5.

**Bacitracin F and related substances.** Prepare a solution containing 30 mg in 100 mL of sulfuric acid (0.05 mol/l) VS. The ratio of the absorbance at 290 nm to that at 252 nm is not greater than 0.15.

**Zinc.** Dissolve 0.20 g in 5 mL of acetic acid (~60 g/l) TS and add 50 mL of water, 50 mg of xylenol orange indicator mixture R, and sufficient methenamine R to produce a red solution. Add 2.0 g of methenamine R in excess and titrate with disodium edetate (0.01 mol/l) VS until the colour changes to yellow. Each mL of disodium edetate (0.01 mol/l) VS is equivalent to 0.6537 mg of Zn; the zinc content is not less than 40 mg/g and not more than 60 mg/g, calculated with reference to the dried substance.

**Assay.** Suspend 0.05 g, accurately weighed, in 5 mL of water and add 0.5 mL of hydrochloric acid (~70 g/l) TS and sufficient water to produce 100 mL. Allow to stand at room temperature for 30 minutes and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Micrococcus luteus* (NCTC 7743 or ATCC 10240) as the test organism, culture medium Cm1 with a final pH of either 7.0-7.1 or 6.5-6.6, sterile phosphate buffer TS of pH either 7.0 or 6.0, an appropriate concentration of bacitracin (usually 1-4 IU per mL), and an incubation temperature of either 35-39°C or 32-35°C. 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 55 IU of bacitracin per mg, calculated with reference to the dried substance.
**Additional Requirements for Bacitracin Zinc for sterile use**

**Storage.** Sterile Bacitracin zinc should be kept in a hermetically closed container, protected from light, and stored at a temperature not exceeding 25 °C.

**Sterility.** Complies with 3.2 Test for sterility.