Protamine sulfate (Protaminii sulfas)


Description. A white or almost white powder; hygroscopic.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Drug affecting blood coagulation.

Storage. Protamine sulfate should be kept in a tightly closed and tamper-evident container.

Labelling. The designation Protamine sulfate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. Protamine sulfate binds with heparin in solution, inhibiting its anticoagulant activity. It is prepared in conditions designed to minimize the risk of microbial contamination.

Requirements

Definition. Protamine sulfate is a mixture of sulfates of purified proteins extracted from the sperm or roe of fish usually belonging to the family Clupidae and Salmonidae.

The quantity of 1mg of Protamine sulfate precipitates not less than 100IU of heparin sodium activity, calculated with reference to the dried substance.

Identity tests

A. Use a 10mg/mL solution in hydrochloric acid (0.1mol/l) VS. Measure the optical rotation and calculate with reference to the dried substance; $[\alpha]_{D}^{20^\circ} = -65^\circ$ to $-85^\circ$.

B. Dissolve 0.1 g in 5ml of water, add 4.5ml of water, 1.0ml of sodium hydroxide (~80 g/l) TS, and 2.0 mL of 1-naphthol TS1. Cool the mixture to 5°C and add 0.5 mL of sodium hypobromite TS; an intense red colour is produced.

C. Dissolve 0.04 g in 2ml of water and heat in a water-bath at 60°C. Add 0.1 mL of mercuric sulfate TS and mix; no precipitate is formed. Cool the mixture in an ice-bath; a precipitate is formed.

D. A 20mg/mL solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20μg/g.

Sulfates. Transfer 0.15 g to a beaker and dissolve in 15 mL of water. Add 5ml of hydrochloric acid (~70 g/l) TS, heat to boiling and slowly add to the boiling solution 10 mL of barium chloride (100 g/l) TS. Cover the beaker and heat in a water-bath for 1 hour. Filter, and wash the precipitate several times with small quantities of hot water. Dry and ignite the residue at 600 °C to constant mass. Each g of residue is equivalent to 0.412 g of sulfates (SO$_4$), calculated with reference to the dried substance; 0.16-0.24 g/g.

Clarity and colour of solution. A solution of 0.50 g in 10 mL of water is not more opalescent than opalescence standard TS2 and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11.1 Colour of liquids.

Loss on drying. Dry at 105 °C for 3 hours; it loses not more than 0.050 g/g.

Light absorbance. Dissolve 0.050g in 5ml of water and measure the absorbance of a 1-cm layer at a wavelength between 260nm and 280nm; not greater than 0.1.

Nitrogen. Proceed as described under 2.10 Determination of nitrogen, Method B, using 10 mg of Protamine sulfate; the content of nitrogen is not less than 0.23 g/g and not more than 0.27 g/g, calculated with reference to the dried substance.

Assay. Prepare the following solutions: for solution (A) dissolve 15.0mg of Protamine sulfate in sufficient water to produce 100 mL; for solution (B) dilute 2.0 mL of solution A to 3.0 mL with water; for solution (C) dilute 1.0 mL of solution A to 3.0 mL with water.

As titrant use a solution of heparin RS in water containing about 170 IU/mL. Titrate each of solutions A, B, and C in duplicate and carry out 3 independent assays. Measure accurately 1.5 mL of one of the solutions and introduce it to a cell of a suitable spectrophotometer set at 420 nm. Add small volumes of the titrant until a sharp change in transmittance is observed and note the volume of titrant added.
For each individual titration, calculate the number of International Units of heparin in the volume of titrant added, per mg of Protamine sulfate. Average the 18 values and test the linearity of the response using the usual statistical methods. The assay is not valid unless the relative standard deviations calculated for the results obtained with each solution are less than 5% of the average result.

**Additional requirements for Protamine sulfate for parenteral use**

Complies with the monograph for "Parenteral preparations".

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 7.0 IU of endotoxin RS per mg.