Heparin sodium (Heparinum natricum)

Chemical name. Heparin sodium; CAS Reg. No. 9041-08-1.

Description. A white or almost white powder.

Solubility. Freely soluble in water.

Category. Anticoagulant.

Storage. Heparin sodium should be kept in a tightly closed container.

Labelling. The designation Heparin sodium for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. The label should also state the name and quantity of any added substances, and the source of the material (lung or mucosal). Expiry date.

Additional information. Heparin sodium is moderately hygroscopic.

Requirements

Definition. Heparin sodium is a preparation containing the sodium salt of a sulfated glucosaminoglycan present in mammalian tissues. It has the characteristic property of delaying the clotting of fresh blood.

Heparin sodium intended for the manufacture of a parenteral dosage form contains not less than **150 IU per mg**, and Heparin sodium not intended for use in the manufacture of a parenteral dosage form contains not less than **120 IU per mg**, both calculated with reference to the dried substance.

Manufacture. Heparin sodium is prepared from the lungs of oxen or from the intestinal mucosa of oxen, pigs or sheep. All stages of production and sourcing are governed by a suitable quality assurance system.

The method of manufacture is designed to minimize or eliminate microbial contamination and substances lowering blood pressure and to ensure freedom from contaminants such as over-sulfated glycosaminoglycans. The method is validated *inter alia* to demonstrate that, if tested, the substance would comply with the following tests.

Nuclear magnetic resonance spectrometry. The ^{1}H NMR spectrum obtained with a frequency of at least 300 MHz complies with the specifications approved by the appropriate national or regional regulatory authority.

Capillary electrophoresis. The electrophoretogram obtained complies with the specifications approved by the appropriate national or regional regulatory authority.

Identity tests

A. Delays the clotting of fresh blood.

B. Specific optical rotation, use a 40 mg/mL solution; $[\alpha]_D^{20\,°C}$ is not less than +35°.

C. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/mL solution.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 30 µg/g.

Sodium. Determine by atomic absorption spectrophotometry under 1.8 Atomic spectrometry: emission and absorption at a wavelength of 330.3 nm, using a sodium hollow cathode lamp and a flame of suitable composition (e.g. 11 litres of air and 2 litres of acetylene per minute). Prepare a solution of 5 mg in 10 mL of hydrochloric acid (0.1 mol/l) VS containing 1.27 mg/mL of caesium chloride R. As a reference solution use sodium standard (200µg of Na per mL) TS and use dilutions containing 25, 50, and 75µg of Na per mL in the same mixture of caesium chloride and hydrochloric acid as prepared above; 95-125 mg of Na per g, calculated with reference to the dried substance.

Nitrogen. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.1 g, accurately weighed, and 5ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each mL of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of nitrogen; not more than 25 mg/g, with reference to the dried substance.

Protein and nucleotidic impurities. Measure the absorbance of a 1-cm layer of a 4mg/mL solution at a wavelength of 260nm and 280nm; at 260nm not greater than 0.20 and at 280 nm not greater than 0.15.

Clarity and colour of solution. A solution containing 5000 IU per mL is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11.1 Colour of liquids.

Sulfated ash. Use 0.2 g; 0.30-0.43 g/g, with reference to the dried substance.

Loss on drying. Dry to constant mass at 60 °C under reduced pressure (not exceeding 0.5 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 0.080 g/g.

pH value. pH of 10 mg/mL solution in carbon-dioxide-free water R, 5.5-8.0.

Assay. The anticoagulant activity of heparin is determined *in vitro* using a biological assay to compare its ability to delay the clotting of recalcified citrated sheep plasma with that of the reference substance. The following method is suitable for carrying out the assay *(other methods may also be applicable)*.

The onset of clotting is determined either as a change in optical density (by direct visual inspection, preferably using indirect illumination against a matt black background, or by spectrophotometry, recording at a wavelength of approximately 600 nm) or as a change in fluidity (by visual detection while manually tilting the tube or by mechanical recording while stirring, taking care to cause the minimum disturbance of the solution during the initial phase of clotting). Use appropriate tubes according to the chosen technique.

Prepare a solution of Heparin sodium and a solution of heparin RS in sodium chloride (9 g/l) TS, each containing an accurately known number of IU of heparin per mL. Using sodium chloride (9 g/l) TS prepare from each solution a series of dilutions in geometric progression such that the clotting time obtained with the lowest concentration is not less than 1.5 times the blank recalcification time, and the clotting time obtained with the highest concentration is such as to give a satisfactory log dose-response curve, as determined in a preliminary test.

Place in an ice-bath 12 labelled tubes for each dilution: T_1, T_2, T_3, etc. for Heparin sodium and S_1, S_2, S_3, etc. for heparin RS. To each tube add 1.0ml of thawed plasma substrate R and 1.0 mL of the appropriate dilution, either from Heparin sodium or heparin RS, mixing each tube carefully, and not allowing bubbles to be formed. (The detection technique employed may require the addition of different volumes of plasma substrate, consequently the appropriate adjustment of all tubes would be needed.) Transfer all the tubes to a water-bath at 37 °C, and allow to equilibrate for about 15 minutes. Add to each tube, mixing after each addition, 1 mL of a dilution of cephalin TS and 1 mL of kaolin suspension TS freshly prepared just before use. (A suitable dilution of cephalin TS is one that, under the conditions of the assay, gives a blank recalcification time of not more than 60 seconds.) After exactly 2 minutes, add 1.0 mL of calcium chloride (3.7 g/l) TS. Record in seconds the interval between this addition and the onset of clotting, determined according to the chosen technique. Similarly determine the blank recalcification time at the beginning and at the end of the procedure, using 1.0 mL of sodium chloride (9 g/l) TS in place of one of the heparin dilutions; the two values for the blank should not differ significantly. Repeat the procedure using fresh dilutions of the initial solutions and carrying out the incubation in the reverse order (first tubes S, then tubes T).

Transform the clotting times to logarithms using the mean values for the duplicate tubes and calculate the results by standard statistical methods.

Carry out not fewer than 3 independent assays. For each assay prepare fresh solutions of Heparin sodium and heparin RS, and use a different, freshlythawed portion of the stored plasma substrate R.

Calculate the potency of Heparin sodium by combining the results of the assays by standard statistical methods. If the variance is significant (P = 0.01), due to differences between assays, it is possible to obtain a combined estimate by calculating the non-weighted mean of potency estimates.

The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error of the estimated potency (P = 0.95) are not less than 80% and not more than 125% of the stated potency.

Additional requirements for Heparin sodium 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; Heparin sodium intended for the manufacture of a parenteral dosage form, without further appropriate procedure for the removal of bacterial endotoxins, contains not more than 0.01 IU of endotoxin RS per IU of heparin activity. The addition of divalent cations may be necessary in order to fulfil the validation criteria.