Protamine Sulfate

» Protamine Sulfate is a purified mixture of simple protein principles obtained from the sperm or testes of suitable species of fish, which has the property of neutralizing heparin. Each mg of Protamine Sulfate, calculated on the dried basis, neutralizes not less than 100 USP Heparin Units.

Packaging and storage—Preserve in tight containers, in a refrigerator.

USP Reference standards ⟨11⟩**—***USP Heparin Sodium RS*.

Loss on drying ⟨731⟩**—**Dry it at 105° for 3 hours: it loses not more than 5% of its weight.

Ultraviolet absorbance—The difference in absorbance of a 1.0% solution in water between 260 nm and 280 nm against a water blank, is not greater than 0.1 (see *Spectrophotometry and Light-Scattering* ⟨851⟩).

Sulfate—Dissolve about 150 mg, accurately weighed, in 75 mL of water, add 5 mL of 3 N hydrochloric acid, heat to boiling, and while maintaining at the boiling point, slowly add 10 mL of barium chloride TS. Cover the vessel, and allow the mixture to stand on a steam bath for 1 hour. Filter, wash the precipitate with several portions of hot water, dry, and ignite to constant weight. The weight of the barium sulfate, multiplied by 0.4117, represents the weight of sulfate in the portion of Protamine Sulfate taken. Not less than 16% and not more than 22%, calculated on the dried basis, is found.

Nitrogen content—Determine the nitrogen content as directed under *Method II* (see *Nitrogen Determination* ⟨461⟩). Not less than 22.5% and not more than 25.5% of N, calculated on the dried basis, is found.

Change to read:

Assay—

Assay preparation—Dissolve a suitable quantity of Protamine Sulfate, accurately weighed, in Water for Injection to obtain a solution having a concentration of 1 mg per mL, calculated on the dried basis.

•*Preparation of plasma*—Collect blood from sheep directly into a vessel containing 8% sodium citrate solution in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly centrifuge the blood, and pool the separated plasma. To a 1-mL portion of the pooled plasma in a clean test tube add 0.2 mL of calcium chloride solution (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 mL in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.• (RB 1-Oct-2009)

Heparin preparation—On the day of the assay, prepare a solution of USP Heparin Sodium RS in saline TS to give a final concentration of •139•(RB 24-Oct-2008) USP Heparin Units per mL.

Calcium–thromboplastin solution—Dissolve in calcium chloride solution (1 in 50) a quantity of thromboplastin that is sufficient, as determined by preliminary trial if necessary, to produce clotting in about 35 seconds in a mixture consisting of equal volumes of plasma and a mixture of 4 volumes of saline TS and 1 volume of the prepared calcium–thromboplastin solution.

Procedure—Into each of 10 meticulously cleansed, 13- × 100-mm test tubes pipet 2.5 mL of *Plasma*. Place the tubes in a water bath at 37 ± 0.2°, and to each of nine of them add 0.5 mL of *Assay preparation*. Into the tenth tube, to provide the control, pipet 2 mL of saline TS and 0.5 mL of *Calcium–thromboplastin solution*, noting the time, to the nearest second, of adding the latter. While mixing with a wire loop, note the time of the first appearance of fibrin fibers, and record it to the nearest second. The elapsed time is the normal clotting time of the plasma. Pipet into the nine remaining tubes the following volumes, in mL, of *Heparin preparation:* 0.43, 0.45, 0.47, 0.49, 0.50, 0.51, 0.53, 0.55, and 0.57, respectively. To each tube add saline TS to make 4.5 mL. Taking the tubes in random order, add 0.5 mL of *Calcium–thromboplastin solution*, and note the clotting time in each tube in the same manner as for the control tube.

Calculation—Calculate the number of USP Heparin Units neutralized per mg taken by the formula:

$$N_S / W_U$$

in which N_S is the number of USP Heparin Units, and W_U is the number of mg of Protamine Sulfate in the last tube prior to the first one in which the clotting time is not less than 2 seconds longer than that in the control tube.