

Change to read:

Heparin Sodium Injection

•DEFINITION

Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits a potency NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

ASSAY

• ANTI-FACTOR IIa POTENCY

NOTE—Allow alternative platforms.

pH 8.4 Buffer: Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 Buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagents Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 Buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water and dilute with *pH 8.4 Buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. To each tube add twice the volume (100–200 μ L), of *Antithrombin solution* and one volume (50–100 μ L), of either the *pH 8.4 Buffer* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 μ L of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 μ L of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just prior to use.

Two different types of measurements can be recorded:

1. **ENDPOINT MEASUREMENT:** Stop the reaction after at least 1 min with 50–100 μ L of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). The RSD over the blank readings is less than 10%.
2. **KINETIC MEASUREMENT:** Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). Calculate the change in absorbance/min (Δ OD/min). The blanks for kinetic measurement are also expressed as Δ OD/min and should give the highest values as they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Sample solutions* and the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the activity of Heparin Sodium/mg, calculated on the dried basis.

Acceptance criteria: NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.03 USP Endotoxin Unit/USP Heparin Unit.

• **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

• **pH** (791): 5.0–7.5

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* (1).

ADDITIONAL REQUIREMENTS

• **LABELING:** Label it to indicate the volume of the total contents and the potency in terms of USP Heparin Units only per mL, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, and store at a temperature below 40°, preferably at room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS

USP Heparin Sodium for Assays RS₅