# Heparin Sodium Injection

## DEFINITION

Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits 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**

**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, NMT 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 having a concentration 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—Reagent Specifications*) in water to obtain a solution having a concentration of 20 thrombin IU/mL. Dilute this solution 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 the *Standard solutions* to obtain similar concentrations of heparin sodium.

**Analysis**

[NOTE—The procedure can also be performed using alternative platforms.] For each dilution of the *Standard solutions* and the *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; at least T1, T2, T3, and T4 in duplicate for the dilutions of the *Sample solutions*; and at least S1, S2, S3, and S4 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. Add twice the volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. All reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100 µL of the *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 (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values because 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 per min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of heparin sodium, in USP Units/mL, using statistical methods for parallel-line assays. Express the potency of heparin sodium per 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 per min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of heparin sodium, in USP Units/mL, using statistical methods for slope-ratio assays. Express the potency of heparin sodium per mg, calculated on the dried basis.

**Acceptance criteria:** 90.0%–110.0%

## SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** 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:** Meets the requirements for *Injections* ⟨1⟩

## ADDITIONAL REQUIREMENTS

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

***Change to read:***

- **LABELING** •*Injections* ⟨**1**⟩ *Labels and Labeling, Strength and Total Volume for Single- and Multiple-Dose Injectable*: For single-dose and multiple-dose injectable drug products, the strength per total volume should be the primary and prominent expression on the principal display panel of the label, followed in close proximity by strength per mL enclosed by parentheses. For containers holding a volume of less than 1 mL, the strength per fraction of an mL should be the only expression of strength. Strength per single mL should be expressed as mg/mL, not mg/1 mL. For further information, see the entirety of *Injections* ⟨1⟩.

Label it to indicate the tissue and the animal species from which it is derived.● (IRA 1-May-2013)

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS
  USP Heparin Sodium for Assays RS