Enoxaparin Sodium Injection

DEFINITION

Enoxaparin Sodium Injection is a sterile solution of Enoxaparin Sodium in Water for Injection. Its appearance is analyzed for clarity and degree of color, using a validated method. Its potency value is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU). It may contain, in multiple-dose containers, a suitable antimicrobial preservative, such as benzyl alcohol.

IDENTIFICATION

- **A.**
  Analysis: Transfer the total contents of a single-dose container or 0.4 mL from a multiple-dose container to a glass test tube, add 2 mL of water and 1 mL of 2% (w/v) protamine sulfate solution, and mix.
  Acceptance criteria: A creamy white precipitate is formed.
- **B. ULTRAVIOLET ABSORPTION ⟨197U⟩**
  Medium: 0.01 N hydrochloric acid
  Standard solution: 500 µg/mL
  Sample solution: Transfer the total content of a single-dose container or 0.4 mL from a multiple-dose container to a 100-mL volumetric flask. Dilute with *Medium* to volume.
  Acceptance criteria: The spectra exhibit maxima at 231 ± 2 nm.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** Meets the requirements

ASSAY

Change to read:

- **ANTI-FACTOR Xa ACTIVITY**
  Acetic acid solution: Glacial acetic acid and water (42:58)
  pH 7.4 polyethylene glycol 6000 buffer: Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000, adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.
  pH 7.4 buffer: Dissolve 6.08 g of tris(hydroxymethyl)-aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.
  pH 8.4 buffer: Dissolve 3.03 g of tris(hydroxymethyl)-aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.
  Human antithrombin III solution: Reconstitute a vial of antithrombin III (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin III Units/mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Antithrombin III Unit/mL.
  Factor Xa solution: Reconstitute a weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min when assayed as described below but using as an appropriate volume, *V*, the volume in µL of *pH 7.4 buffer* instead of *V* µL of the enoxaparin solution.
  Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for Factor Xa in water to obtain a concentration of about 3 mM. Dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM.
  Standard solutions: •Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute• (RB 1-Dec-2011) with *pH 7.4 buffer* to obtain four dilutions in the concentration range between 0.025 and 0.2 Anti-Factor Xa IU/mL.
  Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.
  Analysis
  Samples: *Standard solutions*, *Sample solutions*, *Human antithrombin III solution*, *pH 7.4 buffer*, *Factor Xa solution*, *Chromogenic substrate solution*, and *Acetic acid solution*
  Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions*. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V* (20–50 µL), of *Human antithrombin III solution* and an equal volume, *V*, of either the blank *(pH 7.4 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 1.0 min. Add to each tube 2*V* (40–100 µL) of *Factor Xa solution*, and incubate for 1.0 min. Add a 5*V* (100–250 µL) volume of *Chromogenic substrate solution*. Stop the reaction after 4.0 min with a 5*V* (100–250 µL) volume of *Acetic acid solution*. Measure the absorbance of each solution at 405 nm, using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩) against blank B1. The reading of blank B2 relative to blank B1 is NMT ±0.05 absorbance unit.
  Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor Xa activity/mL, using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated.
  Acceptance criteria: The potency is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU).
- **ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO:** The ratio of the numerical value of the Anti-Factor Xa activity in Anti-Factor Xa IU/mL to the numerical value of the Anti-Factor IIa activity in Anti-Factor IIa IU/mL, as determined by *Anti-Factor Xa Activity* and *Anti-Factor IIa Activity*, respectively, is NLT 3.3 and NMT 5.3.

OTHER COMPONENTS

Change to read:

- **BENZYL ALCOHOL CONTENT (IF PRESENT)**
  Mobile phase: Acetonitrile, methanol, and water (3:1:16)
  Standard solution: 1.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*
  Sample solution: Transfer •exactly• (RB 1-Dec-2011) 5.0 mL of the Injection to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC
Detector: UV 256 nm
Column: 4.6-mm × 15-cm stainless steel; packing L7[1]
Flow rate: 1.0 mL/min, maintained constant to ±10%
Injection volume: 20 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (w/v) of benzyl alcohol in the portion of Injection taken:

$$\bullet\text{Result} = (r_U/r_S) \times C\bullet \text{ (RB 1-Dec-2011)}$$

r_U = peak area of benzyl alcohol from the *Sample solution*
r_S = peak area of benzyl alcohol from the *Standard solution*
$\bullet C$ = concentration of benzyl alcohol in the *Standard solution* (mg/mL)$\bullet$ (RB 1-Dec-2011)
Acceptance criteria: 1.35%–1.65%

SPECIFIC TESTS

- **pH ⟨791⟩:** 5.5–7.5
- **Bacterial Endotoxins Test ⟨85⟩:** It contains less than 0.01 USP Endotoxin Unit/unit of Anti-Factor Xa activity in Anti-factor Xa IU.

Change to read:

- **Anti-Factor IIa Activity**
Acetic acid solution, pH 7.4 polyethylene glycol 6000 buffer, pH 7.4 buffer, pH 8.4 buffer, and **Human antithrombin III solution:** Proceed as directed in the *Assay* for *Anti-Factor Xa Activity*, except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit/mL.
Thrombin human solution: Reconstitute thrombin human (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL.
Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Immediately before use, dilute with *pH 8.4 buffer* to 0.5 mM.
Standard solutions: $\bullet$Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute$\bullet$ (RB 1-Dec-2011) with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.
Standard solutions: Dilute USP Enoxaparin Sodium Solution for Bioassays RS with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.
Sample solutions: Proceed as directed under *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.
Analysis: Proceed as directed in the *Assay* for *Anti-Factor Xa Activity*, except to use *Thrombin human solution* instead of *Factor Xa solution* and to use *Human antithrombin III solution* as described above.
Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor IIa activity/mL, using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits.
Acceptance criteria: $\bullet$The Anti-Factor IIa activity IU (or IU/mL) is NLT 20.0% and NMT 35.0% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU or IU/mL).$\bullet$ (RB 1-Dec-2011)

Change to read:

- **Free Sulfate Content**
Mobile phase: 3.0 mM sodium carbonate solution
System suitability solution: 3 µg/mL of sulfate anion and 5 µg/mL of oxalate anion
Standard sulfate stock solution: Prepare a solution of sodium sulfate in *Mobile phase* in a suitable sulfate-free container such that the concentration of sulfate is accurately known at about 1 mg/mL. Transfer 5 g of the solution to a similar container, and add *Mobile phase* to obtain 25 g of solution.
Standard solution A: 0.1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Standard solution B: 0.5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Standard solution C: 1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Standard solution D: 2 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Standard solution E: 4 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Standard solution F: 5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*
Sample solution: $\bullet$Transfer a known quantity, *m*, of Enoxaparin Sodium Injection, accurately weighed, to a suitable previously tared sulfate-free vial. Add *Mobile phase* to obtain a solution having a known concentration of about 10 mg/g.$\bullet$ (RB 1-Dec-2011)
Chromatographic system
(See *Chromatography ⟨621⟩, System suitability*.)
Mode: Ion chromatography
Detector: Conductivity
Column
Guard: 4-mm × 5-cm; packing L61
Analytical: 4-mm × 25-cm; packing L61
[Note—Use a micromembrane anion autosuppressor[2] or a suitable chemical suppression system.]
Flow rate: 2.0 mL/min
Injection size: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1 between the sulfate and oxalate peaks
Analysis
Samples: *Standard solutions A–F* and *Sample solution*
Plot the standard curve of sulfate peak height as a function of sulfate concentration (in µg/g) in *Standard solutions A–F*. From the sulfate peak height determine the concentration of sulfate, *C*, in µg/g, in the *Sample solution*, using the standard curve. Calculate the percentage of free sulfate content (w/w) in the portion of Injection taken:

$$\text{Result} = [(C \times M_S)/10m)]\ \bullet\bullet \text{ (RB 1-Dec-2011)}$$

M_S = total mass of the *Sample solution* (g)
m = mass of Injection taken to prepare the *Sample solution* (mg)

[1] Available as Lichrospher 100 RP 18, pore size 100 Å, particle size 5 µm, or equivalent.

[2] Available as Anion Self-Regenerating Suppressor (ASRS) from Dionex Inc, or equivalent.

Acceptance criteria: The percentage of free sulfate is NMT 0.12% •(w/w).• (RB 1-Dec-2011)

- **STERILITY TESTS ⟨71⟩:** Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers in Type I glass. Store between 20° and 25°, excursions permitted between 15° and 30°.
- **LABELING:** Label it to indicate the amount (mg) of Enoxaparin Sodium in the total volume of contents. The label states also that the Enoxaparin Sodium starting material is porcine derived.

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Benzyl Alcohol RS
  USP Endotoxin RS
  USP Enoxaparin Sodium RS
  USP Enoxaparin Sodium •• (RB 1-Dec-2011) for Bioassays RS