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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.

Change to read:

• **B.** ▲Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

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

• **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 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 Xasolution*, *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 *Ultraviolet-Visible Spectroscopy ⟨857⟩*) 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

• **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 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:

$$\text{Result} = (r_U/r_S) \times C$$

r_U = peak area of benzyl alcohol from the *Sample solution*

r_S = peak area of benzyl alcohol from the *Standard solution*

C = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

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.

• **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: Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute 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: 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).

• **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: 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.

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:

$$\text{Result} = [(C \times M_S)/10m)]$$

M_S = total mass of the *Sample solution* (g)

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

Acceptance criteria: The percentage of free sulfate is NMT 0.12% (w/w).

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Particulate Matter in Injections ⟨788⟩:** Meets the requirements

• **Other Requirements:** It meets the requirements under *Injections and Implanted Drug Products ⟨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.

• **USP Reference Standards ⟨11⟩**

USP Benzyl Alcohol RS

USP Enoxaparin Sodium RS

USP Enoxaparin Sodium for Bioassays RS

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[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.

Topic/Question	Contact	Expert Committee
ENOXAPARIN SODIUM INJECTION	Jennifer Tong Sun Senior Scientist II	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

Chromatographic Database Information: Chromatographic Database

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