Fondaparinux Sodium Injection

Type of Posting	Revision Bulletin
Posting Date	30-Oct-2020
Official Date	1-Nov-2020
Expert Committee	Biologics Monographs 3 - Complex Biologics & Vaccines

In accordance with the Rules and Procedures of the Council of Experts, the Biologics Monographs 3 - Complex Biologics & Vaccines Expert Committee has revised the Fondaparinux Sodium Injection monograph. The purpose for the revision is to widen the acceptance criteria for fondaparinux related compound C from NMT 0.4% (w/w) to NMT 0.8% (w/w) for 5 mg/mL strength under the test for *Organic Impurities*.

The Fondaparinux Sodium Injection Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Chisty Basha SKM, Scientific Liaison (888-887-6654 or skb@usp.org).

Revision Bulletin
Official: November 1, 2020

Fondaparinux Sodium Injection

DEFINITION

Fondaparinux Sodium Injection is a sterile solution of Fondaparinux Sodium in Water for Injection with sodium chloride added for isotonicity. It is a clear, colorless to slightly yellow solution.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. Identification Tests—General, *Chloride* ⟨191⟩:** Proceed as directed in the chapter. Meets the requirements of the *Chloride and Sulfate* ⟨221⟩ test.

ASSAY

• **Procedure**

5 mM phosphate solution: 0.210 g of monobasic sodium phosphate dihydrate and 0.650 g of dibasic sodium phosphate dihydrate. Dissolve in and dilute with water to 1000 mL. pH is approximately 7.3.

Solution A: 15 ± 10 ppm of dimethylsulfoxide (DMSO) in *5 mM phosphate solution* (1 in 67000, v/v)

Solution B: 2.0 M sodium chloride solution in *5 mM phosphate solution*

Mobile phase: See *Table 1*. [Note—Make adjustments to *Solution A* as necessary, and degas the *Mobile phase* before use. Dissolved gas in the injected solution may lead to baseline interference. Degassing of the *Mobile phase* is critical to obtain a suitable signal-to-noise ratio and higher sensitivity. An eluant generator[1] installed between the injector and the column may reduce the baseline interference.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
5	50	50
25	5	95
30	5	95
35	50	50
50	50	50

System suitability solution: 5.0 mg/mL of USP Fondaparinux Sodium System Suitability Mixture B RS

Standard solution: 5.0 mg/mL of USP Fondaparinux Sodium for Assay RS in water. Prepare in duplicate.

Sensitivity check solution: 0.01 mg/mL of USP Fondaparinux Sodium for Assay RS in water from the *Standard solution*

Sample solution: Transfer the contents of prefilled syringes to a suitable container, and mix well. Dilute with water, if needed, to obtain a 5.0-mg/mL solution of fondaparinux sodium.

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 25-cm; packing L46

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, and *Blank*

Inject the *Blank* in duplicate, the *Sensitivity check solution*, and the *System suitability solution*. Inject the *Standard solution* at least six times consecutively.

Suitability requirements

Specificity and baseline drift: The chromatogram of a second *Blank* injection shows a baseline drift between 0.00 and 0.02 AU over 30 min. If necessary, adjust the DMSO content of the *Mobile phase* until an acceptable baseline is achieved. The chromatogram of a second *Blank* injection does not contain peaks between 3.00 and 30.00 min.

Chromatogram similarity: The chromatogram of the *System suitability solution* corresponds to that of the reference chromatogram provided with USP Fondaparinux Sodium System Suitability Mixture B RS.

Signal-to-noise ratio: NLT 10 for the fondaparinux peak in the chromatogram of the *Sensitivity check solution*

Resolution: NLT 1.2 between fondaparinux related compound C and fondaparinux related compound D, *System suitability solution*; NLT 1.1 between fondaparinux related compound F and fondaparinux related compound G (see *Table 2*), *System suitability solution*

Standard agreement: The difference in the mean response factors for each *Standard solution* is NMT 2.0%.

Relative standard deviation: For six consecutive injections of the *Standard solution* the calculated % RSD of the area of the fondaparinux peak is NMT 2.0%. The retention time of the fondaparinux peak should be ±5% of the mean value. The calculated % RSD of the response factors for six consecutive injections of the *Standard solution* is NMT 2.0%. The calculated % RSD of the pooled response factors for all injections of the *Standard solution* is NMT 2.0%. The % RSD of the mean response factors for the duplicate preparations of the duplicate *Standard solutions* is NMT 2.0%.

Analysis

Samples: *Standard solution* and *Sample solution*

Inject the *Standard solution* at least six times consecutively. Inject duplicate preparations of the *Sample solution*. Record the chromatograms, and measure the retention times and areas for the major peaks (excluding peaks before 3.00 and after 30.00 min).

Calculations: For each injection of the *Standard solution* calculate a response factor (F_R):

$$F_R = (C_S/r_S)$$

C_S = concentration of fondaparinux sodium in the *Standard solution* (mg/mL)

r_S = peak response of fondaparinux sodium from the *Standard solution*

Relative retention times (RRT) are calculated by dividing the retention time of the peak by the retention time of fondaparinux established by the *Standard solution*. Using the mean response factor (F_M), calculate the concentration (mg/mL) of fondaparinux sodium in each injection of the *Sample solution*:

$$\text{Result} = F_M \times r_U \times D_U$$

F_M = mean response factor from the *Standard solution*

= peak response of fondaparinux sodium in the *Sample solution*

r_U

D_U = dilution factor for the *Sample solution*, if needed

Acceptance criteria: 90%–105% (for the 2.5-mg/0.5-mL injection) or 95%–105% (for the 5.0-mg/0.4-mL, 7.5-mg/0.6-mL, and 10-mg/0.8-mL injections)

IMPURITIES

• Free Sulfate Determination

[Note—Regenerate the anion-exchange column for 15 min with 0.1 M sodium hydroxide after each injection of fondaparinux sample, followed by equilibration with *Mobile phase* for 21 min.]

Mobile phase: 3 mM carbonate solution using 0.106 g of sodium carbonate and 0.168 g of sodium hydrogen carbonate in 1000 mL of water

Standard solution 1: Prepare a 1000-ppm sulfate solution, using anhydrous sodium sulfate in water.

Standard solution 2: Prepare a 10-ppm sulfate solution by diluting *Standard solution 1* in water.

Sensitivity check solution: Dilute 1.0 mL of *Standard solution 2* with water to 5.0 mL.

Resolution solution: 0.100 g of anhydrous sodium sulfate and 0.100 g of sodium chloride. Dissolve in and dilute with water to 100.0 mL. Dilute 1.0 mL with water to 100.0 mL.

Sample solution: In triplicate, combine and mix the contents of a suitable number of syringes. Dilute 0.8 mL (strengths of 5.0 mg/0.4 mL, 7.5 mg/0.6 mL, and 10.0 mg/0.8 mL) or 2.0 mL (strengths of 1.5 mg/0.3 mL and 2.5 mg/0.5 mL) with water to 5.0 mL.

Blank: A sample of the water used to prepare other solutions

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*).

Mode: LC

Detector: Conductivity; range 200 µS, suppressor current 300 mA

Column: 4.6-mm × 5-cm; packing L23, coupled with a neutralization micromembrane suppressor[2]

Column temperature: Ambient

Regenerating solvent for the suppressor: Ultrapurified water in a counter current direction

Flow rate: 1.0 mL/min

Injection volume: 50 µL

Run time: 24 min

System suitability

Samples: *Standard solution 2*, *Sensitivity check solution*, *Resolution solution*, and *Blank*

Suitability requirements

Specificity: The chromatogram of a second *Blank* injection does not contain a peak corresponding to the sulfate ion.

Signal-to-noise-ratio: NLT 10, *Sensitivity check solution*

Resolution: NLT 10 between the sulfate and chloride peaks, *Resolution solution*

Relative standard deviation: NMT 5% of the response factors for six consecutive injections of *Standard solution 2*

Standard agreement: NMT 5% difference in the mean response factors for each *Standard solution 2* injection

Analysis: Inject the *Blank* in duplicate, the *Sensitivity check solution*, and the *Resolution solution*. Inject *Standard solution 2* at least six times consecutively. Inject triplicate preparations of the *Sample solution*. Record the chromatograms, and measure the retention times and areas for the sulfate peaks found.

Calculations: For each injection of *Standard solution 2*, calculate a response factor (F):

$$F = (C_S/r_S)$$

C_S = concentration of sodium sulfate in *Standard solution 2* (mg/mL)

r_S = peak response of the sulfate peak from *Standard solution 2*

Using the mean response factor (F_M), calculate the concentration (% w/w) of free sulfate in each injection of the *Sample solution*:

$$\text{Result} = F_M \times r_U \times D_U \times (M_{r1}/M_{r2}) \times (100/C)$$

F_M = mean response factor from *Standard solution 2*

r_U = peak response of the sulfate ion in the *Sample solution*

D_U = dilution factor for the *Sample solution*

M_{r1} = molecular weight of the sulfate ion, 96.1

M_{r2} = molecular weight of sodium sulfate, 142.0

C = nominal concentration of fondaparinux sodium in the content of the syringe

Acceptance criteria: NMT 0.50% (w/w)

Change to read:

• **Organic Impurities**

System suitability solution, Standard solution, Sensitivity check solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, *Sample solution*, and *Blank*

Calculate the percentage (area/area) of each individual unspecified impurity for each injection of the *Sample solution*:

$$\text{Result} = [r_U/(r_T + r_S)] \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses for degradation impurities from the *Sample solution*

r_S = peak response of fondaparinux sodium from the *Sample solution*

Taking into account the response factors for specified impurities (see *Table 2*), calculate the individual content (% w/w) of specified fondaparinux related compounds B, C, and G:

$$\text{Result} = (r_U \times F_i \times 100)/\{[\Sigma(r_U \times F_i)] + r_S\}$$

r_U = peak response of each impurity from the *Sample solution*

F_i = relative response factor for the individual impurity peak (response factor of fondaparinux sodium/response factor of individual impurity [see *Table 2*])

r_S = peak response of fondaparinux sodium from the *Sample solution*

Calculate the total degradation product content by summing the mean unrounded content values for the following peaks: fondaparinux related compounds A, B, C, D, F, and G and any unspecified impurities that are not synthetic impurities. Exclude peaks below the LOQ (0.003% w/w for fondaparinux related compound B, 0.002% w/w for fondaparinux related compound G, and 0.200% for all other degradation products and fondaparinux related compound E).

Individual impurities: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fondaparinux related compound A	0.35	1.0	1.0 (a/a)
Fondaparinux related compound B[a]	0.48	70	0.150 (w/w)
Fondaparinux related compound C[b]	0.76	1.0	0.8 (w/w)▲ ▲ (RB 1-Nov-2020)
Fondaparinux related compound D	0.80	1.0	0.8 (a/a)
Fondaparinux related compound E[c]	0.93	—	0.8 (a/a)
Fondaparinux related compound F[d]	1.29	1.0	2.0 (a/a)
Fondaparinux related compound G[e]	1.34	100	0.10 (w/w)
Fondaparinux sodium	—	1.0	—
Individual Unspecified	—	—	0.5 (a/a)
Total impurities	—	—	5.0

[a] Methyl-*O*-(4-deoxy-2-*O*-sulfo-α-L-threo-hex-4-enopyranosyluronate)-(1→4)-*O*-(2-deoxy-6-*O*-sulfo-2-sulfamino-α-D-glucopyranoside), tetrasodium salt.

[b] Methyl *O*-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronate)-(1→4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-amino-α-D-glucopyranosyl-(1→4)-*O*-2-*O*-sulfo-α-L-idopyranosyluronate)-(1→4)-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranoside), nonasodium salt.

[c] Synthetic impurity included for identification purposes only and excluded from impurities calculations.

[d] The fondaparinux related compound F peak can appear as a complex set of peaks in the region RRT 1.2 to RRT 1.24. These peaks, which may not be fully resolved from each other, appear before the fondaparinux related compound G peak. In such a case, the integration should be performed so that all such peaks are combined. Specified degradation products can be assigned by reference to the specimen chromatogram of the *System suitability solution* associated with USP Fondaparinux Sodium System Suitability Mixture B RS.

[e] 2-Deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-(β-D-glucopyranosyluronate)-(1→4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronate)-(1→4)-(1,2-dideoxy-6-*O*-sulfo-2-(sulfoamino)-D-enoglucopyranoside), decasodium salt.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 3.3 USP Endotoxin Units/mg of fondaparinux sodium
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **Sterility Tests ⟨71⟩:** Where it is labeled as sterile, it meets the requirements.
- **pH ⟨791⟩:** 5.0–8.0, in a solution, at 20°–25°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers in Type I glass or other validated container-closure system. Store at or below 25°.
- **Labeling:** Label it to indicate the amount, in mg, of fondaparinux sodium in the total volume of contents.
- **USP Reference Standards ⟨11⟩**
  USP Fondaparinux Sodium for Assay RS
  USP Fondaparinux Sodium System Suitability Mixture B RS

---

[1] One suitable eluant generator is Dionex DEGAS EG40/50 (12 × 17 cm, thickness 2.2 cm).

[2] One suitable suppressor is Dionex ASRS 300 4 mm.

Page Information:

Not Applicable

DocID: