Fondaparinux Sodium Injection

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<tr>
<td>Expert Committee</td>
<td>Biologics Monographs 3 - Complex Biologics &amp; Vaccines</td>
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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).
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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
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<tr>
<td>35</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

**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°C  
**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):

\[
F_R = \frac{C_S}{r_S}
\]

*CS*  = 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*  
*r_U*  = peak response of fondaparinux sodium in the *Sample solution*
\[ r_U \]

\[ D_U \quad = \text{dilution factor for the Sample solution, if needed} \]

**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 x 5-cm; packing L23, coupled with a neutralization micromembrane suppressor²

**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 \((F)\):

\[
F = \left( \frac{C_S}{r_S} \right)
\]

\(C_S\) = concentration of sodium sulfate in Standard solution 2 (mg/mL)
\[ r_S = \text{peak response of the sulfate peak from Standard solution 2} \]

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

\[
\text{Result} = F_M \times r_U \times D_U \times \left( \frac{M_{r1}}{M_{r2}} \right) \times \left( \frac{100}{C} \right)
\]

- \( F_M \) = 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:

\[
\text{Result} = \left[ \frac{r_U}{(r_T + r_S)} \right] \times 100
\]

- \( r_U \) = 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:

\[
\text{Result} = \left( \frac{r_U \times F_i \times 100}{\Sigma (r_U \times F_i) + r_S} \right)
\]

- \( r_U \) = 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>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fondaparinux related compound A</td>
<td>0.35</td>
<td>1.0</td>
<td>1.0 (a/a)</td>
</tr>
<tr>
<td>Fondaparinux related compound B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48</td>
<td>70</td>
<td>0.150 (w/w)</td>
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<tr>
<td>Fondaparinux related compound C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76</td>
<td>1.0</td>
<td>0.8 (w/w)&lt;sup&gt;▲&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fondaparinux related compound D</td>
<td>0.80</td>
<td>1.0</td>
<td>0.8 (a/a)</td>
</tr>
<tr>
<td>Fondaparinux related compound E&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.93</td>
<td>—</td>
<td>0.8 (a/a)</td>
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<tr>
<td>Fondaparinux related compound F&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.29</td>
<td>1.0</td>
<td>2.0 (a/a)</td>
</tr>
<tr>
<td>Fondaparinux related compound G&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.34</td>
<td>100</td>
<td>0.10 (w/w)</td>
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<tr>
<td>Fondaparinux sodium</td>
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<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Individual Unspecified</td>
<td>—</td>
<td>—</td>
<td>0.5 (a/a)</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Methyl-O-4-deoxy-2-O-sulfo-a-L-threo-hex-4-enopyranosuronate)-(1→4)-O-(2-deoxy-6-O-sulfo-2-sulfoamino-a-D-glucopyranoside), tetrasodium salt.<br><sup>b</sup> Methyl-O-2-deoxy-6-O-sulfo-2-(sulfoamino)-a-D-glucopyranosyl)-(1→4)-O-(β-D-glucopyranosyluronate)-(1→4)-O-(2-deoxy-3,6-di-O-sulfo-2-amino-a-D-glucopyranosyl)-(1→4)-O-2-O-sulfo-a-L-idopyranosylonate)-(1→4)-(2-deoxy-6-O-sulfo-2-(sulfoamino)-a-D-glucopyranoside), nonasodium salt.<br><sup>c</sup> Synthetic impurity included for identification purposes only and excluded from impurities calculations.<br><sup>d</sup> 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 BRS.<br><sup>e</sup> 2-Deoxy-6-O-sulfo-2-(sulfoamino)-a-D-glucopyranosyl-(1→4)-O-(β-D-glucopyranosyluronate)-(1→4)-O-(2-deoxy-3,6-di-O-sulfo-2-(sulfoamino)-a-D-glucopyranosyl)-(1→4)-O-(2-O-sulfo-a-L-idopyranosylonate)-(1→4)-(1,2-dideoxy-6-O-sulfo-2-(sulfoamino)-a-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

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1. One suitable eluant generator is Dionex Degas EG40/50 (12 x 17 cm, thickness 2.2 cm).
2. One suitable suppressor is Dionex ASRS 300 4 mm.

**Page Information:**

- Not Applicable

**DocID:**

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