



## 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<sup>1</sup> 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  $\mu$ S, suppressor current 300 mA

**Column:** 4.6-mm  $\times$  5-cm; packing L23, coupled with a neutralization micromembrane suppressor<sup>2</sup>

**Column temperature:** Ambient

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

**Flow rate:** 1.0 mL/min

**Injection volume:** 50  $\mu$ L

**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:

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

$r_U$  = 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 <sup>a</sup>	0.48	70	0.150 (w/w)
Fondaparinux related compound C <sup>b</sup>	0.76	1.0	0.8 (w/w) <sup>▲</sup> ▲ (RB 1-Nov-2020)
Fondaparinux related compound D	0.80	1.0	0.8 (a/a)
Fondaparinux related compound E <sup>c</sup>	0.93	—	0.8 (a/a)
Fondaparinux related compound F <sup>d</sup>	1.29	1.0	2.0 (a/a)
Fondaparinux related compound G <sup>e</sup>	1.34	100	0.10 (w/w)
Fondaparinux sodium	—	1.0	—
Individual Unspecified	—	—	0.5 (a/a)
Total impurities	—	—	5.0

<sup>a</sup> Methyl-*O*-(4-deoxy-2-*O*-sulfo- $\alpha$ -L-threo-hex-4-enopyranosyluronate)-(1 $\rightarrow$ 4)-*O*-(2-deoxy-6-*O*-sulfo-2-sulfamino- $\alpha$ -D-glucopyranoside), tetrasodium salt.

<sup>b</sup> Methyl *O*-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-*O*-( $\beta$ -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-amino- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-*O*-2-*O*-sulfo- $\alpha$ -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)- $\alpha$ -D-glucopyranoside), nonasodium salt.

<sup>c</sup> Synthetic impurity included for identification purposes only and excluded from impurities calculations.

<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 B RS](#).

<sup>e</sup> 2-Deoxy-6-*O*-sulfo-2-(sulfoamino)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*-( $\beta$ -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2-*O*-sulfo- $\alpha$ -L-idopyranosyluronate)-(1 $\rightarrow$ 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](#)
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<sup>1</sup> One suitable eluant generator is Dionex DEGAS EG40/50 (12 × 17 cm, thickness 2.2 cm).

<sup>2</sup> One suitable suppressor is Dionex ASRS 300 4 mm.

#### **Page Information:**

Not Applicable

#### **DocID:**

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