Protamine Sulfate Injection

DEFINITION

Protamine Sulfate Injection is a sterile, isotonic solution of Protamine Sulfate. Protamine Sulfate used in the manufacture of Protamine Sulfate Injection complies with the compendial requirements stated in the *Protamine Sulfate* monograph. Each mg of Protamine Sulfate, used in the manufacture of the Injection, neutralizes NLT 100 USP Heparin Units, calculated on the dried basis. It contains NLT 90.0% and NMT 120.0% of the labeled amount of protamine sulfate.

IDENTIFICATION

- **A.** The retention times of the four major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Sulfate*:** Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**

Solution A: •0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 1.8.• (IRA 1-Jul-2016) Pass the solution through a membrane filter of 0.45-µm pore size, and degas before use.
Solution B: *Solution A* and acetonitrile (93.5: 6.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	55	45
25	55	45
30	85	15

[NOTE—Initial gradient composition may be adjusted as appropriate to obtain sufficient resolution. The end of the gradient can be increased to re-equilibrate the column for the next injection.]
Standard solution: 0.5 mg/mL of USP Protamine Sulfate RS in 0.01 M hydrochloric acid
Sample solution: 0.5 mg/mL of protamine sulfate in 0.01 M hydrochloric acid
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 55°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Retention time: The chromatogram of the *Standard solution* must show four major peaks (in increasing elution order: protamine peptides 1, 2, 3, and 4), with protamine peptide 4 eluting no later than 15 min. [NOTE—See the reference chromatogram provided with the USP Protamine Sulfate RS certificate.]
Resolution: NLT 2.0 between protamine peptides 1 and 2, calculated by the tangent method
Relative standard deviation: NMT 2.0% for the total integrated areas of the six chromatograms of the *Standard solution*, using vertical drop-down integration
Analysis
Samples: *Standard solution* and *Sample solution*
Separately inject equal volumes of the *Standard solution* (at least six injections) and the *Sample solution* into the chromatograph. Record the chromatograms for approximately 30 min, and measure the responses for all the peaks observed using a full-scale comparable to the height of the largest peak and using vertical drop-down integration.
Calculate the percentage of the labeled amount of protamine sulfate in the portion of Injection taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Protamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of protamine sulfate in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 7.0 USP Endotoxin Units/mg of protamine sulfate.

Add the following:

- ••**pH ⟨791⟩:** 6.0–7.0• (IRA 1-Jan-2016)
- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** Meets the requirements for small-volume injections

Change to read:

- **OTHER REQUIREMENTS:** It meets the requirements in •*Injections and Implanted Drug Products* ⟨1⟩.• (CN 1-May-2016)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature or at 2°–8°.
- **LABELING:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Endotoxin RS
USP Heparin Sodium for Assays RS
USP Protamine Sulfate RS