Protamine Sulfate Injection

Type of Posting	Revision Bulletin
Posting Date	29–May–2015
Official Date	01–Jun–2015
Expert Committee	Monographs—Biologics and Biotechnology
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2010-2015 Council of Experts, the Monographs-Biologics and Biotechnology 1 Expert Committee has revised the Protamine Sulfate Injection monograph based on comments received.

The monograph has been revised to omit specification for the pH test. The pH specification will be re-proposed in an Interim Revision Announcement (IRA) at a later date to allow sufficient implementation time for stakeholders.

Should you have questions, please contact Anita Szajek, Scientific Liaison (301-816-8325 or aey@usp.org).

Protamine Sulfate Injection

DEFINITION

Change to read:

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.▲*USP38* 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

Add the following:

▲• **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*.▲*USP38*

Change to read:

• ▲**B.**▲*USP38* **IDENTIFICATION TESTS—GENERAL** ⟨**191**⟩**,** *Sulfate***:** Meets the requirements

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** 0.3 M sodium phosphate, pH 1.8. 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

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 standard chromatogram provided with USP Protamine Sulfate RS.]

Resolution: The resolution between protamine peptides 1 and 2 calculated by the tangent method is NLT 2.0.

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 (about 100 µL) 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%▲*USP38*

SPECIFIC TESTS

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

Delete the following:

•• **pH** ⟨**791**⟩**:** 6.0–7.0• (RB 1-Jun-2015)

Add the following:

▲• **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements for small-volume injections▲*USP38*

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature ▲or at 2°–8°.▲*USP38*

• **LABELING:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.

Change to read:

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Endotoxin RS

USP Heparin Sodium for Assays RS

▲USP Protamine Sulfate RS▲*USP38*