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## 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

#### • PROCEDURE

**Solution A:** 0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 1.8. Pass the solution through a membrane filter of 0.45- $\mu$ m 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  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 55°

**Flow rate:** 1 mL/min

**Injection volume:** 100  $\mu$ L

#### 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.
- **pH (791):** 6.0–7.0
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

#### 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 Protamine Sulfate RS

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
PROTAMINE SULFATE INJECTION	<a href="#">Julie Zhang</a> Associate Science & Standards Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

**Chromatographic Database Information:** [Chromatographic Database](#)

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