Protamine Sulfate

DEFINITION

Protamine Sulfate is a purified mixture of peptides obtained from the sperm or testes of Salmonidae, which has the property of neutralizing heparin. Each mg of Protamine Sulfate, calculated on the dried basis, neutralizes NLT 100 USP Heparin Units. It contains NLT 90.0% and NMT 110.0% of protamine sulfate, calculated on the dried basis.

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
- **C. BIOIDENTITY**

  Sample solution A: 0.15 mg/mL of Protamine Sulfate in water

  Sample solution B: Dilute 2.0 mL of *Sample solution A* with water to 3.0 mL.

  Sample solution C: Dilute 1.0 mL of *Sample solution A* with water to 3.0 mL.

  Titrant: USP Heparin Sodium for Assays RS in water (about 80–120 USP Heparin Units/mL)

  Analysis: [NOTE—Titrate each *Sample solution* in duplicate.] Transfer a volume of each *Sample solution* to the analytical cell of a suitable colorimeter, and set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range. Add *Titrant* in small volumes until there is a sharp increase in the absorbance, and note the volume of *Titrant* added. Perform the entire *Analysis* in triplicate for a total of 18 determinations.

  Calculate the number of USP Heparin Units per mg of Protamine Sulfate in the volume of *Titrant* added at the endpoint.

  Calculate the USP Heparin Units neutralized per mg of Protamine Sulfate taken:

$$\text{Result} = (V_T \times C_T)/(V_S \times C_U)$$

  V_T = volume of *Titrant* added (mL)
  C_T = concentration of *Titrant* (USP Heparin Units/mL)
  V_S = volume of each *Sample solution* (mL)
  C_U = concentration of Protamine Sulfate in each *Sample solution* (mg/mL)

  Calculate the potency of the Protamine Sulfate as the average of the 18 values. Calculate the three standard deviations for the results obtained with each *Sample solution*. Calculate the three standard deviations for the results obtained with each of the three independent assays. The test is valid if each of the six standard deviations is NMT 5% of the average result.

  Acceptance criteria: Each mg of Protamine Sulfate neutralizes NLT 100 USP Heparin Units on the dried basis.

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 through a membrane filter of 0.45-µm pore size, and degas before use.

  Solution B: •*Solution A* and acetonitrile (93.5: 6.5)• (IRA 1-Jul-2016)

  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 at least six replicate injections, 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*, record the chromatograms for approximately 30 min, and measure the responses for all the peaks using a full scale comparable to the height of the largest peak and using vertical drop down integration.

  Calculate the percentage of protamine sulfate in the portion of Protamine Sulfate 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 = concentration of Protamine Sulfate in the *Sample solution* (mg/mL)

  Acceptance criteria: 90.0%–110.0% on the dried basis

IMPURITIES

- **RESIDUAL SOLVENTS ⟨467⟩:** Meets the requirements
- **CHROMATOGRAPHIC PURITY**

  Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis
Sample: *Sample solution*
Proceed as directed in the *Assay*.
Calculate the percentage purity of Protamine Sulfate from the four protamine peptide peaks:

$$\text{Result} = [(r_{U1} + r_{U2} + r_{U3} + r_{U4})/r_T] \times 100$$

r_{U1} = peak response of protamine peptide 1 from the *Sample solution*
r_{U2} = peak response of protamine peptide 2 from the *Sample solution*
r_{U3} = peak response of protamine peptide 3 from the *Sample solution*
r_{U4} = peak response of protamine peptide 4 from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: NLT 92%

Delete the following:

- •• **Elemental Impurities—Limits** ⟨**232**⟩ and **Elemental Impurities—Procedures** ⟨**233**⟩**:** 20 ppm• (IRA 1-Jul-2016)
- **Iron** ⟨**241**⟩**:** NMT 10 ppm
- **Methylmercury:** NMT 10 ppm, using a validated analytical procedure. [NOTE—Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.]

SPECIFIC TESTS
- **Bacterial Endotoxins Test** ⟨**85**⟩**:** NMT 7.0 USP Endotoxin Units/mg

Delete the following:

- •• **pH** ⟨**791**⟩**:** 4–7• (ERR 1-Jun-2015)
- **Loss on Drying** ⟨**731**⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 5%
- **Ultraviolet Absorbance**
  Sample solution: 1.0% Solution of Protamine Sulfate in water
  Instrumental conditions
  Mode: UV
  Wavelength range: 260–280 nm
  Blank: Water
  Acceptance criteria: The difference between the absorbance of the *Sample solution* at 260–280 nm and the *Blank* is NMT 0.1.
- **Sulfate**
  Sample: 150 mg
  Analysis: Dissolve the *Sample* in 75 mL of water, add 5 mL of 3 N hydrochloric acid, heat to boiling, and while maintaining the boiling point, slowly add 10 mL of barium chloride TS. Cover the vessel, and allow the mixture to stand on a steam bath for 1 h. Filter, wash the precipitate with several portions of hot water, dry, and ignite to constant weight. The weight of the barium sulfate, multiplied by 0.4117, represents the weight of sulfate in the portion of Protamine Sulfate taken.
  Acceptance criteria: 16%–22% on the dried basis

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight and tamper-proof containers. Store at controlled room temperature or at 2°–8°.

Change to read:

- **USP Reference Standards** ⟨**11**⟩
  •USP Endotoxin RS• (IRA 1-Jul-2016)
  USP Heparin Sodium for Assays RS
  USP Protamine Sulfate RS