SPECIFIC TESTS

• **Percent Monomer**

Mobile phase: 34.84 mg/mL of arginine, 158.56 mg/mL of ammonium sulfate, and 100 mL/L of isopropyl alcohol in water. Adjust with phosphoric acid to a pH of 7.3, degas, and pass through a filter of 0.45-µm pore size.

System suitability solution: 1 mg/mL each of chicken ovalbumin and bovine gamma globulin

Standard solution: 1 mg/mL of USP Alteplase RS in water

Sample solution: 1 mg/mL of Alteplase for Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.5-mm × 30-cm; packing L25

Flow rate: 0.5–1.0 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.6 between gamma globulin and ovalbumin, *System suitability solution*

Column efficiency: NLT 1200 theoretical plates, determined from the alteplase peak, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate, as a percentage, the monomer in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the alteplase monomer

r_T = sum of all the peak responses related to alteplase

Acceptance criteria: NLT 95.0%

• **Single-Chain Content**

Mobile phase: 27.6 mg/mL of monobasic sodium phosphate in sodium dodecyl sulfate solution (1 in 1000). Adjust with sodium hydroxide to a pH of 6.8. Filter, and degas.

Dithiothreitol solution: 3.12 mg/mL of dithiothreitol in *Mobile phase*

Standard stock solution: Using an accurately weighed quantity of USP Alteplase RS, make a 1-mg/mL solution in water.

Standard solution: Pipet 1 mL of the *Standard stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Sample stock solution: Using an accurately weighed quantity of Alteplase for Injection, make a 1-mg/mL solution in water.

Sample solution: Pipet 1 mL of the *Sample stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.5-mm × 60-cm; packing L25

Flow rate: 0.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.1 between the single-chain and two-chain alteplase peaks

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.]

Calculate the percentage of single-chain alteplase in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for single-chain alteplase

r_T = sum of all the peak responses of alteplase

Acceptance criteria: No peaks or shoulders in the *Sample solution* that are not present in the *Standard solution* are found; NLT 60%.

• **Injections and Implanted Drug Products** ⟨1⟩**:** Meets the requirements of constituted solutions at the time of use

• **pH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 7.1–7.5

• **Water Determination** ⟨921⟩, *Method I*: NMT 4.0%

• **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 1 USP Endotoxin Unit/mg

• **Sterility Tests** ⟨71⟩**:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **Biological Reactivity Tests, In Vivo** ⟨88⟩**:** Meets the requirements for *Safety Tests—Biologicals*

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in hermetic, light-resistant containers, and store in a refrigerator.

• **Labeling:** Label it to state the biological activity in USP Alteplase Units/vial and the amount of protein/vial.

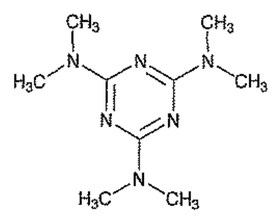
Change to read:

• **USP Reference Standards** ⟨11⟩

USP Alteplase RS

• (CN 1-May-2018)

Altretamine



$C_9H_{18}N_6$ 210.28

1,3,5-Triazine-2,4,6-triamine, *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexamethyl-; Hexamethylmelamine [645-05-6].

DEFINITION

Altretamine contains NLT 98.0% and NMT 102.0% of altretamine ($C_9H_{18}N_6$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Infrared Absorption** ⟨197K⟩

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of 8.0 ± 0.05.