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Alteplase for Injection

DEFINITION

Alteplase for Injection is a sterile lyophilized preparation of Alteplase. Its biological activity is NLT 90% and NMT 115% of that stated on the label in USP Alteplase Units. It contains NLT 95% and NMT 111% of the total protein content stated on the label.

IDENTIFICATION

• **A.**

Standard solution: 1.0–2.5 mg/mL of USP Alteplase RS in water

Sample solution: Prepare similarly to the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-*p*-nitroaniline dihydrochloride. Separately transfer 200 µL of the *Standard solution* and 200 µL of the *Sample solution* to two of the test tubes. To the third test tube add 200 µL of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

Acceptance criteria: A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

• **B. Peptide Mapping**

Solution A: 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
91	70	30
121	40	60
131	40	60

Dialysis solution: 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

Standard solution: Prepare a solution containing 1.0 mg/mL of USP Alteplase RS in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10 µL of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25 µL of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50 µL of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20 µg of trypsin, and incubate for 6–8 h at room temperature. Again add 20 µg of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution. [Note—Store the *Standard solution* in a freezer.]

Sample solution: Using a quantity of Alteplase for Injection, proceed as directed in the *Standard solution*.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

Analysis

Samples: *Standard solution*, *Sample Solution,* and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the USP Alteplase RS Data Sheet.

Acceptance criteria: The retention times of corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the USP Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

ASSAY

• **Biological Potency**

Buffer: 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

Human thrombin solution: 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

Human fibrinogen solution: 2 mg/mL of human fibrinogen in *Buffer*

Human plasminogen solution: 1 mg/mL of human plasminogen in *Buffer*

Standard stock solution: 1.0 mg/mL (580,000 USP Alteplase Units) of USP Alteplase RS in water

Standard solutions: Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

Sample stock solution: 1.0 mg/mL of Alteplase for Injection in water

Sample solutions: Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

Analysis

Samples: *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20 µL of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200 µL of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen-fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time (t_{cl}) from the first addition of the alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units/mL, versus the log values of their clot lysis times in seconds taken:

$$\log t = m(\log U_S) + b$$

t = time to bubble release (s)

m = slope of the line

U_S = activity of the *Standard solution* (USP Alteplase Units/mL)

b = *y*-intercept of the line

The correlation coefficient is NLT −0.9900. From the line equation and using the log of the clot lysis time for the *Sample solution*, calculate the log of the activity (U_A):

$$\log U_A = \{[(\log t) - b]/m\}$$

Calculate the alteplase activity in USP Alteplase Units/mL taken:

$$\text{Result} = D(10^{\log U})$$

D = dilution factor for the *Sample solution*

Calculate the specific activity in the portion of Alteplase for Injection taken:

$$\text{Result} = (U_A/P)$$

P = concentration of protein obtained in the test for *Protein Content*

Acceptance criteria: 90%–115% of the potency stated on the label in USP Alteplase Units

OTHER COMPONENTS

• **Protein Content**

Arginine solution: 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

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

Sample solution: Dilute a volume of *Sample stock solution* with a volume of *Arginine solution* to obtain a solution having an absorbance value of 0.5–1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume (*V*).

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy ⟨857⟩*.)

Mode: UV

Wavelength range: 240–500 nm

Analytical wavelengths: 320 nm and maximum absorbance at about 280 nm

Cell: 1 cm

Blank: *Arginine solution*

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein content in the portion of Alteplase for Injection taken:

$$\text{Result} = [(A_{max} - A_{320})/\varepsilon] \times V$$

A_{max} = absorbance value at the wavelength of maximum absorbance

A_{320} = absorbance of the *Sample solution* at 320 nm

ε = molar absorptivity of alteplase, 1.9

V = volume of *Arginine solution* required to prepare the *Sample solution*

Acceptance criteria: 95%–111% of the total protein content stated on the label

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩**

Acceptance criteria: Meets the requirements for *Content Uniformity*

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.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Alteplase RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ALTEPLASE FOR INJECTION	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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