the membranes, and clamp it to the rigid plastic sheet. Dismantle the dryer, and cut off excess cellophane when dry (about 24 h). Visually examine the gel under light.

System suitability: The 2.5- and 10-ng *Control solutions* must be visible. The nonreduced *Control solutions* migrate with an apparent molecular weight of slightly less than 66,000 Da, as compared with the *Molecular weight standard solution*.

Acceptance criteria: The *Sample solution* exhibits three major bands in the region between 66,000 Da and 31,000 Da, corresponding to the major bands from the *Standard solution*. The *Carboxymethylated sample solution* exhibits six major bands in the region between 92,500 Da and 45,000 Da, corresponding to the major bands from the *Carboxymethylated standard solution*.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 1 USP Endotoxin Unit/mg of Alteplase
- **SINGLE-CHAIN CONTENT**

Mobile phase: 27.6 g of monobasic sodium phosphate in 1000 mL of 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, 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 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%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in the frozen state at a temperature of –20° or below.

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
USP Alteplase RS
• (CN 1-May-2018)

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