Standard stock solution: 0.5 mg/mL of USP Alprostadil RS in dehydrated alcohol

Standard solution: 0.13 mg/mL of USP Alprostadil RS, prepared as follows. Gently evaporate a 0.5-mL portion of the *Standard stock solution* to dryness with a stream of nitrogen. Add 150 µL of *Solution A*, rinse the inside of the container with this solution, and swirl. Add 150 µL of *Solution B* to the container, rinse the inside of the container with this solution, and swirl. Cap and sonicate to dissolve. Heat the container at 45° for 45 min, swirling occasionally. Sonicate again after heating is complete. Discard the specimen if the entire sample does not dissolve. Evaporate the solution using a stream of nitrogen, add 2.0 mL of *Internal standard solution*, and sonicate to dissolve. Discard the specimen if the entire sample does not dissolve.

Sample solution: Nominally 0.13 mg/mL of alprostadil, prepared as follows. Pool the contents of several containers of the Injection. Gently evaporate a volume, equivalent to 0.25 mg of alprostadil, to dryness using a stream of nitrogen. Proceed as directed for the *Standard solution*, beginning with "Add 150 µL of *Solution A*".

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.4-mm × 25-cm; packing L18

Flow rate: 1.5 mL/min

Injection volume: Equal volumes of *Standard solution* and *Sample solution*

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethylparaben and alprostadil are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 9.0 between alprostadil and the internal standard

Relative standard deviation: NMT 2.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprostadil ($C_{20}H_{34}O_5$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alprostadil to the internal standard from the *Sample solution*

R_S = peak response ratio of alprostadil to the internal standard from the *Standard solution*

C_S = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprostadil the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 5 USP Endotoxin Units/100 µg of alprostadil.
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.4%
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

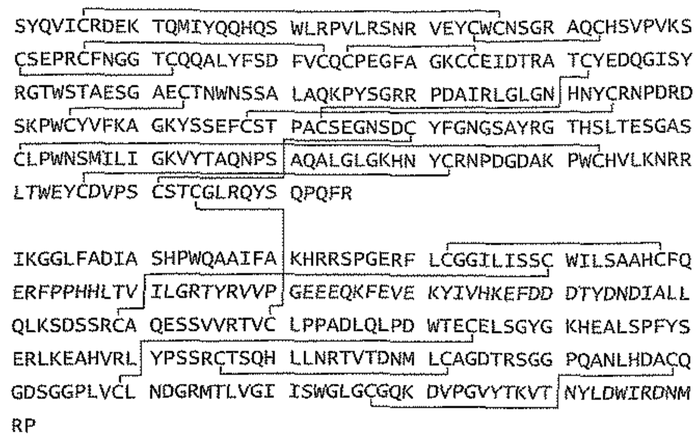
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass. Store in a refrigerator.

Change to read:

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

Alteplase



$C_{2569}H_{3894}N_{746}O_{781}S_{40}$ 59,007.61

[105857-23-6].

DEFINITION

Alteplase is a highly purified glycosylated serine protease with fibrin-binding properties and plasminogen-specific proteolytic activities. It is produced by recombinant DNA synthesis in mammalian cell culture. It has a biological potency of NLT 90.0% and NMT 115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein.

The presence of host cell DNA and host cell protein impurities in Alteplase is process specific; the limits of these impurities are determined by validated methods.

IDENTIFICATION

- **A.**

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

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