# Aprotinin Injection

Aprotinin Injection is a sterile solution of Aprotinin in Water for Injection that also contains Sodium Chloride. One Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.)

Aprotinin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aprotinin, $C_{284}H_{432}N_{84}O_{79}S_7$, expressed in K.I.U. per ml.

**Usual strength**. 20000 K.I.U.per ml.

## Identification

In the limit of *N*-Pyroglutamyl-Aprotinin and Related compounds, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 4.5 to 6.5.

**Limit of *N*-Pyroglutamyl-Aprotinin and Related compounds**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a suitable volume of the injection with mobile phase A to obtain a solution containing 5 Aprotinin units per ml.

*Reference solution.* Dissolve a suitable quantity of *aprotinin system suitability IPRS* in mobile phase A to obtained a solution containing 5 Aprotinin units per ml.

Chromatographic system

- a stainless steel column 7.5 cm x 7.5 mm, packed with a strong cation exchange resin with sulfopropyl groups bonded to porous silica (5 µm) (Such as SupelcosilLC-SCX),
- column temperature: 40º,
- mobile phase: A. a buffer solution prepared by dissolving 3.52 g of *monobasic potassium phosphate* and 7.26 g of *dibasic sodium phosphate* in 1000 ml of *water*,
  B. a buffer solution prepared by dissolving 3.52 g of *monobasic potassium phosphate*, 7.26 g of *dibasic sodium phosphate* and 66.07 g of *ammonium sulphate* in 1000 ml of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 40 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 92 | 8 |
| 21 | 64 | 36 |
| 30 | 0 | 100 |
| 31 | 92 | 8 |
| 40 | 92 | 8 |

The relative retention time with reference to aprotinin for N-pyroglutamyl-aprotinin is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to N-pyroglutamyl-aprotinin and aprotinin is not less than 1.0 and the tailing factor is not more than 2.0 for aprotinin peak.

Inject the test solution. The area of any peak corresponding to N-pyroglutamyl-aprotinin is not more than 1.0 per cent, the area of any other secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks other than N-pyroglutamyl-aprotinin, is not more than 1.0 per cent, calculated by area normalization.

**High molecular weight proteins**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a suitable volume of the injection in *water* to obtain a solution containing 5 Aprotinin Units per ml.

*Reference solution.* A solution containing 5 Aprotinin Units per ml with about 2 per cent w/v of aprotinin oligomers *(Note-This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 hours and dissolving the solid at the specified concentration in water).*

Chromatographic system

- a series of three stainless steel column 30 cm x 7.8 mm, packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da (Such as TSK gel Ultra SW Aggregate),

- mobile phase: a mixture of 20 volumes of *acetonitrile*, 20 volumes of *glacial acetic acid* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 100 µl.

The relative retention times with reference to aprotinin for dimer is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dimer and aprotinin is not less than 1.3 and the tailing factor is not more than 2.5 for aprotinin peak.

Inject the test solution. The sum of the areas of all the peaks with retention time less than that of aprotinin monomer is not more than 1.5 per cent, calculated by area normalization.

**Content of sodium chloride**. 42.5 to 47.5 mg.
Dilute 5.0 ml of the injection with 50.0 ml with *water*. Add 10 ml of 25 per cent v/v of *nitric acid*. Titrate with *0.1 M silver nitrate*, determining the endpoint potentiometrically (2.4.25. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

**Other tests**. Comply with the tests stated under Parenteral Preparations (Injections).

**Bacterial endotoxins** (2.2.3). Not more than 0.14 Endotoxin units per aprotinin unit.

**Sterility** (2.2.11). Complies with the test for sterility.

**Assay**.

*NOTE- Prepare the solutions immediately before use.*

*Solvent mixture.* Dissolve 0.93 g of *boric acid* in 900 ml of *water*, adjusted to pH 8.0 with *sodium hydroxide* and dilute to 1000.0 with *water*. Dilute 100.0 ml of the solution to 1000.0 with *water*.

*Test solution.* Dilute a suitable volume of the injection with the solvent mixture to obtain a solution containing 1.67 Aprotinin Units per ml.

*Reference solution (a).* A solution containing 4300 Trypsin Units per ml of *trypsin crystallized IPRS* in *0.001M hydrochloric acid*. Use a freshly prepared solution, and store in ice-water.

*Reference solution (b).* Dilute 4.0 ml of reference solution (a) and 1.0 ml of the test solution to 40.0 ml with the solvent mixture. Allow to stand the solution at room temperature for 10 minutes and then keep in ice-water. Use the solution within 6 hours of preparation.

*Reference solution (c).* Dilute 0.5 ml of reference solution (a) to 10.0 ml with the solvent mixture. Allow to stand the solution at room temperature for 10 minutes then store in ice-water.

*Reference solution (d).* A 0.69 per cent w/v solution of *N-benzoyl-L-arginine ethyl ester hydrochloride* in the solvent mixture. Use the solution within 2 hours.

Dilute 1.0 ml of reference solution (d) with 9.0 ml of the solvent mixture in a jacketed-glass vessel with a capacity of about 30 ml and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a burette, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjusted to pH 8.0 with *0.1M sodium hydroxide*. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at 25 ± 0.1°, add 1.0 ml of reference solution (b), and start a timer. Maintain at a pH of 8.0 by the addition of *0.1 M sodium hydroxide*, and record the volume added every 30 seconds. Continue the reaction for 6 minutes. Carry out a similar titration using 1.0 ml of reference solution (c).

Calculate the potency in Aprotinin units per ml by using following expression.

$$\text{Potency (Aprotinin units per ml)} = C_1 \times (C_2 \times V_2 - V_1) \times D$$

Where,

$C_1$ = conversion factor, 4000

$C_2$ = difference in the amount of trypsin used in reference solution (b) and reference solution (c), 2

$V_2$= volume of *0.1 M sodium hydroxide* added per second, after adding reference solution (c) (ml per second),

$V_2$= volume of *0.1 M sodium hydroxide* added per second, after adding reference solution (b) (ml per second),

D = dilution factor used to prepare the *test solution*

**Storage**. Store protected from moisture, in a single dose containers, at a temperature not exceeding 25°, do not freeze.