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Edition: BP 2025 (Ph. Eur. 11.6 update)

Urokinase

General Notices

(Ph. Eur. monograph 0695)

9039-53-6

Action and use

Plasminogen activator; fibrinolytic; enzyme.

Ph Eur

DEFINITION

Enzyme, obtained from human urine, that activates plasminogen. It consists of a mixture of low-molecular-mass (LMM) (M_r 33 000) and high-molecular-mass (HMM) (M_r 54 000) forms, the high-molecular-mass form being predominant.

Potency

Minimum 70 000 IU per milligram of protein.

PRODUCTION

It is prepared by suitable collection and extraction procedures followed by purification steps.

The method of preparation includes steps that have been shown to remove and/or inactivate extraneous agents. In addition, the process is designed to minimise microbial contamination and vasoactive substances.

CHARACTERS

Appearance

White or almost white, amorphous powder.

Solubility

Soluble in water.

IDENTIFICATION

A. Place separately in 2 haemolysis tubes 0.5 mL of citrated human plasma and 0.5 mL of citrated bovine plasma and maintain in a water-bath at 37 °C. To each tube add 0.1 mL of a solution containing a quantity of the substance to be examined equivalent to 1000 IU/mL in *phosphate buffer solution pH 7.4 R* and 0.1 mL of a solution containing a quantity of

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human thrombin R equivalent to 20 IU/mL in *phosphate buffer solution pH 7.4 R*. Shake immediately. In both tubes, a clot forms and lyses within 30 min.

B. Carry out identification by a suitable immunodiffusion test.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 10 mg in 10 mL of *water R*.

Thromboplastic contaminants

Test solutions Dissolve suitable quantities of the substance to be examined in *barbital buffer solution pH 7.4 R* to obtain solutions with activities of 5000 IU/mL, 2500 IU/mL, 1250 IU/mL, 625 IU/mL and 312 IU/mL.

To each of 6 haemolysis tubes 1 cm in internal diameter add 0.1 mL of *citrated rabbit plasma R*. Allocate the test solutions 1 to each of 5 of the tubes; add to each tube 0.1 mL of the solution allocated to it and to the 6th tube add 0.1 mL of *barbital buffer solution pH 7.4 R* (blank). Incubate the tubes at 25 ± 0.5 °C for 5 min and add 0.1 mL of a 3.675 g/L solution of *calcium chloride R*. Measure with a stop-watch the coagulation time for each tube. Plot the shortening of the recalcification time (clotting time of the blank minus clotting time measured) against log concentration in International Units. Extrapolate the best-fitting straight line through the 5 points until it reaches the log-concentration axis. The urokinase activity at the intersection point, which represents the limit concentration for coagulant activity (zero coagulant activity), is not less than 150 IU/mL.

Molecular fractions

Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve about 1 mg in 1.0 mL of *0.02 M phosphate buffer solution pH 8.0 R*. Prepare immediately before use.

Column:

— *size*: *l* = 0.9 m, Ø = 16 mm;

— *stationary phase*: *cross-linked dextran for chromatography R3*;

— *temperature*: 5 °C.

Mobile phase 17.5 g/L solution of *sodium chloride R* in *0.02 M phosphate buffer solution pH 8.0 R*.

Flow rate 0.1 mL/min.

Apply the test solution to the head of the column rinsing twice with 0.5 mL portions of the buffer and carry out the elution. The eluate may be collected in fractions of 1 mL. Measure the absorbance (*2.2.25*) of the eluate at the maximum at 280 nm and plot the individual values on a graph. Draw perpendicular lines towards the axis of the abscissae from the minima before the HMM peak, between the HMM and the LMM peaks, and after the LMM peak, thus identifying the fractions to be considered in calculating the HMM/LMM activity ratio. Pool the HMM fractions and, separately, the LMM fractions. Determine separately the urokinase activity in International Units of each of the fraction pools by the method prescribed under Assay. The ratio of the urokinase activity in the HMM fraction pool to that in the LMM fraction pool is not less than 2.0.

Total protein

Determine the nitrogen content, using 10 mg, by the method of sulfuric acid digestion (*2.5.9*) and calculate the quantity of protein by multiplying by 6.25.

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ASSAY

The potency of urokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of urokinase calibrated in International Units; the formation of plasmin is measured by the determination of the lysis time of a fibrin clot under given conditions.

The International Unit is the activity contained in a stated amount of the International Reference Preparation, which consists of freeze-dried urokinase with lactose. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organization.

Unless otherwise prescribed, use *phosphate buffer solution pH 7.4 R* containing 30 g/L of *bovine albumin R* for the preparation of the solutions and dilutions used in the assay.

Test solution Prepare a solution of the substance to be examined expected to have an activity of 1000 IU/mL.

Reference solution Prepare a solution of a reference preparation having an activity of 1000 IU/mL.

Keep the test solution and the reference solution in iced water and use within 6 h. Prepare 3 serial 1.5-fold dilutions of the reference preparation such that the longest clot-lysis time is less than 20 min and the shortest clot-lysis time is greater than 3 min. Prepare 3 similar dilutions of the test solution. Keep the solutions in iced water and use within 1 h. Use 24 tubes 8 mm in diameter. Label the tubes T_1, T_2 and T_3 for the dilutions of the test solution and S_1, S_2 and S_3 for the dilutions of the reference solution, allocating 4 tubes to each dilution. Place the tubes in iced water. Into each tube, introduce 0.2 mL of the appropriate dilution, 0.2 mL of *phosphate buffer solution pH 7.4 R* containing 30 g/L of *bovine albumin R* and 0.1 mL of a solution of *human thrombin R* having an activity of not less than 20 IU/mL. Place the tubes in a water-bath at 37 °C and allow to stand for 2 min to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the 1st tube 0.5 mL of a 10 g/L solution of *bovine euglobulins R*, ensuring mixing. At intervals of 5 s, introduce successively into the remaining tubes 0.5 mL of a 10 g/L solution of *bovine euglobulins R*. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulins solution and the lysis of the clot. Plot the logarithms of the lysis times for the substance to be examined and for the reference preparation against the logarithms of the concentration and calculate the activity of the substance to be examined using the usual statistical methods.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states the potency in International Units per milligram of protein.

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