follicle-stimulating hormone and luteinising hormone or of a reference preparation calibrated in International Units. The International Units of FSH and LH are the activities contained in stated amounts of the International Standard of human urinary follicle-stimulating hormone and luteinising hormone (interstitial-cell-stimulating hormone) which consists of a mixture of a freeze-dried extract of urine of post-menopausal women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Use immature female rats of the same strain, 19 to 28 days old, differing in age by not more than 3 days and having masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 6 equal groups of at least 5 animals. If sets of 6 litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose 3 doses of the reference preparation and 3 doses of the preparation to be examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 1.5 IU, 3.0 IU and 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate-albumin buffered saline pH 7.2 R* such that the daily dose is administered in a volume of about 0.5 ml. The buffer solution shall contain in the daily dose not less than 14 IU of chorionic gonadotrophin to ensure complete luteinisation. Add a suitable antimicrobial preservative such as 4 g/l of phenol or 0.02 g/l of thiomersal. Store the solutions at 5 ± 3 °C.

Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the injection of each dose 24 h and 48 h after the first injection. About 24 h after the last injection, euthanise the rats and remove the ovaries from each animal. Remove any extraneous fluid and tissue from the ovaries and weigh the 2 combined ovaries of each animal immediately. Calculate the results by the usual statistical methods, using the mass of the 2 combined ovaries as the response. (The precision of the assay may be improved by a suitable correction of the organ mass with reference to the mass of the animal from which it was taken; an analysis of covariance may be used).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

In an airtight, tamper-proof container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the activity expressed in International Units of follicle-stimulating hormone per container,
- the potency expressed in International Units of follicle-stimulating hormone per milligram.

01/2008:0695

UROKINASE

Urokinasum

[9039-53-6]

DEFINITION

Urokinase is an 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. The potency is not less than 70 000 International Units per milligram of protein.

PRODUCTION

It is produced by validated methods of manufacturing designed to minimise or eliminate vasoactive substances.

CHARACTERS

A white or almost white, amorphous powder, soluble in water.

IDENTIFICATION

A. Place separately in two 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 *humanthrombin 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. Dissolve 10 mg in 10 ml of *water R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Hepatitis B surface antigen. Examine by a suitably sensitive method such as radio-immunoassay. Hepatitis B surface antigen is not detected.

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 six haemolysis tubes 1 cm in internal diameter add 0.1 ml of *citrated rabbit plasma R*. Allocate the test solutions one to each of five of the tubes; add to each tube 0.1 ml of the solution allocated to it and to the sixth 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 five 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. Examine by size-exclusion chromatography (*2.2.30*).

Test solution. Dissolve about 1 mg of the substance to be examined in 1.0 ml of *0.02 M phosphate buffer pH 8.0 R*. Prepare immediately before use.

The chromatographic procedure may be carried out using:

– a column 0.9 m long and 16 mm in internal diameter packed with *cross-linked dextran for chromatography R3*,
– as mobile phase at a flow rate of 6 ml/h a 17.5 g/l solution of *sodium chloride R* in *0.02 M phosphate buffer solution pH 8.0 R*,

equilibrating the column and operating at 5 °C. 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 sulphuric acid digestion (*2.5.9*) and calculate the quantity of protein by multiplying by 6.25.

Pyrogens (*2.6.8*). If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogen, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1.0 ml of a sterile 9 g/l solution of *sodium chloride R* containing a quantity of the substance to be examined equivalent to 20 000 IU/ml.

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 in 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 Organisation.

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 three 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 three similar dilutions of the test solution. Keep the solutions in iced water and use within 1 h. Use twenty-four 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 four 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 *humanthrombin 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 first 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

Store 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-proof container.

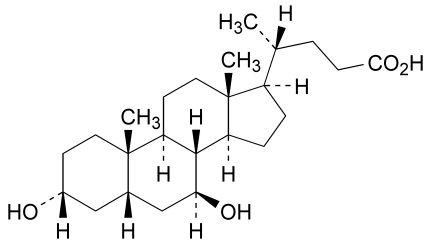
LABELLING

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

01/2008:1275
corrected 6.0

URSODEOXYCHOLIC ACID

Acidum ursodeoxycholicum



$C_{24}H_{40}O_4$ M_r 392.6
[128-13-2]

DEFINITION

Ursodeoxycholic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3α,7β-dihydroxy-5β-cholan-24-oic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in acetone, practically insoluble in methylene chloride.

It melts at about 202 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C.