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.

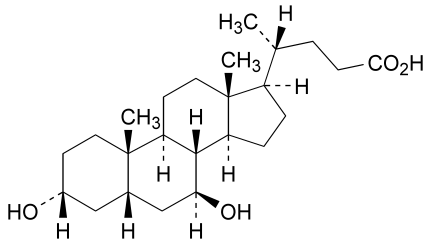
LABELLING

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.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *ursodeoxycholic acid CRS*. Examine the substances as discs prepared using *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 1 ml of *sulphuric acid R*. Add 0.1 ml of *formaldehyde solution R* and allow to stand for 5 min. Add 5 ml of *water R*. The suspension obtained is greenish-blue.

TESTS

Specific optical rotation (*2.2.7*). Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 58.0 to + 62.0, calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a). Dissolve 0.40 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (a). Dissolve 40 mg of *ursodeoxycholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dissolve 20 mg of *lithocholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents. Dilute 2 ml of this solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (c). Dissolve 20 mg of *chenodeoxycholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (d). Dissolve 20 mg of *cholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (e). Dilute 0.5 ml of test solution (a) to 20 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*. Dilute 1 ml of this solution to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (f). Dissolve 10 mg of *ursodeoxycholic acid CRS* in reference solution (c) and dilute to 25 ml with the same solution.

Apply 5 µl of each solution to the plate. Develop in an unsaturated tank over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 30 volumes of *acetone R* and 60 volumes of *methylene chloride R*. Dry the plate at 120 °C for 10 min. Spray the plate immediately with a 47.6 g/l solution of *phosphomolybdic acid R* in a mixture of 1 volume of *sulphuric acid R* and 20 volumes of *glacial acetic acid R* and heat again at 120 °C until blue spots appear on a lighter background. In the chromatogram obtained with test solution (a): any spot corresponding to lithocholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent); any spot corresponding to chenodeoxycholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (1 per cent); any spot corresponding to cholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (d) (0.5 per cent); any spot, apart from the principal spot and any spots corresponding to lithocholic acid, chenodeoxycholic acid and cholic acid, is not more intense than the principal spot in the chromatogram obtained with reference solution (e) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (f) shows two clearly separated principal spots.

Heavy metals (*2.4.8*). 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

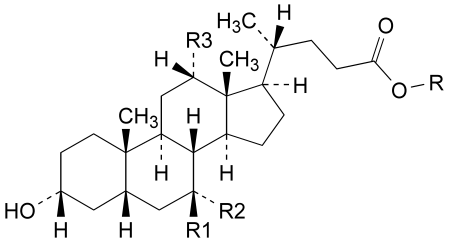
Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 ml of *ethanol (96 per cent) R*, previously neutralised to 0.2 ml of *phenolphthalein solution R*. Add 50 ml of *water R* and titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.

IMPURITIES



A. R = R1 = R3 = H, R2 = OH: chenodeoxycholic acid,

B. R = R1 = H, R2 = R3 = OH: 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (cholic acid),

C. R = R1 = R2 = R3 = H: 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid),

D. R = R2 = H, R1 = R3 = OH: 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid (ursocholic acid),

E. R = R1 = R2 = H, R3 = OH: 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid),

F. R = R3 = H, R1+R2 = O: 3α-hydroxy-7-oxo-5β-cholan-24-oic acid,

G. R = CH_3, R1 = OH, R2 = R3 = H: methyl 3α,7β-dihydroxy-5β-cholan-24-oate.