less than 3 animals are killed.

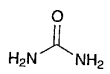
Toxicity Inject intravenously 0.50 mL of Ulinastatin into each of five well-fed, healthy albino mice weighing 18 to 25 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 5 albino mice weighing 19 to 21 g: all the animals survive for 48 hours.

Assay Measure exactly a suitable volume of Ulinastatin, dilute with 2,2′,2″-nitrilotrisethanol buffer solution so that each mL of the solution contains about 150 Units according to the labeled amount, and use this solution as the sample solution. Separately, dilute a suitable volume of Ulinastatin Reference Standard with 2,2′,2″-nitrilotrisethanol buffer solution so that each mL of the solution contains exactly 300, 200, 100, 50 or 0 Units, and use these solutions as the standard solutions. 2,2′,2″-Nitrilotrisethanol buffer solution and *N*-α-benzoyl-L-arginine-4-nitroanilide TS are warmed in a water bath of 25 ± 1°C for use as described below. Take exactly 0.1 mL each of the sample solution and the standard solutions in test tubes, add exactly 1.6 mL of 2,2′,2″-nitrilotrisethanol buffer solution, mix, and put the tubes in the water bath of 25 ± 1°C. One minute after addition of the buffer solution add exactly 0.2 mL of ice-cooled trypsin TS for test of ulinastatin, mix, and put the tubes again in the water bath. One minute later add exactly 1 mL of *N*-α-benzoyl-L-arginine-4-nitroanilide TS, mix, and then put the tubes in the water bath. Exactly 2 minutes later add exactly 0.1 mL of diluted acetic acid (100) (1 in 2) to stop the enzyme reaction, and determine the absorbances of the solutions so obtained at 405 nm using water as the blank. Prepare a calibration curve using the absorbances obtained with the standard solutions, and calculate ulinastatin Units in the sample solution from its absorbance by using this curve.

Containers and storage Containers—Tight containers.
Storage—Preserve at −20°C or lower.

Urea

尿素



CH_4N_2O: 60.06
Urea [*57-13-6*]

Urea contains not less than 99.0% of CH_4N_2O.

Description Urea occurs as colorless to white crystals or crystalline powder. It is odorless, and has a cooling, saline taste.

It is very soluble in water, freely soluble in boiling ethanol (95), soluble in ethanol (95), and very slightly soluble in diethyl ether.

A solution of Urea (1 in 100) is neutral.

Identification (1) Heat 0.5 g of Urea: it liquefies and the odor of ammonia is perceptible. Continue heating until the liquid becomes turbid, then cool. Dissolve the resulting lump in a mixture of 10 mL of water and 2 mL of sodium hydroxide TS, and add 1 drop of copper (II) sulfate TS: a reddish purple color develops.

(2) Dissolve 0.1 g of Urea in 1 mL of water, and add 1 mL of nitric acid: a white, crystalline precipitate is formed.

Melting point 132.5 - 134.5°C

Purity (1) Chloride—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(2) Sulfate—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals—Proceed with 1.0 g of Urea according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Ethanol-insoluble substances—Dissolve 5.0 g of Urea in 50 mL of warm ethanol (95), filter through a tared glass filter (G4), wash the residue with 20 mL of warm ethanol (95), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.2 g of Urea, dissolve in water, and make exactly 200 mL. Measure exactly 5 mL of this solution into a Kjeldahl flask, and proceed as directed under the Nitrogen Determination.

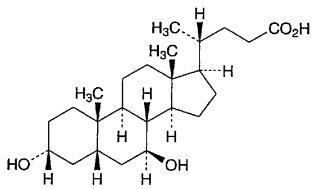
Each mL of 0.005 mol/L sulfuric acid VS
= 0.30028 mg of CH_4N_2O

Containers and storage Containers—Well-closed containers.

Ursodeoxycholic Acid

Ursodesoxycholic Acid

ウルソデオキシコール酸



$C_{24}H_{40}O_4$: 392.57
3α,7β-Dihydroxy-5β-cholan-24-oic acid [*128-13-2*]

Ursodeoxycholic Acid, when dried, contains not less than 98.5% of $C_{24}H_{40}O_4$.

Description Ursodeoxycholic Acid occurs as white crystals or powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95), in ethanol (99.5) and in acetic acid (100), slightly soluble in chloroform, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification Dissolve 0.01 g of Ursodeoxycholic Acid in

1 mL of sulfuric acid, add 1 drop of formaldehyde solution, and allow to stand for 5 minutes. To the solution add 5 mL of water: a blue-green suspended substance is produced.

Optical rotation $[\alpha]_D^{20}$: +59.0 - +62.0° (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

Melting point 200 - 204°C

Purity (1) Odor—To 2.0 g of Ursodeoxycholic Acid add 100 mL of water, and boil for 2 minutes: no odor is perceptible.

(2) Chloride—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100) with shaking, add water to make 200 mL, shake thoroughly, and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 4 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(3) Sulfate—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals—Proceed with 1.0 g of Ursodeoxycholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, boil for 2 minutes, cool, filter, and wash with water until the last washing makes 100 mL. To 10 mL of the solution add 1 mL of dilute sulfuric acid: no turbidity is produced.

(6) Arsenic—Prepare the test solution with 1.0 g of Ursodeoxycholic Acid according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(7) Related substances—Dissolve 0.050 g of Ursodeoxycholic Acid in a mixture of chloform and ethanol (95) (9:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 0.075 g of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 100 mL. To exactly 2 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 0.025 g of lithocholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL. To exactly 1 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL. To exactly 2 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120°C for 30 minutes, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120°C for 2 to 3 minutes: the spot from the sample solution, corresponding to that from the standard solution (1), is not more intense than the spot from the standard solution (1), and the spot other than the principal spot and the above spots from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition Not more than 0.20% (1 g).

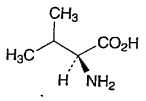
Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, and dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS, and titrate again after adding 100 mL of freshly boiled and cooled water near the end point.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.258 mg of $C_{24}H_{40}O_4$

Containers and storage Containers—Well-closed containers.

L-Valine

L-バリン



$C_5H_{11}NO_2$: 117.15
(2*S*)-2-Amino-3-methylbutanoic acid [*72-18-4*]

L-Valine, when dried, contains not less than 98.5% of $C_5H_{11}NO_2$.

Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly sweet taste, which becomes bitter.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Valine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +26.5 - +29.0° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 0.5 g of L-Valine in 20 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Valine in 20 mL of water: the solution is clear and colorless.

(2) Chloride—Perform the test with 0.5 g of L-Valine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate—Perform the test with 0.6 g of L-Valine. Pre-