Turpentine Oil

Oleum Terebinthinae

テレビン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index n_D^{20}: 1.465 - 1.478

Specific gravity d_{20}^{20}: 0.860 - 0.875

Purity **(1)** Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

Distilling range 150 - 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Urokinase

ウロキナーゼ

[*9010-53-1*]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000. It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

Description Urokinase is a clear and colorless liquid.
The pH is between 5.5 and 7.5.

Identification **(1)** Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop 10 μL of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for overnight: lysis circle is appeared.

(2) Dissolve 1.0 g of Powdered Agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish until the height come to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well place separately 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and 10 μL of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

Purity **(1)** Heavy metals—Proceed with 2.0 mL of Urokinase according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Blood group substances—Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μL each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add 25 μL of the sample solution into the six wells on the first lane and 25 μL of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add 50 μL of A-type erythrocyte suspension, mix, allow to stand for 2 hours, and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes.

Perform the same test by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and inject 0.5 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy mice aged about 5 weeks. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High molecular mass urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas of two peaks eluted closely at about 35 minutes having smaller retention time, A_a, and larger retention time, A_b, by the automatic integration method: the value, $A_a/(A_a + A_b)$, is not less than 0.85.

Operating conditions—

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 365 nm, fluorescence wavelength: 460 nm).

Column: A stainless steel column 7.5 mm in inside di-

ameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 to 12 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Reaction coil: A stainless steel column 0.25 mm in inside diameter and 150 cm in length.

Reaction coil temperature: 37°C

Mobile phase: Gelatin-phosphate buffer solution.

Flow rate of mobile phase: 0.5 mL per minute.

Reaction reagent: 7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS.

Flow rate of reaction reagent: 0.75 mL per minute.

Selection of column: Adjust the pH of Urokinase to 7.5 with sodium hydroxide TS, allow to stand at 37°C for over 24 hours, and add gelatin-phosphate buffer solution to make the solution containing 20,000 Units per mL. Proceed with 100 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of high molecular mass urokinase (mol. wt.: 54,000) and low molecular mass urokinase (mol. wt.: 33,000) in this order with the resolution between these peaks being not less than 1.0.

Assay **(1)** Urokinase—Pipet 1 mL of Urokinase, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the sample solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ampoule of High Molecular Mass Urokinase Reference Standard to dissolve, pipet 1 mL of this solution, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the standard solution. Place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two silicon-coated test tubes about 10 mm in inside diameter, warm them in a water bath at 35 ± 0.2°C for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at 35 ± 0.2°C for exactly 30 minutes, then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, A_T and A_S, of these solutions at 405 nm as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank. Separately place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two test tubes, add 0.50 mL of diluted acetic acid (100) (2 in 5), and 0.50 mL each of the sample solution and the standard solution. Determine the absorbances, A_{TO} and A_{SO}, of these solutions at 405 nm as the same manner, using water as the blank.

$$\text{Amount (Units) of Urokinase} = \frac{A_T - A_{TO}}{A_S - A_{SO}} \times a \times b$$

a: Amount (Units) of urokinase in 1 mL of the standard solution

b: Total volume (mL) of the sample solution

(2) Protein—Measure exactly a volume of Urokinase, equivalent to about 0.015 g of protein, and perform the test as directed under the Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.87544 mg of protein

Containers and storage Containers—Tight containers.

Storage—Not exceeding −20°C.

Uva Ursi Fluidextract

ウワウルシ流エキス

Uva Ursi Fluidextract contains not less than 3.0 w/v% of arbutin.

Method of preparation Prepare an infusion from Bearberry Leaf, in coarse powder, as directed under Fluidextracts, using hot Purified Water. Remove a part of the accompanying tannin, evaporate the mixture under reduced pressure, if necessary, and add Purified Water to adjust the percentage. It may contain an appropriate quantity of Ethanol.

Description Uva Ursi Fluidextract is a yellow-brown to dark red-brown liquid, and has a bitter and astringent taste.

It is miscible with water and with ethanol (95).

Identification To 1 mL of Uva Ursi Fluidextract add 30 mL of a mixture of ethanol (95) and water (7:3), shake, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (2) under Bearberry Leaf.

Component determination Pipet 1 mL of Uva Ursi Fluidextract, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Component determination under Bearberry Leaf.

$$\text{Amount (mg) of arbutin} = \text{amount (mg) of arbutin for component determination} \times \frac{A_T}{A_S}$$

Containers and storage Containers—Tight containers.

Vitamin A Oil

ビタミンA油

Vitamin A Oil is the fatty oil obtained from fresh livers and pyloric caeca of marine animals, or this fatty oil, its concentrate, vitamin A or its fatty acid esters diluted with cod liver oils or edible fixed oils. It contains not less than 30,000 Vitamin A Units per g. It may contain suitable antioxidants.

It contains not less than 90% and not more than 120% of the labeled amount of vitamin A.

Description Vitamin A Oil is a yellow to yellow-brown, clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.

Its decomposition is accelerated upon exposure to air or light.

Identification Dissolve Vitamin A Oil in chloroform, prepare a solution of 30 Vitamin A Units per mL according to the labeled Units, pipet 1 mL of the solution, and add 3 mL of antimony (III) chloridee TS: the color of the solution changes immediately to blue, which fades rapidly.

Purity **(1)** Acid—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl ether