

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-(Glutaryl-glycyl-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 $35 \pm 0.2^\circ\text{C}$ for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at $35 \pm 0.2^\circ\text{C}$ 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_{T0} and A_{S0} , of these solutions at 405 nm as the same manner, using water as the blank.

$$\text{Amount (Units) of Urokinase} = \frac{A_T - A_{T0}}{A_S - A_{S0}} \times a \times b$$

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.

$$\begin{aligned} \text{Each mL of 0.005 mol/L sulfuric acid VS} \\ = 0.87544 \text{ mg of protein} \end{aligned}$$

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.

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

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) chloride 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