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

(1:1), boil gently for 10 minutes under a reflux condenser, cool, and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Rancidity—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

(3) Related substances—Vitamin A Oil meets the conditions determined as directed in Method 1 under the Vitamin A Assay, or its *f* value determined as directed in Method 2 under the Vitamin A Assay is not less than 0.85.

Assay Proceed as directed under the Vitamin A Assay.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Capsules

ビタミンA油カプセル

Vitamin A Oil Capsules contain not less than 90% and not more than 130% of the labeled Units of Vitamin A.

Method of preparation Prepare as directed under Capsules, using Vitamin A Oil.

Tests for Vitamin A Oil The oil obtained by the procedure directed in the Assay meets the requirements of the Description, Identification and Purity under Vitamin A Oil.

Assay Weigh accurately 20 Vitamin A Oil Capsules, cut open, transfer the oil contents, mix well, and proceed with the oil as directed under Vitamin A Assay. Wash the capsules with a small amount of diethyl ether, allow to stand at room temperature to evaporate the diethyl ether, and weigh accurately. Calculate the mass of Vitamin A Oil from the difference between the masses before and after the above-described procedure. Calculate the Vitamin A Units per 1 capsule from the mass and the Vitamin A Units of the oil.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Compound Vitamin B Powder

複方ビタミンB散

Method of preparation

Thiamine Nitrate	10 g
Riboflavin	10 g
Pyridoxine Hydrochloride	10 g
Nicotinamide	100 g
Starch, Lactose or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Vitamin B Powder is orange-yellow in color. It has a slighly bitter taste.

It is slowly affected by light.

Identification (1) Shake 2 g of Compound Vitamin B Powder with 100 mL of water, filter, and to 5 mL of the filtrate add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and observe under ultraviolet light: the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline (thiamine).

(2) Shake 0.1 g of Compound Vitamin B Powder with 100 mL of water, and filter. Perform the following tests with the filtrate (riboflavin).

(i) The filtrate is light yellow-green in color and has an intense yellow-green fluorescence. This color and fluorescence of the solution disappears upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the filtrate, and again appears by shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(ii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, after illuminating with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake thoroughly with 5 mL of chloroform: the chloroform layer shows yellow-green fluorescence.

(3) Shake 1 g of Compound Vitamin B Powder with 100 mL of diluted ethanol (7 in 10), filter, and to 5 mL of the filtrate add 2 mL of sodium hydroxide TS and 40 mg of manganese dioxide. Heat on a water bath for 30 minutes, cool, and filter. Add 5 mL of 2-propanol to 1 mL of the filtrate, and use the solution as the sample solution. To 3 mL of the sample solution add 2 mL of bartibal buffer solution, 4 mL of 2-propanol and 2 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000) prepared when required for use: a blue color develops. To 1 mL of the sample solution add 1 mL of a saturated boric acid solution, and proceed as directed in the same manner as above: no blue color develops (pyridoxine).

(4) Shake 0.5 g of Compound Vitamin B Powder with 10 mL of ethanol (95), filter, and evaporate 1 mL of the filtrate on a water bath to dryness. Add 0.01 g of 2,4-dinitrochlorobenzen to the residue, heat gently for 5–6 seconds to fuse, and after cooling, add 4 mL of potassium hydroxide-ethanol TS: a red color develops (nicotinamide).

(5) Shake 1 g of Compound Vitamin B Powder with 5 mL of diluted ethanol (7 in 10), filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g each of thiamine mononitrate, riboflavin, pyridoxine hydrochloride and nicotinamide in 1 mL, 50 mL, 1 mL and 1 mL of water, respectively, and use these solutions as standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (100:50:1) to a distance of about 10 cm, and air-dry the plate. Examine un-