add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

Assay Weigh accurately about 2.0 g of Nux Vomica Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to disperse the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make exactly 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.075 g of strychnine nitrate for assay (separately determine its loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the ratio, Q_T and Q_S, of the peak area of strychnine to that of the internal standard in each solution.

$$\text{Amount (mg) of strychnine } (C_{21}H_{22}N_2O_2) = \text{amount (mg) of strychnine nitrate for assay, calculated on the dried basis} \times \frac{Q_T}{Q_S} \times \frac{1}{5} \times 0.8414$$

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (6.8 in 1000), acetonitrile and triethylamine (45:5:1), adjusted the pH to 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of strychnine is about 17 minutes.

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nux Vomica Tincture

ホミカチンキ

Nux Vomica Tincture contains not less than 0.097 w/v% and not more than 0.116 w/v% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica, in coarse powder	100 g
70 vol% Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Nux Vomica Tincture is a yellow-brown liquid. It has an extremely bitter taste.

Specific gravity d_{20}^{20}: about 0.90

Identification Heat 20 mL of Nux Vomica Tincture on a water bath to remove ethanol, cool, transfer to a separator, add 2 mL of ammonia TS and 20 mL of chloroform, and shake well for 2 to 3 minutes. Filter the chloroform layer through a pledget of absorbent cotton, warm the filtrate on a water bath to remove most of chloroform, and proceed as directed in the Identification under Nux Vomica.

Alcohol number Not less than 6.7 (Method 2).

Assay Pipet 3 mL of Nux Vomica Tincture into a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to disperse the diethyl ether layer. Repeat this procedure twice with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue with 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, and add the mobile phase to make exactly 50 mL. Filter the solution through a membrane filter of 0.8-μm or finer porosity, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.075 g of strychnine nitrate (determine loss on drying before using), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Nux Vomica.

Amount (mg) of strychnine ($C_{21}H_{22}N_2O_2$)
= amount (mg) of strychnine nitrate for assay, calculated on the dried basis
$\times \frac{Q_T}{Q_S} \times \frac{1}{20} \times 0.8414$

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

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

Olive Oil

Oleum Olivae

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of *Olea europaea* Linné (*Oleaceae*).

Description Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.

It is miscible with diethyl ether, with petroleum diethyl ether and with carbon disulfide.

It is slightly soluble in ethanol (95).

The whole or a part of it congeals between 0°C and 6°C.

Congealing point of the fatty acids: 17 - 26°C

Specific gravity d_{25}^{25}: 0.908 - 0.914

Acid value Not more than 1.0.

Saponification value 186 - 194

Unsaponifiable matters Not more than 1.5%.

Iodine value 79 - 88

Purity **(1)** Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5 hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in acetone to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions. Measure the peak heights, H_T and H_S, of methyl behenate of respective solutions: H_T is not higher than H_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of methyl behenate obtained from 2 μL of the standard solution is 5 to 10 mm.

Containers and storage Containers—Tight containers.

Ophiopogon Tuber

Ophiopogonis Tuber

バクモンドウ

Ophiopogon Tuber is the enlarged part of the root of *Ophiopogon japonicus* Ker-Gawler (*Liliaceae*).

Description Fusiform root, 1 - 2.5 cm in length, 0.3 - 0.5 cm in diameter, somewhat sharp at one end, and somewhat rounded at the other; externally light yellow to light yellow-brown, with longitudinal wrinkles of various sizes; when fractured, cortex flexible and friable, stele strong; fractured surface of cortex light yellow-brown in color, slightly translucent and viscous. Odor, slight; taste, slightly sweet and mucous.

Under a microscope, a transverse section reveals brown, 4- to 5-layer velamen internally adjoining the epidermis; a single-layer exodermis inside the velamen, and cortex of parenchyma cells inside the exodermis; endodermis is distinct; about 20 protoxylems in actionstele; cortex parenchyma contains columnar crystals and needle raphides of calcium oxalate; oil drops in the exodermis.

Purity Rootlets—The amount of the rootlets contained in Ophiopogon Tuber does not exceed 1.0%.

Total ash Not more than 3.0%.