lowing conditions. Measure the peak area A_T of oxygen. Separately, introduce 1.0 mL of oxygen into the gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard gas mixture. Proceed with 1.0 mL of this mixture in the same manner under Nitrogen, and measure the peak area A_S of oxygen.

$$\text{Amount (vol\%) of } N_2 = 100 - \frac{A_T}{A_S}$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with zeolite for gas chromatography (250 to 350 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium

Flow rate: Adjust the flow rate so that the retention time of oxygen is about 3 minutes.

Selection of column: Introduce 1.0 mL of oxygen into the gas mixer, add Nitrogen to make 100 mL, and mix thoroughly. Proceed with 1.0 mL of this mixture under the above operating conditions. Use a column giving well-resolved peaks of oxygen and Nitrogen in this order.

System repeatability: Repeat the test 5 times according to the above conditions with the standard gas mixture. Relative standard deviation of peak area of oxygen is not more than 2.0%.

Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

Nuphar Rhizome

Nupharis Rhizoma

センコツ

Nuphar Rhizome is the longitudinally split rhizome of *Nuphar japonicum* De Candolle.

Description Usually, longitudinally split irregular column, twisted, bent or somewhat pressed, 20 - 30 cm in length, about 2 cm in width; the outer surface, dark brown, and the cross section, white to grayish white in color; one side shows nearly round to blunt triangular scars of petiole about 1 cm in diameter, and the other side numerous scars of roots less than 0.3 cm in diameter; light, spongy in texture, and easily broken; fractured surface flat and powdery. Under a magnifying glass, a transverse section reveals a black outer portion, and porous tissue with scattered vascular bundles in the inner portion. Odor, slight; taste, slightly bitter and unpleasant.

Identification Boil 1 g of pulverized Nuphar Rhizome with 20 mL of methanol under a reflux condenser on a water bath for 15 minutes, cool, and filter. Evaporate the filtrate to dryness, warm the residue with 5 mL of dilute acetic acid on a water bath for 1 minute, cool, and filter. Spot 1 drop of the filtrate on a piece of filter paper, air-dry the paper, spray Dragendorff's TS for spraying on it, and allow it to stand: a yellow-red color appears.

Purity (1) Petiole—The amount of its petioles contained in Nuphar Rhizome does not exceed 3.0%.

(2) Foreign matter—The amount of foreign matter other than petiole contained in Nuphar Rhizome does not exceed 1.0%.

Loss on drying Not more than 15.0% (6 hours).

Total ash Not more than 10.0%.

Acid-insoluble ash Not more than 1.0%.

Nux Vomica

Strychni Semen

ホミカ

Nux Vomica is the seed of *Strychnos nux-vomica* Linné (*Loganiaceae*).

When dried, it contains not less than 1.07% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Description Disk, often slightly bent, 1 - 3 cm in diameter, 0.3 - 0.5 cm in thickness; externally light grayish yellow-green to light grayish brown, covered densely with lustrous appressed hairs radiating from the center to the circumference; on both sides, the margin and the central part bulged a little; the dot-like micropyle situated at one point on the margin, and from which usually a raised line runs to the center on one side; extremely hard in texture; when cracked upon soaking in water, the seed coat thin, the interior consisting of two horny, light grayish yellow endosperms, and leaving a central narrow cavity at the center; a white embryo, about 0.7 cm in length, situated at one end between the inner surfaces of the endosperms. Odorless; taste, very bitter and persisting.

Identification (1) To 3 g of pulverized Nux Vomica 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.

Total ash Not more than 3.0%.

Assay Weigh accurately about 1.0 g of pulverized Nux Vomica, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 1 mL of ammonia solution (28). To this solution add 20 mL of diethyl ether, stopper the centrifuge tube tightly, shake for

15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure three times with the residue using 20-mL portions of diethyl ether. Combine all 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 (determine the loss on drying before use), 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.

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

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: Dissolve 6.8 g of potassium dihydrogenphosphate in water to make 1000 mL, and mix with acetonitrile and triethylamine (45:5:1), and adjust the mixture with phosphoric acid to a pH of 3.0.

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.

Nux Vomica Extract

ホミカエキス

Nux Vomica Extract contains not less than 6.15% and not more than 6.81% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation After defatting 1000 g of coarse powder of Nux Vomica with hexane, digest by the percolation method, using a mixture of 750 mL of Ethanol, 10 mL of Acetic Acid and 240 mL of Purified Water as the first solvent, and 70 vol% ethanol as the second solvent. Combine the extracts, and prepare the dry extract as directed under Extracts. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Nux Vomica Extract occurs as yellow-brown to brown powder. It has a characteristic odor, and an extremely bitter taste.

Identification Extract 0.5 g of Nux Vomica Extract with 0.5 mL of ammonia TS and 10 mL of chloroform with occasional shaking. Filter the chloroform extract, evaporate the filtrate on a water bath until most of the chloroform is removed, and proceed as directed in the Identification under Nux Vomica.

Assay Weigh accurately about 0.2 g of Nux Vomica Extract, 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. Proceed 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
$\times \frac{Q_T}{Q_S} \times \frac{1}{5} \times 0.8414$

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

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

Nux Vomica Extract Powder

ホミカエキス散

Nux Vomica Extract Powder contains not less than 0.61% and not more than 0.68% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica Extract	100 g
Starch, Lactose or their mixture	a sufficient quantity
To make	1000 g

To Nux Vomica Extract add 100 mL of Purified Water, then warm, and soften with stirring. Cool, add 800 g of Starch, Lactose or their mixture little by little, and mix well. Dry, preferably at a low temperature, and dilute with a sufficient additional quantity of Starch, Lactose or their mixture to make 1000 g of the homogeneous powder.

Description Nux Vomica Extract Powder occurs as a yellow-brown to grayish brown powder. It has a slight, characteristic odor and a bitter taste.

Identification (1) To 3 g of Nux Vomica Extract Powder