Acid value Not more than 2.0.

Saponification value 193 - 200

Iodine value 33-50 (When the sample is insoluble in 20 mL of cyclohexane, dissolve it by shaking a glass-stoppered flask in warm water. Then, if insoluble, increase the volume of solvent.)

Purity (1) Moisture and coloration—Beef Tallow (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkali—To 2.0 g of Beef Tallow add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Beef Tallow add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of an ethanolic solution of silver nitrate (1 in 50).

Containers and storage Containers—Well-closed containers.

White Beeswax

Cera Alba

サラシミツロウ

White Beeswax is bleached Yellow Beeswax.

Description White Beeswax occurs as white to yellowish white masses. It has a characteristic odor. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

It is slightly soluble in diethyl ether, and practically insoluble in water and in ethanol (99.5).

Acid value 5 - 9 or 17 - 22 Weigh accurately about 6 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value under the Fats and Fatty Oils. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value 80 - 100 Weigh accurately about 3 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS and 50 mL of ethanol (95), heat for 4 hours on a water bath under a reflux condenser, and proceed as directed in the Saponification value under the Fats and Fatty Oils.

Melting point 60 - 67°C (Method 2).

Purity Paraffin, fat, Japan wax or resin—Melt White Beeswax at the lowest possible temperature, drip the liquid into a vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Yellow Beeswax

Cera Flava

ミツロウ

Yellow Beeswax is the purified wax obtained from honeycombs such as those of *Apis indica* Radoszkowski or *Apis mellifera* Linné (*Apidae*).

Description Yellow Beeswax occurs as light yellow to brownish yellow masses. It has a characteristic odor, which is not rancid. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

Acid value 5 - 9 or 17 - 22 Weigh accurately about 6 g of Yellow Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value under the Fats and Fatty Oils. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value 80 - 100 Weigh accurately about 3 g of Yellow Beeswax, place in a 250-mL glass-stoppered flask, and add 25 mL of 0.5 mol/L potassium hydroxide-ethanol and 50 mL of ethanol (95), insert a reflux condenser, heat for 4 hours on a water bath, and proceed as directed in the Saponification value under the Fats and Fatty Oils.

Melting point 60 - 67°C (Method 2).

Purity Paraffin, fat, Japan wax or resin—Melt Yellow Beeswax at the lowest possible temperature, drip the liquid into a glass vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Belladonna Extract

Extractum Belladonnae

ベラドンナエキス

Belladonna Extract contains not less than 0.85%

and not more than 1.05% of hyoscyamine ($C_{17}H_{23}NO_3$: 389.37).

Method of preparation To 1000 g of a coarse powder of Belladonna Root add 4000 mL of 35 vol% ethanol, and digest for 3 days. Press the mixture, add 2000 mL of 35 vol% ethanol to the residue, and digest again for 2 days. Combine all the extracts, and allow to stand for 2 days. Filter, and prepare the viscous extract as directed under Extracts. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Belladonna Extract has a dark brown color, a characteristic odor and a bitter taste.

Identification Mix 0.5 g of Belladonna Extract with 30 mL of ammonia TS in a flask, transfer the mixture to a separator, then add 40 mL of ethyl acetate, and shake the mixture. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification under Belladonna Root.

Assay Weigh accurately about 0.4 g of Belladonna Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Proceed as directed under Belladonna Root.

$$\text{Amount (mg) of hyoscyamine } (C_{17}H_{23}NO_3) = \text{amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis} \times \frac{Q_T}{Q_S} \times \frac{1}{5} \times 0.855$$

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Belladonna Root

Belladonnae Radix

ベラドンナコン

Belladonna Root is the root of *Atropa belladonna* Linné (*Solanaceae*).

Belladonna Root, when dried, contains not less than 0.4% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37).

Description Cylindrical root, usually 10 - 30 cm in length, 0.5 - 4 cm in diameter; often cut crosswise or lengthwise; externally grayish brown to grayish yellow-brown, with longitudinal wrinkles; periderm often removed; fractured surface is light yellow to light yellow-brown in color and is powdery. Almost odorless; taste, bitter.

Identification Place 2.0 g of pulverized Belladonna Root in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: the principal spot from the sample solution is the same in color tone and *Rf* value with a yellow-red spot from the standard solution.

Purity **(1)** Stem and crown—The amount of stems and crowns contained in Belladonna Root does not exceed 10.0%.

(2) Foreign matter—The amount of foreign matter other than stems and crowns contained in Belladonna Root does not exceed 2.0%.

Total ash Not more than 6.0%.

Acid-insoluble ash Not more than 4.0%.

Assay Weigh accurately about 0.7 g of pulverized Belladonna Root, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Filter this solution through a filter of a porosity of 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 0.025 g of Atropine Sulfate Reference Standard (determine the loss on drying before use), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution. Pipet 5 mL of standard stock solution, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the ratios, Q_T and Q_S, of the peak area of hyoscyamine (atropine), to that of the internal standard in each solution.