

Powdered Glycyrrhiza

Glycyrrhizae Radix Pulverata

カンゾウ末

Powdered Glycyrrhiza is the powder of Glycyrrhiza.

It contains not less than 2.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Powdered Glycyrrhiza is light yellow-brown or light yellow to grayish yellow (powder of peeled Glycyrrhiza) in color. It has a slight odor and a sweet taste.

Under a microscope, Powdered Glycyrrhiza reveals mainly yellow sclerenchymatous fiber bundles accompanied with crystal cell rows; vessels, 80 to 200 μm in diameter, with pitted, reticulate and scalariform pits, and with round perforations; parenchyma cells, containing starch grains and solitary crystals of calcium oxalate, their fragments, and cork tissues; but powder of peeled Glycyrrhiza shows no cork tissue; if any, a very few. Starch grains are simple grains, 2–20 μm in diameter; simple grains of calcium oxalate, 10–30 μm in a diameter.

Identification To 2.0 g of Powdered Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of glycyrrhizic acid for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the with these solutions test as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity Foreign matter—Under a microscope, Powdered Glycyrrhiza shows no stone cells.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 7.0%.

Acid-insoluble ash Not more than 2.0%.

Extract content Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of Powdered Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Glycyrrhizic Acid Reference Standard (separately determine the water content), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 μL each of the sample solu-

tion and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S , of glycyrrhizic acid of each solution.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ & = \text{amount (mg) of Glycyrrhizic Acid Reference} \\ & \quad \text{Standard, calculated on the anhydrous basis} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 ml in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

Selection of column: Dissolve 5 mg of Glycyrrhizic Acid Reference Standard and 1 mg of propyl parahydroxybenzoate in dilute ethanol to make 20 mL. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of glycyrrhizic acid and propyl parahydroxybenzoate in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Glycyrrhiza Extract

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Glycyrrhiza Extract contains not less than 4.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation To 1 kg of fine cuttings of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza add 5 L of Water or Purified Water, and digest for 2 days. Filter the digested solution through a cloth filter. Add 3 L of Water or Purified Water to the residue, digest again for 12 hours, and filter through a cloth filter. Evaporate the combined filtrates until the whole volume becomes 3 L. After cooling, add 1 L of Ethanol, and allow to stand in a cold place for 2 days. Filter, and evaporate the filtrate to a viscous extract.

Description Glycyrrhiza Extract is a brown to blackish brown, viscous extract, and has a characteristic odor and a sweet taste.

It dissolves in water, forming a clear solution, or with a slight turbidity.

Identification To 0.8 g of Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 2

minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered, centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Glycyrrhizic Acid Reference Standard (separately determine the water content), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Glycyrrhiza.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= \text{amount (mg) of Glycyrrhizic Acid Reference} \\ & \quad \text{Standard, calculated on the anhydrous basis} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

Containers and storage Containers—Tight containers.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Glycyrrhiza Extract contains not less than 6.0% of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93).

Method of preparation Boil coarse powder of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza with Water or Purified Water, filter the solution under pressure, and evaporate the filtrate.

Description Crude Glycyrrhiza Extract occurs as lustrous, dark yellow-red to blackish brown plates, rods or masses. It is comparatively brittle when cold, and the fractured surface is dark yellow-red, shell-like, and lustrous. It softens when warmed.

It has a characteristic odor and a sweet taste.

It dissolves in water with turbidity.

Identification To 0.6 g of Crude Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), dissolve by warming if necessary, cool, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity (1) Water-insoluble substances—Boil 5.0 g of pulverized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter the mixture through tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(2) Foreign matter—The filtrate obtained in (1) does not have a strong bitter taste.

(3) Starch—To about 1 g of pulverized Crude Glycyrrhi-

za Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Total ash Not more than 12.0% (1 g, proceed as directed in the Total ash under Crude Drugs).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered, centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Glycyrrhizic Acid Reference Standard (separately determine the water content), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Glycyrrhiza.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= \text{amount (mg) of Glycyrrhizic Acid Reference} \\ & \quad \text{Standard, calculated on the anhydrous basis} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

Containers and storage Containers—Tight containers.

Chorionic Gonadotrophin

胎盤性性腺刺激ホルモン

Chorionic Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of pregnant women or from the placenta. It contains not less than 1500 chorionic gonadotrophin Units per mg.

It contains not less than 80% and not more than 125% of the labeled Units of chorionic gonadotrophin.

Description Chorionic Gonadotrophin occurs as a white to light yellow-brown powder. It is odorless.

It is freely soluble in water and practically insoluble in diethyl ether.

Identification Calculate *b* by the following equation, using *Y*₃ and *Y*₄ obtained in the Assay: *b* is not less than 120.

$$\begin{aligned} b &= \frac{E}{I} \\ E &= \frac{Y_3 - Y_4}{f} \end{aligned}$$

f: Number of test animals per group.

$$I = \log \frac{T_H}{T_L}$$

Purity (1) Clarity and color of solution—Dissolve 0.05 g of Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or light yellow.

(2) Estrogen—Inject subcutaneously into each of three female albino rats or albino mice ovariectomized at least two