

spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** Not more than 0.10% (1 g).

**Assay** Weigh accurately about 0.08 g of Glycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 7.507 \text{ mg of } \text{C}_2\text{H}_5\text{NO}_2 \end{aligned}$$

**Containers and storage** Containers—Well-closed containers.

## Glycyrrhiza

### *Glycyrrhizae Radix*

カンゾウ

Glycyrrhiza is the root and stolon, with (unpeeled) or without (peeled) the periderm, of *Glycyrrhiza uralensis* Fisher or *Glycyrrhiza glabra* Linné (*Leguminosae*).

It contains not less than 2.5% of glycyrrhizic acid ( $\text{C}_{42}\text{H}_{62}\text{O}_{16}$ : 822.93), calculated on the basis of dried material.

**Description** Nearly cylindrical pieces, 0.5–3.0 cm in diameter, over 1 m in length. Glycyrrhiza is externally dark brown to red-brown, longitudinally wrinkled, and often has lenticels, small buds and scaly leaves; peeled Glycyrrhiza is externally light yellow and fibrous. The transverse section reveals a rather clear border between phloem and xylem, and a radial structure which often has radiating splits; a pith in Glycyrrhiza originated from stolon, but no pith from root. Odor, slight; taste, sweet.

Under a microscope, the transverse section reveals several layers of yellow-brown cork layers, and 1- to 3-cellular layer of cork cortex inside the cork layer; the cortex exhibiting medullary rays and obliterated sieve portions radiated alternately; the phloem exhibiting groups of phloem fibers with thick but incompletely lignified walls and surrounded by crystal cells; peeled Glycyrrhiza some times lacks periderm and a part of phloem; the xylem exhibiting large yellow vessels and medullary rays in 3 to 10 rows radiated alternately; the vessels accompanied with xylem fibers surrounded by crystal cells, and with xylem parenchyma cells; the parenchymatous pithonily in Glycyrrhiza originated from stolon. The parenchyma cells contain starch grains and often solitary crystals of calcium oxalate.

**Identification** To 2.0 g of pulverized 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 test with these so-

lutions as directed under the Thin-layer Chromatography. Spot 2  $\mu\text{L}$  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.

**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 pulverized 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  $\mu\text{L}$  each of the sample solution and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas,  $A_r$  and  $A_s$ , of glycyrrhizic acid of each solution.

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

**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 octadecylsilylated silica gel for liquid chromatography (5 to 10  $\mu\text{m}$  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  $\mu\text{L}$  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%.