

## Licorice

Licorice consists of the roots, rhizomes, and stolons of *Glycyrrhiza glabra* Linn é or *Glycyrrhiza uralensis* Fisher (Fam. Leguminosae). It contains not less than 2.5 percent of glycyrrhizic acid ( $C_{42}H_{62}O_{16}$ ), calculated on the dried basis.

**Packaging and storage**— Preserve in well-closed containers. Store in a cool, dry place.

**Labeling**— The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

USP Glycyrrhizic Acid RS.

**Botanic characteristics**—

**Macroscopic**— The terrestrial stem is nearly cylindrical, 0.5 to 3.0 cm in diameter, and over 1 m in length; it is externally dark brown to red-brown and longitudinally wrinkled. It often has lenticels, small buds, and scaly leaves. The transverse section reveals a rather clear border between the phloem and the xylem, and a radial structure that often has radiating splits.

**Microscopic**— The transverse section reveals several yellow-brown cork layers, and a layer of phelloderm that is 1 to 3 cells thick. The cortex exhibits medullary rays, and obliterated sieve portions radiate alternately. The phloem exhibits groups of phloem fibers, which are surrounded by crystal cells, with thick but incompletely lignified walls. The vessels are accompanied by xylem fibers, which are surrounded by crystal cells, and by xylem parenchyma cells. The parenchyma cells contain starch grains and often contain single crystals of calcium oxalate.

[Thin-layer chromatographic identification test](#) [201](#) —

**Test solution**— Add 10 mL of a mixture of alcohol and water (7:3) to 2.0 g of pulverized Licorice, heat by shaking on a water bath for 5 minutes, cool, and filter.

**Standard solution**— Dissolve 5 mg of [USP Glycyrrhizic Acid RS](#) in 1 mL of a mixture of alcohol and water (7:3).

Application volume: 2 µL.

Developing solvent system: a mixture of butyl alcohol, water, and glacial acetic acid (7:2:1).

**Procedure**— Proceed as directed in the chapter, except to develop the chromatogram in an unsaturated chamber to a length of about 10 cm. Examine the plate under UV light at a

wavelength of 254 nm. The chromatograms show a dark purple zone, among other spots, due to glycyrrhizic acid at an RF value of about 0.4.

[Loss on drying](#) [731](#) — Dry it at 105 °C for 6 hours: it loses not more than 12.0% of its weight.

[Foreign organic matter](#) [561](#) : not more than 2.0%.

[Total ash](#) [561](#) : not more than 7.0%.

[Acid-insoluble ash](#) [561](#) : not more than 2.0%.

[Alcohol-soluble extractives, Method II](#) [561](#) : not less than 25.0%.

[Pesticide residues](#) [561](#) : meets the requirements.

[Heavy metals, Method III](#) [231](#) : 0.003%.

#### **Content of glycyrrhizic acid—**

**Solvent:** a mixture of alcohol and water (1:1).

**Mobile phase:** a filtered and degassed mixture of diluted acetic acid (1 in 15) and acetonitrile (3:2).

**Standard solution—** Dissolve an accurately weighed quantity of [USP Glycyrrhizic Acid RS](#) in Solvent to obtain a solution having a known concentration of about 0.25 mg per mL.

**Test solution—** Transfer about 500 mg of Licorice, reduced to a powder and accurately weighed, to a suitable flask, add 70 mL of Solvent, shake for 15 minutes, centrifuge, and decant the supernatant into a 100-mL volumetric flask. Mix the residue with 25 mL of Solvent, shake for 15 minutes, centrifuge, and add the supernatant to the volumetric flask. Dilute with Solvent to volume, mix, and pass through a membrane filter having a 0.45- $\mu$ m porosity.

**Chromatographic system—** The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 0.6 mL per minute.

Chromatograph the Standard solution, and record the peak areas as directed for Procedure: the column efficiency determined from glycyrrhizic acid is not less than 5000 theoretical plates; the tailing factor for the glycyrrhizic acid peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**— Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of glycyrrhizic acid ( $\text{C}_{42}\text{H}_{62}\text{O}_{16}$ ) in the portion of Licorice taken by the formula:

$$10,000(C / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of [USP Glycyrrhizic Acid RS](#) in the Standard solution; W is the weight, in mg, of Licorice taken to prepare the Test solution; and  $r_U$  and  $r_S$  are the peak areas for glycyrrhizic acid obtained from the Test solution and the Standard solution, respectively.