

## Eleuthero

Eleuthero is the dried rhizome with roots of *Eleutherococcus senticosus* (Rupr. et Maxim.) (Fam. Araliaceae) [*Acanthopanax senticosus* Harms]. It contains not less than 0.08 percent of the sum of eleutheroside B and eleutheroside E, calculated on the dried basis.

Packaging and storage— Preserve in well-closed, light-resistant containers.

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

### **Botanic characteristics**—

**Macroscopic**— The rhizome is knotty and of irregular cylindrical form with a diameter of 15 to 40 mm. The heartwood area is light brown, and the connecting splint wood is pale yellow. The bark is approximately 2 mm thick and is firmly affixed to the xylem. The surface is gray-brown or black-brown, coarse, and longitudinally vallecuate and plicate. A broken rhizome is coarse and fibrous, particularly inside of the xylem. The fractured surface of the bark shows short thin fibers. Numerous roots spring from the underside of the rhizome. These roots are 35 to 150 mm long, cylindrical, and knotty, with a diameter of 3 to 15 mm. The surface of the roots is gray-brown to black-brown, is smoother than the rhizome, and has longitudinal stripes. A 0.5-mm thin bark is tightly affixed to the pale yellow xylem. A broken root is sparsely fibrous and appears yellowish gray where the thin epidermis is flaked off.

**Histology**— The roots have five to seven rows of brown cork cells. Secretory canals with brown contents appear in groups of four or five and are not greater than 20  $\mu\text{m}$  in diameter. Phloem fibers with thick lignified walls occur singly or in small groups; crystals of calcium oxalate cluster in the phloem parenchyma. Parenchymatous cells surround the secretory cells, and medullary ray cells contain small starch granules. The xylem shows reticulately thickened and pitted vessels. The rhizome is similar to the roots except for its larger secretory canals, up to 25  $\mu\text{m}$  in diameter, and the presence of a pith with parenchymatous cells containing starch granules.

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

**Test solution**— Comminute about 10 g of Eleuthero, add about 50 mL of alcohol 30% (v/v), and heat under reflux in a water bath for 30 minutes. Cool to room temperature, filter, gently evaporate the solvent, and suspend the residue in 5 mL of methanol.

**Standard solution**— Prepare a solution of eleutheroside B in methanol containing about 1 mg per mL.

Developing solvent system: a mixture of chloroform, methanol, and water (70:30:4).

[**note**—Saturate the chamber with Developing solvent system before the development of the chromatogram.]

**Spray reagent**— Prepare a solution of antimony trichloride in chloroform having a concentration of about 200 mg per mL.

**Procedure**— Develop the chromatogram until the solvent front has moved to 15 cm, dry, and spray the plate with Spray reagent. Heat the plate at 120 °C for 10 minutes, and examine it under UV light at 365 nm and in daylight. The chromatogram of the Test solution shows a brownish to red zone due to eleutheroside B, corresponding in color and RF value to the zone exhibited by the chromatogram of the Standard solution. A blue zone appears directly above, and a yellow zone appears directly below the red zone. In daylight, a violet band is visible in the lower half sector. Some brownish to yellowish bands occur in the upper sector.

[Microbial enumeration](#) [2021](#) — The total aerobic microbial count does not exceed  $10^7$  cfu per g, the total combined molds and yeasts count does not exceed  $10^5$  cfu per g, the coliform count does not exceed  $10^4$  cfu per g, and the count for enterobacteria does not exceed  $10^4$  cfu per g. It meets the requirements of the tests for absence of Salmonella species and Escherichia coli.

[Loss on drying](#) [731](#) — Dry at 105 °C to constant weight: it loses not more than 14.0% of its weight.

[Foreign organic matter](#) [561](#) : not more than 3.0%.

[Total ash](#) [561](#) : not more than 8.0%.

[Water-soluble extractives](#) [561](#) : not less than 4.0%.

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

[Heavy metals, Method III](#) [231](#) : not more than 0.002%.

**Content of eleutherosides B and E**—

**Solvent**— Use a mixture of methanol and water (1:1).

**Solution A**— Prepare a filtered and degassed mixture of water and acetonitrile (95:5).

**Solution B**— Prepare a filtered and degassed mixture of acetonitrile and water (60:40).

**Mobile phase**— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary

**Standard solution**— Transfer an accurately weighed quantity of [USP Powdered Eleuthero Extract RS](#) to a suitable volumetric flask, and add Solvent to obtain a solution having a known concentration of about 5.0 mg of Powdered Extract per mL. Sonicate for 30 minutes, cool to room temperature, decant, and pass through a nylon filter having a 0.45-  $\mu\text{m}$  or finer porosity.

**Test solution**— Transfer about 5.0 g of finely ground Eleuthero, accurately weighed, to a round-bottom flask equipped with a condenser, add 50.0 mL of Solvent, and heat under reflux for 30 minutes. Decant the suspension, and filter the supernatant through cotton wool into a 100-mL volumetric flask. Transfer the cotton wool to the round bottom-flask, and repeat the extraction twice, using 22 mL of Solvent for each extraction. Filter through cotton wool into the volumetric flask, wash the residue and the cotton wool with Solvent, cool to room temperature, and dilute with Solvent to volume.

**Chromatographic system**— The liquid chromatograph is equipped with a 220-nm detector and a 4.0-mm  $\times$  25-cm column that contains 5-  $\mu\text{m}$  packing L1. The flow rate is about 1.0 mL per minute.

The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	97	3	isocratic
5–30	97→60	3→40	linear gradient
30–31	60→5	40→95	linear gradient
31–45	5	95	isocratic
45–45.1	5→97	95→3	linear gradient
45.1–60	97	3	equilibration

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the chromatogram obtained is similar to the Reference Chromatogram provided with [USP Powdered Eleuthero Extract RS](#); and the relative standard deviation for replicate injections determined from the eleutheroside B peak is not more than 2.0%.

**Procedure**— Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatogram, identify the eleutheroside B and eleutheroside E peaks in the chromatogram of the Test solution by comparison with the Reference Chromatogram, and measure the peak responses. Separately calculate the percentages of eleutheroside B and eleutheroside E in the portion of Eleuthero taken by the formula:

$$100P(C/W)(rU / rS)$$

in which P is the percentage of eleutheroside B or eleutheroside E in [USP Powdered Eleuthero Extract RS](#), respectively; C is the concentration, in mg per mL, of [USP Powdered Eleuthero Extract RS](#) in the Standard solution; W is the weight, in mg, of Eleuthero taken to prepare the Test solution; rU is the peak response of the relevant analyte obtained from the Test solution; and rS is the eleutheroside B or eleutheroside E peak response obtained from the Standard solution.

Calculate the percentage of eleutheroside B and eleutheroside E in the portion of Eleuthero taken by adding the individual percentages.