- **BOTANICAL EXTRACTS,** *Residual Solvents* ⟨**565**⟩**:** Meets the requirements

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩
  Sample: 1 g
  Analysis: Dry the *Sample* at 105° for 2 h.
  Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cool place.
- **LABELING:** The label states the Latin binomial and, following the official name, the parts of the plant from which the article was prepared. If derived from root and aerial parts, indicate the corresponding percentages. Label it to indicate the content of total phenols and dodecatetraenoic isobutylamides. The label bears a statement indicating that *Echinacea purpurea* may cause rare allergic reactions, rashes, or aggravate asthma. It meets the requirements for *Botanical Extracts* ⟨565⟩*, Labeling*.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Caftaric Acid RS
  USP Chicoric Acid RS
  USP Chlorogenic Acid RS
  USP Powdered *Echinacea purpurea* Extract RS
  USP Echinacoside RS
  USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS
  USP β-Sitosterol RS

Eleuthero

DEFINITION

Eleuthero is the dried rhizome with roots of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Fam. Araliaceae) [*Acanthopanax senticosus* (Rupr. & Maxim.) Harms]. It contains NLT 0.08% of the sum of eleutheroside B and eleutheroside E, calculated on the dried basis.

IDENTIFICATION

- **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN** ⟨**203**⟩
  Standard solution A: 1 mg/mL of USP Eleutheroside E RS in methanol
  Standard solution B: 1 mg/mL of USP Eleutheroside B RS in methanol
  Standard solution C: 0.1 g of USP Powdered Eleuthero Extract RS in 5 mL of aqueous ethanol 50%. Sonicate for 10 min, centrifuge, and use the supernatant.
  Sample solution: Transfer about 1 g of finely powdered Eleuthero to a centrifuge tube, add 5 mL of aqueous ethanol 50%, and mix well. Sonicate for 10 min. Centrifuge or filter the solution, and use the supernatant or the filtrate.
  Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)
  Application volume: 10 µL, as bands
  Developing solvent system: Chloroform, methanol, and water (35:15:2)
  Derivatization reagent: To 18 mL of ice-cold methanol slowly and carefully add 2 mL of sulfuric acid, and mix well. Allow the mixture to adjust to room temperature.
  Analysis
  Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
  Before the development of the chromatogram, saturate the chamber for 20 min with *Developing solvent system*. If the ambient relative humidity exceeds 50%, condition the plate to about 30% relative humidity, using a suitable device. Develop the plate over a path of 6 cm, dry, and spray with *Derivatization reagent*. Heat the plate at 100° for 5 min, and examine under white light and under UV light (365 nm).
  Acceptance criteria: Under white light, the *Sample solution* exhibits two brown bands due to eleutheroside E and eleutheroside B at R_F values of about 0.34 and 0.45, corresponding in color and R_F to the bands exhibited by *Standard solution A* and *Standard solution B*, respectively. The *Sample solution* also exhibits two additional brown bands near the application zone, corresponding in color and R_F values to the bands exhibited by *Standard solution C*. Other bands may be observed in the *Sample solution* and *Standard solution C* chromatograms. Under UV light, the *Sample solution* shows a brown band due to eleutheroside E corresponding in color and R_F to the band exhibited by *Standard solution A*.
- **B. HPLC:** The chromatogram of the *Sample solution* obtained in the test for *Content of Eleutherosides B and E* shows a peak at the retention time corresponding to that of eleutheroside B in the chromatogram of *Standard solution B* and a peak at the retention time corresponding to that of eleutheroside E in the chromatogram of *Standard solution A*.

COMPOSITION

- **CONTENT OF ELEUTHEROSIDES B AND E**
  Solvent: Methanol and water (1:1)
  Solution A: Acetonitrile and water (5:95)
  Solution B: Acetonitrile and water (60:40)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
5	97	3
30	60	40
31	5	95
45	5	95
45.1	97	3
60	97	3

Standard solution A: 0.1 mg/mL of USP Eleutheroside E RS in methanol. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.
Standard solution B: 0.1 mg/mL of USP Eleutheroside B RS in methanol. Transfer 2.0 mL to a 5-mL volumetric flask, and dilute with *Solvent* to volume.
Standard solution C: 5.0 mg/mL of USP Powdered Eleuthero Extract RS in *Solvent*. Sonicate for 30 min, cool to room temperature, decant, and pass through a nylon filter of 0.45-µm or finer pore size.
Sample solution: Transfer about 5.0 g of finely ground Eleuthero, accurately weighed, to a round-bottom flask equipped with a condenser. Add 50 mL of *Solvent*, and heat under reflux for 30 min. 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, dilute with *Solvent* to volume, and mix. Before injection, pass through a nylon filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution B* and *Standard solution C*
Suitability requirements
Chromatogram similarity: The chromatogram from *Standard solution C* is similar to the reference chromatogram provided with the lot of USP Powdered Eleuthero Extract RS being used.
Relative standard deviation: NMT 2.0% determined from the eleutheroside B peak in repeated injections, *Standard solution B*
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Identify the eleutheroside B and eleutheroside E peaks in the *Sample solution* by comparison with the chromatograms of *Standard solution B* and *Standard solution A*, respectively.
Separately calculate the percentages of eleutheroside B and eleutheroside E in the portion of Eleuthero taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of the relevant analyte in the *Sample solution*
r_S = peak area of eleutheroside E or eleutheroside B in *Standard solution A* or *Standard solution B*, respectively
C_S = concentration of eleutheroside E or eleutheroside B in *Standard solution A* or *Standard solution B*, respectively (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Eleuthero taken to prepare the *Sample solution* (mg)
Acceptance criteria: Add the percentages of eleutheroside B and eleutheroside E: NLT 0.08% on the dried basis.

CONTAMINANTS
- **Articles of Botanical Origin,** *Limits of Elemental Impurities* ⟨**561**⟩**:** Meets the requirements
- **Articles of Botanical Origin,** *Pesticide Residue Analysis* ⟨**561**⟩**:** Meets the requirements
- **Microbial Enumeration Tests** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria do not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS
- **Botanical Characteristics**
Macroscopic: The rhizome is knotty and of irregular cylindrical shape with a diameter of 15–40 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 valleculate and plicate. A broken rhizome is coarse and fibrous, particularly inside the xylem. The fractured surface of the bark shows short, thin fibers. Numerous roots spring from the underside of the rhizome. These roots are 35–150 mm long, cylindrical and knotty, with a diameter of 3–15 mm. The surface of the roots is gray-brown to black-brown, 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.
Microscopic: 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 more than 20 µm in diameter. Phloem fibers with thick lignified walls occur singly or in small groups; there are cluster crystals of calcium oxalate 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 larger secretory canals, up to 25 µm in diameter, and the presence of a pith with parenchymatous cells containing starch granules.
- **Loss on Drying** ⟨**731**⟩**:** Dry a sample at 105° to constant weight; it loses NMT 14.0% of its weight.
- **Articles of Botanical Origin,** *Total Ash* ⟨**561**⟩**:** NMT 8.0%
- **Articles of Botanical Origin,** *Water-Soluble Extractives, Method 2* ⟨**561**⟩**:** NLT 4.0%
- **Articles of Botanical Origin,** *Foreign Organic Matter* ⟨**561**⟩**:** NMT 3.0%

ADDITIONAL REQUIREMENTS
- **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.
- **USP Reference Standards** ⟨**11**⟩
USP Eleutheroside B RS
β-D-Glucopyranoside, 4-(3-hydroxy-1-propenyl)-2,6-dimethoxyphenyl.
$C_{17}H_{24}O_9$ 372.37
USP Eleutheroside E RS
β-D-Glucopyranoside, (tetrahydro-1*H*,3*H*-furo(3,4-c)furan-1,4-diyl)bis(2,6-dimethoxy-4,1-phenylene)bis-.
$C_{34}H_{46}O_{18}$ 742.70
USP Powdered Eleuthero Extract RS

Powdered Eleuthero

DEFINITION
Powdered Eleuthero is Eleuthero reduced to a powder or very fine powder. It contains NLT 0.08% of the sum of eleutheroside B and eleutheroside E, calculated on the dried basis.

IDENTIFICATION
- **A. HPTLC for Articles of Botanical Origin** ⟨**203**⟩
Standard solution A: 1 mg/mL of USP Eleutheroside E RS in methanol
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Standard solution C: 0.1 g of USP Powdered Eleuthero Extract RS in 5 mL of aqueous ethanol 50%. Sonicate for 10 min, centrifuge, and use the supernatant.
Sample solution: Transfer about 1 g of Powdered Eleuthero to a centrifuge tube, add 5 mL of aqueous ethanol 50%, and mix well. Sonicate for 10 min. Centrifuge or filter the solution, and use the supernatant or the filtrate.
Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)
Application volume: 10 µL, as bands
Developing solvent system: Chloroform, methanol, and water (35:15:2)
Derivatization reagent: To 18 mL of ice-cold methanol slowly and carefully add 2 mL of sulfuric acid, and mix well. Allow the mixture to adjust to room temperature.

DS Monographs