Echinacea purpurea Aerial Parts

DEFINITION

Echinacea purpurea Aerial Parts consists of the aerial parts of *Echinacea purpurea* (L.) Moench (Fam. Asteraceae). It is harvested during the flowering stage. It contains NLT 1.0% of the sum of caftaric acid ($C_{13}H_{12}O_9$) and chicoric acid ($C_{22}H_{18}O_{12}$), and NLT 0.01% of dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$), calculated on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Presence of chicoric acid and absence of echinacoside

Standard solution A: 0.2 mg/mL of USP Echinacoside RS in methanol

Standard solution B: 0.2 mg/mL of USP Caftaric Acid RS, 0.1 mg of USP Chlorogenic Acid RS, and 0.2 mg/mL of USP Chicoric Acid RS in methanol

Standard solution C: 20 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in methanol. Shake to disperse, sonicate for 5 min, and centrifuge. Use the supernatant.

Sample solution: Transfer 1 g of finely pulverized *Echinacea purpurea* Aerial Parts to a centrifuge tube, add 10 mL of methanol, mix well, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 5 µL *Standard solution C* and *Sample solution*, and 2 µL *Standard solution A* and *Standard solution B* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of ethyl acetate, methylethyl ketone, water, and formic acid (5:3:1:1)

Developing distance: 6 cm

Derivatization reagent: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Apply the *Samples* as bands to a suitable thin-layer chromatographic plate, and dry in air. Develop the chromatograms in a satutated chamber. Remove the plate from the chamber, heat at 100° for 5 min, derivatize the plate while still warm with *Derivatization reagent*, dry in air, and examine under UV light at 366 nm.

System suitability: *Standard solution A* shows one major blue band in the lower third section of the chromatogram due to echinacoside. *Standard solution B* shows two major blue bands at about the middle of the chromatogram due to caftaric acid (higher R_F) and chlorogenic acid (lower R_F) that are clearly separated, and a blue band for chicoric acid in the upper third section of the chromatogram.

Acceptance criteria: The most prominent band in the *Sample solution* chromatogram is a blue band in the upper third section of the chromatogram at an R_F corresponding to the chicoric acid band in the chromatogram of *Standard solution B* and *Standard solution C.* The second most prominent band in the *Sample solution* chromatogram is a blue band at about the middle of the chromatogram due to caftaric acid, corresponding to a band in the chromatogram of *Standard solution C.* The *Sample solution* chromatogram does not exhibit a band at the R_F of echinacoside in *Standard solution A* (difference from *Echinacea pallida* and *Echinacea angustifolia*). The *Sample solution* chromatogram exhibits minor blue bands corresponding to similar bands in the chromatogram of *Standard solution C.* One of these bands is due to chlorogenic acid at an R_F corresponding to chlorogenic acid in *Standard solution B.* The *Sample solution* chromatogram exhibits a red band due to chlorophyll close to the solvent front.

• **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the chicoric acid peak in *Standard solution A*, and the second most prominent peak corresponds to that of the caftaric acid peak in *Standard solution B*. The *Sample solution* chromatogram shows no peak or a very minor peak at the retention time corresponding to the echinacoside peak in the *Standard solution C* chromatogram, all peaks as obtained in the test for *Content of Chicoric Acid and Caftaric Acid*.

• **C.** The retention times for the relevant peaks of the *Sample solution*, mainly due to dodecatetraenoic isobutyl amides, correspond to those of *Standard solution A*, as obtained in the test for *Content of Dodecatetraenoic Isobutylamides*.

COMPOSITION

• **Content of Chicoric Acid and Caftaric Acid**

Solution A: Phosphoric acid (0.1 in 100) in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
13	78	22
14	60	40
17.5	60	40
18	90	10
30	90	10

Solvent: Alcohol and water (7:3)

Standard solution A: 30 µg/mL of USP Chicoric Acid RS in *Solvent*

Standard solution B: 20 µg/mL of USP Caftaric Acid RS in *Solvent*

Standard solution C: 20 µg/mL of USP Echinacoside RS in *Solvent*

Sample solution: Transfer about 125 mg, accurately weighed, of finely powdered *Echinacea purpurea* Aerial Parts (capable of passing through a 40-mesh sieve) to a round-bottom flask equipped with a condenser. Add 25.0 mL of *Solvent*, and heat under reflux while shaking by mechanical means for 15 min. Centrifuge, or pass through a membrane filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 330 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection size: 5 µL

System suitability

Samples: *Standard solution A*

Suitability requirements

Relative standard deviation: NMT 2.0% for the chicoric acid peak in *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Separately calculate the percentages of caftaric acid ($C_{13}H_{12}O_9$) and chicoric acid ($C_{22}H_{18}O_{12}$) in the portion of *Echinacea purpurea* Aerial Parts taken:

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

r_U = peak area of the relevant analyte from the *Sample solution*
r_S = peak area of the relevant analyte from the corresponding *Standard solution*
C_S = concentration of the relevant analyte in the corresponding *Standard solution* (mg/mL)
V = final volume of the *Sample solution* (mL)
W = weight of *Echinacea purpurea* Aerial Parts taken to prepare the *Sample solution* (mg)

Calculate the percentage of the sum of chicoric acid and caftaric acid in the portion of *Echinacea purpurea* Aerial Parts taken by adding the individual percentages calculated.

Acceptance criteria: NLT 1.0% on the dried basis

• **Content of Dodecatetraenoic Acid Isobutylamides**

Mobile phase: Acetonitrile and water (55:45)

Standard solution A: 5 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in methanol. Dissolve using sonication and shaking for 10 min. After dilution, pass through a membrane filter of 0.45-µm or finer pore size.

Standard solution B: 10 µg/mL of USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS in methanol

Sample solution: Transfer about 2.5 g of finely powdered *Echinacea purpurea* Aerial Parts (capable of passing through a 40-mesh sieve), accurately weighed, into a round-bottom flask. Add 80 mL of methanol, and reflux for 30 min. Cool to room temperature, and filter into a 100-mL volumetric flask, using small portions of methanol to rinse the flask and the filter. Dilute with methanol to volume. Pass through a membrane filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution A* is similar to the Reference Chromatogram for alkamides provided with USP Powdered *Echinacea purpurea* Extract RS.

Resolution: NLT 1.0 between dodecatetraenoic acid isobutylamide peaks, *Standard solution A*

Tailing factor: NMT 2.0 for 2*E*,4*E*-hexadienoic acid isobutylamide, *Standard solution B*

Relative standard deviation: NMT 2.5% for the 2*E*,4*E*-hexadienoic acid isobutylamide peak in repeated injections, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks of the two isomers of dodecatetraenoic acid isobutylamides in the chromatogram from the *Sample solution* by comparison with the chromatogram from *Standard solution A*. Measure the areas for the relevant peaks.

Calculate the percentage of dodecatetraenoic acid isobutylamides in the portion of *Echinacea purpurea* Aerial Parts taken:

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

r_U = sum of the peak areas of the relevant analytes from the *Sample solution*
r_S = peak area of 2*E*,4*E*-hexadienoic acid isobutylamide from *Standard solution B*
C_S = concentration of USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS in *Standard solution B* (mg/mL)
V = final volume of the *Sample solution* (mL)
W = weight of *Echinacea purpurea* Aerial Parts taken to prepare the *Sample solution* (mg)
F = response factor to convert 2*E*,4*E*-hexadienoic acid isobutylamide into dodecatetraenoic acid isobutylamides, 1.353

Acceptance criteria: NLT 0.01% of dodecatetraenoic acid isobutylamides on the dried basis

CONTAMINANTS

• **Elemental Impurities—Procedures** ⟨233⟩

Acceptance criteria

Arsenic: NMT 1.0 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 1.0 µg/g

Change to read:

• ▲**Articles of Botanical Origin,** *Pesticide Residue Analysis* ⟨561⟩▲ (CN 1-May-2019)**:** Meet the requirements

• **Microbial Enumeration Tests** ⟨2021⟩**:** The total aerobic microbial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the enterobacterial count does not exceed 10^3 cfu/g.

• **Absence of Specified Microorganisms** ⟨2022⟩**:** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

SPECIFIC TESTS

• **Botanic Characteristics**

Macroscopic: The herb is an erect, coarse, rough-hairy perennial, usually up to 90 cm tall, rarely up to 180 cm. The leaves are alternate and simple; the lowermost leaves are slender, long, and petioled, ovate to broadly lanceolate, mostly penta-nerved, acute or acuminated at the apex, abruptly narrowed or rarely cordate at the base, usually sharply dentate, and 7–20 cm long and 2.5–7.5 cm wide; the petioles are mostly winged at the summit. The upper leaves are narrower, often almost entirely sessile, lanceolate or ovate lanceolate, and usually with 3 veins.

The flower heads are radiate, up to 15 cm across, solitary or few, and long-peduncled, with 12–20 rays, purple, crimson, or rarely pale; the bristle disks are often orange, 3.5–7.5 cm long; the involucre is depressed-hemispheric; the bracts are lanceolate, spreading or appressed, imbricated in 2–4 series, and hairy on the outer surface with ciliate margins; the receptacle is conical, the scales of the receptacle stiff, spinescent, and conspicuously longer than the disc flowers; the chaff is carinate and cuspidate; the achenes are 3–4 mm in length, tetrasided, obypyramidal, and thick; the pappus has a short, dentate crown.

Microscopic

Leaf: The leaf has a thickness of 200–350 µm, with an epidermis 9–13 µm thick, largely without chloroplasts; the stomata are 28–35 µm, abundant on the ventral surface and fewer on the dorsal surface; the mesophyll is clearly divided into palisade parenchyma and sponge parenchyma. The palisade parenchyma is one layer thick, with elongated cells 50–65 µm in length, oriented at right angles to the leaf surface, containing numerous chloroplasts. The sponge parenchyma is 150–250 µm thick, with cells of irregular shape, and has multiple cell layers, few chloroplasts, and large intercellular spaces. The phloem bundles of the lateral veins within the sponge parenchyma are bound by a one-layer sheath of small parenchymous cells, with vascular elements of the midrib surrounded by large-celled parenchyma. The uniseriate trichomes are few in the ventral surface, numerous on the dorsal surface, typically tricelled, occasionally tetra- or pentacelled, 250–500 µm in length, each arising from an epidermal cell; the epidermal cell walls appear with moderate thickening; the vessels are various, scalariform, with variable reticulated width.

Petiole: The parenchyma appear without chloroplasts, in several layers adjacent to a layer of collenchyma; 5–7 phloem bundles of small- to medium-sized vessels are weakly lignified and embedded in the parenchyma in the form of an arc; the wing ribs of the upper surface of the slightly hollowed petiole are marginal.

Inflorescence: The epidermal cells of the ray florets are square, 50 µm, with a transparent, beaded cell wall; various elements of the *Asteraceous* exhibit inflorescence; numerous multicellular jointed trichomes of the involucral bracts are 500–800 µm in length; tangential sections of the paleae with numerous fiber bundles are 10–15 µm in diameter and 100–150 µm in length; cell walls are thin. The epidermis of ray florets is reddish to violet; the epidermal cells from the end of the corolla form rounded papillae; a stigma of papillary cells is present; *Asteraceous* pollen grains are 20–30 µm and spherical with a warty exine.

Calcium oxalate is negative; crystals of inulin and starch granules are rare.

- **ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matter* ⟨561⟩**:** NMT 3.0%
- **LOSS ON DRYING** ⟨731⟩

Sample: 1 g of the powdered plant material
Analysis: Dry the *Sample*.
Acceptance criteria: NMT 12%

- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩**:** NMT 10.0%, determined on 3 g
- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨561⟩**:** NMT 2.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tight, light-resistant containers at controlled room temperature.
- **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 Chlorogenic Acid RS
USP Caftaric Acid RS
USP Chicoric Acid RS
USP Echinacoside RS
USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS
USP Powdered *Echinacea purpurea* Extract RS