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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 saturated 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 (C₁₃H₁₂O₉) and chicoric acid (C₂₂H₁₈O₁₂) 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 2E,4E-Hexadienoic Acid Isobutylamide RS](#) in methanol