

Powdered *Echinacea purpurea*

DEFINITION

Powdered *Echinacea purpurea* is *Echinacea purpurea* Root reduced to a powder or very fine powder.

IDENTIFICATION

• A. PRESENCE OF CHICORIC ACID AND ABSENCE OF ECHINACOSIDE

Standard solution A: 20 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in methanol

Standard solution B: 1 mg/mL of 1,3-dicaffeoylquinic acid in methanol

Sample solution: Transfer about 1 g of Powdered *Echinacea purpurea* to a suitable extraction thimble. Transfer the thimble to a continuous extraction apparatus, and extract with 50 mL of chloroform for 1 h. Retain the chloroform extract for *Identification test B*. Continue the extraction with 50 mL of methanol, and concentrate to a small volume at 40° in vacuum. With the aid of methanol, transfer the extract to a 10-mL volumetric flask, and dilute with methanol to volume.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture (TLC plates)

Application volume: 10 μ L

Developing solvent system: Ethyl acetate, formic acid, and water (17:2:1)

Spray reagent A: 10 mg/mL of diphenylborinic acid, ethanolamine ester in methanol

Spray reagent B: 50 mg/mL of polyethylene glycol 4000 in alcohol

Analysis

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

Develop the chromatograms until the solvent front has moved NLT 18 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent A* followed by *Spray reagent B*, and examine the plate under UV light at 365 nm.

Acceptance criteria: The chromatogram from the *Sample solution* shows a yellowish-green zone at an R_f value of 0.75 due to chicoric acid and another yellowish-green zone at an R_f value of 0.45 due to caftaric acid, both zones corresponding in color and R_f value to zones in the chromatogram from *Standard solution A*. The chromatogram from the *Sample solution* does not show or shows only traces of a zone at an R_f value of 0.1 due to echinacoside (present in *Echinacea angustifolia* and in *Echinacea pallida*), and does not show a zone that corresponds in color and R_f value to the spot for 1,3-dicaffeoylquinic acid (cynarin) (present in *Echinacea angustifolia*) in the chromatogram from *Standard solution B*. Other colored zones of varying intensities may be observed in the chromatogram from the *Sample solution*.

• B. PRESENCE OF ISOBUTYLALKENYLAMIDES

Standard solution A: 100 mg/mL USP *Echinacea purpurea* Extract RS in methanol

Standard solution B: 1 mg/mL of β -sitosterol in methanol

Sample solution: Evaporate the chloroform extract retained from preparation of the *Sample solution* in *Identification test A* to dryness at 40° in vacuum. To the residue, add 1 mL of alcohol, and pass through a nylon membrane filter having a pore size of 0.45 μ m.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture (TLC plates)

Application volume: 10 μ L

Developing solvent system: Hexane and ethyl acetate (2:1)

Spray reagent: Prepare a mixture of glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (10:5:0.5) in an ice bath.

Analysis

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

Develop the chromatograms until the solvent front has moved NLT 12 cm, and dry the plate in a current of air. Examine the plate under UV light at 254 nm, then spray the plate with *Spray reagent*, and heat the plate at 100° for 5 min. Examine the plate under long-wavelength UV light.

Acceptance criteria

Under UV light at 254 nm: The chromatogram from the *Sample solution* shows one main zone corresponding in R_f value to the zone due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide in the chromatogram of *Standard solution A*, and below this zone there are several other zones due to α , β , γ , δ -unsaturated isobutylamides.

After treatment with *Spray reagent* and heating: The zone due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide turns blue-black, and below this zone there are several other zones due to α , β , γ , δ -unsaturated isobutylamides (not detectable in *Echinacea pallida*) that turn violet (unlike the corresponding zones in the chromatogram of *Echinacea angustifolia* that are mostly yellowish due to α , β -unsaturated isobutylamides). A zone due to β -sitosterol that corresponds in R_f value to the principal spot in the chromatogram of *Standard solution B* is also observed.

- **C.** The retention times for the relevant peaks of the *Sample solution*, mainly due to caftaric acid and chicoric acid, correspond to those of *Standard solution A*, as obtained in the test for *Content of Total Phenols*. An echinacoside peak is not detectable or is very weak.

COMPOSITION

• CONTENT OF TOTAL PHENOLS

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: 5 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in *Solvent*. Dissolve by shaking for 1 min. After dilution, pass through a membrane filter of 0.45- μ m or finer pore size.

Standard solution B: 40 μ g/mL of USP Chlorogenic Acid RS in *Solvent*

Sample solution: Transfer about 125 mg of Powdered *Echinacea purpurea* (capable of passing through a 40-mesh sieve), accurately weighed, 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- μ m 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* and *Standard solution B*
Suitability requirements

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

Relative standard deviation: NMT 2% for chlorogenic acid peak in repeated injections, *Standard solution B*

Analysis

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

Identify the relevant analytes in the chromatogram obtained from the *Sample solution* by comparison with the chromatogram obtained from *Standard solution A*. Measure the areas for the relevant peaks.

Separately calculate the percentage of caftaric acid (C₁₃H₁₂O₉), chicoric acid (C₂₂H₁₈O₁₂), and chlorogenic acid (C₁₆H₁₈O₉) in the portion of Powdered *Echinacea purpurea* taken:

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

r_U = peak area for the relevant analyte from the *Sample solution*

r_S = peak area for chlorogenic acid from *Standard solution B*

C_S = concentration of USP Chlorogenic Acid RS in *Standard solution B* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered *Echinacea purpurea* used to prepare the *Sample solution* (mg)

F = response factor: chicoric acid, 0.695; caftaric acid, 0.881; and chlorogenic acid, 1.000

Calculate the percentage of total phenols in the portion of Powdered *Echinacea purpurea* taken by adding the individual percentages calculated.

Acceptance criteria: NLT 0.5% of total phenols on the dried basis

• **CONTENT OF ALKAMIDES**

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 having a 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 Powdered *Echinacea purpurea* (capable of passing through a 40-mesh sieve) 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 having a 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 obtained 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 peak, *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 10 major alkamides in the chromatogram obtained from the *Sample solution* by comparison with the chromatogram obtained from *Standard solution A*.

Calculate the percentage of alkamides in the portion of Powdered *Echinacea purpurea* 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 = volume of the *Sample solution* (mL)

W = weight of Powdered *Echinacea purpurea* used to prepare the *Sample solution* (mg)

F = response factor for 2*E*,4*E*-hexadienoic acid isobutylamide, 1.353

Acceptance criteria: NLT 0.025% on the dried basis

CONTAMINANTS

- **HEAVY METALS**, *Method III* <231>: NMT 10 ppm
- **ARTICLES OF BOTANICAL ORIGIN**, *General Procedure for Pesticide Residues Analysis* <561>: Meets the requirements

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS:** Under a microscope, the following characteristics are observed: vessels (80 × 30 μm) with slanted end walls and spiral or pitted secondary walls; rectangular cork cells (150 × 60 μm) with brown inclusions; rectangular parenchymatous cells (120 × 30 μm), some pitted; elongated fiber cells having a narrow lumen with funnel-shaped end (20 to 40 μm wide); polygonal sclereids; a melanogenic layer of variable thickness, interspersed between the cell walls of the parenchyma; and lignified sclereids, vessels, and fibers. Starch is present; calcium oxalate and inulin crystals are absent.
- **LOSS ON DRYING** <731>: Dry a sample at 105° for 2 h: it loses NMT 10.0%.
- **ARTICLES OF BOTANICAL ORIGIN**, *Total Ash* <561>: NMT 7.0%
- **ARTICLES OF BOTANICAL ORIGIN**, *Acid-Insoluble Ash* <561>: NMT 4.0%

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 part of the plant from which the article was derived.
- **USP REFERENCE STANDARDS** <11>
USP Chlorogenic Acid RS
USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS
USP Powdered *Echinacea purpurea* Extract RS

Powdered *Echinacea purpurea* Extract

DEFINITION

Powdered *Echinacea purpurea* Extract is prepared from dried *Echinacea purpurea* Root, *Echinacea purpurea* Aerial Parts, or a mixture of them, by extraction with hydroalcoholic mixtures or other suitable solvents. The ratio of the starting crude plant material to Powdered Extract is between 2:1 and 8:1. It contains NLT 4.0% of total phenols, calculated as the sum of caftaric acid (C₁₃H₁₂O₉), chicoric acid (C₂₂H₁₈O₁₂), and chlorogenic acid (C₁₆H₁₈O₉), on the dried basis. It contains NLT 0.025% of dodecatetraenoic acid isobutylamides (C₁₆H₂₅NO), calculated on the dried basis.

IDENTIFICATION

A. PRESENCE OF ISOBUTYLALKENYLAMIDES

Standard solution A: 100 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in methanol

Standard solution B: 1 mg/mL of β-sitosterol in methanol

Sample solution: Transfer about 1 g of Powdered *Echinacea purpurea* Extract to a suitable extraction thimble.

Transfer the thimble to a continuous extraction apparatus, and extract with 50 mL of chloroform for 1 h. Evaporate the chloroform extract to dryness at 40° in vacuum. To the residue add 1 mL of alcohol, and pass through a nylon membrane filter of 0.45-μm pore size.

Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 μm (TLC plates)

Application volume: 10 μL

Developing solvent system: Hexane and ethyl acetate (2:1)

Spray reagent: Prepare a mixture of glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (10:5:0.5) in an ice bath.

Analysis

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

Develop the chromatograms until the solvent front has moved NLT 12 cm, and dry the plate in a current of air. Examine the plate under UV light at 254 nm, and then spray the plate with *Spray reagent*, and heat the plate at 100° for 5 min.

Acceptance criteria

Under UV light at 254 nm: The chromatogram from the *Sample solution* shows one main zone corresponding in *R_f* value to the zone due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide in the chromatogram of *Standard solution A*, and below this zone there are several other zones due to α,β,γ,δ-unsaturated isobutylamides.

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- **B.** The retention times of the peaks for chicoric and caftaric acids of the *Sample solution* correspond to those of *Standard solution A*, as obtained in the test for *Content of Total Phenols*. An echinacoside peak is not detectable or is very weak.

COMPOSITION

CONTENT OF TOTAL PHENOLS

Solvent: Alcohol and water (7:3)

Standard solution A: 5 mg/mL of USP Powdered *Echinacea purpurea* Extract RS in *Solvent*. Dissolve by shaking for 1 min. After dilution, pass through a membrane filter having a 0.45-μm or finer pore size.

Standard solution B: 40 μg/mL of USP Chlorogenic Acid RS in *Solvent*

Sample solution: Transfer 60 mg of Powdered *Echinacea purpurea* Extract to a round-bottom flask equipped with a condenser. Add 25 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.

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

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

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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

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

r_U = peak response for the relevant analyte from the *Sample solution*

r_S = peak response for chlorogenic acid from *Standard solution B*

C_S = concentration of USP Chlorogenic Acid RS in *Standard solution B* (mg/mL)