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Powdered *Echinacea angustifolia*

DEFINITION

Powdered *Echinacea angustifolia* consists of the dried rhizome and roots of *Echinacea angustifolia* DC. (Fam. Asteraceae), harvested in the fall after one or more years of growth, and reduced to powder. It contains NLT 0.5% of total phenols, calculated on the dried basis as the sum of echinacoside ($C_{35}H_{46}O_{20}$), dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$), and chlorogenic acid ($C_{16}H_{18}O_9$). It contains NLT 0.075% of dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$) on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Presence of echinacoside and dicaffeoylquinic acid (cynarin(e))

Standard solution A: A mixture of 0.2 mg/mL of USP Echinacoside RS and 0.2 mg/mL of dicaffeoylquinic acid (cynarin) in methanol

Standard solution B: 0.05 mg/mL of USP Caftaric Acid RS, 0.1 mg/mL of USP Chlorogenic Acid RS, and 0.05 mg/mL of USP Chicoric Acid RS in methanol

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

Sample solution: Transfer 1 g of Powdered *Echinacea angustifolia* 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⟩, Thin-Layer Chromatography*.)

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 two major blue bands, one in the lower third of the chromatogram due to echinacoside, and the other band in the middle section of the chromatogram due to dicaffeoylquinic acid (cynarin). *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 lower third section of the chromatogram at an R_F corresponding to the echinacoside band in the chromatogram of *Standard solution A* and *Standard solution C* (absent in *Echinacea purpurea*). The *Sample solution* chromatogram exhibits a prominent greenish blue band in the middle section of the chromatogram at an R_F corresponding to the dicaffeoylquinic acid (cynarin) band in the chromatogram of *Standard solution A* and *Standard solution C* (absent in *Echinacea pallida* and *Echinacea purpurea*). The *Sample solution* chromatogram does not exhibit, or exhibits very faint blue bands at an R_F corresponding to the caftaric acid and chicoric acid bands in *Standard solution B* (difference from *Echinacea pallida* and *Echinacea purpurea*). The *Sample solution* chromatogram exhibits minor bands between the positions of echinacoside and cynarin. One of these is due to chlorogenic acid at an R_F corresponding to that of chlorogenic acid in *Standard solution B*.

• **B. Thin-Layer Chromatography**

Presence of alkylamides

Standard solution A: 0.2 mg/mL of USP β-Sitosterol RS in methanol

Standard solution B: 100 mg/mL of USP Powdered *Echinacea angustifolia* Extract RS in dichloromethane. Shake to disperse, sonicate for 5 min, and centrifuge. Use the supernatant.

Sample solution: Transfer 1 g of Powdered *Echinacea angustifolia* to a centrifuge tube, add 10 mL of dichloromethane, mix well, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

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(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

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

Application volume: 5 µL *Standard solution B* and *Sample solution*, and 2 µL *Standard solution A* 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 toluene, ethyl acetate, cyclohexane, and formic acid (8:2:1:0.3)

Developing distance: 6 cm

Derivatization reagent: Place 85 mL of methanol in a 100-mL glass bottle, and cool it down in a water–ice cubes–salt bath or in a freezer. To the ice-cold methanol, slowly and carefully add 10 mL of acetic acid and 5 mL of sulfuric acid, and mix well. Allow the mixture to cool to room temperature, then add 0.5 mL of *p*-anisaldehyde.

Analysis

Samples: *Standard solution A*, *Standard solution B*, 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, dry in air, derivatize with *Derivatization reagent*, heat at 100° for 3–5 min, dry in air, and examine under visible light.

System suitability: The β-sitosterol band of the *Standard solution B* chromatogram and the two bands underneath are clearly separated from one another. These two bands, in decreasing R_F, include a major blue violet band and a yellow band.

Acceptance criteria: The most prominent band of the *Sample solution* chromatogram is a blue violet band in the lower-third section of the chromatogram (much less prominent in *Echinacea purpurea* and absent in *Echinacea pallida*). This blue violet band is between two bands: a less prominent blue violet band at a higher R_F corresponding to the β-sitosterol band in the chromatograms of *Standard solution A* and *Standard solution B*, and a characteristic yellow band at a lower R_F (absent in *Echinacea purpurea* and *Echinacea pallida*). The yellow band turns reddish pink when the plate is heated at 100° for more than 10 min. The *Sample solution* chromatogram exhibits a minor pink-violet band at about the middle of the chromatogram (much more prominent in *Echinacea purpurea*), a minor pink-violet band at about two-thirds of the chromatogram (much more prominent in *Echinacea pallida*), and a broad pink-violet band close to the solvent front.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the echinacoside peak of *Standard solution A* and *Standard solution C*, and the retention time of the 1,3-dicaffeoylquinic acid peak from the *Sample solution* corresponds to that of *Standard solution A*, all peaks as obtained in the test for *Content of Total Phenols*.

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
3	90	10
16	78	22
17	60	40
20	60	40
20.5	90	10
25	90	10

Solvent: Alcohol and water (7:3)

Standard solution A: Dissolve USP Powdered *Echinacea angustifolia* Extract RS in *Solvent*, shaking and heating in a water bath. Dilute with *Solvent* to obtain a solution having a known concentration of 1 mg/mL. 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*

Standard solution C: 80 µg/mL of USP Echinacoside RS in *Solvent*

Sample solution: Transfer about 125 mg of Powdered *Echinacea angustifolia* (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 having a 0.45-µm or finer pore size.

Chromatographic system

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

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