

ChromaDex®

Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0040 - Echinacea for Phenolic Acids and Isobutylamides by HPLC

Botanical Name: *Echinacea* spp.; *Echinacea purpurea*; *Echinacea pallida*; *Echinacea angustifolia*.

Common Names: Narrow-leaf echinacea and narrow-leaf purple echinacea for *Echinacea angustifolia*; pale purple coneflower for *Echinacea pallida*; purple coneflower for *Echinacea purpurea*.

Parts of Plant Used: Aerial parts and roots

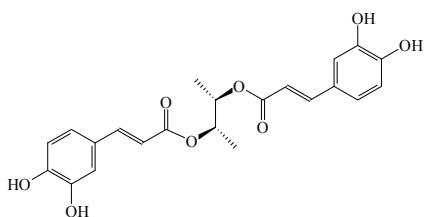
Uses: As an immunostimulating agent; treatment of infections of the upper respiratory tract.

Modes of Action:

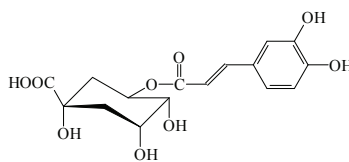
More than 20 clinical trials have been performed on echinacea; most of them found positive effects for symptoms related to colds, upper respiratory tract infections, and flu. There is the widespread belief that echinacea has immunomodulatory activity; however, it is still somewhat unclear which compounds are responsible for this activity. Polysaccharides, alkamides, or the caffeic acid derivatives may be active components.¹

Chemical Markers:

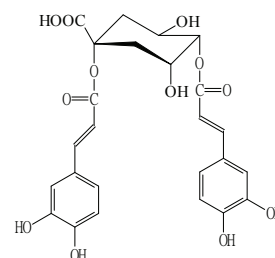
Various chemical compounds have been identified from *Echinacea* spp. including alkamides, polyalkenes, polyalkynes, volatile oils, phenolic compounds, and polysaccharides. More than 20 alkamides have been identified in *Echinacea* spp. Most of them are isobutylamides of C11–C16 long-chain unsaturated fatty acids, with a mixture of isomeric dodeca-2,4,8,10-tetraenoic acid isobutylamides as the major ones. The root contains higher amounts of alkamides than other parts and the highest concentration is found in *Echinacea angustifolia*. Echinacea is also known to contain phenolic compounds (flavonoids and caffeic acid derivatives). The well-known caffeic acid derivatives found in echinacea are cichoric acid, echinacoside, 1,3-dicaffeoylquinic acid, and chlorogenic acid; caftaric acid with cichoric acid is the major phenolic compound in *Echinacea purpurea* and echinacoside as the main phenolic compound in *Echinacea angustifolia* and *E. pallida*.² Currently, the alkamides and caffeic acid derivatives are used as marker compounds for quality control of echinacea products.



Cichoric acid

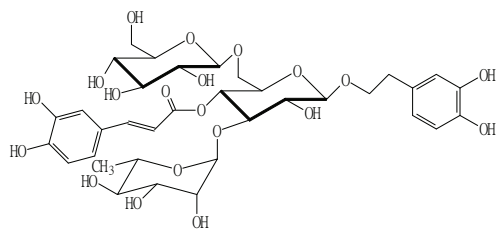


Chlorogenic acid

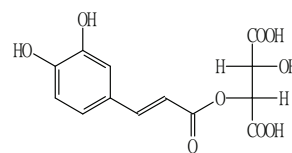


1,3-Dicaffeoylquinic acid

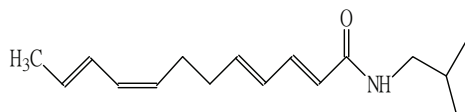




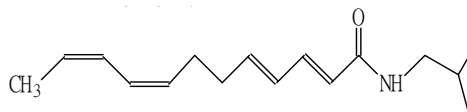
Echinacoside



Caftaric acid



Dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide



Dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide

Methods of Analysis

As echinacea is one of the most popular herbs in the U.S. and European markets, various analytical techniques including TLC, HPLC, micellar electrokinetic chromatography, and ATR-IR and NIR spectroscopy have been used to analyze the compounds in echinacea.³⁻⁶ Currently, HPLC is the most accepted method for analysis of alkaloids and caffeic acid derivatives in *Echinacea* spp.

Method 1:

The method found at www.nsfina.org determines 2-O-caffeoyltartaric acid (caftaric acid), 2,3-O-dicaffeoyltartaric acid (cichoric acid or chicoric acid), 3-O-caffeoylquinic acid (chlorogenic acid), and echinacoside in plant materials and powdered extracts of *Echinacea angustifolia*, *E. pallida*, and *E. purpurea*.

Sample Preparation:

For plant material, shake 0.125 g of ground material with 25 mL of ethanol-water (70:30) for 15 minutes.

Chromatography:

Column: Phenomenex Prodigy ODS(3), 5 μm , 100Å, 4.6 \times 250 mm.

Mobile phase: Solvent A = water (0.1% phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	90	10
13	78	22
14	60	40
14.5	60	40
15	90	10

Injection volume: 5 μL

Run time: 20 minutes

Flow rate: 1.5 mL/minute

Column temperature: 35°C

Detection wavelength: 330 nm

Validation Data:

Not available

Method 2:

The method of Molgaard et al.⁷ can be used to analyze cichoric acid, 1,3-dicaffeoylquinic acid, echinacoside, and alkamides in Echinacea purpurea simultaneously.

Sample Preparation:

For plant material and capsules, sonicate about 400 mg of sample in 10 mL of methanol–water (7:3) for 30 minutes, and follow by 120 minutes on a blood turner.

For liquid samples, dilute the samples with extraction solvent.

Chromatography:

Column: Merck LiChroCART Superspher 100 RP-18 column, 5 µm, 125 × 4.1 mm.

Mobile phase: Solvent A = water–acetonitrile (95:5), solvent B = water–acetonitrile (5:95), solvent C = water–acetonitrile (95:5) with 0.1% trifluoroacetic acid.

Gradient:

Time (minutes)	%A	%B	%C
0	75	5	20
4	75	5	20
4	curve-2*		
7	68	12	20
19	60	20	20
30	49	31	20
30	curve-2*		
55	27	53	20

Flow rate: 1.3 mL/minute
 Injection volume: 5 µL
 Column temperature: 40°C
 Detection wavelength: 0 to 35 minutes = 290 nm, 35 to 55 minutes = 260 nm.

*Convex/concave curves as described in Shimadzu's Class-LC-10 software.

Validation Data:

Linearity: Not available

Accuracy: The percent recoveries were 95 for cichoric acid, 98 for dodeca-2E,4E-8Z, 10E/Z-tetraenisobutylamide, and more than 80 for undeca-2Z,4E-dien-8,10-diynisobutylamide.

Precision: The RSD was less than 2% for all four compounds.

Selectivity: Peak identification was determined against standards.

Ruggedness: The RSD was less than 1%.

Robustness: The RSD was less than 5%.

LOD/LOQ: LOD for cichoric acid, dodeca-2E,4E-8Z,10E/Z-tetraenisobutylamide, and undeca-2Z,4E-dien-8,10-diynisobutylamide were 0.011, 0.000074, and 0.0011 mg/mL, respectively. The LOQs were 0.038, 0.000025, and 0.0037, respectively.

Method 3:

The method of Laasonen et al.^{3,4} can be used for the simultaneous analysis of alkamides and caffeic acid derivatives in *Echinacea purpurea*, *E. angustifolia*, *E. pallida*, and *Parthenium integrifolium*.

Sample Preparation:

Extract 100 mg of herbal powder with 8 mL of ethanol–water (70:30) three times by sonication for 5 minutes and then centrifuge. Combine the supernates and adjust to a volume of 25 mL.

Chromatography:

Column: Phenomenex Luna C18, 3 μ m, 100 \times 4.6 mm.

Mobile phase: Solvent A = water (0.1% phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	95	5
2	82	18
4	82	18
7	30	70
20	10	90

Flow rate: 1.0 mL/minute

Injection volume: 25 μ L

Detection wavelength: 255 nm

Validation Data:

Not available.

Method 4:

The method of Luo et al.⁸ also can be used to analyze alkamides and caffeic acid derivatives in *Echinacea purpurea* simultaneously.

Sample Preparation:

Sonicate 1 g of ground plant materials in 10 mL of methanol–water (0.1% phosphoric acid) (70:30) for 20 minutes.

Chromatography:

Column: Johnson Spherigel C18, 5 μ m, 250 \times 4.6 mm.

Mobile phase: Solvent A = water (0.1% formic acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	90	10
9	81.5	18.5
9.5	55	45
39.5	20	80
42	0	100
45	90	10

Flow rate: 1 mL/minute

Injection volume: 10 μ L

Detection wavelength: 330 nm for caffeic acid derivatives and 254 nm for alkamides.

Column temperature: 30°C

Validation Data:

Linearity: 600 to 1200 ng at 330 nm for cichoric acid, 300 to 900 ng under 254 nm for dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide.

Accuracy: The percent recoveries were 98.7 for cichoric acid and 95.7 for dodeca-2E,4E-8Z, 10E/Z-tetraenisobutylamide.

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not available

Robustness: Not available

LOD/LOQ: LODS for cichoric acid and dodeca-2E,4E-8Z,10E/Z-tetraenisobutylamide were 40 and 35 ng, respectively.

Method 5:

The unpublished method of Mingfu Wang was used to determine the phenolic compounds in echinacea.

Sample Preparation:

Accurately weigh approximately 0.25 g of ground raw material or 100 mg of dried extract and place into a 50-mL volumetric flask. Add exactly 40 mL of methanol–water (70:30) and sonicate for 30 minutes. Then dilute to volume with methanol–water (70:30).

Chromatography:

Column: Phenomenex Phenylhexyl, 3 μ m, 150 \times 4.6 mm.

Mobile phase: Solvent A = water (0.2% phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	94	6
8	84	16
20	40	60
25	40	60

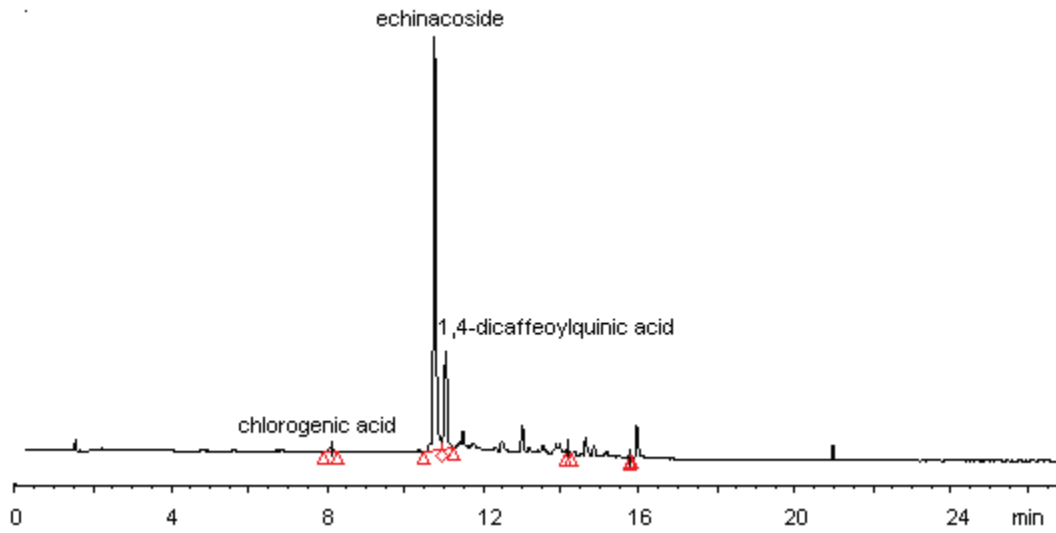
Injection volume: 5 μ L

Detection wavelength: 330 nm

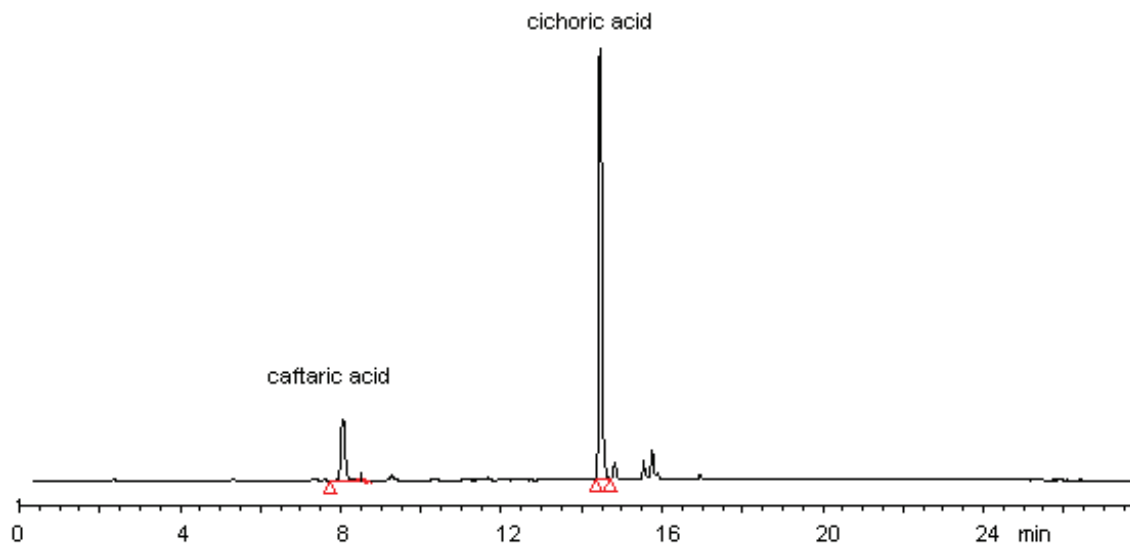
Flow rate: 1.1 mL/minute

Validation Data:

Not available.



Representative HPLC chromatogram of *Echinacea angustifolia* run by method 5.



Representative HPLC chromatogram of *Echinacea purpurea* run by method 5.

References:

1. Perry NB, Van Klink JW, Burgess EJ, et al. Alkamide levels in *Echinacea purpurea*. A rapid analytical method revealing differences among roots, rhizomes, stems, leaves, and flowers. *Planta Med.* 1997;63(1):58–62.
2. Perry NB, Burgess EJ, Glennie VL. Echinacea standardization: analytical methods for phenolic compounds and typical levels in medicinal species. *J Agric Food Chem.* 2001;49(4):1702–6.
3. Laasonen M, Harmia-Pulkkinen T, Simard CL, et al. Fast identification of *Echinacea purpurea* dried roots using near-infrared spectroscopy. *Anal Chem.* 2002;74(11):2493–9.
4. Laasonen M, Wennberg T, Harmia-Pulkkinen T, et al. Simultaneous analysis of alkamides and caffeic acid derivatives for the identification of *Echinacea purpurea*, *Echinacea angustifolia*, *Echinacea pallida* and *Parthenium integrifolium* roots. *Planta Med.* 2002;68(6):572–4.
5. Bergeron C, Livesey JF, Awang DV, et al. A quantitative HPLC method for the quality assurance of Echinacea products on the North American market. *Phytochem Anal.* 2000;11(4):207–15.
6. Schulz H, Pfeffer S, Quilitzsch R, et al. Rapid and non-destructive determination of the echinacoside content in Echinacea roots by ATR-IR and NIR spectroscopy. *Planta Med.* 2002;68(10):926–9.
7. Molgaard P, Johnsen S, Christensen P, et al. HPLC method validated for the simultaneous analysis of cichoric acid and alkamides in *Echinacea purpurea* plants and products. *J Agric Food Chem.* 2003;51(24):6922–33.
8. Luo XB, Chen B, Yao SZ, et al. Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of *Echinacea purpurea* by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. *J Chromatogr A.* 2003;986(1):73–81.