

ChromaDex®

Application Note

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

0033 - Black Cohosh for Triterpenes by HPLC

Botanical Name: *Cimicifuga racemosa* [L.] Nutt.; *Actace racemosa* L.

Common Names: Black Snakeroot

Parts of Plant Used: Dried rhizomes and roots

Uses: Treatment of symptoms of menopause, relief from premenstrual discomfort.

Modes of Action:

Several clinical trials have proven the efficacy of black cohosh for menopausal symptoms. Earlier research found black cohosh improved neurovegetative symptoms and psychological complaints associated with hormonal deficiencies in women. The authors proposed that an estrogen like effect was the primary mechanism of action. But current research shows that black cohosh's mode of action is not identical to estrogen and that black cohosh extract shows no estrogenic effects.¹



Chemistry and Chemical Markers for Quality Control:

Triterpene glycosides are the main chemical compounds in black cohosh root extract with 23-epi-26-deoxyactein (27-deoxyactein), actein, cimigenol-3-O-β-D-xylopyranoside, and cimircemoside C (cimicifugoside M) as the main saponins. Recent research efforts have led to the isolation and identification of various novel triterpene glycosides:

Cimircemosides A to P (Two new compounds named cimircemoside A were reported in 2000. In the study of Shao et al.,² cimircemoside A is 21-hydroxycimigenol-3-α-L-arabinopyranoside; in the paper by Bedir and Khan,³ cimircemoside A is 16β:23;22:25-diepoxy-12β-acetoxy-3β,23,24-trihydroxy-9,19-cycloanosta-7-ene-3-O-β-D-xylopyranoside, the same as cimircemoside F in the Shao et al.² report.)

25-O-Methylcimigenol-3-O-α-L-arabinopyranoside (also named cimircemoside B by Bedir and Khan,³ but it is different from cimircemoside B reported by Shao et al.²).

23-O-Acetylshengmanol-3-O-α-L-arabinopyranoside

Shengmanol-3-O-β-L-arabinopyranoside

25-O-Acetyl-12β-hydroxycimigenol-3-O-α-L-arabinopyranoside

12β,21-Dihydroxycimigenol-3-O-α-L-arabinopyranoside

(22R,23R,24R)-12β-Acetyloxy-16β:23;22:25-diepoxy-23,24-dihydroxy-9,19-cycloanosta-7-ene-3-O-α-L-arabinopyranoside.

Current research²⁻⁹ also identified several known triterpene glycosides:

26-Deoxycimifugoside

25-O-Acetylshengmanol-3-O-β-D-xylopyranoside

Cimircemoside A

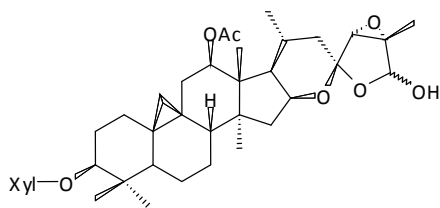
12β-Hydroxycimigenol-3-O-β-D-xylopyranoside

12β-Hydroxycimigenol-3-O-α-L-arabinopyranoside

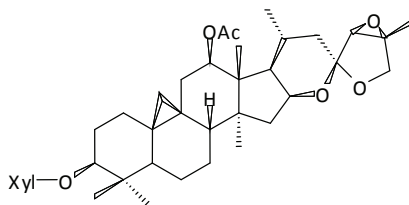


25-Anhydrocimigenol-3-O- β -D-xyloside
 25-Anhydrocimigenol-3-O- α -L-arabinoside
 25-O-Acetylcimigenol-3-O- β -D-xyloside
 25-O-Acetylcimigenol-3-O- α -L-arabinoside
 Cimicifugoside H-1
 Cimicifugoside H-2
 2'-Acetylacetin
 25-O-Methylcimigenol-3-O- β -D-xylopyranoside

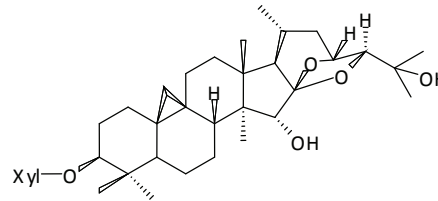
Phenolic compounds also were isolated from black cohosh. The isolated phenolic compounds include formononetin, kaempferol, isoferulic acid, ferulic acid, caffeic acid, methyl caffeate, fukinolic acid, cimicifugic acid A, cimicifugic acid B, cimicifugic acid F, and new compounds cimiracemate A to D.^{10,11} Currently, the triterpene glycosides are used as marker compounds for quality control of black cohosh extract.



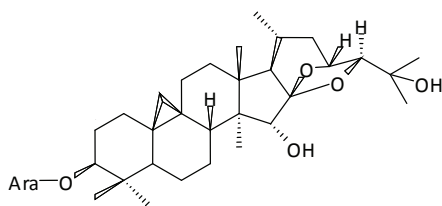
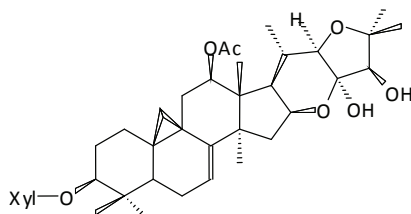
26-S and 26R-Actein



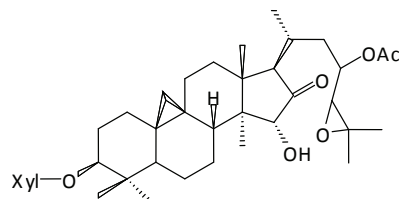
23-Epi-26-deoxyactein



Cimigenol-3-xyloside

Cimircemoside C
(cimicifugoside M)

Cimircemoside F

23-O-Acetylshengmanol
-3-O- β -D-xyloside

Methods of Analysis:

Several methods have been reported for the analysis of the triterpene glycosides in black cohosh, including HPLC coupled with an evaporative light-scattering detector (ELSD) or a MS detector.

Method 1:

The method found at www.nsfina.org can be used to determine triterpene glycosides including actein, 26-deoxycimifugoside, cimircemoside A, 26-deoxyactein, acetylshengmanolxyloside, cimicifugoside (cimigenol-3-O- β -D-xylopyranoside), cimircemoside F, cimircemoside C, and cimircemoside E.

Sample Preparation:

For root material, mix 1.0 g of ground plant material with 40 mL of ethanol–water (50:50). Shake on an orbital or wrist-action shaker for 24 hours.

For the extract, sonicate 300 mg of powdered extract with 7 mL of methanol in a 10-mL volumetric flask for 30 minutes. Cool to room temperature and dilute to volume with methanol.

Chromatography:

Column: Phenomenex Prodigy ODS3, 5 μ m, 4.6% 250 mm.

Mobile phase: Water (0.1% formic acid)–acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	70	30
30	60	40
60	40	60
70	70	30

Column temperature: Room temperature ($\approx 25^{\circ}\text{C}$)

Injection volume: 20 μ L

Flow rate: 1.0 mL/minute

Detector: ELSD

Validation Data:

Not available, but this method is known to be a validated method.

Method 2:

The method of Li et al.¹² can be used to analyze 16 compounds in black cohosh including caffeic acid, ferulic acid, isoferulic acid, cimicifugoside H-1, cimicracemoside F, cimicifugoside H-2, (26R)-actein, 26-deoxycimicifugoside, (26S)-actein, 23-epi-26-deoxyactein, 23-O-acetylshengmanol-3-O- β -D-xyloside, 26-deoxyactein, 25-O-acetylshengmanol-3-O- α -L-arabinoside, 25-O-acetylshengmanol-3-O- β -D-xyloside, cimigenol-3-O- α -L-arabinoside (cimicracemoside C), and cimigenol-3-O- β -D-xyloside (cimicifugoside).

Sample Preparation:

For the liquid extract, directly filter the solution through a 0.22- μ m membrane filter and use for HPLC analysis.

For the dried powdered extract, dissolve 30 mg in 1 mL of methanol using sonication.

For herbal powders, extract 0.5 to 1.0 g of sample with 15 mL of methanol twice using sonication for 30 minutes. Combine the methanol extracts and evaporate to dryness under reduced pressure. Dissolve the residue in 10 mL of methanol.

For commercial dietary supplements, extract one dosage unit of the product with 25 mL of methanol (100% methanol for capsules, 80% methanol for caplets, and 50% methanol for tablets) twice using sonication for 30 minutes. Combine the methanol extracts and use for HPLC analysis.

Chromatography:

Column: Waters YMC ODS-AQ RP18, 5 μ m, 250 \times 4.6 mm, 5 μ m with a Waters Delta-Pak RP18 guard column, 4.6 \times 5 mm.

Mobile phase: Solvent A = water (0.05% trifluoroacetic acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	80	20
8	80	20
15	68	32
55	36	64
65	5	95
70	5	95

Flow rate: 1.6 mL/minute

Injection volume: 10 μ L

Detection wavelength: ELSD detector

Validation Data:

Linearity: 10 to 100 mcg/mL for caffeic acid, ferulic acid, and isoferulic acid, 10 to 340 mcg/mL for cimicifugoside H-2, 11 to 110 mcg/mL for cimircemoside F, 10 to 340 mcg/mL for cimicifugoside H-2, 3.5 to 12 mcg/mL for (26R)-actein, 5.3 to 53 mcg/mL for 26-deoxycimicifugoside, 8 to 80 mcg/mL for (26S)-actein, 10 to 320 mcg/mL for 23-epi-26-deoxyactein, 9 to 90 mcg/mL for 23-O-acetylshengmanol-3-O- β -D-xyloside, 10 to 100 mcg/mL for 26-deoxyactein, 9 to 90 mcg/mL for 25-O-acetylshengmanol-3-O- α -L-arabinoside, 10 to 100 mcg/mL for 25-O-acetylshengmanol-3-O- β -D-xyloside, 6.5 to 125 mcg/mL for cimigenol-3-O- α -L-arabinoside (cimircemoside C), and 6.5 to 65 mcg/mL for cimigenol-3-O- β -D-xyloside (cimicifugoside). The correlation coefficients were more than 0.9949 for all compounds.

Accuracy: The percent recoveries were from 91.75 to 99.96 for six selected compounds.

Precision: Not specified

Selectivity: Peak identification was determined against standards.

References:

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