

Status: Currently Official on 14-Feb-2025
 Official Date: Official as of 01-Jan-2018
 Document Type: Dietary Supplement Monographs
 DocId: GUID-F4137E6E-6DBD-40DF-A6B7-BF6231279C08_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M2304_03_01
 DOI Ref: 2lp3o

© 2025 USPC
 Do not distribute

Black Cohosh Fluidextract

DEFINITION

Black Cohosh Fluidextract is prepared from Black Cohosh by extraction with hydroalcoholic mixtures or isopropanol–water mixtures. Each mL contains the extracted constituents of 1 g of the plant material. It contains NLT 90.0% and NMT 110.0% of the labeled amount of triterpene glycosides, calculated as 23-*epi*-26-deoxyactein ($C_{37}H_{56}O_{10}$).

IDENTIFICATION

• A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST](#)

Standard solution A: 100 mg/mL of [USP Powdered Black Cohosh Extract RS](#) in methanol

Standard solution B: 1 mg/mL each of [USP Actein RS](#), [USP 23-*epi*-26-Deoxyactein RS](#), and isoferulic acid in methanol

Sample solution: Fluidextract

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

Application volume: 10 µL

Developing solvent system: Use the upper phase of a mixture of butyl alcohol, glacial acetic acid, and water (5:1:4).

Spray reagent: Methanol, glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (85:10:5:0.5). [NOTE—Store in a refrigerator. The reagent is colorless; discard if color appears.]

Analysis

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

Develop the chromatograms until the solvent front has moved about 15 cm, and dry the plate with the aid of a current of air.

Acceptance criteria: Examine the plate under UV light at 365 nm. The *Sample solution* exhibits main zones similar in position and color to the main zones of *Standard solution A*. In the upper third of the plate, the *Sample solution* exhibits a blue fluorescent zone at the level of the zone due to isoferulic acid of *Standard solution B*. Spray the plate with *Spray reagent*, heat at 100° for 5 min, and examine in daylight. The *Sample solution* exhibits main zones similar in position and color to the main zones of *Standard solution A*. *Standard solution B* exhibits red-violet zones due to actein and 23-*epi*-26-deoxyactein. The *Sample solution* exhibits several greenish-brown spots in the lower third of the plate and several violet zones above; two of these violet zones occur at R_f values similar to those due to actein and 23-*epi*-26-deoxyactein of *Standard solution B*.

• B. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST](#)

Standard solution A: 0.5 mL of *Standard solution A* prepared in *Identification test A*, diluted with methanol to 2.0 mL

Standard solution B: 1.0 mL of *Standard solution B* prepared in *Identification test A*, diluted with methanol to 5.0 mL

Sample solution: Use the Fluidextract, diluting if necessary with a suitable solvent to obtain a concentration of 0.25 mg/mL of triterpene glycosides.

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

Application volume: 2 µL as an 8-mm band

Developing solvent system: Toluene, ethyl formate, and formic acid (5:3:2)

Spray reagent: Proceed as directed for *Identification test A*.

Analysis

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

Develop until the solvent front has moved two-thirds of the length of the plate, and dry the plate with the aid of a current of air. Spray the plate with *Spray reagent*, heat at 100° for 5 min, and examine in daylight.

Acceptance criteria: The *Sample solution* exhibits main zones similar in position and color to the main zones of *Standard solution A*. *Standard solution B* exhibits red-violet zones due to actein and 23-*epi*-26-deoxyactein at R_f values of about 0.5 and 0.4, respectively. The *Sample solution* exhibits zones similar in color and R_f values to those due to actein and 23-*epi*-26-deoxyactein of *Standard solution B*.

• C. The *Sample solution* exhibits peaks for cimracemoside A, 26-deoxycimicifugoside, (26S)-actein, 23-*epi*-26-deoxyactein, cimigenol–arabioside, and cimigenol–xyloside at retention times corresponding to those compounds in the *Standard solution*, as obtained in the test for *Content of Triterpene Glycosides*. The ratio of the peak areas of cimigenol–arabioside to cimigenol–xyloside is NLT 0.4 (distinction from *Cimicifuga foetida*).

COMPOSITION

• CONTENT OF TRITERPENE GLYCOSIDES

Standard solution: Dissolve a quantity of [USP Powdered Black Cohosh Extract RS](#) in methanol with shaking for 1 min, and dilute with methanol to obtain a solution having a known concentration of 30 mg/mL. Pass through a membrane filter of 0.45 µm or finer pore size.

23-*epi*-26-Deoxyactein standard solutions: Dissolve [USP 23-*epi*-26-Deoxyactein RS](#) in methanol with shaking for 1 min. Dilute quantitatively, and stepwise if necessary, to obtain solutions having known concentrations of 500, 100, 50, 25, and 12.5 µg/mL. Pass through a membrane filter of 0.45-µm or finer pore size.

System suitability solution: 0.1 mg/mL each of [USP Actein RS](#) and [USP 23-*epi*-26-Deoxyactein RS](#) in methanol

Sample solution: Use the Fluidextract, diluting if necessary with methanol to obtain a concentration of about 0.75 mg/mL of triterpene glycosides. Centrifuge, or pass through a filter of 0.45-µm or finer pore size.

Solution A: 0.05% trifluoroacetic acid in water

Solution B: Acetonitrile

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Water (%)	Solution A (%)	Solution B (%)
0	0	80	20
8	0	80	20
15	68	0	32
55	36	0	64
65	5	0	95
70	5	0	95
85	0	80	20

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: Evaporative light-scattering

[NOTE—The detector is set up according to the manufacturer's instruction in order to achieve a signal-to-noise ratio of NLT 10 for the 12.5-µg/mL 23-*epi*-26-Deoxyactein standard solution.]

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1.6 mL/min

Injection size: 20 µL

System suitability

Samples: System suitability solution, Standard solution, and 100-µg/mL 23-*epi*-26-Deoxyactein standard solution

Suitability requirements

Chromatogram similarity: The chromatogram of the Standard solution is similar to the Reference Chromatogram provided with the lot of [USP Powdered Black Cohosh Extract RS](#) being used.

Resolution: NLT 1.0 between the (26S)-actein and the 23-*epi*-26-deoxyactein peaks, System suitability solution

Tailing factor: NMT 2.0 for the 23-*epi*-26-deoxyactein peak, 100-µg/mL 23-*epi*-26-Deoxyactein standard solution

Relative standard deviation: NMT 2.0% of the logarithm of the area response of the 23-*epi*-26-deoxyactein peak in repeated injections, 100-µg/mL 23-*epi*-26-Deoxyactein standard solution

Analysis

Samples: System suitability solution, Standard solution, 23-*epi*-26-Deoxyactein standard solutions, and Sample solution

Using the chromatogram of the Standard solution and the Reference Chromatogram provided with the lot of [USP Powdered Black Cohosh Extract RS](#), identify the retention times of the peaks corresponding to the triterpene glycosides. The approximate relative retention times of the triterpene glycosides are provided in [Table 2](#).

Table 2

Compound	Relative Retention Time
Cimicifugoside H-1	0.61
Cimiracemoside A	0.78



Documents

Document Sharing Hub