

Status: Currently Official on 14-Feb-2025
 Official Date: Official Prior to 2013
 Document Type: Dietary Supplement Monographs
 DocId: GUID-43096B26-90F0-41C0-B490-9435710E8616_1_en-US
 DOI: https://doi.org/10.31003/USPNF_M9826_01_01
 DOI Ref: sj02g

© 2025 USPC
 Do not distribute

Black Cohosh Tablets

DEFINITION

Black Cohosh Tablets contain Powdered Black Cohosh Extract or Black Cohosh Fluidextract. Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of Powdered Extract or Fluidextract, represented by the content of triterpene glycosides, calculated as 23-*epi*-26-deoxyactein ($C_{37}H_{56}O_{10}$).

IDENTIFICATION

• A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#)

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

Sample solution: 10 mL of the *Sample solution* prepared for *Identification test B*. Evaporate to dryness, and redissolve in 1 mL of methanol.

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

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 until the solvent front has moved 15 cm, and dry the plate with the aid of a current of air. Examine the plate under UV light at a wavelength of 365 nm. 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*.

Examined under UV light, the *Sample solution* exhibits a blue fluorescent zone at the level of the zone due to isoferulic acid in *Standard solution B*, in the upper third of the plate. Examined after treatment with *Spray reagent*, 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 in *Standard solution B*.

• B. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#)

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

Sample solution: Transfer the equivalent of the labeled amount of Powdered Extract or Fluidextract, containing 25 mg of triterpene glycosides from a portion of powdered Tablets, to 25 mL of water; shake to disperse; and sonicate for 10 min. Add 75 mL of methanol, and sonicate for 10 min. Allow to stand for 15 min, and use the clear supernatant.

Standard solution A: Methanol and *Standard solution A* prepared in *Identification test A* (3:1)

Standard solution B: Methanol and *Standard solution B* prepared in *Identification test A* (4:1)

Application volume: 2 µL as an 8-mm band

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

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 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*, two of which are red-violet zones at R_f values of 0.5 and 0.4, similar in color and R_f values to those due to actein and 23-*epi*-26-deoxyactein in *Standard solution B*.

• C. The *Sample solution* exhibits peaks for cimracemoside A, 26-deoxycimicifugoside, (26*S*) 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*).

STRENGTH

• CONTENT OF TRITERPENE GLYCOSIDES

Solution A: Filtered and degassed 0.05% trifluoroacetic acid in water

Solution B: Filtered and degassed acetonitrile

Mobile phase: See the gradient table below.

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

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

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 having a 0.45-µm or finer porosity.

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 having a 0.45-µm or finer porosity.

Sample solution: Weigh NLT 20 Tablets, and finely powder. Transfer a quantity of the powder, equivalent to 8 mg of triterpene glycosides, to a suitable polytef-capped centrifuge tube. Add 3 mL of water, shake to disperse, and sonicate for 10 min at 60°. Add 3 mL of methanol, and sonicate for 10 min. Centrifuge, and transfer the clear supernatant to a 10-mL volumetric flask. Wash the residue twice with 1.5 mL of a mixture of methanol and water (1:1), and transfer the washings to the volumetric flask. Dilute with a mixture of methanol and water (1:1) to volume, and pass through a membrane filter having a 0.45-µm or finer porosity.

Chromatographic system

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

Mode: LC

Detector: Evaporative light-scattering

[NOTE—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 and 100 µg/mL of 23-*epi*-26-Deoxyactein standard solution

Suitability requirements

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

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% for the logarithm of the area responses for replicate 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 the following table.

Name	Relative Retention Time
Cimicifugoside H-1	0.61



Documents

Document Sharing Hub