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Powdered Black Cohosh

DEFINITION

Powdered Black Cohosh is Black Cohosh reduced to a powder or a very fine powder. It contains NLT 0.4% of triterpene glycosides, calculated as 23-*epi*-26-deoxyactein¹ ($C_{37}H_{56}O_{10}$) on the dried basis.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

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: Transfer 5 g of Powdered Black Cohosh to a screw-cap centrifuge tube, add 10 mL of a mixture of alcohol and water (7:3), and heat on a steam bath for 10 min. Centrifuge, and use the clear supernatant.

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).

Derivatization 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 in a stream of air. Examine the plate under UV light at 365 nm. Treat the plate with *Derivatization reagent*, heat at 100° for 5 min, and examine in white light.

Acceptance criteria: Under UV light at 365 nm, the chromatogram of 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*. Under white light, 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. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).

Standard solution A: 0.5 mL of *Standard solution A* prepared in *Identification test A*, diluted with methanol to 2 mL

Standard solution B: 1.0 mL of *Standard solution B* prepared in *Identification test A*, diluted with methanol to 5 mL

Sample solution: Transfer 0.5 g of Powdered Black Cohosh to a screw-cap tube, add 5 mL of methanol, sonicate for 10 min, and filter into a 10-mL volumetric flask. Wash the residue on the filter paper four times, using 1 mL of methanol for each washing; add the washings to the volumetric flask; and dilute with methanol to volume.

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

Application volume: 2 µL as 8-mm bands

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

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

Analysis

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

Develop the chromatograms until the solvent front has moved about two-thirds of the length of the plate, and dry the plate with in a current of air. Treat the plate with *Derivatization reagent*, heat at 100° for 5 min, and examine in white light.

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–arabinoside, 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–arabinoside 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 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: Accurately weigh about 750 mg of Powdered Black Cohosh, and place in a 20-mL PTFE-capped centrifuge tube. Add 15 mL of methanol, sonicate for 30 min, centrifuge, and retain the supernatant. Repeat the extraction twice. Evaporate the combined extracts under vacuum at 45°–50°. Dissolve the residue in methanol, and quantitatively transfer to a 10-mL volumetric flask. Dilute with methanol to volume, and pass through a membrane 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 instructions 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 volume: 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 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](#) being used, 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