

Status: Currently Official on 13-Feb-2025
 Official Date: Official as of 01-May-2019
 Document Type: Dietary Supplement Monographs
 DocId: GUID-43057A36-34DF-4B91-9458-BF4CA58205D4_2_en-US
 DOI: https://doi.org/10.31003/USPNF_M2788_02_01
 DOI Ref: 9is4x

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Change to read:

▲Ashwagandha Root Powder ▲ (USP 1-May-2019)

DEFINITION

Change to read:

▲Ashwagandha Root Powder is the dried mature root of *Withania somnifera* (L.) Dunal (Fam. Solanaceae), ▲ (USP 1-May-2019) reduced to a fine or very fine powder. It contains NLT 0.3% of withanolides, calculated on the dried basis as the sum of withanolide aglycones, calculated as withanolide A, and withanolide glycosides, calculated as withanoside IV.

IDENTIFICATION

Change to read:

• **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).**

Standard solution A: 0.2 mg/mL each of [USP β-Sitosterol RS](#) and [USP Withanolide A RS](#) in [methanol](#)

Standard solution B: 20 mg/mL of [USP Powdered Ashwagandha Root Extract RS](#) in [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant. [NOTE—Retain the supernatant for use in the test for *Content of Withanolides*.]

Sample solution: Suspend about 1 g of ▲Ashwagandha Root Powder ▲ (USP 1-May-2019) in 10 mL of [methanol](#), and sonicate for 15 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate)¹

Application volume: 2 μL each of *Standard solution A* and *Standard solution B*, and 10 μL of the *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: [Toluene](#), [ethyl acetate](#), and glacial acetic acid (55:45:3)

Developing distance: 6 cm

Derivatization reagent: 20 mL of [sulfuric acid](#) combined with 180 mL of ice-cold [methanol](#)

Analysis

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

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Treat the plate with the *Derivatization reagent*, heat at 100° for 5 min, and examine under UV light at 365 nm and under white light.

System suitability: Under UV light at 365 nm, the derivatized chromatogram of *Standard solution A* displays in its lower third a blue band due to withanolide A and in its middle third a grayish-blue band due to β-sitosterol. The chromatogram of *Standard solution B* displays a light gray to whitish band due to withanone below the withanolide A band, and a faint light gray band above the β-sitosterol band; there is also a light gray band close to the solvent front. A reddish band slightly above the application line is due to withaferin A. Under white light, the bands due to β-sitosterol and withanolide A appear violet–gray. Additional faint bands may appear.

Acceptance criteria: Under UV light at 365 nm and under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional bands may be observed, in particular a band just above that due to withanolide A (light brown under UV light at 365 nm, dark brown under white light), and a thin band below that due to β-sitosterol (light blue under UV light at 365 nm, violet–gray under white light). Bands vary in intensity, and some of those seen in the chromatogram of *Standard solution B* may be very faint or absent from the *Sample solution*.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Withanolides*.

Acceptance criteria: The *Sample solution* shows main peaks at retention times corresponding to those of withanolide A and withanoside IV in *Standard solution A*. The *Sample solution* shows some of the withanolides listed in [Table 2](#).

COMPOSITION

Change to read:

• **CONTENT OF WITHANOLIDES**

Solution A: Dissolve 0.14 g of [potassium dihydrogen phosphate](#) in 900 mL of water, add 0.5 mL of [phosphoric acid](#), dilute with [water](#) to 1000 mL, and mix.

Solution B: [Acetonitrile](#), filtered and degassed

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Standard solution A: A composite solution containing 0.1 mg/mL of [USP Withanolide A RS](#) and 0.1 mg/mL of [USP Withanoside IV RS](#) in [methanol](#), accurately calculated. Use gentle heat to aid dissolution.

Standard solution B: Dilute a portion of *Standard solution B* from *Identification A* with [methanol](#) (1:1), and mix well. Before injection, pass through a ▲polyethersulfone▲ (USP 1-May-2019) membrane filter of 0.45-μm or finer pore size.

Sample solution: Transfer about 5.00 g of accurately weighed ▲Ashwagandha Root Powder▲ (USP 1-May-2019) to a 250-mL round-bottom flask fitted with a reflux condenser. Add 50 mL of [methanol](#), reflux on a water bath for 15 min, cool to room temperature, decant, and retain the solvent. Repeat until the solvent is colorless. Combine the retained solvents, filter, concentrate under vacuum to about 40 mL, transfer to a 50-mL volumetric flask, and adjust with [methanol](#) to volume. Before injection, pass through a ▲polyethersulfone▲ (USP 1-May-2019) filter of 0.45-μm or finer pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm, end-capped; ▲5-μm▲ (USP 1-May-2019) packing [L1](#)

Column temperature: 27°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

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

Suitability requirements

Resolution: NLT 1.0 between the withanolide A and withanone peaks, and NLT 3.0 between the withaferin A peak and the peak corresponding to coeluting withanoside V and withanoside VI, *Standard solution B*

Tailing factor: NMT 1.5 for the withanolide A peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the withanolide A peak in replicate injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Powdered Ashwagandha Root Extract RS](#) being used.

Analysis

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

Using the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of [USP Powdered Ashwagandha Root Extract RS](#) being used, identify the retention times of the peaks corresponding to withanolide aglycones and glycosides. The approximate relative retention times are provided in [Table 2](#).

Table 2

Analyte	Relative Retention Time
Withanoside IV	0.70
Physagulin D	0.75
27-Hydroxywithanone	0.80