

Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: Dietary Supplement Monographs
DocId: GUID-A77016D8-DCBB-4B79-8587-829967F4C0B8_4_en-US
DOI: https://doi.org/10.31003/USPNF_M3541_04_01
DOI Ref: o7z8m

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Powdered Andrographis Extract

DEFINITION

Powdered Andrographis Extract is prepared from Andrographis by extraction with methanol or alcohol. The ratio of plant material to extract is between 15:1 and 10:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of diterpene lactones, calculated on the dried basis as the sum of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin. The content of 14-deoxy-11,12-didehydroandrographolide is NMT 15% of the total diterpene lactones. It may contain suitable added substances as carriers.

IDENTIFICATION

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

Standard solution 1: Use *Standard solution A*, prepared as directed in the test for *Content of Diterpene Lactones*.

Standard solution 2: Sonicate for 10–15 min a quantity of [USP Powdered Andrographis Extract RS](#), equivalent to about 15 mg of diterpene lactones, in 25 mL of methanol. Centrifuge, and use the supernatant.

Sample solution: Sonicate for 10–15 min a quantity of Powdered Andrographis Extract, equivalent to about 15 mg of diterpene lactones, in 25 mL of methanol. Centrifuge, and use the supernatant.

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

Application volume: 10 µL, as 5–10 mm bands

Developing solvent system: Chloroform, acetone, and toluene (2:2:1)

Spray reagent: A mixture containing 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1)

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Use a saturated chamber. Develop until the solvent front has moved up about 90% of the plate. Remove the plate from the chamber. Dry, and spray with *Spray reagent*. Heat for 5–10 min at 100°, and examine under visible light.

Acceptance criteria: The *Sample solution* exhibits three main grayish blue zones with R_f values of approximately 0.4, 0.6, and 0.8 that correspond in position and color to zones in *Standard solution 2*. *Standard solution 1* exhibits a grayish blue zone due to andrographolide at an R_f of about 0.4. The *Sample solution* exhibits a zone similar in color and R_f value to that due to andrographolide in *Standard solution 1*.

• **B.** The *Sample solution* in the test for *Content of Diterpene Lactones* shows a main peak at a retention time corresponding to that of andrographolide in *Standard solution A*. Identify other diterpene lactone peaks in the *Sample solution* by comparison with *Standard solution B* and the reference chromatogram provided with the lot of [USP Powdered Andrographis Extract RS](#). The *Sample solution* shows additional peaks corresponding to neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin.

COMPOSITION

• **CONTENT OF DITERPENE LACTONES**

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, mix, filter, and degas.

Solution B: Use filtered and degassed acetonitrile.

Standard solution A: Dissolve a weighed quantity of [USP Andrographolide RS](#) in methanol to obtain a solution having a known concentration of about 1.0 mg/mL. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Standard solution B: Transfer an amount of [USP Powdered Andrographis Extract RS](#), equivalent to about 25 mg of diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first 5 mL of the filtrate.

Sample solution: Transfer a weighed amount of Powdered Andrographis Extract, equivalent to about 25 mg of diterpene lactones, to a 50-mL volumetric flask, add 25 mL of methanol, heat gently for 15–20 min, dilute with acetonitrile to volume, and mix. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first 5 mL of the filtrate.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	

Time (min)	Solution A (%)	Solution B (%)
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Chromatographic system(See [Chromatography \(621\), System Suitability.](#))**Mode:** LC**Detector:** UV 223 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1.5 mL/min**Injection size:** 20 μL**System suitability****Samples:** *Standard solution A* and *Standard solution B***Suitability requirements**

The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Powdered Andrographis Extract RS](#).

Column efficiency: NLT 5000 theoretical plates, *Standard solution A***Tailing factor:** NMT 1.5 for the andrographolide peak, *Standard solution A***Relative standard deviation:** NMT 2.0%, determined from the andrographolide peak for replicate injections, *Standard solution A***Resolution:** NLT 5 between the neoandrographolide and 14-deoxy-11,12-didehydroandrographolide peaks, *Standard solution B***Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Using *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Powdered Andrographis Extract RS](#) being used, identify the retention times of the peaks corresponding to different diterpene lactones. The approximate relative retention times of the different diterpene lactones are provided in the following table:

Analyte	Relative Retention Time
Andrographolide	1.00
Neoandrographolide	1.16
14-Deoxy-11,12-didehydroandrographolide	1.31
Andrograpanin	1.50

Separately calculate the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin in the portion of Powdered Andrographis Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times 5F$$

r_U = peak response for each diterpene lactone from the *Sample solution*

r_S = peak response for andrographolide from *Standard solution A*

C_S = concentration of [USP Andrographolide RS](#) in *Standard solution A* (mg/mL)

W = weight of Powdered Andrographis Extract taken to prepare the *Sample solution* (g)

F = conversion factor for each analyte (1.00 for andrographolide, 3.90 for neoandrographolide, 1.45 for 14-deoxy-11,12-didehydroandrographolide, and 2.65 for andrograpanin)

Acceptance criteria: 90.0%–110.0%, on the dried basis, of the labeled amount of diterpene lactones calculated as the sum of the percentages of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin