

Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Dec-2015
Document Type: Dietary Supplement Monographs
DocId: GUID-F654C17B-4729-409D-9868-F289FE28E2E5_1_en-US
DOI: https://doi.org/10.31003/USPNF_M3531_01_01
DOI Ref: 2f4o1

© 2025 USPC
Do not distribute

Powdered *Phyllanthus amarus*

DEFINITION

Powdered *Phyllanthus amarus* is *Phyllanthus amarus* reduced to a powder or very fine powder. It contains NLT 0.25% of lignans, calculated as the sum of phyllanthin and hypophyllanthin, on the dried basis.

IDENTIFICATION

• **A.** Powdered *Phyllanthus amarus* meets the requirements in *Specific Tests for Botanical Characteristics*.

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

Standard solution A: 0.1 mg/mL of [USP Phyllanthin RS](#) in methanol

Standard solution B: 10 mg/mL of [USP Powdered *Phyllanthus amarus* Extract RS](#) in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Sonicate about 0.5 g of Powdered *Phyllanthus amarus* in 5 mL of methanol for 10 min, centrifuge, and use the supernatant.

Adsorbent: Chromatographic silica gel with an average particle size of 10–15 µm (TLC plates) or 5 µm (HPTLC plates)

Application volume: 10 µL (TLC plates) or 4 µL (HPTLC plates)

Developing solvent system: Hexane and ethyl acetate (2:1)

Derivatization reagent: A solution of 10% sulfuric acid in methanol. [NOTE—Prepare fresh.]

Analysis

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

Apply the *Samples* as bands. Use a saturated chamber. Develop until the solvent front has moved up about three-fourths of the plate.

Remove the plate from the chamber, dry in air, treat with the *Derivatization reagent*, heat for 3 min at 120°, and examine under white light.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a blue band in the lower third of the plate due to phyllanthin, corresponding in color and R_f to that in the chromatogram of *Standard solution A*; a violet band due to hypophyllanthin at an R_f higher than that of phyllanthin; a blue band at an R_f higher than that of hypophyllanthin; and a violet band in the upper third of the plate. Bands detected in the chromatogram of the *Sample solution* correspond in position and color to bands in the chromatogram of *Standard solution B*. Other minor bands may be observed in the chromatograms of the *Sample solution* and *Standard solution B*.

• **C. HPLC:** The chromatogram of the *Sample solution* obtained in the test for *Content of Lignans* shows a peak at a retention time corresponding to that of phyllanthin in the chromatogram of *Standard solution A*. Identify other lignan peaks in the chromatogram of the *Sample solution* by comparison with the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of [USP Powdered *Phyllanthus amarus* Extract RS](#) being used. The chromatogram of the *Sample solution* shows an additional peak corresponding to hypophyllanthin.

COMPOSITION

• CONTENT OF LIGNANS

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.

Mobile phase: Acetonitrile and *Solution A* (4:6)

Standard solution A: 0.1 mg/mL of [USP Phyllanthin RS](#) in methanol

Standard solution B: Sonicate a portion of [USP Powdered *Phyllanthus amarus* Extract RS](#) in methanol to obtain a solution having a concentration of about 5.0 mg/mL. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer about 3.0 g of Powdered *Phyllanthus amarus*, accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux for about 20 min, allow to settle, and decant the supernatant. Repeat until the extract is colorless. Combine the extracts, concentrate under vacuum, transfer quantitatively into a 100-mL volumetric flask, and adjust to volume with methanol. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

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

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

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

Suitability requirements

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

Resolution: NLT 1.5 between the phyllanthin and hypophyllanthin peaks, *Standard solution B*

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

Relative standard deviation: NMT 2.0% determined from the phyllanthin peak in replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*. [NOTE—*Standard solution A*, *Standard solution B*, and the *Sample solution* are stable for 48 h at room temperature.]

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Powdered *Phyllanthus amarus* Extract RS](#) being used, identify the retention times of the peaks corresponding to phyllanthin and hypophyllanthin.

Separately calculate the percentages of phyllanthin and hypophyllanthin in the portion of Powdered *Phyllanthus amarus* taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of phyllanthin from *Standard solution A*

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

V = volume of the *Sample solution* (mL)

W = weight of Powdered *Phyllanthus amarus* taken to prepare the *Sample solution* (mg)

F = conversion factor: 1.00 for phyllanthin; 0.75 for hypophyllanthin

Acceptance criteria: Add the percentages of phyllanthin and hypophyllanthin: NLT 0.25% is found on the dried basis.

CONTAMINANTS

- [ARTICLES OF BOTANICAL ORIGIN, Limits of Elemental Impurities\(561\)](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN, Pesticide Residue Analysis\(561\)](#): Meets the requirements
- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10^3 cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#): Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• BOTANICAL CHARACTERISTICS

Macroscopic: Greenish to greenish-brown in color; taste bitter

Microscopic: Fragments of epidermal cells of the leaves with wavy walls, showing anisocytic and paracytic stomata; parenchyma cells, some showing clusters of calcium oxalate crystals; narrow fibers from the stem; pitted vessels of the stem; fragments of the epicarp of the fruits showing anomocytic stomata

• [LOSS ON DRYING \(731\)](#)

Sample: 1.0 g of Powdered *Phyllanthus amarus*

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 12.0%

• [ARTICLES OF BOTANICAL ORIGIN, Total Ash\(561\)](#)

Sample: 1.0 g of Powdered *Phyllanthus amarus*

Acceptance criteria: NMT 8.0%

• [ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash\(561\)](#)