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Powdered Bacopa

DEFINITION

Powdered Bacopa is Bacopa reduced to a powder or very fine powder. It contains NLT 2.5% of triterpene glycosides, calculated on the dried basis as the sum of bacopaside I, bacoside A₃, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C.

IDENTIFICATION

• **A.** Powdered Bacopa meets the requirements under *Specific Tests, Botanical Characteristics*.

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

Standard solution: Transfer about 10 mg of [USP Powdered Bacopa Extract RS](#) to a 10-mL volumetric flask, and add about 8 mL of methanol. Sonicate and heat gently for 15–20 min, dilute with methanol to volume, mix, centrifuge, and use the supernatant.

Sample solution: Use the *Sample solution*, prepared as directed in the test for *Content of Triterpene Glycosides*.

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

Application volume: 15 µL, as 5–10 mm bands

Developing solvent system: Ethyl acetate, methanol, and water (7:2:1)

Spray reagent: 1% Vanillin in alcohol and 10% sulfuric acid in alcohol (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the samples as bands (see [Chromatography \(621\)](#)). Use a saturated chamber. Develop the chromatograms until the solvent front has moved up about three-fourths of the plate. Remove the plate from the chamber, dry, spray with *Spray reagent*, heat for 5–10 min at 70°, and examine under white light.

Acceptance criteria: The *Sample solution* exhibits a main dark blue zone due to mixture of bacoside A₃, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C at an *R_F* value of approximately 0.6 and a faint pink spot due to bacopaside I at an *R_F* value of approximately 0.4, both of which correspond in position and color to zones in the chromatogram of the *Standard solution*. Other zones are observed for the *Sample solution* and *Standard solution*.

• **C. HPLC IDENTIFICATION TEST:** The *Sample solution* from the test for *Content of Triterpene Glycosides* shows a main peak at a retention time corresponding to that of bacoside A₃ in the chromatogram of *Standard solution A*. Identify other triterpene glycoside peaks in the *Sample solution* by comparison with the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of [USP Powdered Bacopa Extract RS](#) being used. The *Sample solution* shows additional peaks corresponding to bacopaside I, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C.

COMPOSITION

• CONTENT OF TRITERPENE GLYCOSIDES

Solution A: Dissolve 0.14 g of anhydrous 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.

Mobile phase: See the [gradient table](#) below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	60	40
26	70	30
30	70	30

Standard solution A: Sonicate an accurately weighed quantity of [USP Bacoside A₃ RS](#) in methanol to obtain a solution having a known concentration of about 0.5 mg/mL.

Standard solution B: Transfer about 10 mg of [USP Powdered Bacopa Extract RS](#) to a 10-mL volumetric flask, and add about 8 mL of methanol. Sonicate and heat gently for 15–20 min, dilute with methanol 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 about 2.5 g of Powdered Bacopa, accurately weighed, to a 100-mL round-bottom flask fitted with a reflux condenser. Add 25 mL of methanol, reflux on a water bath for 10 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum, and adjust the volume to 100 mL using methanol. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first 5 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm, endcapped, base-deactivated 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

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

Resolution: NLT 1.0 between the bacopaside II and bacoside A₃ peaks, *Standard solution B*

Tailing factor: NMT 1.5 for the bacoside A₃ peak, *Standard solution A*

Relative standard deviation: NMT 2% determined from the bacoside A₃ peak for replicate injections, *Standard solution A*

Analysis

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

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

Analyte	Relative Retention Time
Bacopaside I	0.73
Bacoside A ₃	1.00
Bacopaside II	1.04
The jujubogenin isomer of bacopasaponin C	1.15
Bacopasaponin C	1.22

Separately calculate the percentages of bacopaside I, bacoside A₃, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C in the portion of Powdered Bacopa taken:

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

r_U = peak response for each triterpene glycoside from the *Sample solution*

r_S = peak response for bacoside A₃ from *Standard solution A*

C_S = concentration of [USP Bacoside A₃ RS](#) in *Standard solution A* (mg/mL)

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

W = weight of Powdered Bacopa used to prepare the *Sample solution* (mg)

F = conversion factor for each analyte: 1.00 for bacoside A₃, 1.03 for bacopaside I, 0.81 for bacopaside II, 0.99 for the jujubogenin isomer of bacopasaponin C, and 0.75 for bacopasaponin C

Acceptance criteria: Add the percentages of bacopaside I, bacoside A₃, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C: NLT 2.5% is found on the dried basis.

IMPURITIES

• [ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash\(561\)](#): NMT 6.0%