Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-May-2018
Document Type: Dietary Supplement Monographs
DocId: GUID-746AD0FD-4A11-4B7C-B82D-A1DE8403E086_3_en-US
DOI: https://doi.org/10.31003/USPNF_M3534_03_01
DOI Ref: amd2u

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Powdered *Centella asiatica*

DEFINITION

Powdered *Centella asiatica* is *Centella asiatica* reduced to a powder or very fine powder. It contains NLT 2.0% of triterpene derivatives, calculated on the dried basis.

IDENTIFICATION

• **A.** Powdered *Centella asiatica* meets the requirements for *Specific Tests, Botanical Characteristics*.

• **B. Thin-Layer Chromatography**

Standard solution A: 0.5 mg/mL of USP Asiaticoside RS in methanol

Standard solution B: 10 mg/mL of USP Powdered Centella asiatica Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: About 0.5 g of Powdered *Centella asiatica* in 5 mL of methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

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

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

Developing solvent system: Methylene chloride, methanol, and water (14:6:1)

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 the chromatograms until the solvent front has moved up about three-fourths of the plate. Remove the plate from the chamber, dry, treat with *Derivatization reagent*, heat for 3 min at 120°, and examine under white light.

Acceptance criteria: The *Sample solution* chromatogram exhibits a violet band in the lower third of the plate due to asiaticoside, corresponding in color and R_F to that in *Standard solution A*; a violet band due to madecassoside at an R_F lower than that of asiaticoside; and two additional violet bands in the upper third of the plate due to asiatic acid and madecassic acid. Bands detected in the *Sample solution* correspond in position and color to bands in *Standard solution B*. Other minor bands may be observed in the *Sample solution* and *Standard solution B*.

• **C. HPLC:** The *Sample solution* chromatogram from the test for *Content of Triterpene Derivatives* shows a peak at the retention time corresponding to that of asiaticoside in *Standard solution A*. Identify other triterpene derivative 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 Centella asiatica Extract RS being used. The *Sample solution* shows additional peaks corresponding to madecassoside and asiaticoside B (these two peaks may co-elute), madecassic acid, terminolic acid, and asiatic acid.

COMPOSITION

• **Content of Triterpene Derivatives**

Solution A: Dilute 3 mL of phosphoric acid with water to 1000 mL, mix, filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	78	22
65	45	55
66	5	95
75	5	95

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Time (min)	Solution A (%)	Solution B (%)
76	78	22
85	78	22

Standard solution A: 0.05 mg/mL of USP Asiaticoside RS in methanol

Standard solution B: Sonicate a portion of USP Powdered Centella asiatica Extract RS in methanol to obtain a solution with 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 stock solution: Transfer about 1.0 g of Powdered *Centella asiatica*, accurately weighed, to a Soxhlet apparatus. Add 100 mL of methanol, extract for 8 h, cool, and dilute with methanol to 100 mL. Pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate. [Note—Use a thimble of a suitable size such that the volume of methanol used in the Soxhlet extraction is at least twice the volume of the thimble.]

Sample solution: Dilute 5.0 mL of *Sample stock solution* with methanol to 10.0 mL.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 200 nm

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

Flow rate: 1.0 mL/min

Injection volume: 10 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 Centella asiatica Extract RS being used.

Tailing factor: Between 0.8 and 2.0 for the asiaticoside peak, *Standard solution A*

Resolution: NLT 1.5 between the madecassic acid and terminolic acid peaks, *Standard solution B*

Relative standard deviation: NMT 2.0% determined from the asiaticoside 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 *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 Centella asiatica Extract RS being used, identify the retention times of the peaks corresponding to different triterpene derivatives. The approximate relative retention times of the different triterpene derivatives are provided in *Table 2*.

Table 2

Analyte	Approximate Relative Retention Time
Madecassoside	0.71
Asiaticoside B	0.72
Asiaticoside	1.00
Madecassic acid	2.40
Terminolic acid	2.44
Asiatic acid	3.12

Separately calculate the percentages of the sum of madecassoside and asiaticoside B (these two peaks may co-elute), asiaticoside, the sum of madecassic acid and terminolic acid, and asiatic acid in the portion of Powdered *Centella asiatica* taken:

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

r_U = peak areas of the triterpene derivatives from the *Sample solution*

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

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