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Centella asiatica Triterpenes

DEFINITION

Centella asiatica Triterpenes is a fraction enriched in *Centella asiatica* triterpenes derivatives. It is prepared from *Centella asiatica* Extract using suitable solvents or other means. It contains NLT 90.0% of triterpene derivatives, calculated on the anhydrous basis, as the sum of two or more of the following: madecassoside, asiaticoside B, asiaticoside, madecassic acid, terminolic acid, and asiatic acid.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

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: Transfer an amount of *Centella asiatica* Triterpenes, equivalent to about 5 mg of triterpene derivatives, to a centrifuge tube. Add 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: A mixture of methylene chloride, methanol, and water (14:6:1)
Spray 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 to a suitable thin-layer chromatographic plate (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 3 min at 120°, and examine under visible 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*. The *Sample solution* shows additional bands corresponding to some or all of the following triterpene derivatives: a violet band due to madecassoside at an R_f lower than that of asiaticoside, a violet band in the upper third of the plate due to asiatic acid, and a violet band due to madecassic acid at an R_f lower than that of asiatic 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*.
• **B. HPLC IDENTIFICATION TEST:** 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 some or all of the following: 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 the gradient table below.

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

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

Standard solution A: 0.2 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 solution: About 1.0 mg/mL of *Centella asiatica* Triterpenes in methanol; sonicate if necessary. 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 200 nm

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

Flow rate: 1.0 mL/min

Injection size: 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 repeated 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 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 the following table.

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 triterpene derivatives in the portion of *Centella asiatica* Triterpenes taken:

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

r_U = peak response(s) of the triterpene derivative(s) from the *Sample solution*

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

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

C_U = concentration of *Centella asiatica* Triterpenes in the *Sample solution* (mg/mL)

F = conversion factors for analytes: 1.00 for asiaticoside, 1.017 for madecassoside, 1.017 for asiaticoside B, 0.526 for madecassic acid, 0.526 for terminolic acid, and 0.509 for asiatic acid

Acceptance criteria: Add the percentages of different triterpene derivatives: NLT 90.0% on the anhydrous basis.