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 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* Extract 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 Powdered *Centella asiatica* Extract in the *Sample solution* (mg/mL)
F = conversion factors for analytes: 1.00 for asiaticoside, 1.017 for the sum of madecassoside and asiaticoside B, 0.526 for the sum of madecassic acid and terminolic acid, and 0.509 for asiatic acid

Acceptance criteria: Add the percentages of different triterpene derivatives: NLT 90.0% and NMT 110.0% of the labeled amount of triterpene derivatives; the labeled amount of triterpene derivatives is NMT 40%, calculated on the dried basis.

CONTAMINANTS

- **HEAVY METALS,** *Method III* ⟨**231**⟩**:** NMT 20 ppm
- **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements
- **MICROBIAL ENUMERATION TESTS** ⟨**2021**⟩**:** The total aerobic microbial count does not exceed 10^4 cfu per g. The total combined yeast and mold count does not exceed 10^3 cfu per g.
- **MICROBIOLOGICAL PROCEDURES FOR ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry 1.0 g of Powdered *Centella asiatica* Extract at 105° for 2 h: it loses NMT 5% of its weight.
- **OTHER REQUIREMENTS:** Meets the requirements of the test for *Residual Solvents* under *Botanical Extracts* ⟨565⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was derived. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Asiaticoside RS
USP Powdered *Centella asiatica* Extract RS

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
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.

CONTAMINANTS

- **HEAVY METALS,** *Method III* ⟨**231**⟩**:** NMT 20 ppm
- **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements
- **MICROBIAL ENUMERATION TESTS** ⟨**2021**⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g. The total combined yeast and mold count does not exceed 10^2 cfu/g.
- **MICROBIOLOGICAL PROCEDURES FOR ABSENCE OF SPECIFIED MICROORGANISMS** ⟨**2022**⟩**:** Meets the requirements of the tests for absence of *Escherichia coli*

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 5%
- **OTHER REQUIREMENTS:** Meets the requirements of the test for *Residual Solvents* under *Botanical Extracts* ⟨565⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was derived.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Asiaticoside RS
USP Powdered *Centella asiatica* Extract RS

Chamomile

DEFINITION
Chamomile consists of the dried flower heads of *Matricaria recutita* L. (*Matricaria chamomilla* L., *Matricaria chamomilla* L. var. *courrantiana*, *Chamomilla recutita* L.) Rauschert (Fam. Asteraceae alt. Compositae). It contains NLT 0.4% of blue volatile oil, NLT 0.3% of apigenin-7-glucoside, and NLT 0.15% of bisabolol derivatives, calculated as levomenol.

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**
Standard solution: 1.0 mg/mL of borneol, 2.0 mg/mL of bornyl acetate, and 0.4 mg/mL of guaiazulene in toluene
Sample solution: Reduce 1.0 g of Chamomile to a coarse powder, using a porcelain pestle and mortar. Transfer to a 1.5-cm × 15-cm chromatographic column, and tamp lightly with a short length of rubber hose. Rinse the pestle and mortar twice, each time with 10 mL of methylene chloride. Pour the rinsings into the column. Collect the percolate in a flask with a long, narrow neck, and remove the solvent by evaporation on a water bath. Dissolve the residue in 0.5 mL of toluene.
Adsorbent: 0.25-mm layer of chromatographic silica gel
Developing solvent: Chloroform
Spray reagent: Mix anisaldehyde, glacial acetic acid, and methanol (0.5: 10: 85). Then carefully add 5 mL of sulfuric acid to this solution.
Application volume: 10 µL, as 3-mm × 20-mm bands
Analysis
Samples: *Standard solution* and *Sample solution*
Examine the plate under short-wavelength UV light: the *Sample solution* exhibits a number of quenching areas, the largest of which is due to en-yne-dicycloether and has the same R_F value as the band due to bornyl acetate in the *Standard solution*. There is also a band due to matricin near the line of application. Spray the plate evenly with the *Spray reagent*. Examine the plate in daylight while heating at 100°–105° for 5–10 min. The chromatogram obtained from the *Standard solution* shows in the lower third a brownish yellow zone that becomes violet-gray after a few hours and is due to borneol; in the middle a yellowish brown to gray zone due to bornyl acetate; and in the upper third a deep red zone with a blue edge due to guaiazulene.
Acceptance criteria: The *Sample solution* exhibits a blue zone due to matricin near the starting point; several violet-red zones, one of which is due to bisabolol, at R_F values between those of borneol and bornyl acetate; a brownish zone, due to en-yne-dicycloether, at an R_F value corresponding to that of bornyl acetate; red zones, due to terpenes, at R_F values similar to those of guaiazulene; and other zones that appear in the middle and lower parts of the chromatogram.

• **B.**
Analysis: Dissolve 0.25 g of dimethylaminobenzaldehyde in a mixture of 5 mL of phosphoric acid, 45 mL of acetic acid, and 45 mL of water. Transfer 2.5 mL of this solution and 0.1 mL of the *Sample solution*, prepared as directed for *Identification* test *A*, to a test tube. Heat on a water bath for 2 min, and allow to cool. Add 5 mL of solvent hexane, and shake.
Acceptance criteria: The aqueous layer has a distinct greenish blue or blue color.

COMPOSITION
• **CONTENT OF APIGENIN-7-GLUCOSIDE**
Dilute phosphoric acid: Mix 5.0 mL of phosphoric acid in 50 mL of water. Dilute with water to 100 mL.
Solution A: 0.005 M solution of monobasic potassium phosphate. Adjust with *Dilute phosphoric acid* to a pH of 2.55 ± 0.05.
Solution B: Acetonitrile and methanol (13:7)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	74	26
3	74	26
22	15	85
27	74	26
30	74	26

Standard solution: 25.0 µg/mL of USP Apigenin-7-glucoside RS and 10.0 µg/mL of 7-methoxycoumarin in methanol and water (1:1)
Sample solution: Transfer 1.0 g of Chamomile to a suitable flask fitted with a reflux condenser and a stirrer. Add 80.0 mL of methanol, and reflux the mixture with stirring for 1 h. Cool the flask to room temperature, pass the extract through a folded filter paper, and collect the filtrate in a 100-mL volumetric flask. Rinse the flask with 3 mL of methanol, pour the methanolic rinsings through the filter paper, and add the filtrate to the volumetric flask. Dilute with methanol to volume, and filter. Transfer 25.0 mL of the filtered solution to a round-bottom flask fitted with a reflux condenser and a stirrer; add 5.0 mL of sodium hydroxide solution, prepared by dissolving 0.4 g of sodium hydroxide in 5.0 mL of water; and reflux the mixture for 25 min. Cool the flask, and adjust the solution with hydrochloric acid to a pH of 5.0–6.2. Quantitatively transfer the solution to a 50-mL volumetric flask, dilute with methanol to volume, and filter, discarding the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 335 nm
Column: 4-mm × 12.5-cm; packing L1
Flow rate: 1 mL/min
[NOTE—Make adjustments, if necessary, to obtain relative retention times of 0.63 for apigenin-7-glucoside and 1.0 for 7-methoxycoumarin.]
Injection size: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for apigenin-7-glucoside, 7-methoxycoumarin, apigenin, *trans*-spiroether, and *cis*-spiroether are about 0.63, 1.0, 1.2, 1.6, and 1.8, respectively.]
Suitability requirements
Resolution: NLT 3.5 between apigenin-7-glucoside and 7-methoxycoumarin
Relative standard deviation: NMT 2.0% for apigenin-7-glucoside
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Allow the *Sample solution* to elute for NLT 6 times the retention time of apigenin-7-glucoside.]
Calculate the percentage of apigenin-7-glucoside in the portion of Chamomile taken:

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

r_U = peak response of apigenin-7-glucoside from the *Sample solution*