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Lagerstroemia speciosa Leaf Powder

Final Authorized Version 1.0

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DEFINITION

The article consists of the dried leaves of *Lagerstroemia speciosa* (L.) Pers. (Family Lythraceae) reduced to a powder or very fine powder. It contains NLT 0.2% of corosolic acid, on the dried basis.

POTENTIAL CONFOUNDING MATERIALS

Terminalia cuneata Roth

CONSTITUENTS OF INTEREST

Pentacyclic triterpene acids: Corosolic acid, virgatic acid, ursolic acid, and oleanolic acid

IDENTIFICATION

- **A. Botanical Characteristics**

Macroscopic: Grayish-green powder

Microscopic: Fragments of upper epidermis, polygonal cells, some contain calcium oxalate crystals, no stomata; fragments of lower epidermis cells, irregular shapes, with slightly wavy walls, showing anomocytic stomata; fragments of upper epidermal cells with underlying palisade cells; fragments of parenchyma cells, some contain prisms of calcium oxalate, other cells contain cluster crystals of calcium oxalate; oil cells; fragments of lignified fibers; fragments of spiral vessels; fragments of pitted vessels associated with fibers

- **B. Thin-Layer Chromatography**

Standard solution A: 0.2 mg/mL of USP Corosolic Acid RS [1] in methanol

Standard solution B: 10 mg/mL of USP *Lagerstroemia speciosa* Leaf Dry Extract RS [1] in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Sonicate about 0.2 g of *Lagerstroemia speciosa* Leaf Powder in 10 mL of methanol for 10 min, centrifuge, and use supernatant.

Chromatographic system

(See *Chromatography* <621> [2], *Thin-Layer Chromatography*.)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 6 µL each of *Standard solution A* and *Standard solution B* and 8 µL of *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: 25°

Developing solvent system: Toluene, ethyl acetate, and glacial acetic acid (55: 45: 0.5)

Developing distance: 6 cm

Derivatization reagent: Anisaldehyde reagent—85 mL of ice-cooled methanol mixed with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

Analysis

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

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in an un-saturated chamber, remove the plate from the chamber, and dry. Treat with *Derivatization reagent*, and heat for 3 min at 100°. Examine under visible light.

System suitability: Under visible light, the chromatogram of *Standard solution B* exhibits the most intense band, a violet or blue band, with similar R_F and color as the corosolic acid band in the chromatogram of *Standard solution A*; a blue band close to the start (about R_F 0.1), consistent with asiatic acid; two minor blue bands in between corosolic and asiatic; above the band due to corosolic acid a minor blue band due to virgatic acid; and just below the latter, a minor brown band. *Standard solution B* also exhibits at about three-fourths of the chromatogram two minor violet bands, separated, the band with the lower R_F corresponds to oleanolic acid.

Acceptance criteria: Under visible light, the chromatogram of the *Sample solution* exhibits the most intense band as a violet band corresponding in color and R_F to the band due to corosolic acid in the chromatogram of *Standard Solution A*, and the following bands corresponding to similar bands in *Standard solution B*: a minor blue band close to the start (about R_F 0.1); a minor brownish band

above the corosolic acid; and a minor violet band at about three-fourths of the chromatogram.

- **C. HPLC**

Analysis: Proceed as directed in the test for *Content of Corosolic Acid*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a group of three peaks, the one in the center is the most intense of the group and occurs at a retention time corresponding to that of corosolic acid in the chromatogram of *Standard solution A,* the peak that elutes before corosolic acid has about one-half to one-third of the intensity of that of corosolic acid, and the peak eluting after corosolic acid has the lesser intensity of the three and is consistent with virgatic acid. A minor peak due to oleanolic acid elutes later in the chromatogram.

ASSAY

- **Content of Corosolic Acid**

Solution A: 0.1% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: A mixture of *Solution A* and *Solution B* (4:6)

Standard solution A: 0.1 mg/mL of USP Corosolic Acid RS [1] in methanol

Standard solution B: 5.0 mg/mL of USP *Lagerstroemia speciosa* Leaf Dry Extract RS [1] in methanol, sonicate if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

Sample solution: Transfer about 5.0 g of *Lagerstroemia speciosa* Leaf Powder, accurately weighed, to a round-bottom flask. Add 75 mL of methanol and reflux for 15 min, set aside to settle, and decant the supernatant. Repeat the extraction three more times and combine the extract, flter, concentrate under reduced pressure, transfer to a 100-mL volumetric flask, adjust with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* <621> [2], *System Suitability*.)

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Hibar® 250-4,6; Lichrospher® 100, RP-18e; or Luna C18 100A)

Flow rate: 1.6 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 *Lagerstroemia speciosa* Leaf Dry Extract RS [1] being used.

Resolution: NLT 1.5 between the corosolic acid peak and the peak before, *Standard solution B*

Tailing factor: NMT 2.0 for the corosolic acid peak, *Standard solution A*

Relative standard deviation: NMT 2.0%, determined from the corosolic acid peak in repeated injections, *Standard solution A*

Analysis

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

Using the chromatograms of *Standard solution A, Standard solution B,* and the reference chromatogram provided with the lot of USP *Lagerstroemia speciosa* Leaf Dry Extract RS [1] being used, identify the retention time of the peaks corresponding to corosolic acid, virgatic acid, and oleanolic acid in the *Sample solution* chromatogram. The approximate relative retention times of the different peaks for corosolic acid, virgatic acid, and oleanolic acid are 1.0, 1.1, and 3.2, respectively.

Calculate the percentage of corosolic acid in the portion of *Lagerstroemia speciosa* Leaf Powder taken:

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

r_U = peak area of corosolic acid from the *Sample solution*

r_S = peak area of corosolic acid from *Standard solution A*

C_S = concentration of corosolic acid in *Standard solution A* (mg/mL)

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

W = weight of *Lagerstroemia speciosa* Leaf Powder taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 0.2% corosolic acid, calculated on dried basis

CONTAMINANTS

- **Elemental Impurities [3]—Procedures <233>**

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 0.5 µg/g
Lead: NMT 5.0 µg/g
Mercury: NMT 0.2 µg/g

- **Articles of Botanical Origin [4],** *General Method for Pesticide Residues Analysis* **<561>:** Meets the requirements
- **Microbial Enumeration Tests <61> [5]:** 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 bacteria does not exceed 10^3 cfu/g.
- **Tests for Specified Microorganisms <62> [6]:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Articles of Botanical Origin [4],** *Alcohol-Soluble Extractives*, *Method 1* **<561>:** NLT 10.0%
- **Articles of Botanical Origin [4],** *Water-Soluble Extractives*, *Method 1* **<561>:** NLT 18.0%
- **Loss on Drying <731> [7]**
  Analysis: Dry 2.0 g of *Lagerstroemia speciosa* Leaf Powder at 105° for 2 h.
  Acceptance criteria: NMT 10.0%
- **Articles of Botanical Origin [4],** *Total Ash* **<561>**
  Analysis: 2.0 g of *Lagerstroemia speciosa* Leaf Powder
  Acceptance criteria: NMT 7.0%
- **Articles of Botanical Origin [4],***Acid-Insoluble Ash* **<561>**
  Analysis: 4.0 g of *Lagerstroemia speciosa* Leaf Powder
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and the part of the plant from which the article was derived.
- **USP Reference Standards <11> [8]**
  USP Corosolic Acid RS [1]
  USP *Lagerstroemia speciosa* Leaf Dry Extract RS [9]
