Herbal Medicines Compendium

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Aegle marmelos Fruit Dry Extract

Proposed For Comment Version 0.2

***Aegle marmelos* Fruit Dry Extract**

DEFINITION

The article is prepared from the dried ripe or unripe fruit of *Aegle marmelos* (L.) Corréa (Family Rutaceae) by extraction with a hydroalcoholic mixture. It contains NLT 90.0% and NMT 110.0% of the labeled amount of marmelosin on the dried basis.

POTENTIAL CONFOUNDING MATERIALS

Limonia acidissima Groff

CONSTITUENTS OF INTEREST

Furocoumarin: Marmelosin

IDENTIFICATION

- **A. HPTLC for Articles of Botanical Origin <203>**

Standard solution A: 0.1 mg/mL of USP Marmelosin RS in methanol

Standard solution B: 10 mg/mL of USP *Aegle marmelos* Fruit Dry Extract RS in methanol. Sonicate for 5 min, centrifuge or filter, and use the supernatant or the filtrate.

Sample solution: Sonicate about 100 mg of *Aegla marmelos* Fruit Dry Extract in 10 mL of methanol for 10 min, centrifuge or filter, and use the supernatant or the filtrate.

Chromatographic system

Adsorbent: Chromatographic silica gel F_{254} mixture with an average particle size of 5 µm

Application volume: 10 µL of *Standard solution A* and 5 µL each of *Standard solution B* and *Sample solution,* as 8-mm bands

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

Developing solvent system: Toluene, ethyl acetate, methanol, and acetic acid (40: 10: 1.25: 1)

Developing distance: 6 cm

Analysis

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

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber (20 min with filter paper), remove the plate from the chamber, and dry in air. Examine under UV light at 254 and 365 nm.

System suitability

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

Suitability requirements: Under UV 254 nm, the chromatogram of *Standard solution B* exhibits about 7 black bands with the most intense and uppermost band similar in R_F and color to the marmelosin

band in the chromatogram of *Standard solution A*. Under UV 365 nm, a pale greenish-blue band appears near the R_F of 0.6, similar to the color of the marmelosin band in the chromatogram of *Standard solution A*. One pale blue and one pale green band appear above the marmelosin band. Below the marmelosin band, one blue band appears right below the marmelosin band and several bands, with decreasing R_F, one pale green band, two pale blue bands, one bright blue band, two pale blue bands, one green band, one pale green band, and one light blue band.

Acceptance criteria: Under UV 254 nm, the chromatogram of the *Sample solution* exhibits the most intense and uppermost band similar in R_F and color to the marmelosin band in the chromatogram of *Standard solution A*. Six minor black bands appear below the marmolesin band. Under UV 365 nm, a pale greenish blue band appears near the R_F of 0.6 in the *Sample solution* similar to the color of the marmelosin band in the chromatogram of *Standard solution A*. One pale blue and one pale green band appears above the marmelosin band. Below the marmelosin band, one blue band appears right below the marmelosin band and several bands, with decreasing R_F, one pale green band, two pale blue bands, one bright blue band, two pale blue bands, one green band, one pale green band, and one light blue band.

- **B. HPLC**

Analysis: Proceed as directed in the *Assay* for *Content of Marmelosin*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at retention times corresponding to the peaks due to marmelosin in *Standard solution B*.

ASSAY

- **Content of Marmelosin**

Solution A: 0.2% acetic acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	40	60
24	40	60
27	60	40
30	60	40

Standard solution A: 0.01 mg/mL of USP Marmelosin RS in methanol

Standard solution B: Dissolve 0.1 g of USP *Aegle marmelos* Fruit Dry Extract RS in 100 mL of methanol, sonicate, and pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: Transfer 0.1 g of *Aegle marmelos* Fruit Dry Extract to a 100-mL beaker and add 50 mL of methanol. Sonicate for 30 min and transfer the solution to a 100-mL volumetric flask. Rinse the beaker with 20 mL of methanol and add the extract to the volumetric flask. Dilute with methanol to volume. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

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

Detector: UV 300 nm

Column: 4.6-mm × 10-cm; 2.7-µm packing L1

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

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

Relative standard deviation: NMT 2.0% for the marmelosin peak in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Aegle marmelos* Fruit Dry Extract RS being used.

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 *Aegle marmelos* Fruit Dry Extract RS being used, identify the retention times of the peaks corresponding to marmelosin in the *Sample solution*.

Calculate the percentages of marmelosin in the portion of *Aegle marmelos* Fruit Dry Extract taken:

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

r_U = peak area of marmelosin from the *Sample solution*

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

C_S = concentration of USP Marmelosin RS in *Standard solution A* (mg/mL)

C_U = concentration of *Aegle marmelos* Fruit Dry Extract in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of marmelosin in the portion of Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of marmelosin as determined above (%)

L = labeled amount of marmelosin (%)

Acceptance criteria: 90.0%–110.0% on the dried basis

CONTAMINANTS

• **Elemental Impurities—Procedures <233>**

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 1.0 µg/g

• **Articles of Botanical Origin <561>,** *Pesticide Residue Analysis***:** Meets the requirements

• **Microbial Enumeration Tests <61>:** 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>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• **Loss on Drying <731>**

Sample: 2 g of *Aegle marmelos* Fruit Dry Extract

Analysis: Dry the *Sample* at 105° for 5 h.

Acceptance criteria: NMT 6%

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(s) of the plant contained in the article.

• **USP Reference Standards <11>**

USP *Aegle marmelos* Fruit Dry Extract RS

USP Marmelosin RS
