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Add the following:

^Bael Tree Fruit Powder

DEFINITION

Bael Tree Fruit Powder consists of the dried ripe or unripe fruit of *Aegle marmelos* (L.) Corrêa (Fam. Rutaceae), reduced to powder or very fine powder. It contains NLT 0.2% of marmelosin and NMT 0.05% of egeline, both on the dried basis.

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 1 g of Bael Tree Fruit Powder in 10 mL of methanol for 10 min, centrifuge or filter, and use the supernatant or the filtrate.

Chromatographic system

(See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\)](#), [Table 1](#).)

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

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

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 for 30 min with filter paper, remove the plate from the chamber, and dry in air. Examine under both short-wave and long-wave UV light.

System suitability

Under short-wave UV light: *Standard solution A* exhibits a dark band due to marmelosin in the middle of the chromatogram. *Standard solution B* exhibits five to seven dark bands with the most intense and uppermost band similar in R_f and color to the marmelosin band in *Standard solution A*.

Under long-wave UV light: *Standard solution A* exhibits a greenish-blue band due to marmelosin in the middle of the chromatogram. *Standard solution B* exhibits a greenish-blue band corresponding in R_f and color to the marmelosin band in *Standard solution A*. One greenish-brown band and one or more blue and/or green bands are observed above the marmelosin band. Right below the marmelosin band, one blue band is observed along with the following other bands at decreasing R_f : one green band, two or three blue bands, one bright blue band, and several blue and green bands. A greenish-blue band right below the bright blue band may or may not be observed due to the coelution with the bright blue band in some case. The intensities of blue and green colors may vary.

Acceptance criteria

Under short-wave UV light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: the most intense and uppermost band observed due to marmelosin and also corresponding in R_f and color to the marmelosin band in *Standard solution A*, and four to six minor dark bands observed below the marmelosin band.

Under long-wave UV light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: a greenish-blue band also corresponding in R_f and color to the marmelosin band in *Standard solution A*; one greenish-brown band and few blue and/or green bands above the marmelosin band; and several blue and green bands below the marmelosin band.

• B. HPLC

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

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

COMPOSITION

• CONTENT OF MARMELOSIN

Solution A: 0.2% glacial acetic acid in water

Solution B: Acetonitrile

Mobile phase: See [Table 1](#).

Table 1

	Solution A	Solution B
Standard solution A: 0.01 mg/mL of USP Marmelosin RS in methanol		
Standard solution B: 1 mg/mL of USP Aegle marmelos Fruit Dry Extract RS in methanol. Sonicate and pass through a membrane filter of 0.45-µm or finer pore size.		
Sample solution: Transfer 0.2 g of Bael Tree Fruit Powder to a 250-mL flask and add 30 mL of methanol. Reflux at 80° for 30 min and decant the extract into a 100-mL volumetric flask. Repeat the extraction process with another 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 .)	40	60
Mode: LC	60	40
Detector: UV 300 nm		
Column: 4.6-mm × 10-cm; 2.7-µm packing L1	60	40
Column temperature: 40°		

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between the marmelosin peak and the peak before marmelosin, *Standard solution B*

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* and *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 percentage of marmelosin in the portion of Bael Tree Fruit Powder taken:

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

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)

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

W = weight of Bael Tree Fruit Powder used to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 0.2% on the dried basis

CONTAMINANTS

- [ARTICLES OF BOTANICAL ORIGIN \(561\), Limits of Elemental Impurities](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN \(561\), Pesticide Residue Analysis](#): Meets the requirements
- [MICROBIAL ENUMERATION TESTS \(2021\)](#): 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 bacterial count does not exceed 10^3 cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\), Test Procedures, Test for Absence of Salmonella Species](#) and [Test for Absence of Escherichia coli](#): Meets the requirements

SPECIFIC TESTS

- **LIMIT OF EGELINE, Method 1**

[NOTE—This method can be performed simultaneously with *Identification A*.]

Standard solution: 0.1 mg/mL each of [USP Marmelosin RS](#) and [USP Egeline RS](#) in methanol

Sample solution and Chromatographic system: Proceed as directed in *Identification A*.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber for 30 min with filter paper, remove the plate from the chamber, and dry in air. Examine under short-wave UV light.

System suitability: The *Standard solution* exhibits two dark bands clearly separated in the chromatogram: one in the lower-third section corresponding to egeline and the other slightly above the middle corresponding to marmelosin.