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Terminalia chebula Fruit Powder

Proposed For Comment Version 0.2

***Terminalia chebula* Fruit Powder**

DEFINITION

The article consists of the dried fruit of *Terminalia chebula* Retz. (Family Combretaceae) reduced to a powder or very fine powder. It contains NLT 15% of hydrolyzable tannins calculated as the sum of chebulagic acid and chebulinic acid, on the dried basis.

POTENTIAL CONFOUNDING MATERIALS

Terminalia citrine

CONSTITUENTS OF INTEREST

Hydrolyzable tannins: Chebulagic acid and chebulinic acid

Triterpene: Arjungenin

IDENTIFICATION

- **A. Botanical Characteristics**

  Macroscopic: Yellow-brown powder

  Microscopic: Fibers, polygonal epidermal cells, stone cells, and parenchyma containing starch grains

- **B. Thin-Layer Chromatography**

  Standard solution A: 0.1 mg/mL each of USP Chebulagic Acid RS and USP Chebulinic Acid RS in methanol

  Standard solution B: 100 mg/mL of USP *Terminalia chebula* Fruit Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

  Sample solution: Sonicate about 1.0 g of *Terminalia chebula* Fruit Powder in 100 mL of methanol for 10 min, centrifuge, and use the supernatant.

  Chromatographic system

  (See *Chromatography <621>, Thin-Layer Chromatography* [1].)

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

  Application volume: 5 µL of *Standard solution A*, and 1 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.

  Temperature: 25°

  Developing solvent system: Ethyl formate, toluene, formic acid, and water (30: 1.5: 4: 3)

  Developing distance: 7 cm

  Derivatization reagent: Natural products reagent (NP reagent): 1 g of diphenylboinic acid aminoethylester dissolved in 200 mL of ethyl acetate

  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 a saturated chamber, remove the plate from the chamber, and dry. Examine under UV 254 nm before derivatization. Heat the plate at 100° for 3 min, then treat with *Derivatization reagent*. Examine under UV 366 nm.

  System suitability: Under UV 254 nm prior to derivatization, the chromatogram of *Standard solution B* exhibits two intense bands in the lower half similar in R_F and color to the chebulagic acid and chebulinic bands in the chromatogram of *Standard solution A*, with chebulagic acid having a lower R_F. About four or five additional bands appear with increasing R_F: two bands below chebulagic acid, a band above chebulinic acid, and one intense band at an R_F of approximately 0.7. Under UV 366 nm after derivatization, about four intense blue fluorescence bands appear in the lower half and two blue fluorescence bands appear between R_F of 0.6 and 0.7.

  Acceptance criteria: Under UV 254 nm prior to derivatization, the chromatogram of *Sample solution* exhibits two intense bands in the lower half corresponding in R_F and color to the chebulagic acid and chebulinic bands in the chromatogram of *Standard solution A*, with chebulagic acid having a lower R_F. Two additional bands appear, one near the origin and another near an R_F of approximately 0.7. Under UV 366 nm after derivatization, about four pale blue fluorescence bands appear in the lower half, and one blue fluorescence band appears with an R_F of approximately 0.7.

- **C. HPLC**

Analysis: Proceed as directed in the *Assay* for *Content of Constituents of Hydrolyzable Tannins*.

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

ASSAY

- **Content of Constituents of Hydrolyzable Tannins**

Solution A: Dissolve 0.136 g of monobasic potassium phosphate in 900 mL of water, add 0.5 mL of *o*-phosphoric acid, dilute with water to 1 L, and mix.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	65	35
25	45	55
28	45	55
35	65	35
40	95	5
45	95	5

Standard solution A: 1.0 mg/mL of USP Chebulagic Acid RS in boiling water

Standard solution B: Dissolve 0.1 g of USP *Terminalia chebul*a Fruit Dry Extract RS in 100 mL of boiling water, sonicate, and pass through a membrane filter of 0.45-µm pore size.

Sample solution: Transfer 0.1 g of *Terminalia chebula* Fruit Powder, accurately weighed, to a 100-mL volumetric flask. Add 50 mL of boiling water and sonicate for 10 min. Dilute with water to 100 mL, mix well, and pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

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

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Merck kGaA Purospher Star LP HPLC Column, RP-18)

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

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

Resolution: NLT 11.4 between chebulagic acid and chebulinic acid peaks, *Standard solution B*

Tailing factor: NMT 1.5, S*tandard solution A*

Relative standard deviation: NMT 2.5%, *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 *Terminalia chebula* Fruit Dry Extract RS being used, identify the retention times of the peaks corresponding to chebulagic acid and chebulinic acid. The approximate relative retention times of the peaks for chebulagic acid and chebulinic acid are 1.0 and 1.15, respectively.

Separately calculate the percentages of chebulagic acid and chebulinic acid in the portion of *Terminalia chebula* Fruit Powder taken:

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

r_U = peak area of the relevant analyte from the *Sample solution*

r_S = peak area of the relevant analyte from *Standard solution A*

C_S = concentration of the relevant analyte in *Standard solution A* (mg/mL)

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

W = weight of *Terminalia chebula* Fruit Powder taken to prepare the *Sample solution* (mg)

F = conversion factor for the analytes (1 for chebulagic acid and 0.79 for chebulinic acid)

Add the percentages of chebulagic acid and chebulinic acid.

Acceptance criteria: NLT 15% on the dried basis

CONTAMINANTS

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

  Acceptance criteria

  Arsenic: NMT 2.0 µg/g

  Cadmium: NMT 1.0 µg/g

  Lead: NMT 5.0 µg/g

  Mercury: NMT 0.2 µg/g

- **Articles of Botanical Origin,** *General Method for Pesticide Residues Analysis* **<561>:** 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

- **Identification of Arjungenin**

  Standard solution: 0.2 mg/mL of USP Arjungenin RS in methanol

  Sample solution: Sonicate about 10 g of *Terminalia chebula* Fruit Powder in 10 mL of dichloromethane for 10 min, centrifuge, and use the supernatant.

  Chromatographic system

  (See *Chromatography* [1]<621>, [1]*Thin-Layer Chromatography* [1].)

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

  Application volume: 15 µL each of *Standard solution* and *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: Ethyl acetate, toluene, and glacial acetic acid (55:45:2)

  Developing distance: 6 cm

  Derivatization reagent: Anisaldehyde-sulfuric acid reagent created by combining 0.5 mL of anisaldehyde with 10 mL of glacial-acetic acid, 85 mL of methanol, and 5 mL of sulfuric acid.

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with *Derivatization reagent*. Examine under visible light.

  Acceptance criteria: Under visible light, the *Sample solution* exhibits the most intense band in the middle, as a dark blue band corresponding in color and R_F to the band due to arjungenin in the *Standard solution*.

- **Articles of Botanical Origin,** *Foreign Organic Matter* **<561>:** NMT 1.0%
- **Articles of Botanical Origin,** *Alcohol-Soluble Extractives, Method 1* **<561>:** NLT 40.0%
- **Articles of Botanical Origin,** *Water-Soluble Extractives, Method 2* **<561>:** NLT 60.0%
- **Loss on Drying <731>**

  Sample: 1 g of *Terminalia chebula* Fruit Powder

  Analysis: Dry the *Sample* at 105° for 2 h.

  Acceptance criteria: NMT 8%

- **Articles of Botanical Origin,** *Total Ash* **<561>:** NMT 15%
- **Articles of Botanical Origin,** *Acid-Insoluble Ash* **<561>:** NMT 5%

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 Arjungenin RS
  USP Chebulagic Acid RS
  USP Chebulinic Acid RS
  USP *Terminalia chebula* Fruit Dry Extract RS
