of 0.45-µm or finer pore size, discarding the first few mL of filtrate.

Analysis

Sample: *Standard solution*

Calculate the percentage of citric acid in the portion of Powdered *Garcinia cambogia* taken:

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

r_U = peak area of citric acid from the *Sample solution* in the test for *Content of (–)-Hydroxycitric Acid and (–)-Hydroxycitric Acid Lactone*

r_S = peak area of citric acid from the *Standard solution*

C_S = concentration of USP Citric Acid RS in the *Standard solution* (mg/mL)

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

W = weight of Powdered *Garcinia cambogia* used to prepare the *Sample solution* in the test for *Content of (–)-Hydroxycitric Acid and (–)-Hydroxycitric Acid Lactone* (mg)

Acceptance criteria: NMT 2% of citric acid, calculated on the dried basis

- **LOSS ON DRYING ⟨731⟩:** Dry 2.0 g of Powdered *Garcinia cambogia* at 105° for 3 h: it loses NMT 12.0% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* **⟨561⟩:** Determined on 1.0 g of Powdered *Garcinia cambogia*: NMT 3.0%; and NMT 8.0% if sodium chloride was added as a preservative during collection of the fruits
- **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 bacteria do not exceed 10^3 cfu/g.
- **MICROBIOLOGICAL PROCEDURES FOR ABSENCE OF SPECIFIED MICROORGANISMS ⟨2022⟩:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

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, following the official name, the part of the plant contained in the article.
- **USP REFERENCE STANDARDS ⟨11⟩**

  USP Calcium (–)-Hydroxycitrate RS

  USP Citric Acid RS

  USP Powdered Garcinia Hydroxycitrate Extract RS

Powdered Garcinia Hydroxycitrate Extract

DEFINITION

Powdered Garcinia Hydroxycitrate Extract is prepared from *Garcinia cambogia* or *Garcinia indica* by extraction with water, alcohol, or mixtures of these solvents, followed by stabilization of the (–)-hydroxycitric acid content in the form of a calcium, potassium, magnesium, and/or sodium salt. The ratio of plant material to extract is about 5:1 to 10:1. It contains NLT 40% of (–)-hydroxycitric acid, calculated on the dried basis. It may contain suitable added substances.

IDENTIFICATION

- **A. HPLC IDENTIFICATION TEST:** The *Sample solution* chromatogram exhibits a peak for hydroxycitric acid at a retention time corresponding to that of *Standard solution A*, as obtained in the test for *Content of (–)-Hydroxycitric Acid and Limit of (–)-Hydroxycitric Acid Lactone*.

COMPOSITION

- **CONTENT OF (–)-HYDROXYCITRIC ACID AND LIMIT OF (–)-HYDROXYCITRIC ACID LACTONE**

Solution A: 30% phosphoric acid in water

Mobile phase: Dissolve 1.36 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, adjust with *Solution A* to a pH of 2.5, complete to 1000 mL with water, mix, filter, and degas.

Solvent: A mixture of *Solution A* and water (1:9)

Standard solution A: A solution of USP Calcium (–)-Hydroxycitrate RS equivalent to about 2.5 mg/mL of (–)-hydroxycitric acid in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Standard solution B: 5 mg/mL of USP Powdered Garcinia Hydroxycitrate Extract RS in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: 5 mg/mL of Powdered Garcinia Hydroxycitrate Extract in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 25 ± 1°

Flow rate: 1.0 mL/min

Injection size: 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 Powdered Garcinia Hydroxycitrate Extract RS being used.

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

Relative standard deviation: NMT 2.0%, determined from the hydroxycitric acid peak, *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 6 h.]

Calculate the percentage of (–)-hydroxycitric acid and the limit of (–)-hydroxycitric acid lactone, if present, in the portion of Powdered Garcinia Hydroxycitrate Extract taken:

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

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

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

C_S = concentration of (–)-hydroxycitric acid in *Standard solution A* (mg/mL)

C_U = concentration of Powdered Garcinia Hydroxycitrate Extract in the *Sample solution* (mg/mL)

F = conversion factor for each analyte: 2.17 for (–)-hydroxycitric acid lactone, and 1.00 for (–)-hydroxycitric acid

Acceptance criteria: NLT 40% of (–)-hydroxycitric acid and NMT 8% of (–)-hydroxycitric acid lactone on the dried basis

IMPURITIES

Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* **⟨561⟩:** NMT 3.0%
- **HEAVY METALS,** *Method III* **⟨231⟩:** NMT 20 ppm

Organic Impurities

- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residues* **⟨561⟩:** Meets the requirements

SPECIFIC TESTS

- **LIMIT OF CITRIC ACID**

Solvent: Prepare as directed in the test for *Content of (–)-Hydroxycitric Acid and Limit of (–)-Hydroxycitric Acid Lactone*.

Standard solution: 0.5 mg/mL of USP Citric acid RS in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Analysis

Sample: *Standard solution*

Calculate the percentage of citric acid in the portion of Powdered Garcinia Hydroxycitrate Extract taken:

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

r_U = peak area of citric acid, using the peak area of citric acid from the *Sample solution* in the test for *Content of (–)-Hydroxycitric Acid and Limit of (–)-Hydroxycitric Acid Lactone*

r_S = peak area of citric acid from the *Standard solution*

C_S = concentration of USP Citric Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Powdered Garcinia Hydroxycitrate Extract in the *Sample solution* in the test for *Content of (–)-Hydroxycitric Acid and Limit of (–)-Hydroxycitric Acid Lactone* (mg/mL)

Acceptance criteria: NMT 5% of citric acid on the dried basis

- **IDENTIFICATION TESTS—GENERAL ⟨191⟩:** Test for the presence of calcium, magnesium, potassium, and/or sodium.
- **LOSS ON DRYING ⟨731⟩:** Dry 2.0 g of Powdered Extract at 105° for 3 h: Powdered Extract containing calcium hydroxycitrate loses NMT 5.0% of its weight; Powdered Extract containing other salts loses NMT 9.0% of its weight.
- **MICROBIAL ENUMERATION TESTS ⟨2021⟩:** The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **MICROBIOLOGICAL PROCEDURES FOR ABSENCE OF SPECIFIED MICROORGANISMS ⟨2022⟩:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **OTHER REQUIREMENTS:** It 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 prepared. It meets other *Labeling* requirements under *Botanical Extracts* ⟨565⟩.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Calcium (–)-Hydroxycitrate RS
USP Citric Acid RS
USP Powdered Garcinia Hydroxycitrate Extract RS

Garcinia indica

DEFINITION

Garcinia indica consists of the dried pericarp of the fruits of *Garcinia indica* (Thouars) Choisy (Fam. Clusiaceae). It contains NLT 12% of the sum of (–)-hydroxycitric acid and (–)-hydroxycitric acid lactone, on the dried basis.

IDENTIFICATION

- **A.** *Garcinia indica* meets the requirements under *Specific Tests, Botanic Characteristics*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

Standard solution: 0.5 mg/mL of garcinol in alcohol

Sample solution: Transfer about 2.0 g of *Garcinia indica*, finely powdered, to a Soxhlet apparatus, add 100 mL of alcohol, and extract for 6 h. Filter and concentrate under vacuum to about 10 mL. [NOTE—Use a thimble of suitable size such that the volume of alcohol used in the Soxhlet extraction is at least twice the volume of the thimble.]

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

Application volume: 5 µL, as 8-mm bands

Developing solvent system: Toluene, ethyl acetate, and formic acid (4:1:0.5)

Spray reagent: A mixture of 1% vanillin in alcohol and 10% sulfuric acid in alcohol (1:1)

Analysis

Samples: *Standard solution* 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 length of the plate. Remove the plate from the chamber, dry, spray with *Spray reagent*, heat for 5–10 min at about 105°, and examine under visible light.

Acceptance criteria: The *Sample solution* chromatogram exhibits a main greenish-grey band due to garcinol at an R_F value of approximately 0.6, which corresponds in position and color to the main band in the chromatogram of the *Standard solution*. The *Sample solution* exhibits the following additional bands: two purple bands, two greenish-grey bands, two blue bands and a purple band at R_F values of approximately 0.31, 0.34, 0.37, 0.47, 0.54, 0.83, and 0.93, respectively. Other bands may be observed for the *Sample solution*.

- **C. HPLC IDENTIFICATION TEST:** The *Sample solution* chromatogram exhibits a peak for hydroxycitric acid at a retention time corresponding to that of *Standard solution A*, as obtained in the test for *Content of (–)-Hydroxycitric Acid and (–)-Hydroxycitric Acid Lactone*. The *Sample solution* also exhibits a peak for hydroxycitric acid lactone. The hydroxycitric acid and the hydroxycitric acid lactone peaks are the main peaks in the *Sample solution* chromatogram.

COMPOSITION

- **CONTENT OF (–)-HYDROXYCITRIC ACID AND (–)-HYDROXYCITRIC ACID LACTONE**

Solution A: 30% phosphoric acid in water

Mobile phase: Dissolve 1.36 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, adjust with *Solution A* to a pH of 2.5, complete with water to 1000 mL, mix, filter, and degas.

Solvent: A mixture of *Solution A* and water (1:9)

Standard solution A: A solution of USP Calcium (–)-Hydroxycitrate RS equivalent to about 4 mg/mL of (–)-hydroxycitric acid in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Standard solution B: 8 mg/mL of USP Powdered Garcinia Hydroxycitrate Extract RS in *Solvent*. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: Transfer about 5 g of *Garcinia indica*, finely powdered and accurately weighed, to a 250-mL round-bottom flask fitted with a reflux condenser. Add 50 mL of *Solvent*, reflux while stirring on a water bath for 30 min, set aside to settle, and decant the supernatant. Repeat the extraction using four 50-mL portions of water, combine all extracts, cool, filter into a 250-mL volumetric flask, and complete with water to volume.