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# Coffee Fruit Dry Extract

## DEFINITION

Coffee Fruit Dry Extract is prepared from the dried semi-ripened light red entire fruit of *Coffea arabica* L. (Fam. Rubiaceae) by extraction with hydroalcoholic mixtures. It contains NLT 40% of caffeoylquinic acids, calculated as the sum of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, isochlorogenic acid B, isochlorogenic acid A, and isochlorogenic acid C on the dried basis; and NLT 15% of chlorogenic acid on the dried basis.

## IDENTIFICATION

### • A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).

**Standard solution A:** 0.2 mg/mL of [USP Chlorogenic Acid RS](#) in methanol

**Standard solution B:** 4 mg/mL of [USP Coffee Fruit Dry Extract RS](#) in methanol. Sonicate for 10 min, centrifuge or filter, and use the supernatant or filtrate.

**Sample solution:** 4 mg/mL of Coffee Fruit Dry Extract in methanol. Sonicate for 10 min, centrifuge or filter, and use the supernatant or filtrate.

#### Chromatographic system

**Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm

**Application volume:** 5 µL, as 8-mm bands

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

**Temperature:** About 25°

**Developing solvent system:** The upper layer solution of a mixture of *n*-butyl acetate, formic acid, and water (7:5:5)

**Developing distance:** 6 cm

**Derivatization reagent A:** 10 mg/mL of 2-aminoethyl diphenylborinate in methanol

**Derivatization reagent B:** 50 mg/mL of polyethylene glycol 4000 in alcohol

#### 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, remove the plate from the chamber, and dry in air. Treat the plate with *Derivatization reagent A*, and dry in air. Immediately treat the plate with *Derivatization reagent B*, dry in air, and examine under UV light at 366 nm.

**Suitability requirements:** Under UV light at 366 nm, *Standard solution A* exhibits a blue fluorescent band due to chlorogenic acid in the lower-third section of the chromatogram. *Standard solution B* exhibits an intense blue fluorescent band corresponding in  $R_f$  to chlorogenic acid in *Standard solution A*; a band above chlorogenic acid in deeper blue color; and a blue fluorescent band below chlorogenic acid. In the middle-third section, *Standard solution B* exhibits at least three blue fluorescent bands.

**Acceptance criteria:** Under UV light at 366 nm, the *Sample solution* exhibits an intense band corresponding in  $R_f$  and color to chlorogenic acid in *Standard solution A*. The *Sample solution* exhibits additional bands corresponding to similar bands in *Standard solution B*, including a band above chlorogenic acid in deeper blue color; a blue fluorescent band below chlorogenic acid; three blue fluorescent bands in the middle-third section with the top one due to isochlorogenic acid A, and below that, a pair of bands corresponding to other isochlorogenic acids; and several faint blue fluorescent bands above isochlorogenic acid A.

### • B. HPLC

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

**Acceptance criteria:** The chromatogram of the *Sample solution* exhibits the most intense peak corresponding to chlorogenic acid in *Standard solution A*, and peaks corresponding to neochlorogenic acid; cryptochlorogenic acid; 5-*O*-feruloylquinic acid, which typically coelutes with another constituent and sometimes can be resolved into two peaks; isochlorogenic acid B; isochlorogenic acid A; and isochlorogenic acid C in the chromatogram of *Standard solution B*. The peaks of neochlorogenic acid and cryptochlorogenic acid are of comparable medium intensity. The peaks of 5-*O*-feruloylquinic acid, isochlorogenic acid B, isochlorogenic acid A, and isochlorogenic acid C are of lower intensity.

## COMPOSITION

### • CONTENT OF CAFFEYOYLQUINIC ACIDS

**Solution A:** 2% acetic acid in water

**Solution B:** Acetonitrile

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

Table 1

| Time<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 0             | 95                | 5                 |
| 9             | 95                | 5                 |
| 11.5          | 91                | 9                 |
| 21.5          | 91                | 9                 |
| 60            | 75                | 25                |
| 75            | 75                | 25                |
| 75.1          | 95                | 5                 |
| 80            | 95                | 5                 |

[NOTE—Protect from light and proceed under low-actinic light. Each *Standard solution* and the *Sample solution* are stable for 24 h at room temperature.]

**Solvent:** Methanol and water (1:1)

**Standard solution A:** 0.2 mg/mL of [USP Chlorogenic Acid RS](#) in *Solvent*

**Standard solution B:** 1.0 mg/mL of [USP Coffee Fruit Dry Extract RS](#) in *Solvent*. Sonicate for 10 min, and pass through a hydrophilic polypropylene (GHP) membrane filter of 0.45-µm or finer pore size.

**Sample solution:** Accurately transfer a quantity of Coffee Fruit Dry Extract, equivalent to about 10 mg of total caffeoylquinic acids calculated based on the labeled content, into a 25-mL volumetric flask, and add 15 mL of *Solvent*. Sonicate the sample for 10 min, cool to room temperature, dilute with *Solvent* to volume, and mix. Before injection, pass through a GHP membrane filter of 0.45-µm or finer pore size and discard the first portion of the filtrate.

#### Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC

**Detector:** UV 325 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

#### Temperatures

**Autosampler:** 4°

**Column:** 25°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

#### System suitability

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

#### Suitability requirements

**Resolution:** NLT 1.5 between the peaks of chlorogenic acid and cryptochlorogenic acid; and peaks of isochlorogenic acid B and isochlorogenic acid A, *Standard solution B*

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

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

**Chromatographic similarity:** The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Coffee 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 Coffee Fruit Dry Extract RS](#) being used, identify the peaks for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 5-O-feruloylquinic acid, isochlorogenic acid B, isochlorogenic acid A, and isochlorogenic acid C in the *Sample solution*. [NOTE—See [Table 2](#) for relative retention times. These values may vary due to differences in the chromatographic conditions allowed by the *Suitability requirements*.]

**Table 2**