Cinnamomum cassia Twig

Proposed For Comment Version 0.2

Cinnamomum cassia Twig

**DEFINITION**
The article consists of the dried twigs of *Cinnamomum cassia* (L.) J.Presl (Family Lauraceae), collected in spring or summer. It contains NLT 0.6% and NMT 2.0% of cinnamaldehyde (C₉H₈O), calculated on the anhydrous basis.

**SYNONYMS**
*Camphorina cassia* (Nees & T.Nees) Farw.
*Cinnamomum aromaticum* Nees
*Cinnamomum longifolium* Lukman
*Cinnamomum medium* Lukman
*Laurus cassia* L.
*Persea cassia* (L.) Spreng

**POTENTIAL CONFOUNGING MATERIALS**
*Cinnamomum burmannii* (C. G. et Th. Nees) Bl., bark
*Cinnamomum verum* J.Presl, bark
*Cinnamomum bejolghota* (Buch.-Ham.) Sweet, bark
*Cinnamomum cassia* (L.) J. Presl, bark

**SELECTED COMMON NAMES**
Chinese: 桂枝（Gui Zhi）
English: Cassis Twig
Korean: 계수나무가지
Spanish: Cassia Ramita
Swedish: Cassia Twig

**CONSTITUENTS OF INTEREST**
Phenylpropanoids: Coumarin, cinnamic alcohol, cinnamic acid, 2-methoxycinnamic acid, cinnamaldehyde and 2-methoxycinnamaldehyde

**IDENTIFICATION**
- **A. BOTANICAL CHARACTERISTICS**
  - **Macroscopic:** Long cylindrical, much-branched, 30–75 cm long, 0.3–1 cm in diameter at the thick end.
Externally brown to reddish-brown, with longitudinal ridges, fine wrinkles, dotted leaf-scars, branch-scars, bud-scars, and dotted lenticels. Texture hard and fragile, easily broken. Slices 2–4 mm thick, cut surface showing reddish-brown in bark, yellow-white to pale yellow-brown in wood, pith in subsquare.

**Microscopic**

**Transverse section:** Epidermis consists of one layer of cells, unicellular non-glandular hairs are visible in young branches. Cork consists of 3–5 layers of cells, the inmost cells with thickened outer walls. Oil cells and stone cells are scattered in cortex. Groups of stone cells in the pericycle are interruptedly arranged in a ring, accompanied by fiber bundles. Secretory cells and fibers are scattered in phloem. Cambium is distinct. In xylem, xylem rays are 1–2 cells wide, containing brown contents; vessels are scattered single or two to several aggregated; lignified fibers have relatively thin walls and are difficult to differentiate from lignified parenchymatous cells. In pith, the walls of cells are slightly thickened and lignified; cells of rays contain fine needle crystals of calcium oxalate.

**B. Thin-Layer Chromatography**

**Standard solution A:** 0.5 mg/mL of USP Cinnamaldehyde RS in methanol

**Standard solution B:** 100 mg/mL of USP *Cinnamomum cassia* Twig Dry Extract RS in toluene, sonicate for 10 min, centrifuge, and use the supernatant.

**Sample solution:** Sonicate 2.0 g of *Cinnamomum cassia* Twig, finely powdered, in 10 mL of methanol for 10 min, centrifuge or filter, and transfer the extract to a round-bottom flask. Wash the remaining plant material with 5 mL of methanol and combine the washing with the extract. Evaporate the solution under reduced pressure to dryness. Dissolve the residue in 2 mL of toluene, sonicate for about 2 min, centrifuge, and use the supernatant.

**Chromatographic system**

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

- **Adsorbent:** Chromatographic silica gel 

- **Application volume:** 6 μL, as 8-mm bands
- **Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.
- **Developing solvent system:** Toluene and ethyl acetate (95:5)
- **Developing distance:** 7 cm
- **Derivatization reagent:** Methanol, acetic acid, sulfuric acid, and p-anisaldehyde (170:20:10:1). [Note—Prepare fresh. Slowly add sulfuric acid to ice-cold methanol, followed by acetic acid and p-anisaldehyde.]

**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 (20 min with filter paper), remove the plate from the chamber, and air-dry. Examine under UV light at 254 nm. Then dip (time 0, speed 5) the plate with the Derivatization reagent, heat at 100° for 2 min, and examine under UV light at 366 nm.

**System suitability:** Under UV light at 254 nm, Standard solution B exhibits, in the middle-third section, an intense quenching band corresponding in *Rf* to the band for cinnamaldehyde in Standard solution A. In the lower-third section, Standard solution B exhibits one weak quenching band in the upper part due to coumarin, one quenching band close to the starting position due to cinnamic acid, and one quenching band between coumarin and cinnamic acid due to cinnamic alcohol. After derivatization, under UV light at 366 nm, Standard solution B exhibits a band corresponding in *Rf* to the band for cinnamaldehyde in Standard solution A, and one yellow band immediately below the band of cinnamaldehyde.

**Acceptance criteria:** Under UV light at 254 nm, the Sample solution exhibits, in the middle-third...
section, an intense quenching band corresponding in $R_F$ to the band for cinnamaldehyde in *Standard solution A*. The *Sample solution* exhibits additional bands corresponding to similar bands in *Standard solution B*; these include one weak quenching band in the upper part of the lower-third section due to coumarin, one quenching band close to the starting position due to cinnamic acid, and one quenching band between the bands of coumarin and cinnamic acid. After derivatization, under UV light at 366 nm, the *Sample solution* exhibits a band corresponding in $R_F$ and color to the band for cinnamaldehyde in *Standard solutions A* and *B*, and one yellow band immediately below the band of cinnamaldehyde. There is no red band immediately above the cinnamaldehyde band (distinguished from *Cinnamomum verum* bark).

### C. HPLC Analysis

**Acceptance criteria:** The *Sample solution* exhibits an intense peak with a retention time corresponding to cinnamaldehyde in *Standard solution A*; and exhibits peaks related to coumarin and cinnamic alcohol (co-eluted), cinnamic acid, and 2'-methoxycinnamaldehyde at retention times corresponding to the same phenylpropanoids in *Standard solution B* and the reference chromatogram provided with the lot of USP *Cinnamomum cassia* Twig Dry Extract RS being used. The peak area ratios for each peak versus the cinnamaldehyde peak are listed in *Table 1*.

### ASSAY

** CONTENT OF CINNAMALDEHYDE**

- **Solution A:** 0.01% Phosphoric acid in water
- **Solution B:** Acetonitrile

**Mobile phase:** *Solution A* and *Solution B* (65:35). [*Note*—Protect from light and proceed under low actinic light. The *Standard solutions* and *Sample solution* are stable for 24 h at room temperature.]

- **Standard solution A:** 0.06 mg/mL of USP Cinnamaldehyde RS in methanol
- **Standard solution B:** 1 mg/mL of USP *Cinnamomum cassia* Twig Dry Extract RS in methanol, sonicate, centrifuge, and pass through a membrane filter of 0.45-μm or finer pore size.

**Sample solution:** Accurately transfer about 250 mg of *Cinnamaldehyde cassia* Twig, finely powdered, into a suitable flask and accurately add 25 mL of methanol. Sonicate for 30 min and filter into a 50-mL volumetric flask. Rinse the flask and the residue left in the flask with 10 mL of methanol, and wash the residue and paper on the filter using the rinsing. Repeat the rinse procedure one more time. Continue to wash the paper on the filter with about 4 mL of methanol, dilute with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45-μm or finer pore size and discard the first portion of the filtrate.

**Chromatographic system**

(See *Chromatography <621>*, [1] System Suitability.)

- **Mode:** LC
- **Detector:** UV 290 nm
- **Column:** 4.6-mm × 10-cm; 3.5-μm packing L1 (similar to Agilent Zorbax SB C18)
- **Column temperature:** 30°
- **Flow rate:** 1.2 mL/min
- **Injection volume:** 5 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 *Cinnamomum cassia* Twig Dry Extract RS being used.
Resolution: NLT 1.5 between the peak of coumarin and cinnamic alcohol (co-eluted) and the peak of cinnamic acid, Standard solution B

Tailing factor: NMT 2.0 for cinnamaldehyde peak, Standard solution A

Relative standard deviation: NMT 2.0% for cinnamaldehyde, 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 Cinnamomum cassia Twig Dry Extract RS being used, identify the retention times of the peaks corresponding to coumarin and cinnamic alcohol (co-eluted), cinnamic acid, cinnamaldehyde, and 2’-methoxycinnamaldehyde in the Sample solution. [Note—The approximate relative retention times for each peak are provided in Table 1.]

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Times</th>
<th>Peak Area Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumarin and cinnamic alcohol</td>
<td>0.58</td>
<td>1.0–3.0</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.63</td>
<td>2.0–10</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>1.00</td>
<td>100</td>
</tr>
<tr>
<td>2’-methoxycinnamaldehyde</td>
<td>1.35</td>
<td>6.0–13</td>
</tr>
</tbody>
</table>

Calculate the percentage of cinnamaldehyde in the portion of Cinnamomum cassia Twig taken:

\[
\text{Result} = \left( \frac{r_u}{r_s} \right) \times C_s \times \left( \frac{V}{W} \right) \times 100
\]

\( r_u \) = peak area of cinnamaldehyde from the Sample solution

\( r_s \) = peak area of cinnamaldehyde from Standard solution A

\( C_s \) = concentration of USP Cinnamaldehyde RS in Standard solution A (mg/mL)

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

\( W \) = weight of Cinnamomum cassia Twig taken to prepare the Sample solution (mg)

Acceptance criteria: 0.6%–2.0% on the anhydrous basis

CONTAMINANTS

- **Elemental Impurities—Procedures <233> [2]**

  Acceptance criteria

  - Arsenic: NMT 2.0 µg/g
  - Cadmium: NMT 0.3 µg/g
  - Lead: NMT 5.0 µg/g
  - Mercury: NMT 0.2 µg/g


- **Microbial Enumeration Tests [5]<61>: [6] [6]** 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** [7][8][9][10] Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

- **Articles of Botanical Origin** [4], Aflatoxins [9] [561]: Meets the requirements

**Specific Tests**

- **Articles of Botanical Origin** [4], Foreign Organic Matter [561]: NMT 1.0%
- **Articles of Botanical Origin** [4], Alcohol-Soluble Extractives, Method 1 [561]: NLT 6.0%
- **Water Determination** [10], Method II [921]: NMT 12.0%
- **Articles of Botanical Origin** [4], Total Ash [561]: NMT 3.0%
- **Articles of Botanical Origin** [4], Acid-Insoluble Ash [561]: NMT 0.5%

**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 the part(s) of the plant contained in the article.

**USP Reference Standards** [11]

- USP Aflatoxins RS [13]
- USP Cinnamaldehyde RS
- USP *Cinnamomum cassia* Twig Dry Extract RS