Schisandra chinensis Fruit Powder

Proposed For Comment Version 0.2

**Schisandra chinensis** Fruit Powder

**DEFINITION**
The article consists of dried ripe fruits of *Schisandra chinensis* (Turcz.) Baill. (Family Schisandraceae) collected in the fall, reduced to a fine powder. It contains NLT 0.40% of schisandrin (C_{24}H_{32}O_{7}), on the dried basis; NLT 0.95% of total lignans calculated as the sum of schisandrin, schisandrol B (C_{23}H_{28}O_{7}), schisandrin A (deoxyschisandrin, C_{24}H_{32}O_{6}), and schisandrin B (γ-schisandrin, C_{23}H_{28}O_{6}), on the dried basis.

**POTENTIAL CONFOUNDING MATERIALS**
*Kadsura japonica* (L.) Dunal
*Schisandra sphenanthera* Rehder & E. H. Wilson

**CONSTITUENTS OF INTEREST**
Lignans: Schisandrin, schisandrol B, schisandrin A (deoxyschisandrin), and schisandrin B (γ-schisandrin)

**IDENTIFICATION**

- **A. Botanical Characteristics**
  - **Macroscopic:** Dark purple in color
  - **Microscopic:** The epidermal stone cells of testa are polygonal or elongated-polygonal in surface view, 18–50 μm in diameter, thickened walls with very fine and dense pit canals; the cells contain dark brown contents. The inner layer stone cells of testa are polygonal, subrounded, or irregular, up to 83 μm in diameter, slightly thickened walls with relatively large pits. Epidermal cells of pericarp are polygonal in surface view, anticlinal walls are slightly beaded with cuticle striations (anticlinal walls are not beaded in *Schisandra sphenanthera*), scattered with oil cells. Cells in mesocarp are shriveled, containing dark brown contents and starch granules.

- **B. Thin-Layer Chromatography**
  - **Standard solution A:** 1.0 mg/mL of USP Schisandrin RS and 1.0 mg/mL of USP Schisandrin B RS in methanol
  - **Standard solution B:** Sonicate 1.0 g of USP *Schisandra chinensis* Fruit Dry Extract RS in 10 mL of methanol for 10 min. Centrifuge and use the supernatant.
  - **Sample solution:** Sonicate 2.5 g of *Schisandra chinensis* Fruit Powder in 10 mL of methanol for 10 min. Centrifuge and use the supernatant.

**Chromatographic system**
(See Chromatography <621>, Thin-Layer Chromatography.)
  - **Adsorbent:** Chromatographic silica gel F254 mixture with an average particle size of 5 μm (HPTLC plates)
  - **Application volume:** 3 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, ethyl acetate, and glacial acetic acid (23:6:1)

Developing distance: 6 cm

Derivatization reagent: 10% Sulfuric acid in ethanol [Note: slowly add sulfuric acid to ice-cold ethanol]

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, air-dry, and examine under UV light at 254 nm. Then treat the plate with Derivatization reagent, heat at 120° for 7 min, examine under UV light at 366 nm.

System suitability: Under UV light at 254 nm, the chromatogram of Standard solution B exhibits up to six bands in the lower-half section, the most intense band corresponds in Rf to the band of schisandrin in Standard solution A. Standard solution B also exhibits the band due to schisandrin A in the middle of the chromatogram. In the upper-half section, Standard solution B exhibits a band corresponds in Rf to the band of schisandrin B in Standard solution A.

Acceptance criteria: Under UV light at 254 nm, the chromatogram of the Sample solution exhibits an intense band at an Rf corresponding to the band due to schisandrin (distinguished from Schisandra sphenanthera Fruit) in Standard solution A. The Sample solution exhibits additional bands including one or two bands below the position of schisandrin; a band due to schisandrin A in the middle of the chromatogram; four or five bands between the positions of the bands of schisandrin A and schisandrin; and two or three bands in the upper-half section, the most intense band at an Rf corresponding to the band of schisandrin B in Standard solution A. Under UV light at 366 nm after derivitization, the chromatogram of the Sample solution does not exhibit a blue fluorescent band (distinguished from Schisandra sphenanthera Fruit) in the upper-third of the chromatogram.

C. HPLC

Analysis: Proceed as directed in the Assay for Content of Lignans.

Acceptance criteria: The chromatogram of Sample solution exhibits the most intense peak with a retention time corresponding to schisandrin in Standard solution A, and peaks for schisandrol B, schisandrin A, and schisandrin B corresponding to the retention times for the same lignans in Standard solution B. A big intense peak due to schisantherin A (distinguished from Schisandra sphenanthera Fruit) does not appear at about 2.16 of relative retention time versus schisandrin.

ASSAY

Content of Lignans

Solution A: Water

Solution B: Acetonitrile and methanol (1:1)

Mobile phase: See Table 1.

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>
**Standard solution A:** 0.06 mg/mL of USP Schisandrin RS in methanol

**Standard solution B:** 8.5 mg/mL of USP *Schisandra chinensis* Fruit Dry Extract RS in methanol. Mix and pass through a polytetrafluoroethylene filter of 0.2-μm pore size before injection.

**Sample solution:** Accurately transfer about 250 mg of *Schisandra chinensis* Fruit Powder to a 50-mL centrifuge tube. Add 10 mL of methanol and sonicate for 10 min (140 W, 42 kHz). Centrifuge and transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the extracts in the 25-mL volumetric flask and dilute with methanol to volume. Mix, pass through a polytetrafluoroethylene filter of 0.2-μm pore size before injection, and discard the first portion of the filtrate.

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

Mode: UPLC
Detector: UV 251 nm
Column: 2.1 mm × 15 cm; 1.8-μm packing L1 (similar to ACQUITY UPLC® HSS T3)
Column temperature: 35°C
Flow rate: 0.3 mL/min
Injection volume: 3 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 *Schisandra chinensis* Fruit Dry Extract RS being used.
- **Resolution:** NLT 1.5 between the schisandrol B peak and the peak following it, Standard solution B
- **Tailing factor:** NMT 2.0 for schisandrin peak, Standard solution A
- **Relative standard deviation:** NMT 2.0% for the schisandrin peak, 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 *Schisandra chinensis* Fruit Dry Extract RS being used, identify the peaks corresponding to schisandrin, schisandrol B, schisandrin A, and schisandrin B in the Sample solution. [Note-The approximate relative retention times of the analytes are provided in Table 2.]

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schisandrin</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Schisandrol B</td>
<td>1.29</td>
<td>1.21</td>
</tr>
<tr>
<td>Schisandrin A</td>
<td>2.90</td>
<td>1.00</td>
</tr>
<tr>
<td>Schisandrin B</td>
<td>3.31</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Separately calculate the percentages of schisandrin, schisandrol B, schisandrin A, and schisandrin B in the portion of *Schisandra chinensis* Fruit Powder taken:

\[ \text{Result} = \left( \frac{r_i}{r_S} \right) \times C_s \times \left( \frac{V}{W} \right) \times F \times 100 \]
\( r_u \) = peak area of the relevant analyte from the Sample solution
\( r_s \) = peak area of schisandrin from Standard solution A
\( C_s \) = concentration of USP Schisandrin RS in Standard solution A (mg/mL)
\( V \) = volume of the Sample solution (mL)
\( W \) = weight of Schisandra chinensis Fruit Powder taken to prepare the Sample solution (mg)
\( F \) = conversion factors for analytes (See Table 2)

Calculate the percentage of total lignans as the sum of schisandrin, schisandrol B, schisandrin A, and schisandrin B.

Acceptance criteria
Schisandrin: NLT 0.40%, on the dried basis
Total lignans: NLT 0.95%, on the dried basis

CONTAMINANTS
• Articles of Botanical Origin [2], General Method for Pesticide Residues Analysis <561 [2]>: Meets the requirements

• Elemental Impurities—Procedures <233> [3]
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 <61>: [4] 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.


SPECIFIC TESTS
• Loss on Drying <731> [7]
Sample: 2.0 g of Schisandra chinensis Fruit Powder
Analysis: Dry the Sample at 105° for 5 h.
Acceptance criteria: NMT 16.0%

• Articles of Botanical Origin [2], Total Ash <561 [2]>
Analysis: 2.0 g of Schisandra chinensis Fruit powder
Acceptance criteria: NMT 7.0%

• Articles of Botanical Origin [2], Water-Soluble Extractives <561 [2]>
Analysis: Cold extraction method
Acceptance criteria: NLT 35%

• Articles of Botanical Origin [2], Total Alcohol-Soluble Extractives <561 [2]>
Analysis: Cold extraction method
Acceptance criteria: NLT 40%
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>** [8]
  - USP *Schisandra chinensis* Fruit Dry Extract RS
  - USP Aflatoxins RS [6]
  - USP Schisandrin RS
  - USP Schisandrin B RS