Schisandra chinensis Fruit Dry Extract

Proposed For Development Version 0.1

Schisandra chinensis Fruit Dry Extract

**DEFINITION**

The article is prepared from the dried ripe fruits of *Schisandra chinensis* (Turcz.) Baill. (Family Schisandraceae) collected in the fall, by extraction with hydroalcoholic mixtures. It contains NLT 90.0% and NMT 110.0% of the labeled amount of schisandrin (schisandrol A, C\textsubscript{24}H\textsubscript{32}O\textsubscript{7}), on the dried basis; and NLT 90.0% and NMT 110.0% of the labeled amount of total lignans, calculated as the sum of schisandrin, schisandrol B (C\textsubscript{23}H\textsubscript{28}O\textsubscript{7}), schisandrin A (deoxyschisandrin, C\textsubscript{26}H\textsubscript{32}O\textsubscript{6}), and schisandrin B (γ-schisandrin, C\textsubscript{23}H\textsubscript{28}O\textsubscript{6}), on the dried basis.

**POTENTIAL CONFOUNDING MATERIALS**

*Kadsura japonica* (L.) Dunal

*Schisandra sphenanthera* Rehder & E. H. Wilson

**CONSTITUENTS OF INTEREST**

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

**IDENTIFICATION**

- **A. Thin-Layer Chromatography**
  
  **Standard solution A:** 0.5 mg/mL of USP Schisandrin RS and 0.5 mg/mL USP Schisandrin B RS in methanol
  
  **Standard solution B:** Sonicate 100 mg/mL of USP *Schisandra chinensis* Fruit Dry Extract RS in methanol for 10 min. Centrifuge and use the supernatant.
  
  **Sample solution:** Sonicate about 250 mg (adjust the amount properly if necessary) of *Schisandra chinensis* Fruit Dry Extract in 5 mL of methanol for 10 min. Centrifuge and use the supernatant.

  **Chromatographic system**
  
  (See Chromatography <621>, Thin-Layer Chromatography.)
  
  **Adsorbent:** Chromatographic silica gel F\textsubscript{254} 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 an intense band corresponding in R\textsubscript{F} to the band of schisandrin in Standard solution A. Standard solution B also exhibits a band due to schisandrin A in the middle of the chromatogram, four or five bands between the positions of the bands of schisandrin and schisandrin A. In the upper half section, Standard solution B exhibits a band corresponding in R\textsubscript{F} 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 R\textsubscript{F} corresponding to the band due to schisandrin (distinguished from Schisandra sphenanthera Fruit) in Standard solution A. The Sample solution exhibits additional bands corresponding in R\textsubscript{F} to similar bands in Standard solution B, including 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 R\textsubscript{F} 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 an intense blue fluorescent band (distinguished from Schisandra sphenanthera Fruit) in the upper-third of the chromatogram.

- **B. HPLC**

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

  **Acceptance criteria:** The chromatogram of the 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**: 10 mg/mL of USP Schisandra chinensis Fruit Dry Extract RS in methanol. Sonicate and pass through a polytetrafluoroethylene filter of 0.2-μm pore size before injection.

**Sample solution**: Accurately transfer an amount, equivalent to about 4 mg of total lignans according to the labeled content, of Schisandra chinensis Fruit Extract 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>, 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°

**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 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
Chinaensis Fruit Dry Extract taken:

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

- \( r_u \) = 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 Dry Extract 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.

Calculate the percentage of the labeled amount of schisandrin in the portion of Schisandra chinensis Fruit Dry Extract taken:

\[
\text{Result} = (P/L) \times 100
\]

- \( P \) = content of schisandrin as determined above (%)
- \( L \) = labeled amount of schisandrin (%)

**Acceptance criteria:** 90.0%–110.0% on the dried basis

Calculate the percentage of the labeled amount of total lignans in the portion of Schisandra chinensis Fruit Dry Extract taken:

\[
\text{Result} = (P/L) \times 100
\]

- \( P \) = content of total lignans as determined above (%)
- \( L \) = labeled amount of total lignans (%)

**Acceptance criteria:** 90.0%–110.0% 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 [4]<61>:[4] The total aerobic bacterial count does not exceed 10^4 cfu/g, the total combined molds and yeasts count does not exceed 10^2 cfu/g.

**SPECIFIC TESTS**

- **Loss on Drying <731> [7]**
  - **Sample**: 2.0 g of Schisandra chinensis Fruit Dry Extract
  - **Analysis**: Dry the Sample at 105° for 5 h.
  - **Acceptance criteria**: NMT 8.0%
- **Articles of Botanical Origin [2]**, *Total Ash <561 [2]>*
  - **Analysis**: 2.0 g of Schisandra chinensis Fruit Dry Extract
  - **Acceptance criteria**: NMT 5.0%

**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 from which the article was prepared. It meets other labeling requirements under *Botanical Extract* <565>.

• **USP Reference Standards** <11> [8]
  - USP Aflatoxins RS [8]
  - USP *Schisandra chinensis* Fruit Dry Extract RS
  - USP Schisandrin RS
  - USP Schisandrin B RS

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