Schisandra chinensis Fruit Dry Extract

Final Authorized Version 1.0

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_{24}H_{32}O_{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_{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 [2] (schisandrol A), schisandrol B, schisandrin A (deoxyschisandrin), and schisandrin B (γ-schisandrin)

IDENTIFICATION
• A. HPTLC
(See High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin <203> [3].)
Standard solution A: 0.5 mg/mL of USP Schisandrin RS in methanol
Standard solution B: 100 mg/mL of USP Schisandra chinensis Fruit Dry Extract RS in methanol, sonicate for 10 min. Centrifuge and use the supernatant.
Sample solution: About 250 mg (adjust the amount properly if necessary) of Schisandra chinensis Fruit Dry Extract in 5 mL of methanol, sonicate for 10 min. Centrifuge and use the supernatant.
Chromatographic system
Adsorbent: Chromatographic silica gel F_{254}, mixture with an average particle size of 5 µm
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 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, and examine under UV light at 366 nm.
System suitability: Under UV light at 254 nm, Standard solution B exhibits an intense band corresponding in R_f to the band of schisandrin [2] in Standard solution A. Standard solution B also exhibits a band due to schisandrin A in the middle, and four or five bands between the positions of the bands of schisandrin [2] and schisandrin A. In the upper-half, Standard solution B exhibits an intense band due to schisandrin B.
Acceptance criteria: Under UV light at 254 nm, the Sample solution exhibits an intense band corresponding in R_f to the band due to schisandrin [2] (distinction from Schisandra sphenanthera Fruit) in Standard solution A. The Sample solution exhibits additional bands corresponding in R_f to similar bands in Standard solution B, including: a band due to schisandrin A in the middle; four or five bands between the positions of the bands of schisandrin A and schisandrin [2]; two or three bands in the upper half, the most intense band due to schisandrin B. Under UV light at 366 nm after derivitization, the Sample solution does not exhibit an intense blue fluorescent band (distinction from Schisandra sphenanthera Fruit) in the upper-third.
• 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 [2] 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. There is no principal peak due to schisantherin A at a relative retention time of about 2.1 relative to schisandrin [2] (distinction from Schisandra sphenanthera Fruit).

ASSAY

• CONTENT OF LIGNANS

Solution A: Water
Solution B: Acetonitrile and methanol (1:1)
Mobile phase: See Table 1.

| Table 1 |
|---|---|
| Time (min) | Solution A (%) | Solution B (%) |
| 0 | 47 | 53 |
| 30 | 20 | 80 |

Standard solution A: 0.06 mg/mL of USP Schisandrin RS [1] in methanol
Standard solution B: 10 mg/mL of USP Schisandra chinensis Fruit Dry Extract RS [4] 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 Dry Extract to a 50-mL round-bottom 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>, [5] System Suitability [6].)
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 [7] 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 the 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 and Standard solution B, and the reference chromatogram provided with the lot of USP Schisandra chinensis Fruit Dry Extract RS [7] being used, identify the peaks corresponding to schisandrin, schisandrol B, schisandrin A, and schisandrin B in the Sample solution. [Note—See Table 2 for the approximate relative retention times.]

| Table 2 |
|---|---|---|
| Analyte | Approximate Relative Retention Time | Conversion Factor |
| Schisandrin | 1.00 | 1.00 |
| Schisandrol B | 1.29 | 1.21 |
| Schisandrin A | 2.90 | 1.00 |
| Schisandrin B | 3.31 | 1.23 |

Separately calculate the percentages of schisandrin, schisandrol B, schisandrin A, and schisandrin B in the portion of Schisandra
$\text{Result} = \left( \frac{r_U}{r_S} \right) \times C_S \times \left( \frac{V}{W} \right) \times F \times 100$

$r_U$ = 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 [2] 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 content of total lignans as the sum of schisandrin [2], schisandrol B, schisandrin A, and schisandrin B.

Calculate the percentage of the labeled amount of schisandrin [2] in the portion of Schisandra chinensis Fruit Dry Extract taken:

$\text{Result} = \left( \frac{P}{L} \right) \times 100$

$P$ = 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:

$\text{Result} = \left( \frac{P}{L} \right) \times 100$

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

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

### CONTAMINANTS

- **Microbial Enumeration Tests** [10]<61>: [10] 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, and the bile-tolerant Gram-negative bacteria does not exceed $10^2$ cfu/g.
- **Tests for Specified Microorganisms** [62]: [11] Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

### SPECIFIC TESTS

- **Loss on Drying** <731> [12]
  - 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** <561>, [8] *Total Ash* [8]
  - 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 in *Botanical Extracts* <565>. [13]
- **USP Reference Standards** <11> [14]
  - USP *Schisandra chinensis* Fruit Dry Extract RS [4]
  - USP Schisandrin RS [1]