Schisandrae Sphenantherae Fructus

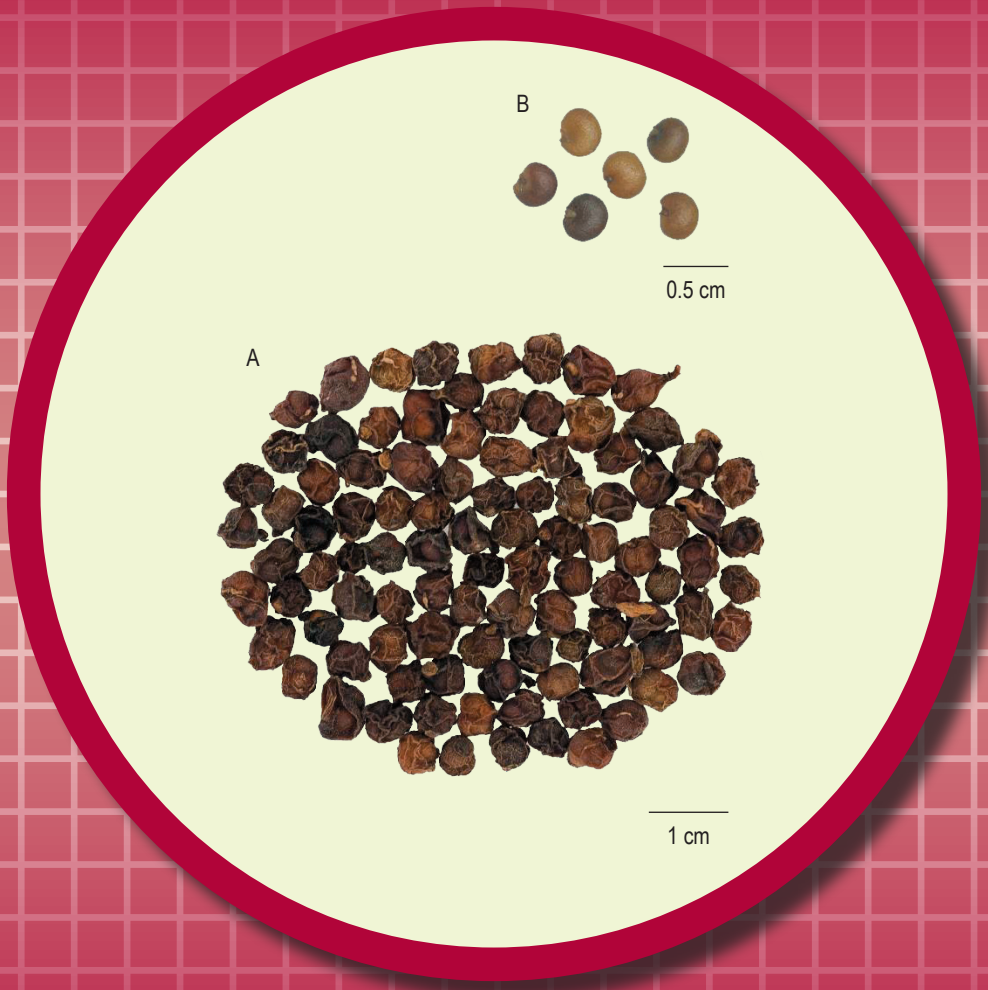


Figure 1 A photograph of Schisandrae Sphenantherae Fructus
A: Fruits B: Seeds

1. NAMES

Official Name: Schisandrae Sphenantherae Fructus

Chinese Name: 南五味子

Chinese Phonetic Name: Nanwuweizi

2. SOURCE

Schisandrae Sphenantherae Fructus is the dried ripe fruit of *Schisandra sphenanthera* Rehd. et Wils. (Magnoliaceae). The ripe fruit is collected in autumn, stalk and foreign matter removed, then dried under the sun to obtain Schisandrae Sphenantherae Fructus.

3. DESCRIPTION

Spheroidal to compressed-spheroidal, 4-7 mm in diameter. Externally brownish-red to dark brown, shrunken and shrivelled; pulp usually adhered closely to seeds. Seeds 1-2, kidney-shaped, externally brownish-yellow, the surface minutely granular, testa thin and fragile. Odour of pulp slight; taste slightly sour. Odour of seeds fragrant when broken; taste pungent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of square to rectangular epidermal cells, covered with cuticle; oil cells scattered. Mesocarp parenchymatous cells shrunken, with indistinct boundaries, containing scattered starch granules, with small collateral vascular bundles. Endocarp consists of 1 layer of small, square parenchymatous cells. The outermost layer of testa consists of radially elongated stone cells, thick-walled, with fine and dense pits and pit canals; inside showing several layers of stone cells, polygonal to subrounded or irregular in shape, with slightly thickened walls and distinct pits and pit canals. The cells in oil cell layer square to rectangular, containing oil droplets. Inner epidermal cells of testa flat and shrunken. Endosperm contains oil droplets and aleurone grains (Fig. 2).

Powder

Colour brown. Stone cells of outer layer of testa 18-30 µm in diameter, polygonal to elongated-polygonal in surface view; rectangular in longitudinal section view; walls thickened with very fine and dense pit canals; lumen contains dark brown contents. Inner layer stone cells of testa vary in size, up to 60 µm in diameter, polygonal to subrounded or irregular in surface view, with slightly thickened walls and distinct pits and pit canals. Epidermal cells of exocarp polygonal in surface view, with striated cuticle, scattered with oil cells. Mesocarp cells shrivelled, containing brown contents and starch granules. Endosperm cells contain oil droplets and aleurone grains (Fig. 3).

4.2 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solutions

Schisandrin A standard solution

Weigh 1.0 mg of schisandrin A CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Schisantherin A standard solution

Weigh 1.0 mg of schisantherin A CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and formic acid (15:5:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 1 h. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately schisandrin A standard solution (2 µL), schisantherin A standard solution (2 µL) and the test solution (1 µL) to the plate. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of schisandrin A and schisantherin A.

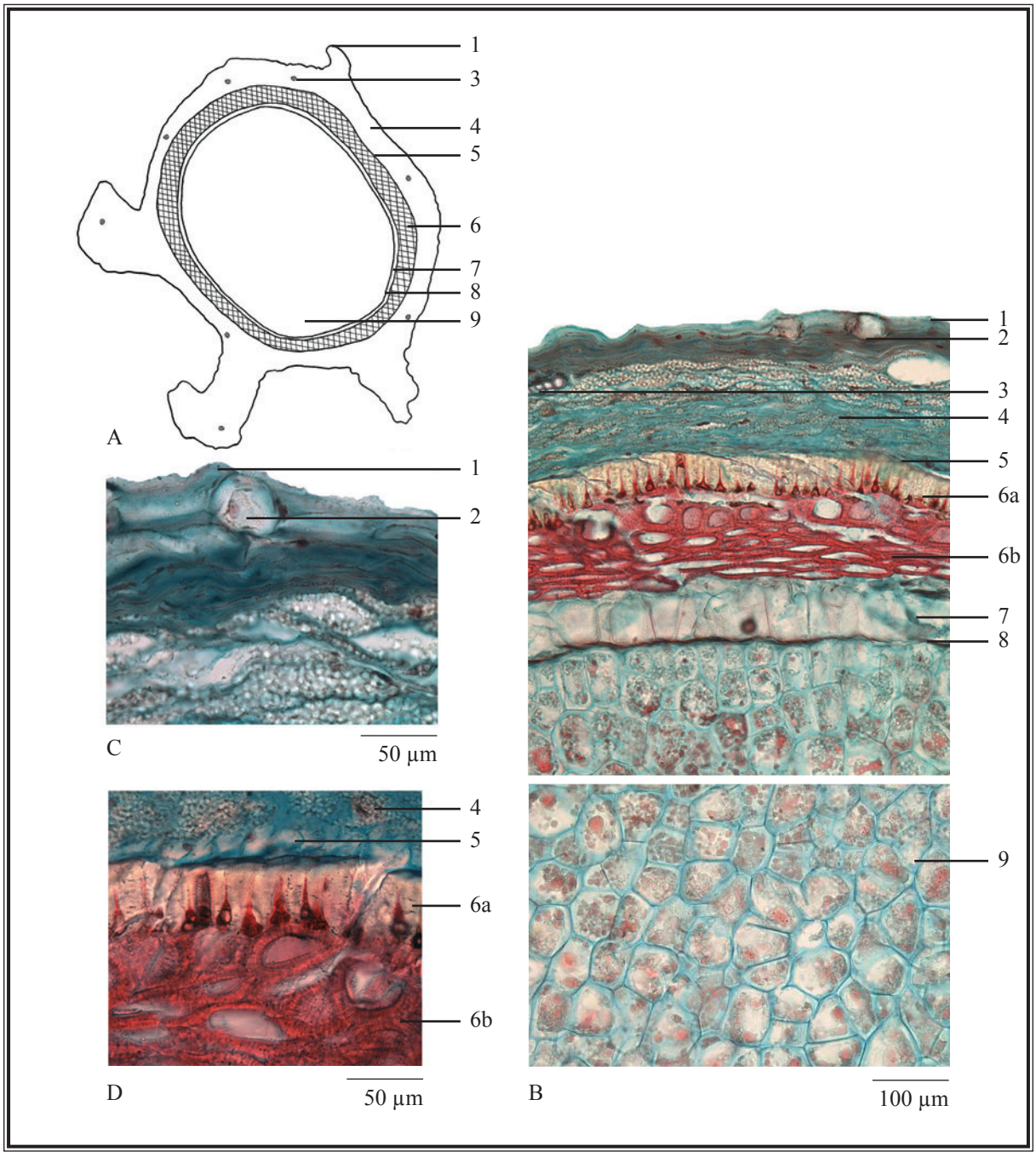


Figure 2 Microscopic features of transverse section of Schisandrae Sphenantherae Fructus

A. Sketch B. Section illustration C. Exocarp, oil cell and mesocarp D. Mesocarp, endocarp and testa

1. Exocarp 2. Oil cell 3. Vascular bundle 4. Mesocarp 5. Endocarp
6. Testa (6a. The outermost layer of testa 6b. Inner layers of testa with several layers of stone cells)
7. Oil cell layer 8. Inner epidermal cells of testa 9. Endosperm

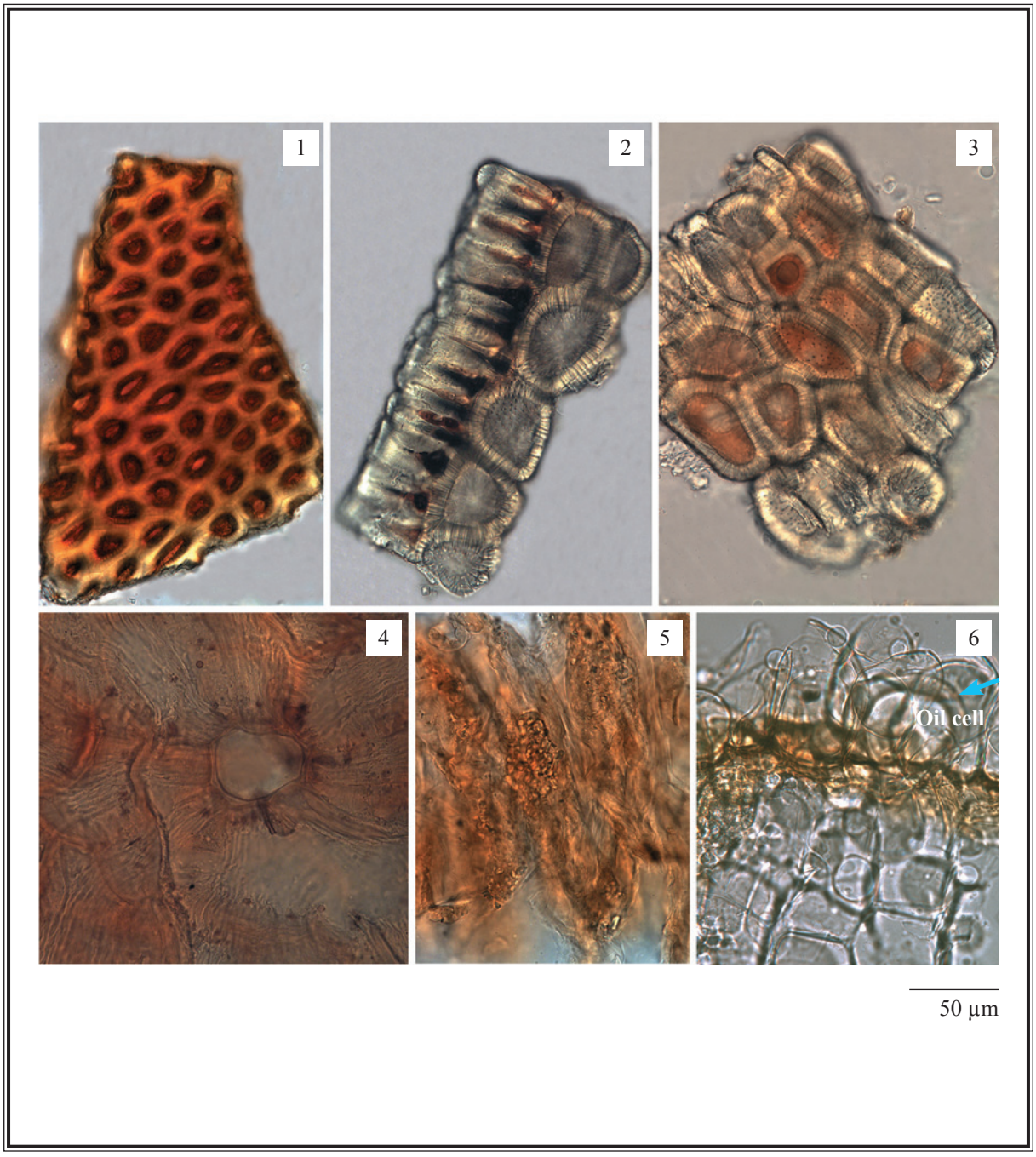
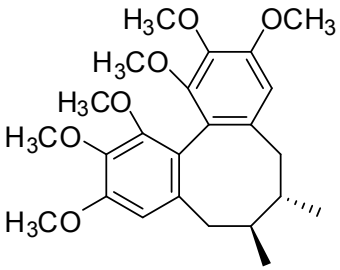


Figure 3 Microscopic features of powder of Schisandrae Sphenantherae Fructus (under the light microscope)

1. Stone cells of outer layer of testa in surface view
2. Stone cells of outer layer of testa and stone cells of inner layer in longitudinal section view
3. Inner layer stone cells of testa 4. Epidermal cells of exocarp 5. Mesocarp cells
6. Oil cell layer and endosperm cells

(i)



(ii)

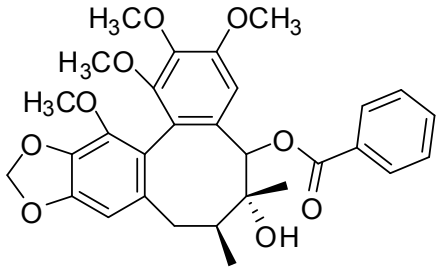


Figure 4 Chemical structures of (i) schisandrin A and (ii) schisantherin A

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

Schisandrin A standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 2.5 mg of schisandrin A CRS and dissolve in 25 mL of ethanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates. Make up to the mark with ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	50	50	isocratic
10 – 45	50 → 15	50 → 85	linear gradient
45 – 50	15 → 0	85 → 100	linear gradient
50 – 60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of schisandrin A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of schisandrin A should not be more than 5.0%; the RSD of the retention time of schisandrin A peak should not be more than 2.0%; the column efficiency determined from schisandrin A peak should not be less than 20000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject schisandrin A Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of schisandrin A peak in the chromatogram of schisandrin A Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify schisandrin A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of schisandrin A Std-FP. The retention times of schisandrin A peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the four characteristic peaks of Schisandrae Sphenantherae Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Schisandrae Sphenantherae Fructus extract

Peak No.	RRT	Acceptable Range
1	0.75	± 0.03
2 (schisantherin A)	0.78	± 0.03
3	0.86	± 0.03
4 (marker, schisandrin A)	1.00	-

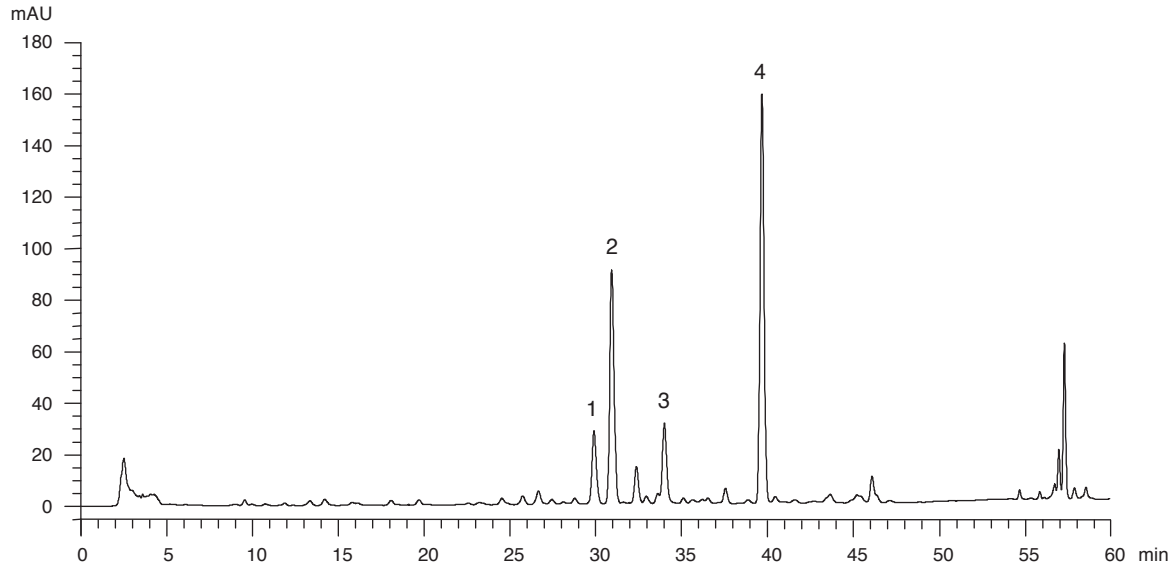


Figure 5 A reference fingerprint chromatogram of Schisandrae Sphenantherae Fructus extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 20.0%.
Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(B).

Standard solution

Mixed schisandrin A and schisantherin A standard stock solution, Std-Stock (500 mg/L each)
Weigh accurately 5.0 mg of schisandrin A CRS and 5.0 mg of schisantherin A CRS, and dissolve in 10 mL of ethanol.
Mixed schisandrin A and schisantherin A standard solution for assay, Std-AS
Measure accurately the volume of the mixed schisandrin A and schisantherin A Std-Stock, dilute with ethanol to produce a series of solutions of 5, 50, 100, 150, 200 mg/L for both schisandrin A and schisantherin A.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	50	50	isocratic
10 – 45	50 → 15	50 → 85	linear gradient
45 – 50	15 → 0	85 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed schisandrin A and schisantherin A Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of schisandrin A and schisantherin A should not be more than 5.0%; the RSD of the retention times of schisandrin A and schisantherin A peaks should not be more than 2.0%; the column efficiencies determined from schisandrin A and schisantherin A peaks should not be less than 10000 theoretical plates.

The *R* value between schisandrin A peak and the closest peak; and the *R* value between schisantherin A peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed schisandrin A and schisantherin A Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of schisandrin A and schisantherin A against the corresponding concentrations of the mixed schisandrin A and schisantherin A Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify schisandrin A and schisantherin A peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed schisandrin A and schisantherin A Std-AS. The retention times of schisandrin A and schisantherin A peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of schisandrin A and schisantherin A in the test solution, and calculate the percentage contents of schisandrin A and schisantherin A in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.67% of the total content of schisandrin A ($C_{24}H_{32}O_6$) and schisantherin A ($C_{30}H_{32}O_9$), calculated with reference to the dried substance.