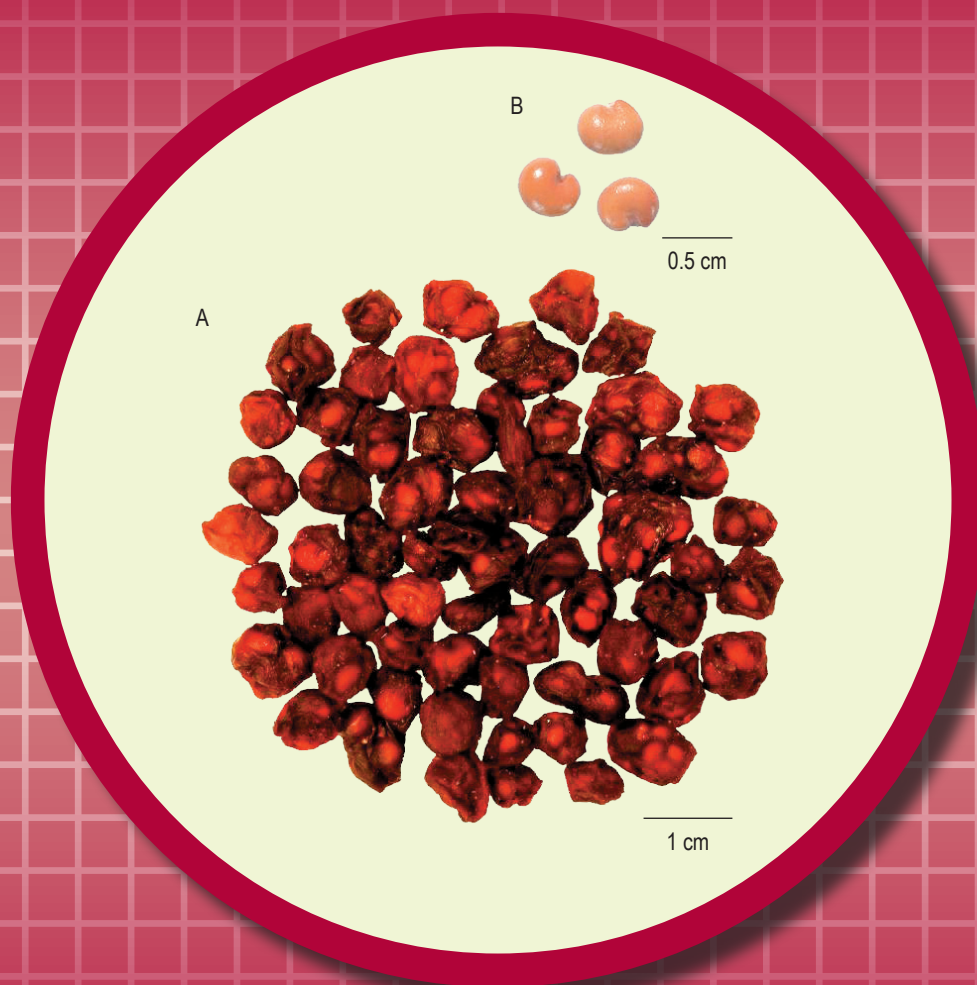


# Schisandrae Chinensis Fructus



**Figure 1** A photograph of Schisandrae Chinensis Fructus  
A: Fruits B: Seeds

## 1. NAMES

Official Name: Schisandrae Chinensis Fructus

Chinese Name: 五味子

Chinese Phonetic Name: Wuweizi

## 2. SOURCE

Schisandrae Chinensis Fructus is the dried ripe fruit of *Schisandra chinensis* (Turcz.) Baill. (Magnoliaceae). The ripe fruit is collected in autumn, stalk and foreign matter removed, then dried under the sun or after steamed to obtain Schisandrae Chinensis Fructus.

## 3. DESCRIPTION

Irregularly spheroidal to compressed-spheroidal, 5-8 mm in diameter. Externally red to dull red, shrunken, oily, with soft pulp, sometimes externally blackish-red or covered with “white frost”. Seeds 1-2, kidney-shaped, externally brownish-yellow, lustrous, 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, walls relatively thickened, covered with striated 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 the oil cell layer square to rectangular, containing oil drops. Inner epidermal cells of testa flat and shrunken. Endosperm contains oil droplets and aleurone grains (Fig. 2).

## Powder

Colour brown to dark brown. Stone cells of the outer layer of testa 18-40  $\mu\text{m}$  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 83  $\mu\text{m}$  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, anticlinal walls slightly beaded, with striated cuticle and scattered 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 standard solution*

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

#### *Schisandrin B standard solution*

Weigh 1.0 mg of schisandrin B 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).

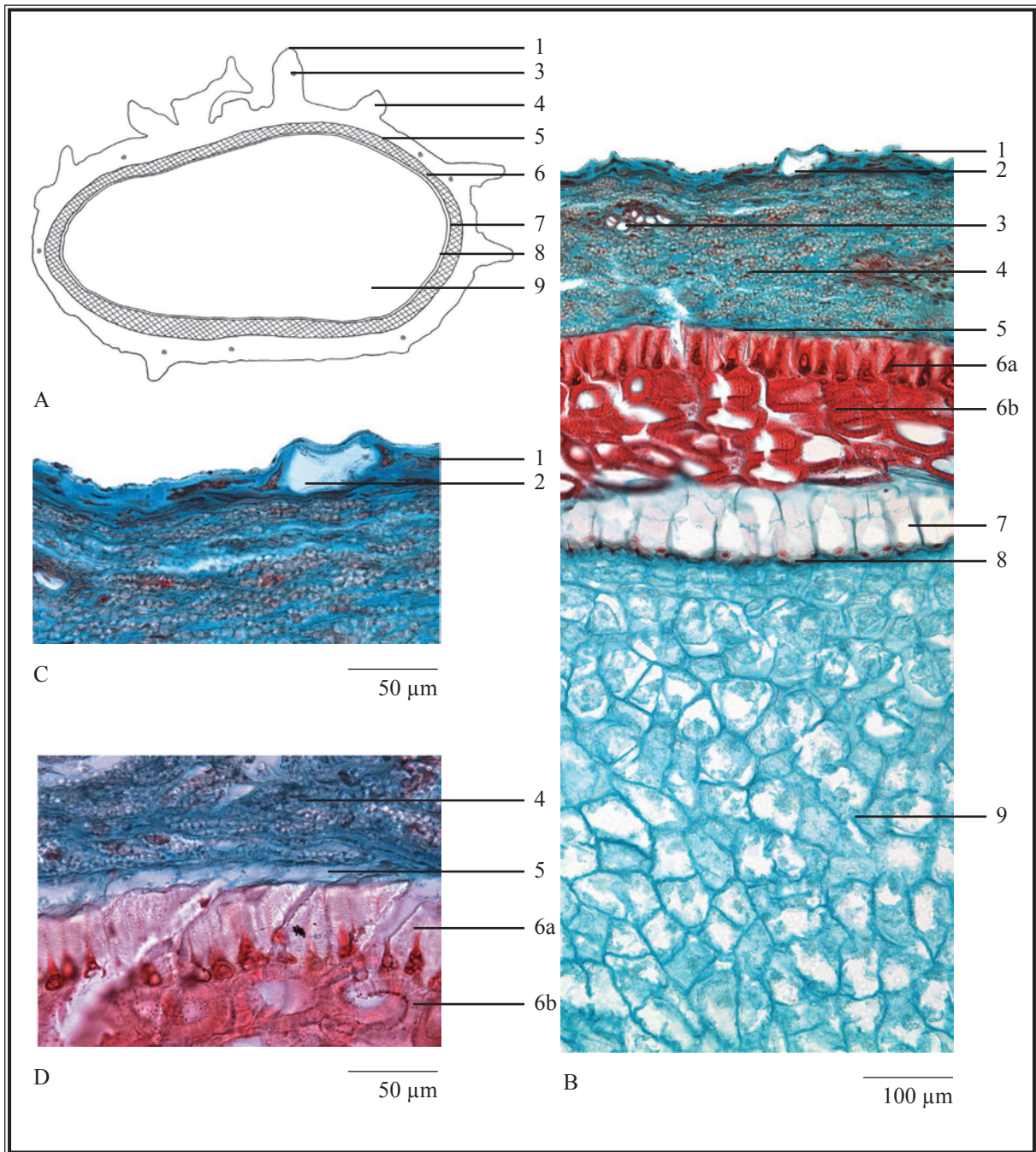
### Test solution

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 standard solution (2  $\mu\text{L}$ ), schisandrin B standard solution (2  $\mu\text{L}$ ) and the test solution (1  $\mu\text{L}$ ) 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).

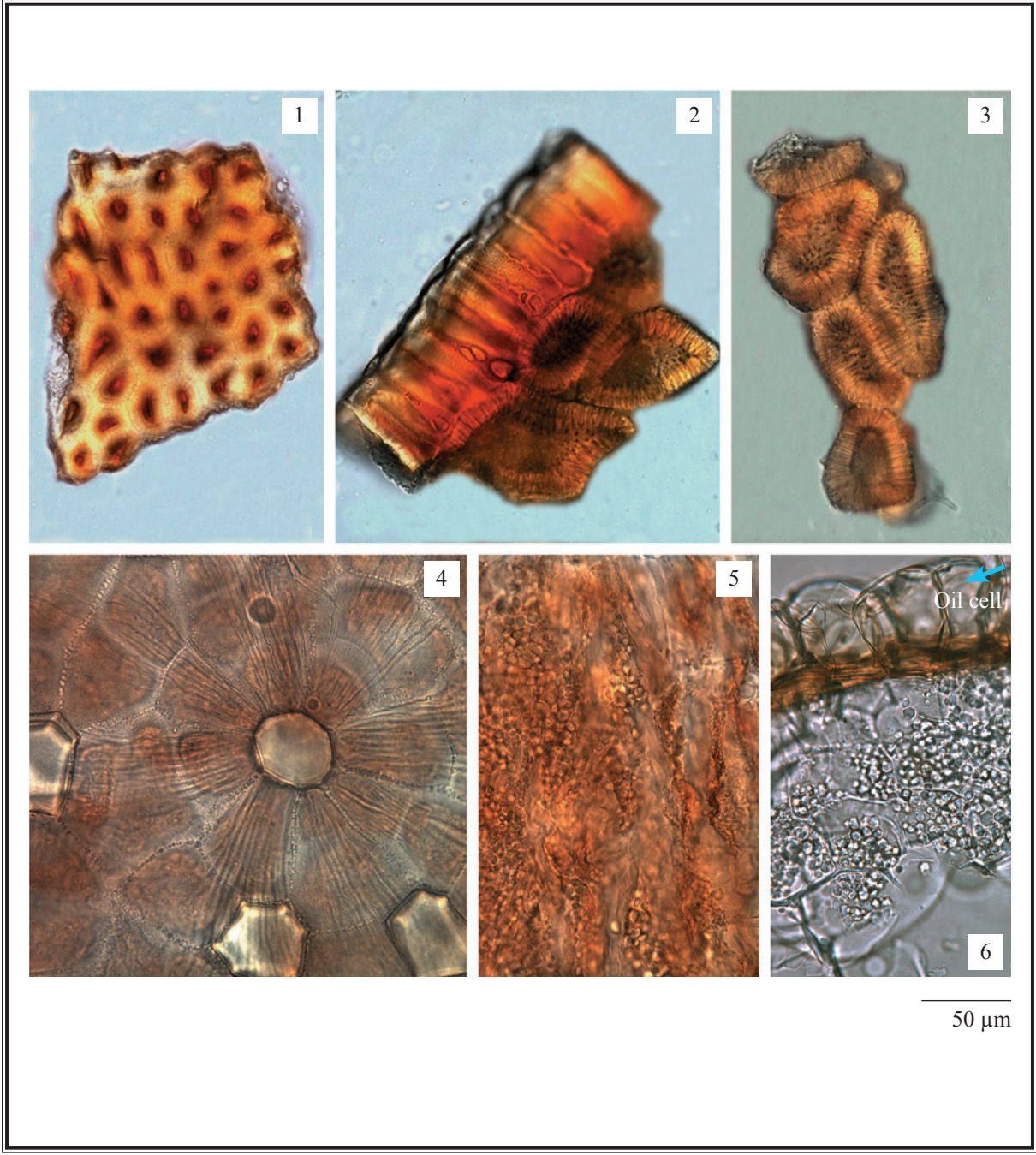
For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the  $R_f$  values, corresponding to those of schisandrin and schisandrin B.



**Figure 2** Microscopic features of transverse section of *Schisandrae Chinensis Fructus*

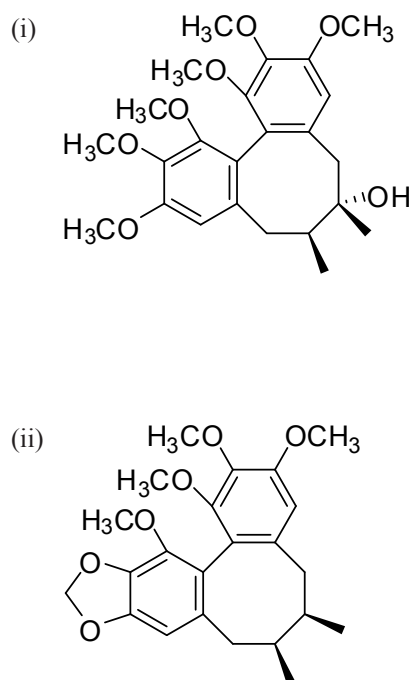
A. Sketch    B. Section illustration    C. Exocarp, oil cell and mesocarp    D. Mesocarp, endocarp and testa

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 Chinensis 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 of stone cells of testa
4. Epidermal cells of exocarp
5. Mesocarp cells
6. Oil cell layer and endosperm cells



**Figure 4** Chemical structures of (i) schisandrin and (ii) schisandrin B

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

#### Standard solutions

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

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

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

Weigh 2.5 mg of schisandrin B 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  $5000 \times g$  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- $\mu\text{m}$  RC filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu\text{m}$  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 Std-FP and schisandrin B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of schisandrin and schisandrin B should not be more than 5.0%; the RSD of the retention times of schisandrin and schisandrin B peaks should not be more than 2.0%; the column efficiencies determined from schisandrin and schisandrin B peaks should not be less than 10000 theoretical plates.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

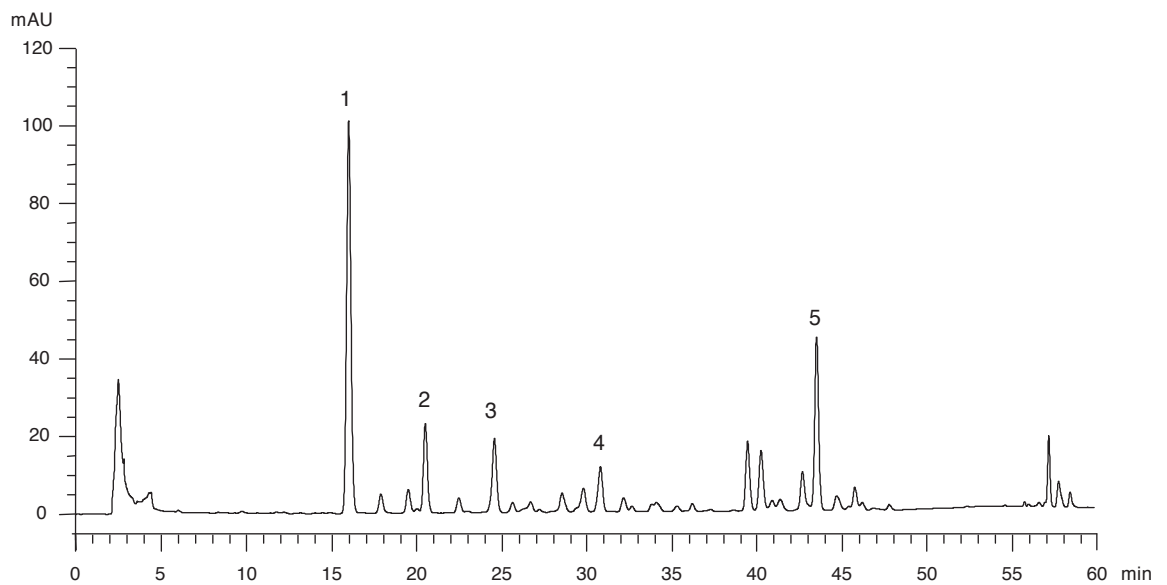
**Procedure**

Separately inject schisandrin Std-FP, schisandrin B Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of schisandrin and schisandrin B peaks in the chromatograms of schisandrin Std-FP, schisandrin B Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify schisandrin and schisandrin B peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of schisandrin Std-FP and schisandrin B Std-FP. The retention times of schisandrin and schisandrin B peaks in the chromatograms of the test solution and the corresponding Std-FP 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 five characteristic peaks of *Schisandrae Chinensis Fructus* extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the five characteristic peaks of Schisandrae Chinensis Fructus extract

| Peak No.                    | RRT              | Acceptable Range |
|-----------------------------|------------------|------------------|
| 1 (marker 1, schisandrin)   | 1.00             | -                |
| 2                           | 1.27 (vs peak 1) | ± 0.03           |
| 3                           | 1.52 (vs peak 1) | ± 0.03           |
| 4                           | 1.90 (vs peak 1) | ± 0.05           |
| 5 (marker 2, schisandrin B) | 1.00             | -                |



**Figure 5** A reference fingerprint chromatogram of Schisandrae Chinensis Fructus extract

For positive identification, the sample must give the above five 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 4.0%.

Acid-insoluble ash: not more than 0.5%.

**5.7 Water Content** (*Appendix X*)

Toluene distillation method: not more than 16.0%.

## 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 39.0%.

Ethanol-soluble extractives (cold extraction method): not less than 40.0%.

## 7. ASSAY

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

### Standard solution

*Mixed schisandrin and schisandrin B standard stock solution, Std-Stock (500 mg/L each)*

Weigh accurately 5.0 mg of schisandrin CRS and 5.0 mg of schisandrin B CRS, and dissolve in 10 mL of ethanol.

*Mixed schisandrin and schisandrin B standard solution for assay, Std-AS*

Measure accurately the volume of the mixed schisandrin and schisandrin B Std-Stock, dilute with ethanol to produce a series of solutions of 5, 50, 100, 150, 200 mg/L for both schisandrin and schisandrin B.

### 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 and schisandrin B Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of schisandrin and schisandrin B should not be more than 5.0%; the RSD of the retention times of schisandrin and schisandrin B peaks should not be more than 2.0%; the column efficiencies determined from schisandrin and schisandrin B peaks should not be less than 10000 theoretical plates.

The *R* value between schisandrin peak and the closest peak; and the *R* value between schisandrin B 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 and schisandrin B Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of schisandrin and schisandrin B against the corresponding concentrations of the mixed schisandrin and schisandrin B Std-AS. Obtain the slopes, y-intercepts and the *r*<sup>2</sup> 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 and schisandrin B peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed schisandrin and schisandrin B Std-AS. The retention times of schisandrin and schisandrin B 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 and schisandrin B in the test solution, and calculate the percentage contents of schisandrin and schisandrin B in the sample by using the equations indicated in Appendix IV(B).

Aurantii Fructus  
枳殼

Orpiment  
雌黃

Cistanches Herba  
仙茅 肉苻蓉

雄黃  
Realgar

Houttuyniae Herba  
魚腥草

墨旱蓮  
Ecliptae Herba

Smilacis Glabrae Rhizoma  
土茯苓

五味子

Calomelas

Curculiginis Rhizoma

前胡

Peucedani Radix

蛇床子

Cnidii Fructus

Scutellariae Barbatae Herba

半枝蓮

Sophorae Flavescentis Radix

苦參

*Schisandrae Chinensis Fructus*

Schisandrae Chinensis Fructus

紅粉

Isatidis Radix

板藍根

### Limits

The sample contains not less than 0.65% of the total content of schisandrin ( $C_{24}H_{32}O_7$ ) and schisandrin B ( $C_{23}H_{28}O_6$ ), calculated with reference to the dried substance.