# Menispermi Rhizoma

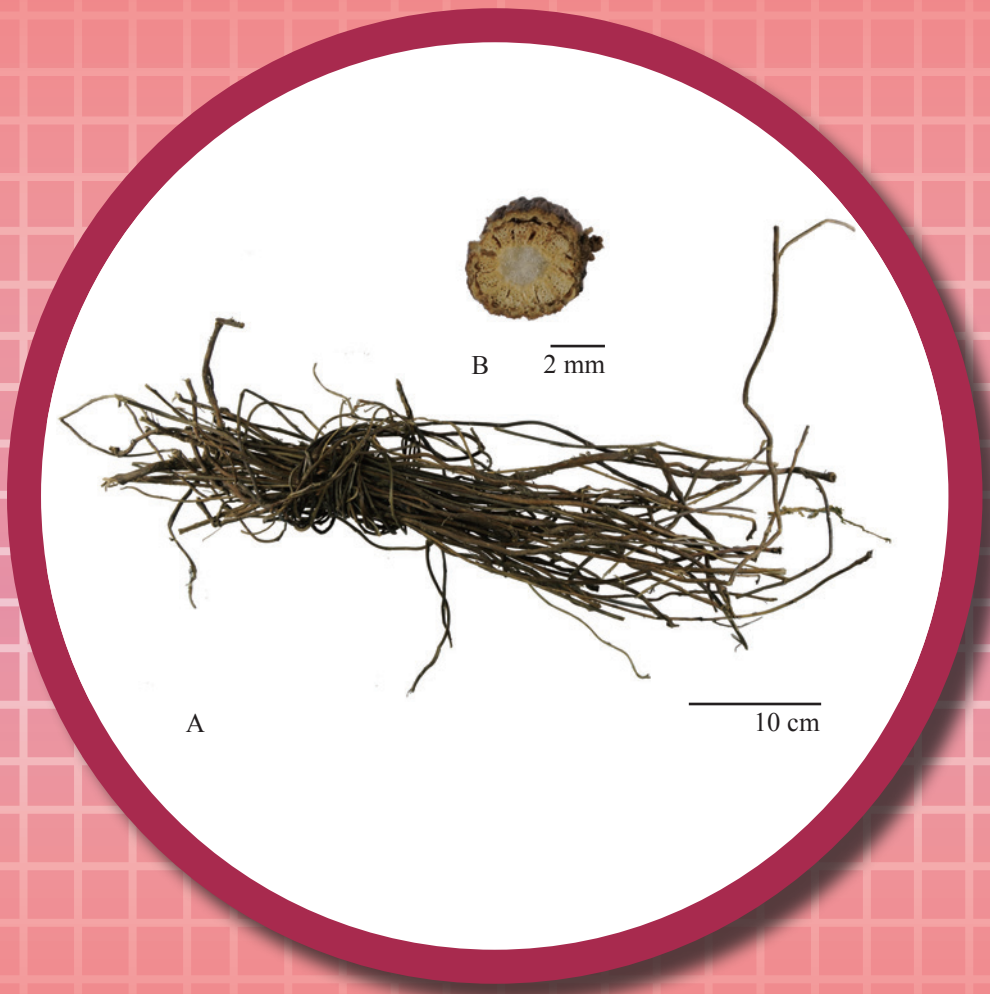


**Figure 1** A photograph of Menispermi Rhizoma

A. Menispermi Rhizoma

B. Magnified image of transverse section of rhizome

## 1. NAMES

Official Name: Menispermi Rhizoma

Chinese Name: 北豆根

Chinese Phonetic Name: Beidougen

## 2. SOURCE

Menispermi Rhizoma is the dried rhizome of *Menispermum dauricum* DC. (Menispermaceae). The rhizome is collected in spring and autumn, foreign matter removed, then dried under the sun to obtain Menispermi Rhizoma.

## 3. DESCRIPTION

Slender cylindrical, tortuous and branched, 12-120 cm long, 1-12 mm in diameter. Externally yellowish-brown to dark brown, mostly bearing numerous curved rootlets, and exhibiting prominent rootlet scars and longitudinal wrinkles, the outer bark easily exfoliated. Texture tough, uneasily broken, fracture uneven and fibrous, wood yellowish, arranged radially, medullated. Odour slight; taste bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse section**

Epidermis consists of 1 layer of cells, some cells suberized. Cortex relatively broad. Vascular bundles collateral. Pericyclic fibres cresent, located in the outer side of vascular bundles. Phloem relatively narrow. Cambium distinct. Xylem lignified. Pith relatively large, parenchymatous cells arranged loosely. Stone cell rare, singly scattered in cortex and pith (Fig. 2).

**Powder**

Colour pale yellowish-brown. Pericyclic fibres pale yellow, long-fusiform, 11-43 μm in diameter, tapering, blunt at the end, sometimes with septa, pits dotted. Xylem fibres relatively long, 8-32 μm in diameter, walls slightly thickened and lignified, with pits, pits obliquely slit-shaped or cruciform. Vessels mainly bordered-pitted, 18-99 μm in diameter. Cork cells colourless to yellowish-brown, rectangular, subsquare or polygonal in surface view. Stone cells scattered singly, pale yellow to yellow, rectangular or subrounded, 19-263 μm in diameter; yellowish-white under the polarized microscope (Fig. 3).

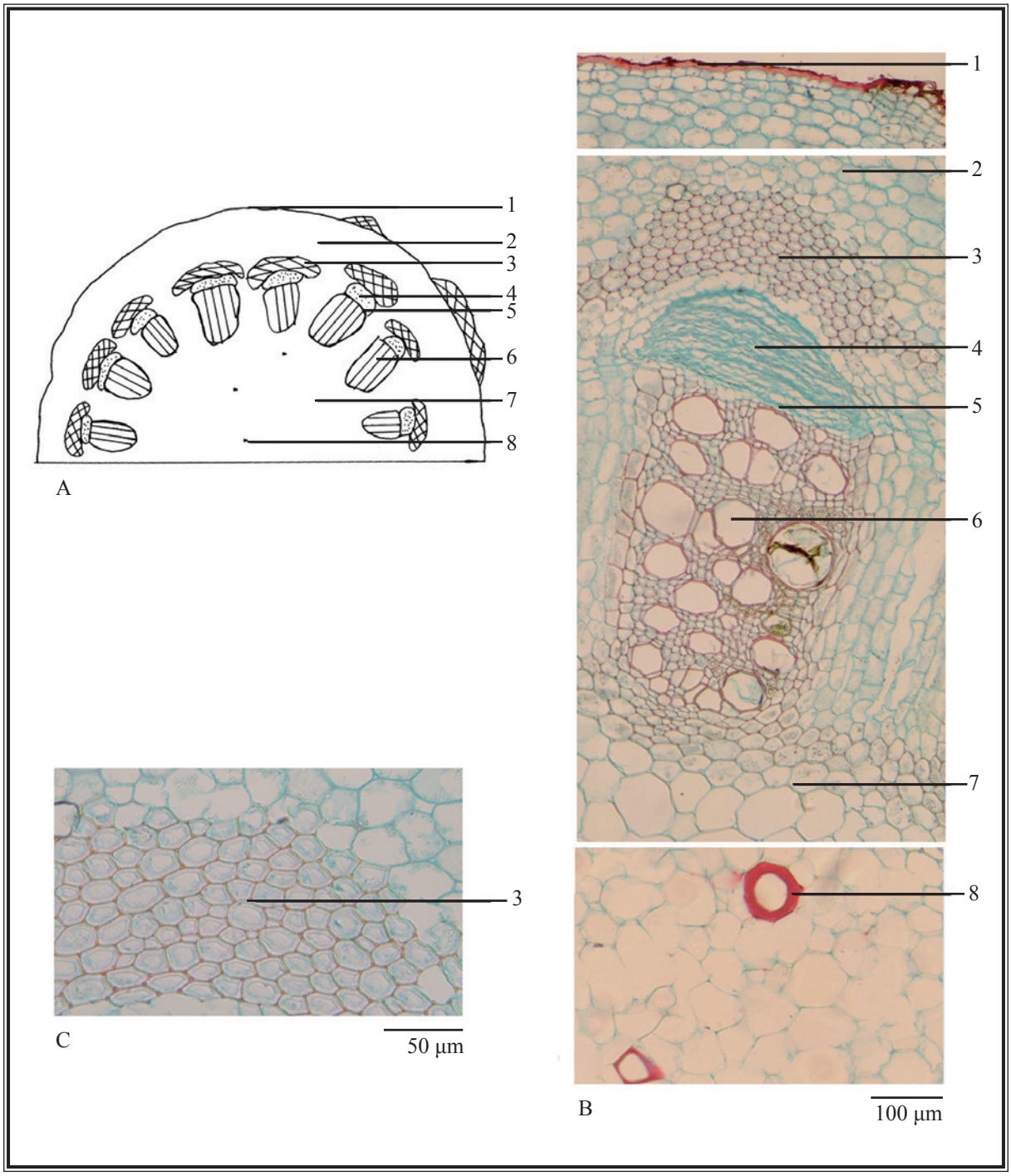


**Figure 2** Microscopic features of transverse section of Menispermi Rhizoma

A. Sketch B. Section illustration C. Pericyclic fibres

1. Epidermis 2. Cortex 3. Pericyclic fibres 4. Phloem 5. Cambium 6. Xylem 7. Pith 8. Stone cell

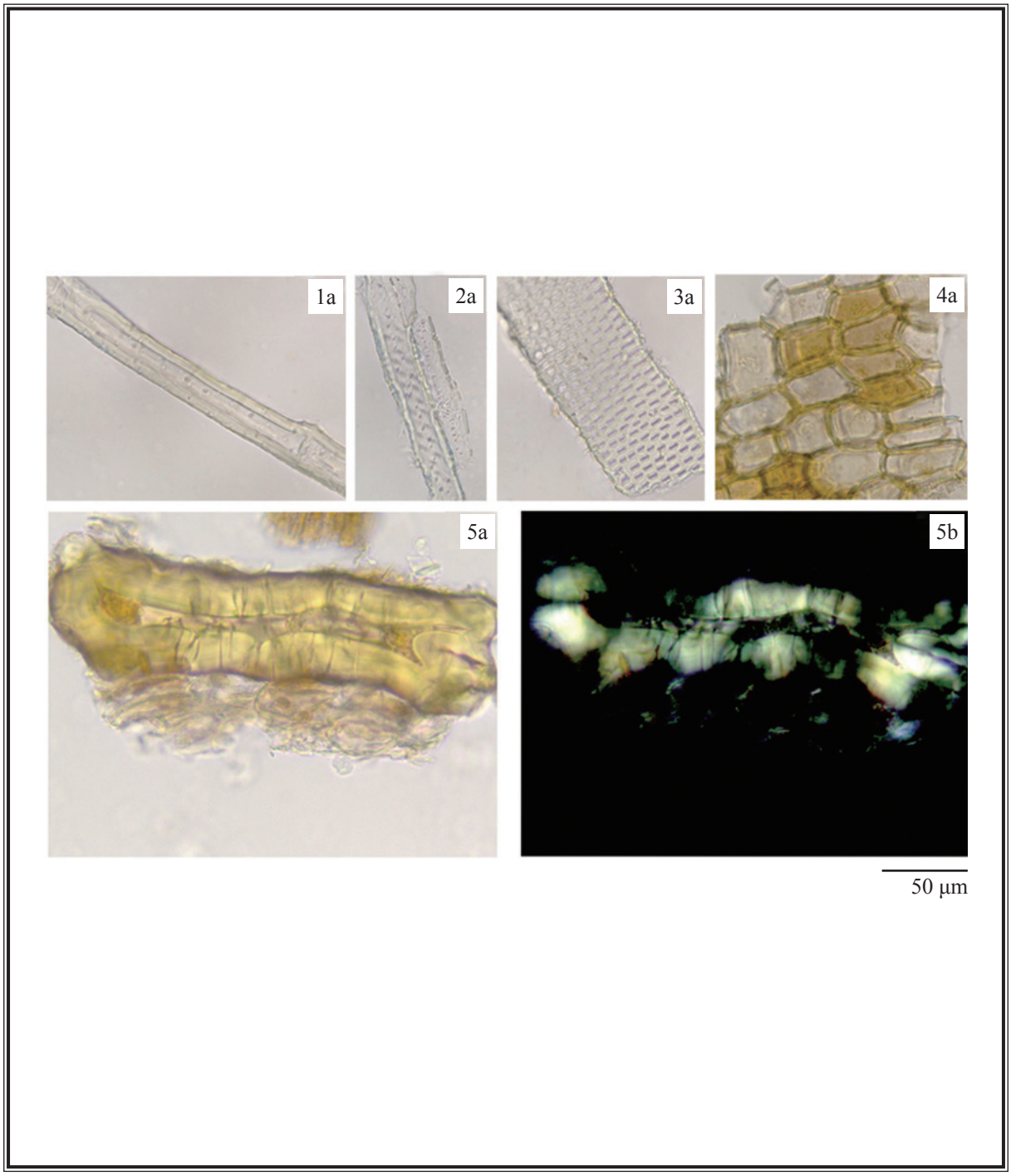


**Figure 3** Microscopic features of powder of Menispermi Rhizoma

1. Pericylic fibre 2. Xylem fibres 3. Bordered-pitted vessel 4. Cork cells 5. Stone cell

a. Features under the light microscope b. Features under the polarized microscope

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

**Standard solutions**

*Dauricine standard solution*

Weigh 1.0 mg of dauricine CRS (Fig. 4) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with ethanol.

*Daurisoline standard solution*

Weigh 1.0 mg of daurisoline CRS (Fig. 4) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with ethanol.

**Developing solvent system**

Prepare a mixture of ammonium hydroxide solution (25%, v/v), methanol and dichloromethane (0.1:1:8, v/v).

**Staining reagent**

Iodine.

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask wrapped in aluminium foil, then add 10 mL of methanol. Sonicate (150 W) the mixture for 1 h. Filter the mixture.

**Procedure**

Carry out the method by using a HPTLC silica gel $F_{254}$ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately dauricine standard solution (1 μL), daurisoline standard solution (1 μL) and the test solution (5 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Fumigate the plate with iodine vapour chamber for about 10 min until the spots or bands become visible. Examine the plate under visible light. Calculate the $R_f$ values by using the equation as indicated in Appendix IV (A).

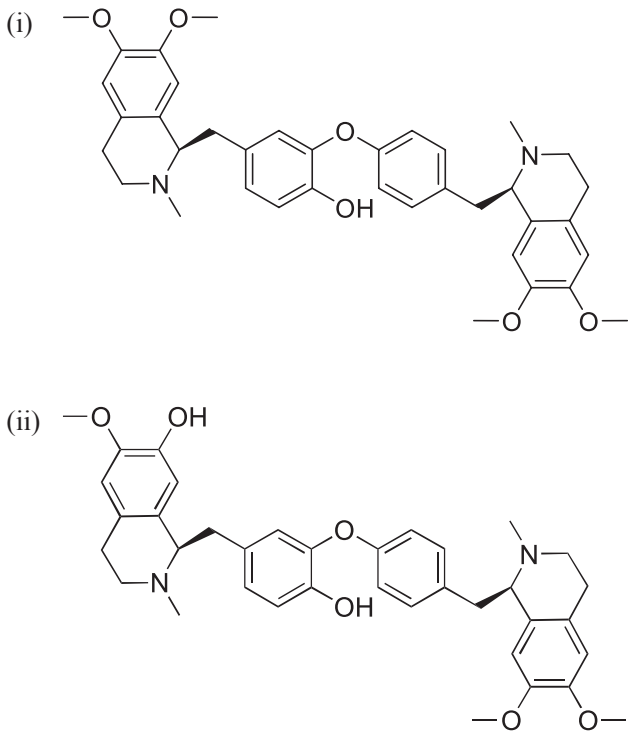


**Figure 4** Chemical structures of (i) dauricine and (ii) daurisoline

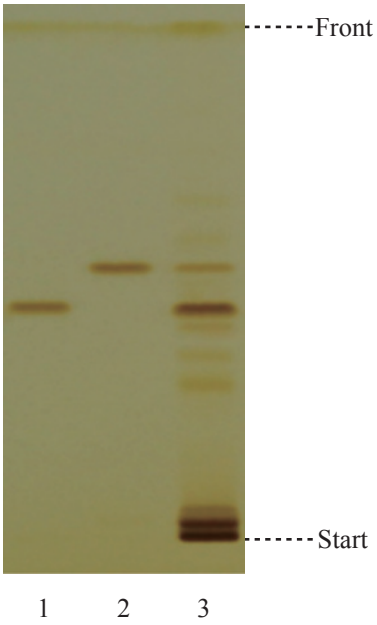


**Figure 5** A reference HPTLC chromatogram of Menispermi Rhizoma extract observed under visible light after staining

1. Daurisoline standard solution 2. Dauricine standard solution
3. Test solution

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 dauricine and daurisoline (Fig. 5).

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

**Standard solutions**

*Dauricine standard solution for fingerprinting, Std-FP (40 mg/L)*

Weigh 0.4 mg of dauricine CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with ethanol (50%).

*Daurisoline standard solution for fingerprinting, Std-FP (90 mg/L)*

Weigh 0.9 mg of daurisoline CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with ethanol (50%).

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL amber glass volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (282 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

**Table 1** Chromatographic system conditions

| Time (min) | 0.07% Trifluoroacetic acid (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 30 | 95 → 83 | 5 → 17 | linear gradient |
| 30 – 60 | 83 → 75 | 17 → 25 | linear gradient |

**System suitability requirements**

Perform at least five replicate injections, each using 5 μL of dauricine Std-FP and daurisoline Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of dauricine and daurisoline should not be more than 5.0%; the RSD of the retention times of dauricine and daurisoline peaks should not be more than 2.0%; the column efficiencies determined from dauricine and daurisoline peaks should not be less than 200000 theoretical plates.

The *R* value between peak 4 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. 6).

**Procedure**

Separately inject dauricine Std-FP, daurisoline Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of dauricine and daurisoline peaks in the chromatograms of dauricine Std-FP, daurisoline Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify dauricine and daurisoline peaks in the chromatogram of the test solution by comparing

its retention time with that in the chromatograms of dauricine Std-FP and daurisoline Std-FP. The retention times of dauricine and daurisoline 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 Menispermi Rhizoma extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the five characteristic peaks of Menispermi Rhizoma extract

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 | 0.66 | ± 0.03 |
| 2 | 0.73 | ± 0.03 |
| 3 | 0.84 | ± 0.03 |
| 4 (marker, daurisoline) | 1.00 | - |
| 5 (dauricine) | 1.07 | ± 0.03 |

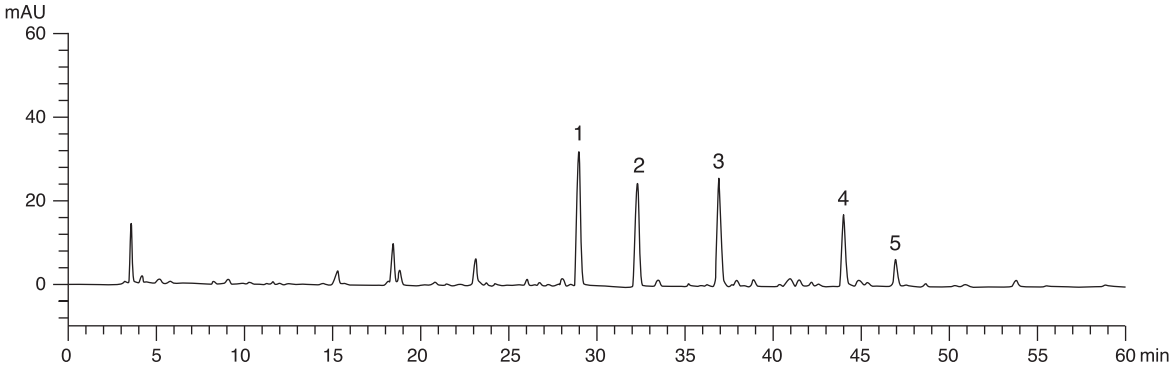


**Figure 6** A reference fingerprint chromatogram of Menispermi Rhizoma extract

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. 6).

## 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 XVI)*: meet the requirements.

**5.5 Foreign Matter** *(Appendix VIII)*: not more than 2.0%.

**5.6 Ash** *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 2.0%.

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

Oven dried method: not more than 12.0%.

## 6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 13.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

## 7. ASSAY

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

**Standard solution**

*Mixed dauricine and daurisoline standard stock solution, Std-Stock (400 mg/L for dauricine and 1000 mg/L for daurisoline)*

Weigh accurately 4.0 mg of dauricine CRS and 10.0 mg of daurisoline CRS, and place it in a 10-mL amber glass volumetric flask. Make up to the mark with ethanol (50%).

*Mixed dauricine and daurisoline standard solution for assay, Std-AS*

Measure accurately the volume of the mixed dauricine and daurisoline Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 2, 6, 24, 36, 60 mg/L for dauricine and 15, 30, 60, 120, 150 mg/L for daurisoline.

**Test solution**

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL amber glass volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (282 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

**Table 3** Chromatographic system conditions

| Time (min) | 0.07% Trifluoroacetic acid (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 30 | 95 → 83 | 5 → 17 | linear gradient |
| 30 – 60 | 83 → 75 | 17 → 25 | linear gradient |

**System suitability requirements**

Perform at least five replicate injections, each using 5 μL of the mixed dauricine and daurisoline Std-AS (24 mg/L for dauricine and 60 mg/L for daurisoline). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of dauricine and daurisoline should not be more than 5.0%; the RSD of the retention times of dauricine and daurisoline peaks should not be more than 2.0%; the column efficiencies determined from dauricine and daurisoline peaks should not be less than 200000 theoretical plates.

The *R* value between dauricine peak and the closest peak; and the *R* value between daurisoline 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 dauricine and daurisoline Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dauricine and daurisoline against the corresponding concentrations of the mixed dauricine and daurisoline Std-AS. Obtain the slopes, y-intercepts and the $r^2$ values from the corresponding 5-point calibration curves.

**Procedure**

Inject 5 μL of the test solution into the HPLC system and record the chromatogram. Identify dauricine and daurisoline peaks in the chromatogram of the test solution by comparing their retention times

with those in the chromatogram of the mixed dauricine and daurisoline Std-AS. The retention times of dauricine and daurisoline 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 dauricine and daurisoline in the test solution, and calculate the percentage contents of dauricine and daurisoline in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The sample contains not less than 1.0% of the total content of dauricine ($C_{38}H_{44}N_2O_6$) and daurisoline ($C_{37}H_{42}N_2O_6$), calculated with reference to the dried substance.