Asteris Radix et Rhizoma

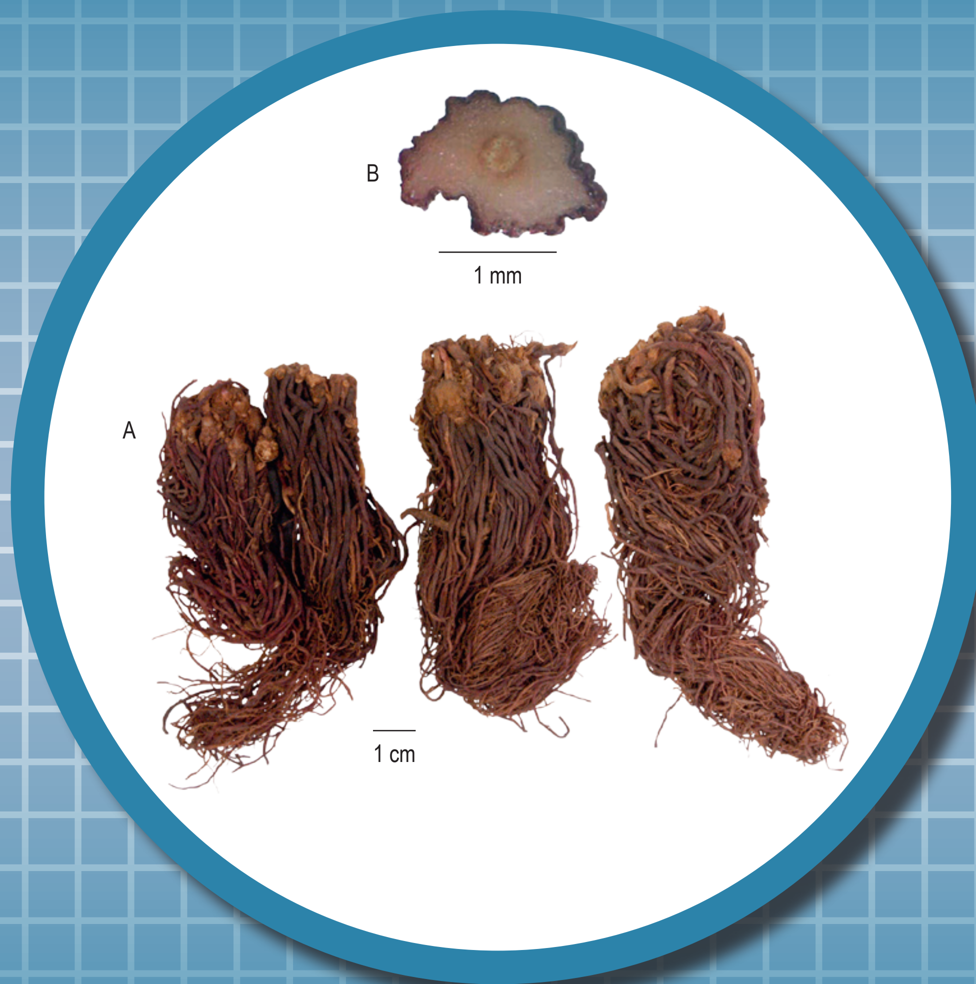

Figure 1 A photograph of Asteris Radix et Rhizoma

A. Asteris Radix et Rhizoma B. Magnified transverse section of root

1. NAMES

Official Name: Asteris Radix et Rhizoma

Chinese Name: 紫菀

Chinese Phonetic Name: Ziwan

2. SOURCE

Asteris Radix et Rhizoma is the dried root and rhizome of *Aster tataricus* L. f. (Asteraceae). The root and rhizome are collected in spring and autumn, knotty rhizome and soil removed and washed, then braided and dried under the sun to obtain Asteris Radix et Rhizoma.

3. DESCRIPTION

Rhizome in irregular masses, varying in size, apex with scar remnants of stems and leaves; texture slightly hard. Rhizomes bearing numerous, fascicular rootlets, 3-15 cm long, 1-3 mm in diameter, frequently braided, externally purplish-red to greyish-red, with longitudinal wrinkles; texture flexible. Odour slightly aromatic; taste sweet and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermal cells frequently withered or sometimes sloughing off, containing purplish-red pigments. Hypodermal cells 1 layer, somewhat elongated tangentially, lateral and inner walls slightly thickened, some containing purplish-red pigments. Cortex broad, consisting 19-21 layers of cells, with intercellular spaces. Secretory ducts 4-5, located in the inner side of cortex, near the endodermis, opposite the vascular bundle. Endodermis distinct. Stele small; each phloem bundles arranged alternate with the arc of xylem respectively. Primary xylem tetrarch to polyarch, small vessels arranged closely; Metaxylem vessels up to 40 µm in diameter, arranged irregularly. Pith mainly consists of parenchymatous cells [Fig. 2 (i)].

Rhizome: Cork cells rectangular or subsquare, with slightly thickened wall. Cortex broad, secretory duct subrounded, several scattered in cortex, usually is located near the corresponding phloem. Parenchymatous cells sometimes contain clusters of calcium oxalate [Fig. 2 (ii)].

Powder

Colour pale brown to reddish-brown. Inulin fan-shaped or irregular, with weak radial striation; slightly bright blue under the polarized microscope. Hypodermal cells rectangle in surface view, anticlinal wall sinuously zigzag, frequently containing purplish-red pigment. Vessels mainly bordered-pitted, spiral or reticulate. Fibres mostly in bundle. Cork cells rectangular, occasionally polygonal or subsquare. Sclerenchymatous cells rectangular or oblong, wall slightly thickened. Clusters of calcium oxalate sometimes visible (Fig. 3).

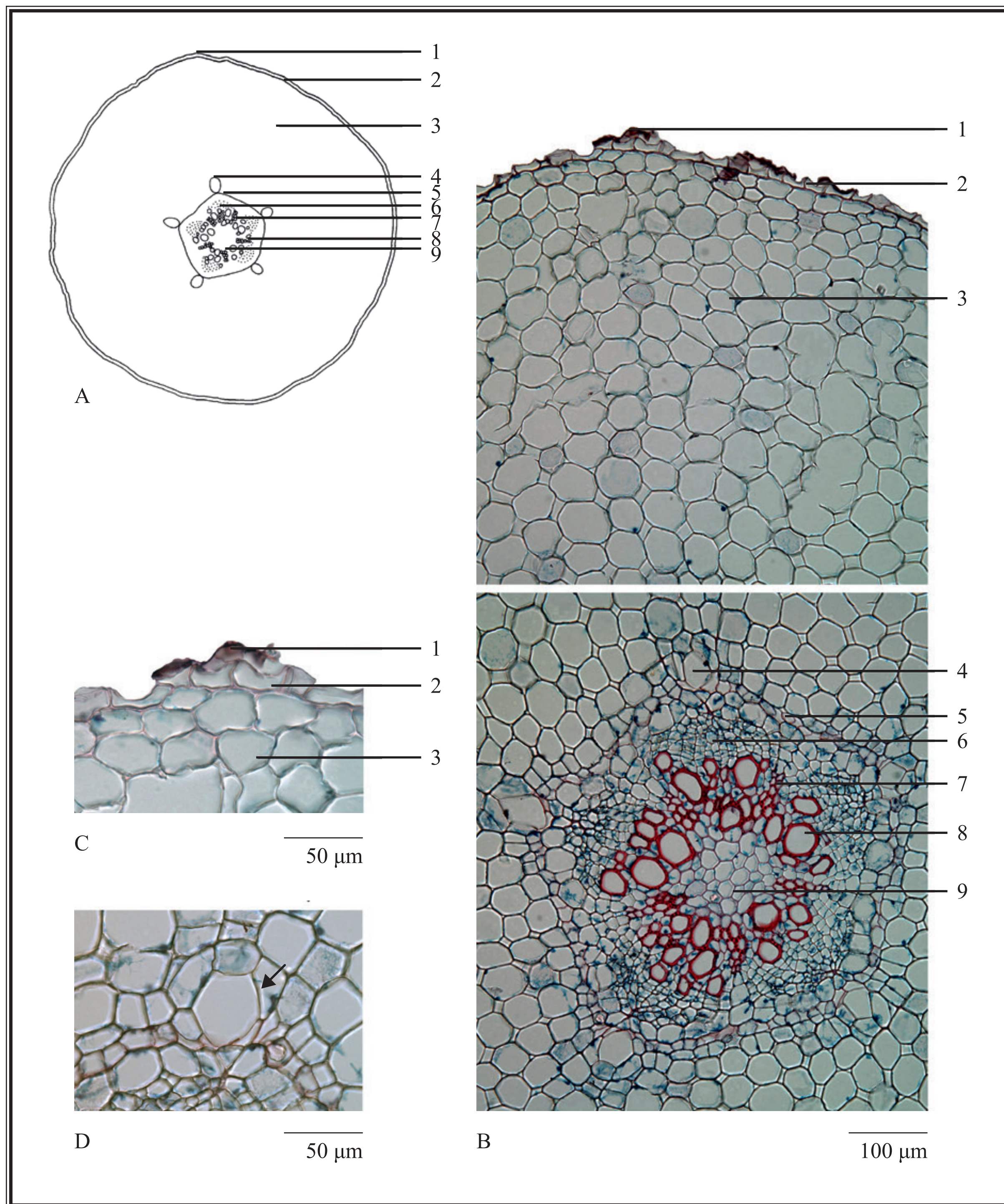

Figure 2 (i) Microscopic features of transverse section of root of Asteris Radix et Rhizoma

A. Sketch B. Section illustration C. Epidermis, hypodermis and cortex
D. Secretory duct

1. Epidermis 2. Hypodermis 3. Cortex 4. Secretory duct 5. Endodermis
6. Phloem 7. Primary xylem 8. Metaxylem 9. Pith

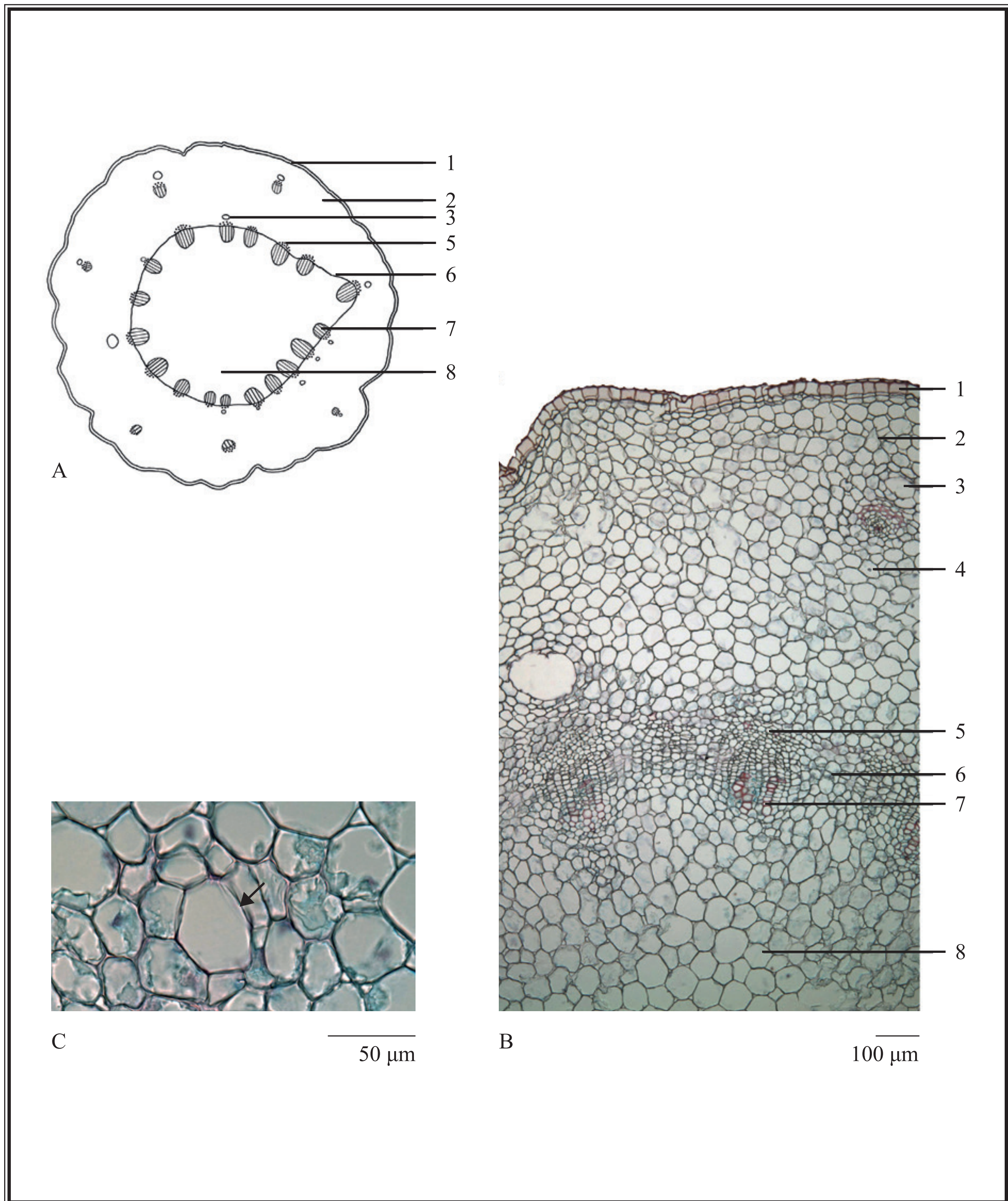

Figure 2 (ii) Microscopic features of transverse section of rhizome of Asteris Radix et Rhizoma

A. Sketch B. Section illustration C. Secretory duct

1. Cork 2. Cortex 3. Secretory duct 4. Clusters of calcium oxalate 5. Phloem
6. Cambium 7. Xylem 8. Pith

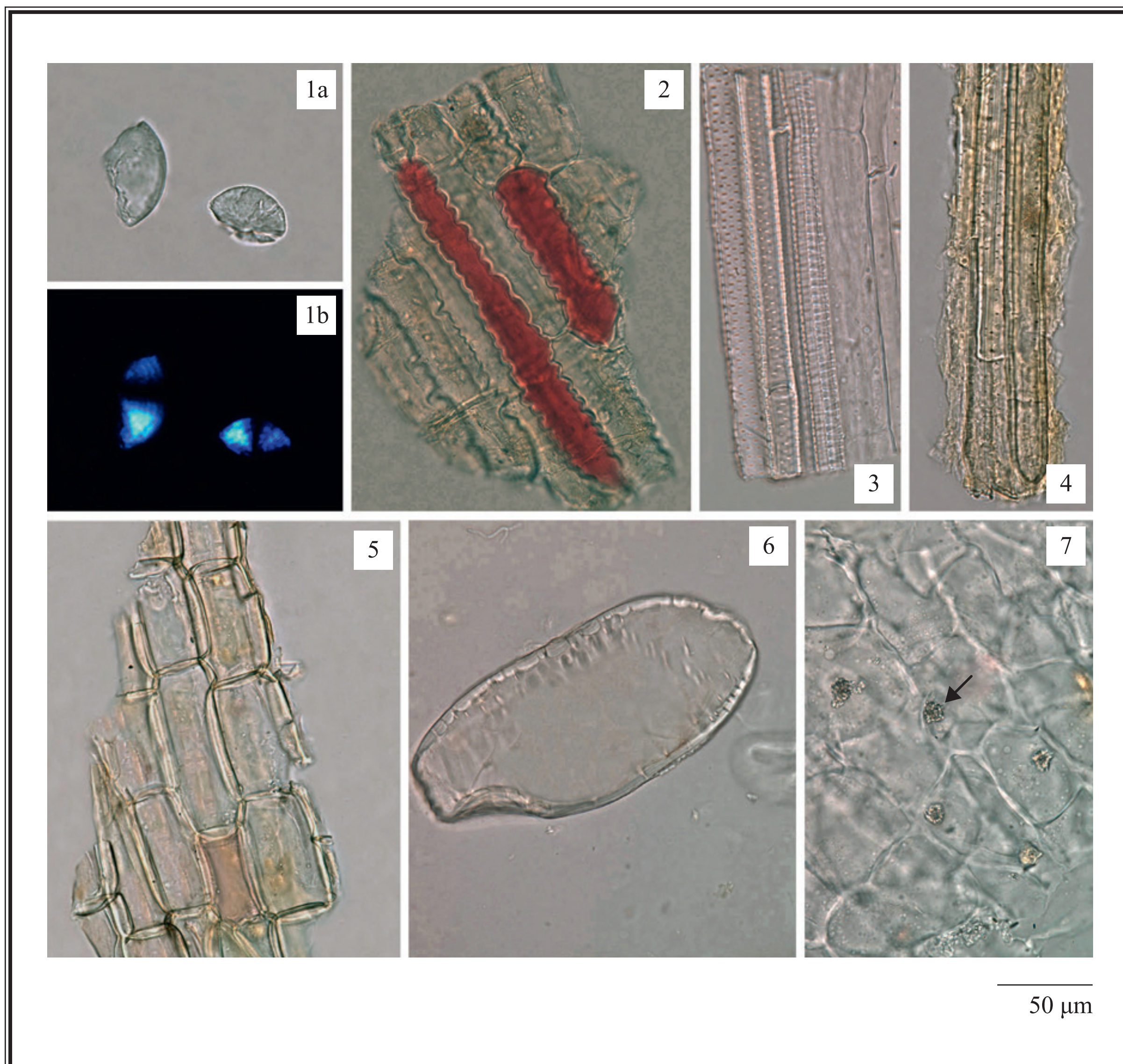

Figure 3 Microscopic features of powder of Asteris Radix et Rhizoma

1. Inulin 2. Hypodermal cells with purplish-red pigments 3. Vessels
4. Fibres 5. Cork cells 6. Sclerenchymatous cell 7. Clusters of calcium oxalate

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

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

Standard solution

Shionone standard solution

Weigh 1.0 mg of shionone CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of n-hexane and acetone (15:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol. Sonicate (160 W) the mixture for 30 min. 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 ethyl acetate.

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 shionone standard solution and the test solution (3 μL each) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of shionone.

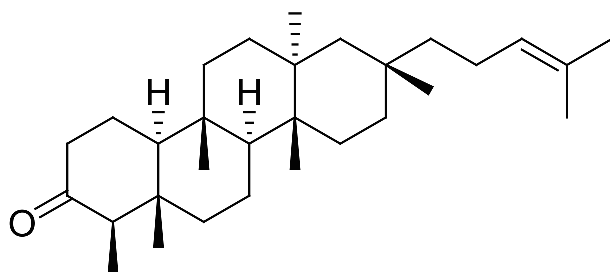

Figure 4 Chemical structure of shionone

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

Standard solution

Shionone standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 0.6 mg of shionone CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (160 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 methanol. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (200 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 - 2.5 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Flow rate (mL/min)	Elution
0 – 20	70 → 0	30 → 100	1.0 → 1.5	linear gradient
20 – 25	0	100	1.5 → 2.5	linear gradient
25 – 45	0	100	2.5	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of shionone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of shionone should not be more than 5.0%; the RSD of the retention time of shionone peak should not be more than 2.0%; the column efficiency determined from shionone peak should not be less than 25000 theoretical plates.

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 shionone Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of shionone peak in the chromatogram of shionone Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify shionone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of shionone Std-FP. The retention times of shionone 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 five characteristic peaks of Asteris Radix et Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Asteris Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.23	± 0.03
2	0.39	± 0.03
3	0.51	± 0.03
4	0.67	± 0.03
5 (marker, shionone)	1.00	-

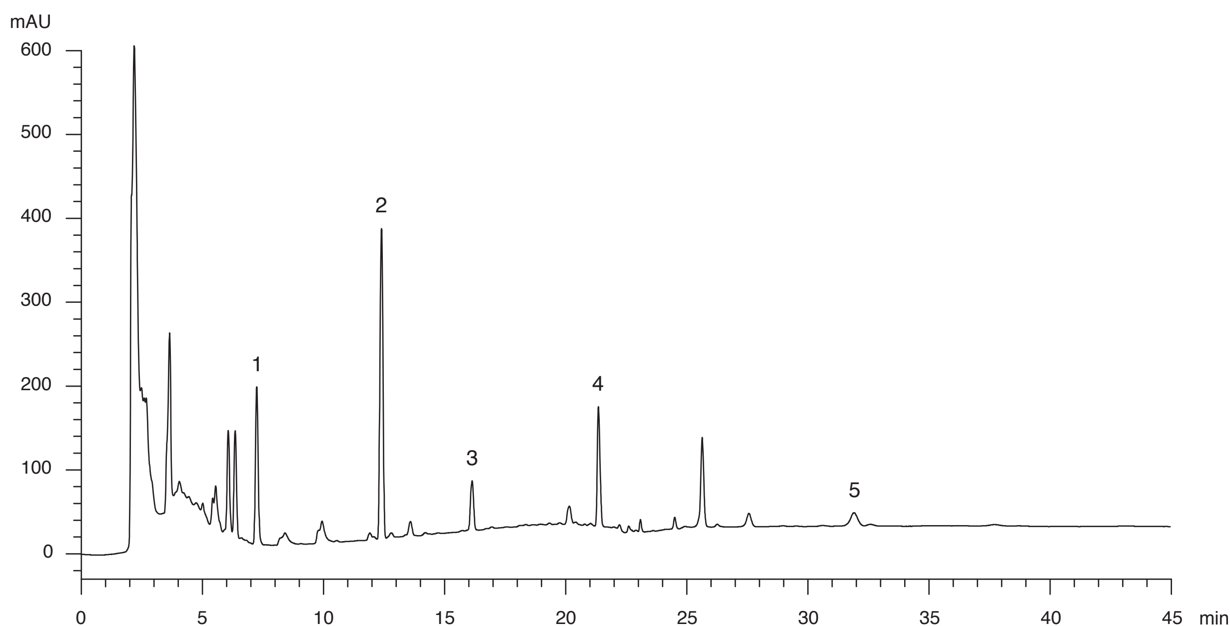

Figure 5 A reference fingerprint chromatogram of Asteris Radix et Rhizoma extract

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. 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 XVII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.5%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 53.0%.
Ethanol-soluble extractives (hot extraction method): not less than 33.0%.

7. ASSAY

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

Standard solution

Shionone standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 1.0 mg of shionone CRS and dissolve in 1 mL of methanol.

Shionone standard solution for assay, Std-AS

Measure accurately the volume of the shionone Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 25, 60, 200, 375 mg/L for shionone.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (160 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 methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (200 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 - 2.5 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Flow rate (mL/min)	Elution
0 – 20	70 → 0	30 → 100	1.0 → 1.5	linear gradient
20 – 25	0	100	1.5 → 2.5	linear gradient
25 – 45	0	100	2.5	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of shionone Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of shionone should not be more than 5.0%; the RSD of the retention time of shionone peak should not be more than 2.0%; the column efficiency determined from shionone peak should not be less than 25000 theoretical plates.

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

Calibration curve

Inject a series of shionone Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of shionone against the corresponding concentrations of shionone Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify shionone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of shionone Std-AS. The retention times of shionone peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of shionone in the test solution, and calculate the percentage content of shionone in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.15% of shionone ($C_{30}H_{50}O$), calculated with reference to the dried substance.