Figure 29　A photograph of Radix Salviae Miltiorrhizae

1. NAMES

Official Name: Radix Salviae Miltiorrhizae

Chinese Name: 丹參

Chinese Phonetic Name: Danshen

2. SOURCE

Radix Salviae Miltiorrhizae is the dried root and rhizome of *Salvia miltiorrhiza* Bge. (Fam. Lamiaceae) (Labiatae). The root and rhizome is collected or harvested in spring and autumn. The stems, rootlets and soil removed, then dried to obtain Radix Salviae Miltiorrhizae.

3. DESCRIPTION

Rhizomes thick and short; sometimes with remains of a stem at the apex. Roots several, long and cylindrical, slightly curved, some branched and with rootlets; 10–20 cm in length and 3–10 mm in diameter. Externally brownish-red or dull red, rough with longitudinal wrinkles. The bark of old roots loose, mostly purplish-brown, usually scaling off. Texture hard and fragile, easily broken, fracture loose, and with clefts, fairly even and dense, with brownish-red bark and greyish-yellow or dull purplish-brown wood, showing bundles of vessels, yellowish-white, arranged radially. Odour, slight; taste, slightly bitter and astringent. (Fig. 29)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Cork consisting of 4–8 layers of cork cells with brown contents, rhytidome tissue sometimes visible. Cortex broad, parenchymatous cells contain reddish-brown granules. Phloem narrow and crescent-shaped. Cambium in a ring. Xylem vessels lignified, mainly scalariform and reticulated, occuring near the cambium ring and gradually reduced near the central part of xylem. Xylem fibres in bundles, scattered radially. Pith in center. (Fig. 30)

Powder

Reddish-brown. Surface view of cork cells subrectangular or polygonal, containing yellowish-brown pigment, 12–151 μm in diameter. Parenchymatous cells in cortex subsquare or polygonal, containing reddish-brown pigmental sediments. Xylem fibers usually in bundles, long fusiform, with oblique or criss-cross striations, 11–60 μm in diameter, vivid yellow when examined under a polarizing microscope. Numerous mainly bordered or reticulated vessels, 3–120 μm in diameter. (Fig. 31)

4.2 Physicochemical Identification

Reagent

Iron (III) chloride solution

Dissolve 9.0 g of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in 100 mL of water.

Procedure

Weigh 3.0 g of the powdered sample and put into a 100-mL round-bottomed flask, then add 30 mL of water. Assemble a reflux setup and boil the mixture for 15–20 min. Cool, filter and collect the filtrate in an evaporating dish. Evaporate to dryness on a water bath maintained at about 80 ˚C. Dissolve the residue in 3–5 mL of ethanol. Transfer 2 mL of the test solution to a test tube and add 2 drops of iron (III) chloride solution. A dark green solution is observed.

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

4.3.1 Thin-Layer Chromatographic Identification of Lipid-Soluble Components

Standard solutions

Cryptotanshinone standard solution

Weigh 1.0 mg of cryptotanshinone and dissolve in 1 mL of methanol.

Tanshinone II_A standard solution

Weigh 1.0 mg of tanshinone II_A and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60–80 ˚C), ethyl acetate and cyclohexane (5:3:2, v/v).

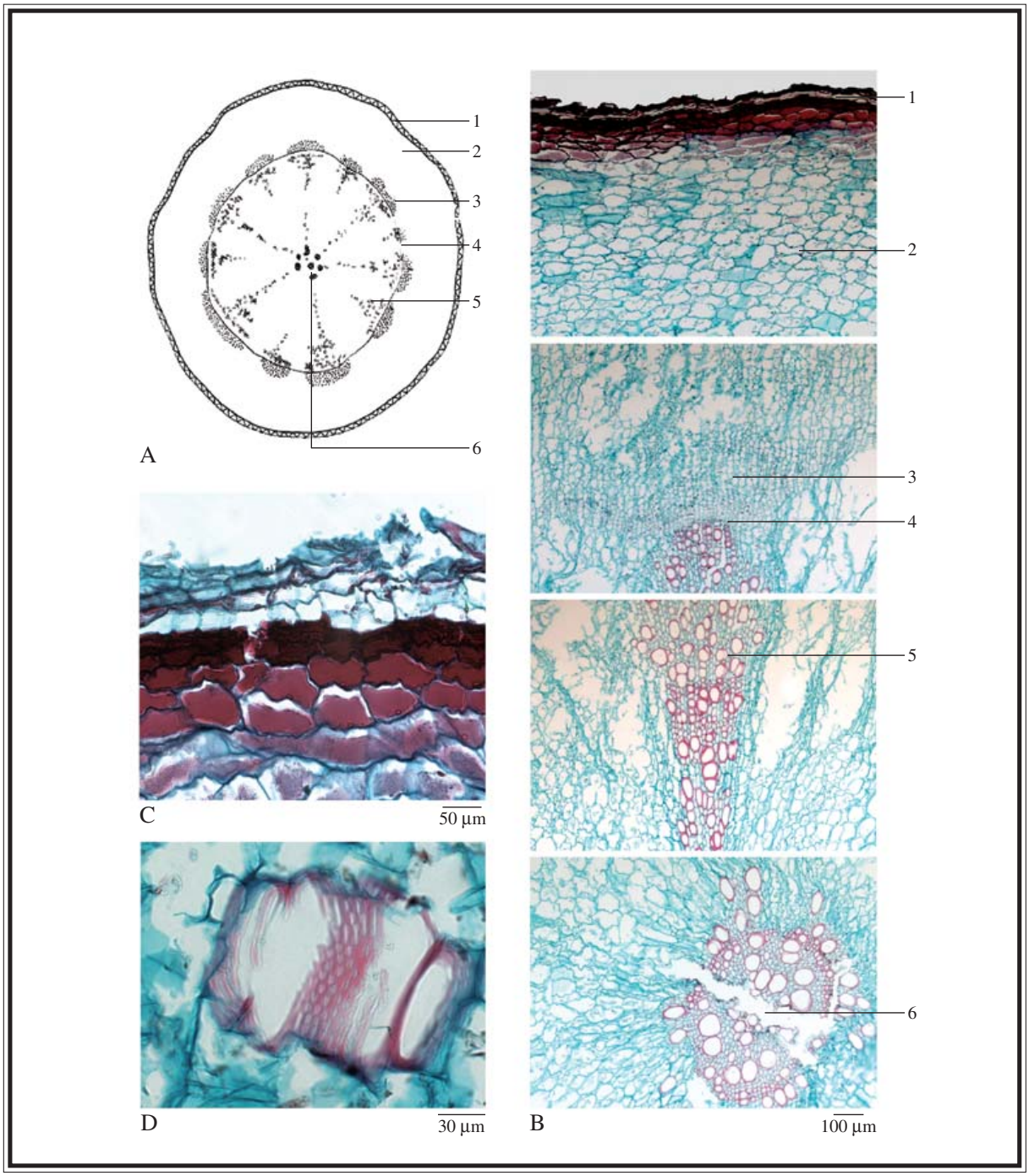

Figure 30 Microscopic features of transverse section of Radix Salviae Miltiorrhizae

A. Sketch B. Section illustration C. Cork D. Reticulated vessel

1. Cork 2. Cortex 3. Phloem 4. Cambium 5. Xylem 6. Pith

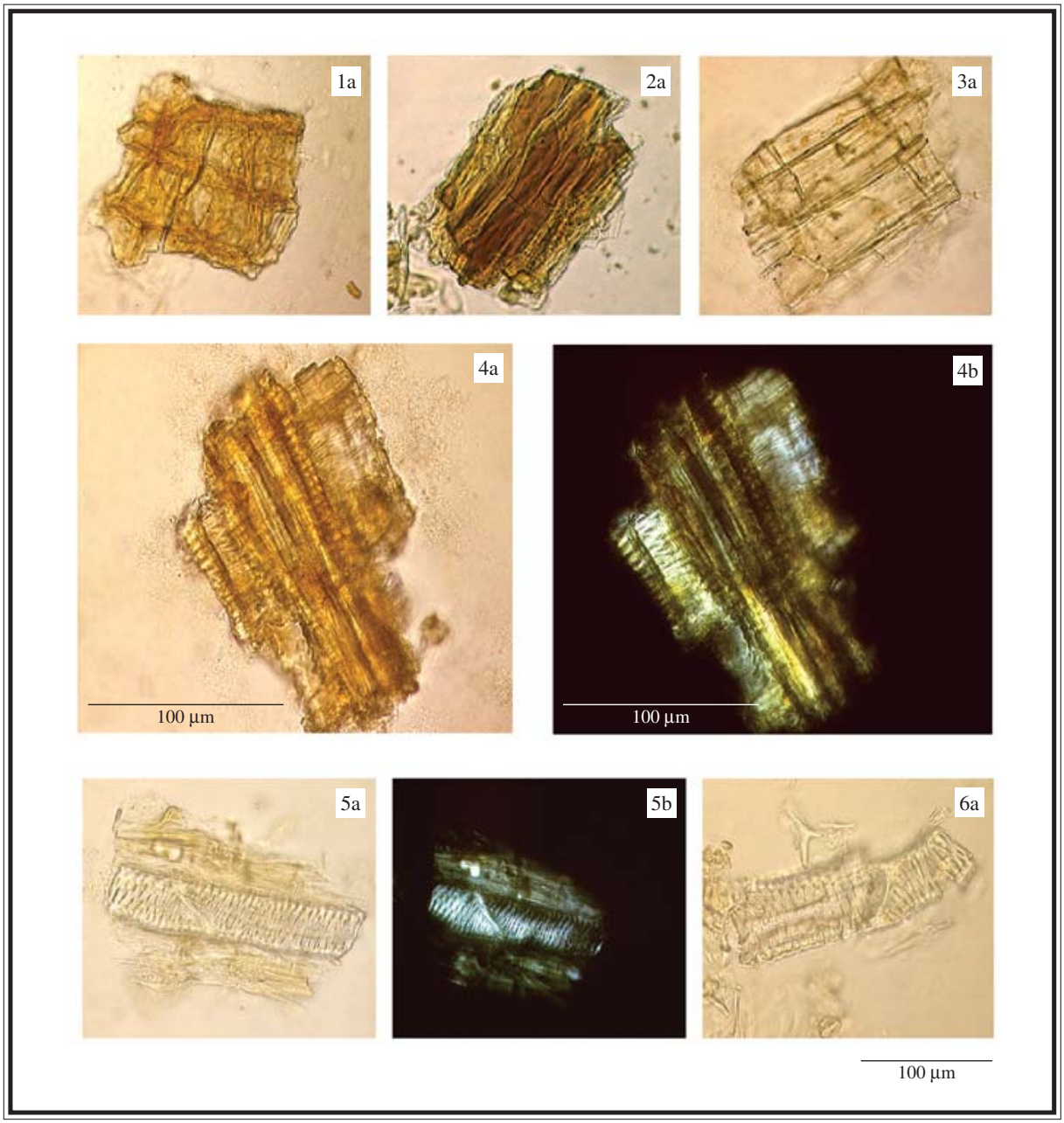

Figure 31 Microscopic features of powder of Radix Salviae Miltiorrhizae

1. Cork cells (surface view) 2. Cork cells (lateral view) 3. Parenchymatous cells in cortex
4. Xylem fibres in a bundle 5. Reticulated vessel 6. Bordered vessels

a. Features under a light microscope b. Features under a polarizing microscope

Test solution

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of diethyl ether. Close the cap of the tube, shake occasionally for 1 h and then centrifuge at about 1200 x *g* for 10 min. Transfer the supernatant to a new tube and evaporate to dryness on a warm water bath maintained at a temperature below 50 ˚C. Dissolve the residue in 0.5 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Develop over a path of about 4 cm. Apply separately cryptotanshinone standard solution, tanshinone II_A standard solution and the test solution (4 µL each) to the plate. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate in 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 cryptotanshinone and tanshinone II_A.

4.3.2 Thin-Layer Chromatographic Identification of Water-Soluble Components

Standard solutions

Rosmarinic acid standard solution

Weigh 2.0 mg of rosmarinic acid and dissolve in 1 mL of methanol.

Salvianolic acid B standard solution

Weigh 4.0 mg of salvianolic acid B and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of chloroform, ethyl acetate, toluene, formic acid and methanol (15:20: 10:10:1, v/v).

Spray reagent

Dissolve 3.3 g of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in 100 mL of ethanol (96%).

Test solution

Weigh 1.0 g of the powdered sample and put into a 200-mL beaker, then add 50 mL of water. Cover the beaker with a watch glass. Boil gently for 30 min. Cool to room temperature and transfer to a 50-mL centrifugal tube. Centrifuge at about 1200 x *g* for 10 min. Collect the supernatant and adjust the pH to about 2 with 2 M hydrochloric acid.

After filtration, extract the water solution three times each with 10 mL of ethyl acetate. Combine the extracts and concentrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 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. Develop over a path of about 8 cm. Apply separately rosmarinic acid standard solution, salvianolic acid B standard solution and the test solution (2 µL each) to the plate. 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, then heat at about 110 ˚C for 10 min. Examine the plate in visible light. 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 rosmarinic acid and salvianolic acid B.

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

Standard solution

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

Weigh 5.0 mg of rosmarinic acid and dissolve in 5 mL of methanol. Store at about -10 ˚C in the dark.

Rosmarinic acid standard solution for fingerprinting, Std-FP (100 mg/L)

Pipette 0.5 mL of rosmarinic acid Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate the mixture for 30 min. Centrifuge at about 1200 x *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with 10 mL of water in a 50-mL beaker and weigh. Cover the beaker with a watch glass. Extract the residue at about 90 ˚C for 30 min. After cooling, weigh again. Add an appropriate amount of water to compensate the weight loss, if any. Filter the extract to the same volumetric flask. Make up to the mark with methanol. Keep the solution overnight at around 4 ˚C. Filter through a 0.2-µm PTFE filter. Allow to stand to room temperature.

Chromatographic system

The liquid chromatograph is equipped with a detector (280 nm) and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Water : acetonitrile : formic acid (90:10:0.4, v/v) (%, v/v)	Acetonitrile (%, v/v)	Elution
0–40	100 ➔ 70	0 ➔ 30	linear gradient
40–50	70 ➔ 20	30 ➔ 80	linear gradient
50–70	20 ➔ 15	80 ➔ 85	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should be not more than 3.0%; the RSD of the retention time of rosmarinic acid peak should be not more than 2.0%; the column efficiency determined from rosmarinic acid peak should be not less than 50,000 theoretical plates.

The *R* value between rosmarinic acid peak and peak 3 (Fig. 32) in the test solution should be not less than 1.5.

Procedure

Separately inject rosmarinic acid Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of rosmarinic acid peak in the chromatogram of rosmarinic acid Std-FP and the retention times of the seven characteristic peaks (Fig. 32) in chromatogram of the test solution. Under the same HPLC conditions, identify the rosmarinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-FP. The retention times of rosmarinic acid 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 seven characteristic peaks of Radix Salviae Miltiorrhizae extract are listed in Table 10.

Table 10 The RRTs and acceptable ranges of the seven characteristic peaks of Radix Salviae Miltiorrhizae extract

Peak No.	RRT	Acceptable Range
1 (danshensu)	0.23	±0.03
2 (marker, rosmarinic acid)	1.00	-
3	1.03	±0.03
4 (salvianolic acid B)	1.10	±0.03
5	1.15	±0.03
6 (cryptotanshinone)	1.88	±0.03
7 (tanshinone II_A)	2.02	±0.04

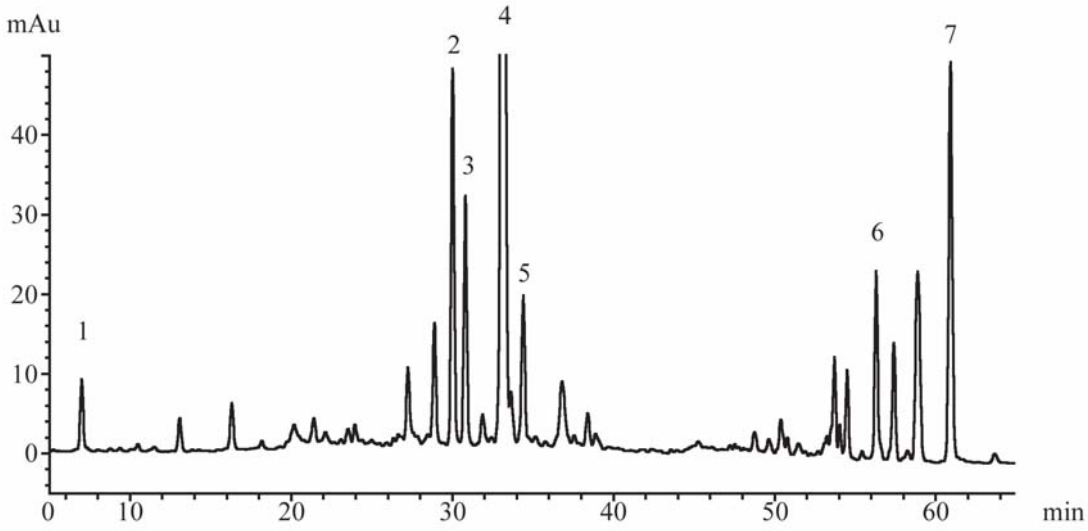

Figure 32 A reference fingerprint chromatogram of Radix Salviae Miltiorrhizae extract

For positive identification, the sample must give the above seven characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 32).

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 XIV)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 57.0%.
Ethanol-soluble extractives (cold extraction method): not less than 52.0%.

7. ASSAY

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

7.1 Assay of Tanshinone II_A

Standard solution

Tanshinone II_A standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 5.0 mg of tanshinone II_A and dissolve in 50 mL of methanol. Store at about -10 ˚C in the dark.

Tanshinone II_A standard solution for assay, Std-AS

Measure accurately the volume of the tanshinone II_A Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 20, 50, 100 mg/L for tanshinone II_A.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 10-mL centrifugal tube, then add 5 mL of a mixture of methanol and dichloromethane (8:2, v/v) and mix. Sonicate the mixture for 30 min. Centrifuge at about 540 x *g* for 5 min and transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for four more times. Combine the extracts. Make up to the mark with the mixed solvent described above. Mix and filter through a 0.2-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (270 nm) and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (75:25, v/v).

System suitability requirements

Perform at least five replicate injections each with 20 µL of tanshinone II_A Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of tanshinone II_A should be not more than 3.0%; the RSD of the retention time of tanshinone II_A peak should be not more than 2.0%; the column efficiency determined from tanshinone II_A peak should be not less than 2,000 theoretical plates.

The *R* value between tanshinone II_A peak and the closest peak in the test solution should be not less than 1.5.

Calibration curve

Inject a series of tanshinone II_A Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of tanshinone II_A against the corresponding concentrations of tanshinone II_A Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify tanshinone II_A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of tanshinone II_A Std-AS. The retention times of tanshinone II_A 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 tanshinone II_A in the test solution, and calculate the percentage content of tanshinone II_A in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.12% of tanshinone II_A ($C_{19}H_{18}O_3$), calculated with reference to the dried substance.

7.2 Assay of Rosmarinic Acid and Salvianolic Acid B

Standard solution

Rosmarinic acid standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 10.0 mg of rosmarinic acid and dissolve in 5 mL of methanol. Store at about -10 ˚C in the dark.

Salvianolic acid B standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 10.0 mg of salvianolic acid B and dissolve in 5 mL of methanol. Store at about -10 ˚C in the dark.

Mixed rosmarinic acid and salvianolic acid B standard intermediate solution, Std-Int (20 mg/L for rosmarinic acid and 400 mg/L for salvianolic acid B)

Pipette 0.1 mL of rosmarinic acid Std-Stock and 2 mL of salvianolic acid B Std-Stock in a 10-mL volumetric flask. Flush with nitrogen to evaporate the solvent. Make up to the mark with methanol (50%).

Mixed rosmarinic acid and salvianolic acid B standard solution for assay, Std-AS

Measure accurately the volume of the mixed rosmarinic acid and salvianolic acid B Std-Int, dilute with methanol (50%) to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for rosmarinic acid and 20, 40, 100, 200, 400 mg/L for salvianolic acid B.

Test solution

Weigh accurately 0.1 g of the powdered sample and put into a 10-mL centrifugal tube, then add 5 mL of methanol (60%) and mix. Sonicate the mixture for 30 min. Centrifuge at about 540 x *g* for 5 min and transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for three more times. Combine the extracts. Make up to the mark with the mixed solvent described above. Mix and filter through a 0.2-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (330 nm) and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of aqueous formic acid solution (0.4%, v/v) and acetonitrile (78:22, v/v).

System suitability requirements

Perform at least five replicate injections each with 20 μL of rosmarinic acid Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should be not more than 3.0%; the RSD of the retention time of rosmarinic acid

peak should be not more than 2.0%; the column efficiency determined from rosmarinic acid peak should be not less than 5,000 theoretical plates.

The *R* value between rosmarinic acid peak and the closest peak in the test solution should be not less than 1.5.

Calibration curves

Inject a series of the mixed rosmarinic acid and salvianolic acid B Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of rosmarinic acid and salvianolic acid B against the corresponding concentrations of the mixed rosmarinic acid and salvianolic acid B Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify rosmarinic acid peak and salvianolic acid B peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed rosmarinic acid and salvianolic acid B Std-AS. The retention times of rosmarinic acid peaks and salvianolic acid B peaks in both chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of rosmarinic acid and salvianolic acid B in the test solution, and calculate the percentage content of rosmarinic acid and salvianolic acid B in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.17% of rosmarinic acid ($C_{18}H_{16}O_8$) and not less than 4.4% of salvianolic acid B ($C_{36}H_{30}O_{16}$), calculated with reference to the dried substance.