Figure 13　A photograph of Radix Angelicae Sinensis

1. NAMES

Official Name: Radix Angelicae Sinensis

Chinese Name: 當歸

Chinese Phonetic Name: Danggui

2. SOURCE

Radix Angelicae Sinensis is the dried root of *Angelica sinensis* (Oliv.) Diels (Fam. Apiaceae) (Umbelliferae). The root is generally collected in late autumn after 2 years of plantation. The stems, leaf sheaths, rootlets and soil removed, slightly dried and tied up in small bundles, then placed on a shelf and smoke-dried to obtain Radix Angelicae Sinensis.

3. DESCRIPTION

The root is slightly cylindrical, externally yellowish-brown to reddish-brown, exhibiting longitudinal wrinkles and transversely elongated lenticels. The root stock is known as "guitou" ("Angelica head"), the main root is known as "guishen" ("Angelica body"), the branch root is known as "guiwei" ("Angelica tails"), the entire root is known as "quangui" ("entire Angelica"). Root stocks 15–40 mm in diameter, annulated, apex obtuse, showing purple or yellowish-green remains of stems and leaf sheaths. Thick and short main roots bear with numerous branch roots in the lower part; upper portion of branch root thick and lower portion thin, mostly twisted, with a few rootlets scars. Texture flexible, fracture yellowish-white or pale yellowish-brown. Thick bark, showing numerous brown spotted secretory cavities. Wood paler in colour than the bark, exhibiting with radial lines. Cambium ring yellowish-brown. Core of root stocks showing pith and cavity. Odour, strongly aromatic; taste, sweet, pungent and slightly bitter. (Fig. 13)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The transverse section shows cork consisting of 5–16 layers of cells. Cortex consists of 4–10 layers of cells, with a few scattered oil cavities. Phloem clefts, broad, oil cavities suborbicular, 16–460 μm in diameter, relatively large on the outer side, gradually becoming small inward, surrounded by 5–22 secretory cells; phloem rays 5–10 cells wide. Cambium in a ring. Xylem clefts; xylem rays 3–10 of cells wide. Vessels scattered singly or in groups of 2–5, arranged radially. (Fig. 14)

Powder

Yellowish-brown. Surface view of cork cells subpolygonal, yellowish in colour, varying in size. Sometimes fragments of oil cavities containing yellowish oil droplets or oil mass secretions visible, 16–460 μm in diameter. Annular, scalariform and reticulate vessels frequent. Pholem parenchymatous cells fusiform, walls fairly thick, with surface view showing very fine oblique crisscross striations, sometimes thin transverse septa visible. Most of starch grains composed of subspheroidal simple granules; showing a black, cross-shape under a polarizing microscope. (Fig. 15)

4.2 Physicochemical Identification

Procedure

Weigh 0.2 g of the powdered sample and put into a test tube, then add 2 mL of ethanol (70%). Sonicate the mixture for 60 min. Allow the solid residue to settle. Spot the supernatant onto a filter paper with a capillary tube. Examine the spot in UV light (254 nm), a fluorescent blue spot is observed.

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

4.3.1 Thin-Layer Chromatographic Identification of Lipid-Soluble Components

Standard solution

Z-ligustilide standard solution

Weigh 1.0 mg of Z-ligustilide and dissolve in 1 mL of methanol.

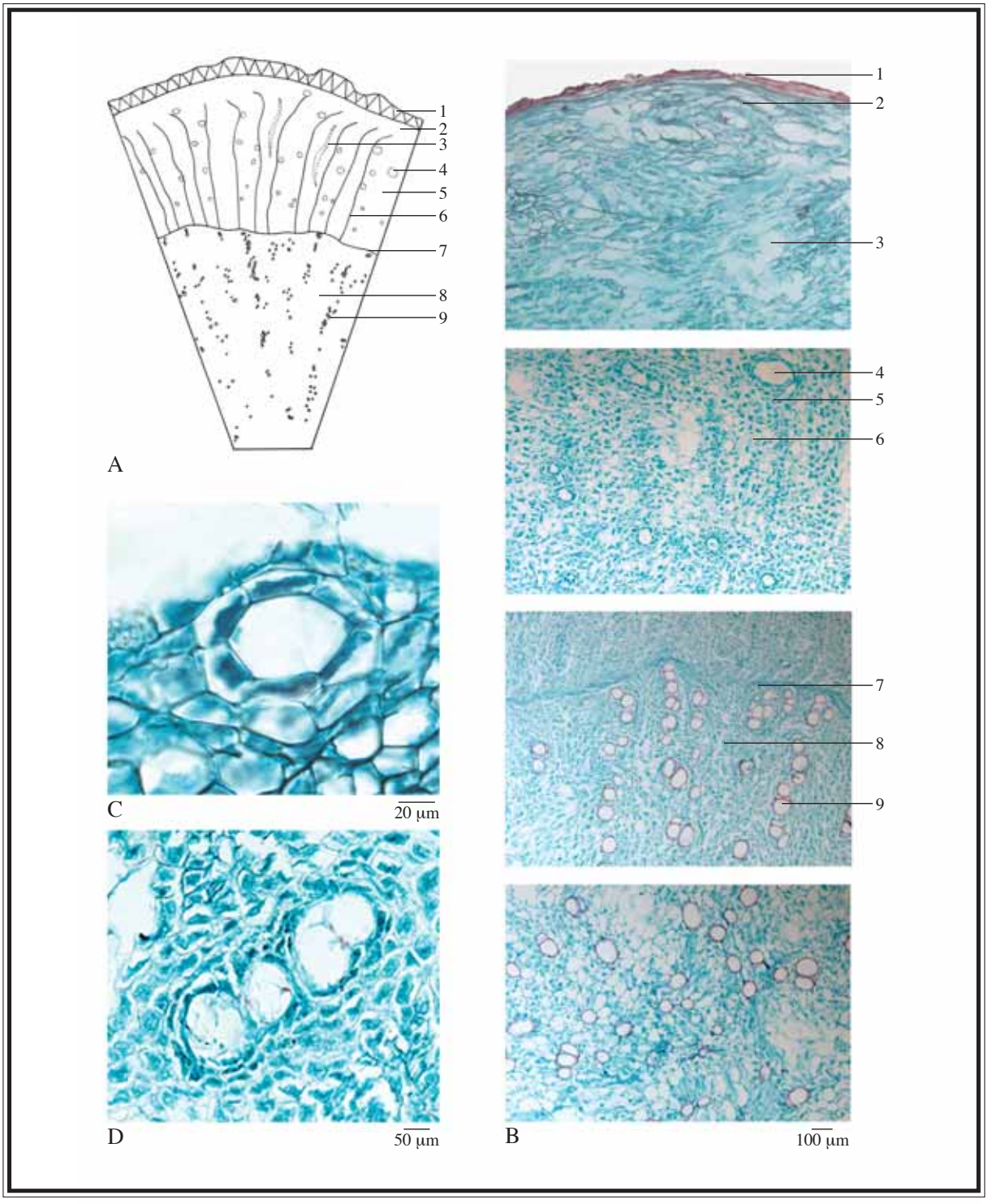

Figure 14 Microscopic features of transverse section of Radix Angelicae Sinensis

A. Sketch B. Section illustration C. Single oil cavity D. Oil cavities in a group

1. Cork 2. Cortex 3. Clefts 4. Oil cavity 5. Phloem 6. Phloem rays 7. Cambium 8. Xylem rays 9. Xylem

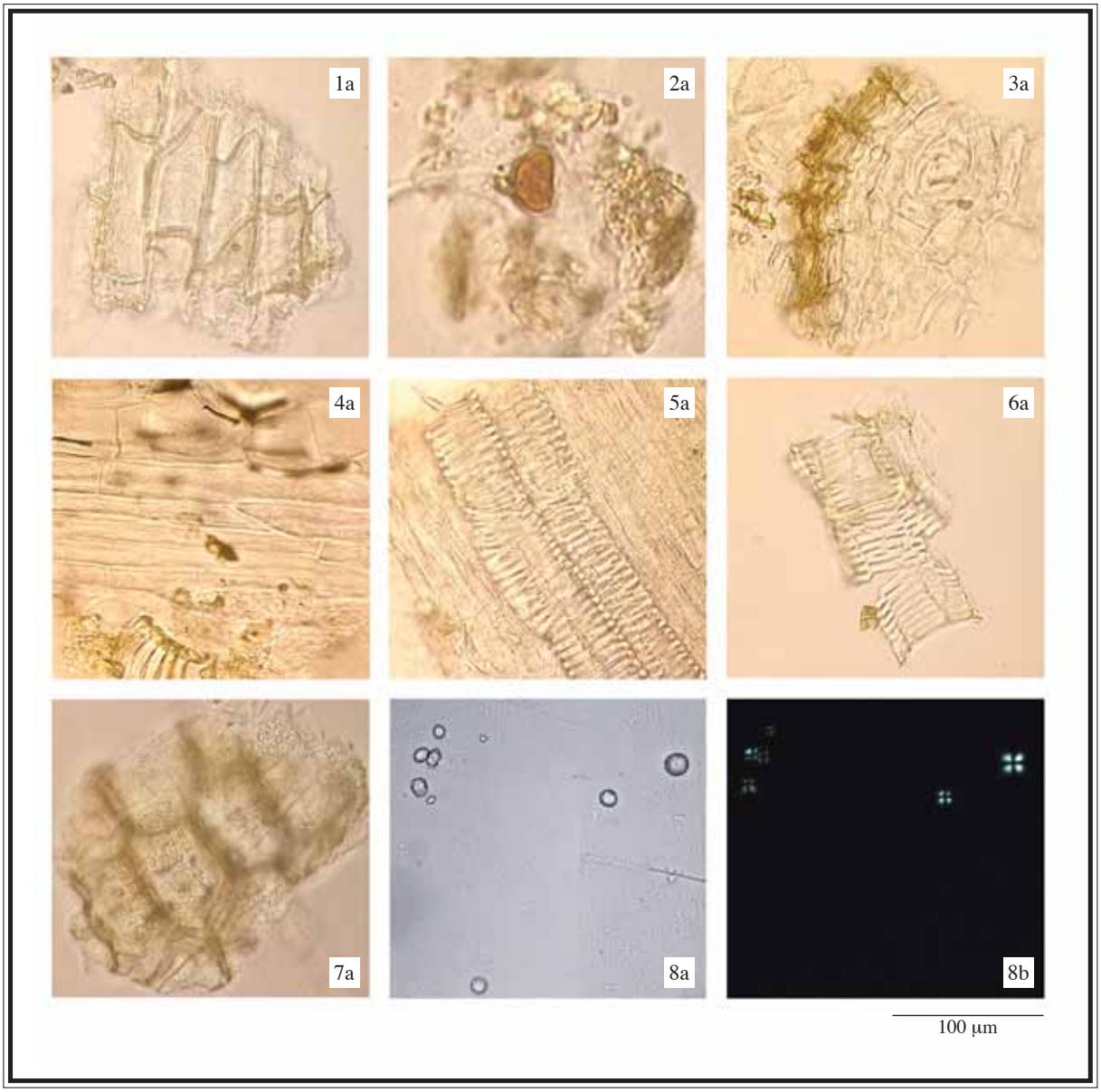

Figure 15 Microscopic features of powder of Radix Angelicae Sinensis

1. Cork cells 2. Secretion from oil cavity 3. Oil cavity 4. Phloem parenchymatous cells
5. Reticulate vessels in a bundle 6. Single scalariform vessel 7. Starch grains in parenchymatous cells
8. Starch grains scattered

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

Developing solvent system

Prepare a mixture of hexane and ethyl acetate (5:1, 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 put into a 50-mL centrifugal tube, then add 10 mL of diethyl ether. Close the cap of the tube, shake occasionally for 2 h and 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 1 mL of ethyl acetate.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} and a freshly prepared developing solvent system as described above. Develop over a path of about 8 cm. Apply separately Z-ligustilide 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. Spray the plate evenly with the spray reagent and heat at about 110 ˚C for about 10 min. Examine the plate in UV light (366 nm). 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 Z-ligustilide.

4.3.2 Thin-Layer Chromatographic Identification of Water-Soluble Components

Standard solution

Ferulic acid standard solution

Weigh 0.5 mg of ferulic acid and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of chloroform, ethyl acetate and formic acid (10:5:0.5, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol and mix. Sonicate the mixture for 30 min and centrifuge at about 1200 x *g* for 10 min. Transfer the supernatant to a round-bottomed flask and evaporate to dryness at reduced pressure in a rotary evaporator. Add 20 mL of water to the residue. Extract the aqueous solution twice each with 20 mL of hexane and discard the hexane layer. Adjust the pH of the aqueous layer to 2 with 2 M hydrochloric acid, then extract three times each with 10 mL of diethyl ether. Combine the ether extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 0.5 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 8.5 cm. Apply separately ferulic acid 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 allow to dry. Spray the plate with the spray reagent and heat at about 110 ˚C for about 10 min. Examine the plate in UV light (366 nm). 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 ferulic acid.

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

Standard solution

Z-ligustilide standard solution for fingerprinting, Std-FP (400 mg/L)

Weigh 10.0 mg of Z-ligustilide and dissolve in 25 mL of acetonitrile. Store at about -10 ˚C in the dark.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of a mixture of methanol and formic acid (95:5, v/v) and weigh. Sonicate the mixture for 100 min and weigh again. Add an appropriate amount of the mixed solvent as described above to compensate the weight loss, if any. Mix and centrifuge at about 540 x *g* for 5 min. Filter through a 0.2-µm PTFE filter.

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 column temperature is maintained at 30 ˚C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Water : acetic acid (99:1, v/v)(%, v/v)	Acetonitrile (%, v/v)	Elution
0–18	81	19	isocratic
18–60	81 → 0	19 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of Z-ligustilide. The requirements of the system suitability parameters are as follows: the RSD of the peak area of Z-ligustilide should be not more than 3.0%; the RSD of the retention time of Z-ligustilide peak should be not more than 3.0%; the column efficiency determined from Z-ligustilide peak should be not less than 300,000 theoretical plates.

The *R* value between Z-ligustilide peak and peak 3 (Fig. 16) in the test solution should be not less than 2.0.

Procedure

Separately inject Z-ligustilide Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of Z-ligustilide peak in the chromatogram of Z-ligustilide Std-FP and the retention times of the four characteristic peaks (Fig. 16) in the chromatogram of the test solution. Under the same HPLC conditions, identity Z-ligustilide peak in the test solution by comparing its retention time with that in the chromatogram of Z-ligustilide Std-FP. The retention times of Z-ligustilide peaks from the two chromatograms should not differ by more than 3.0%. 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 Radix Angelicae Sinensis extract are listed in Table 6.

Table 6 The RRTs and acceptable ranges of the four characteristic peaks of Radix Angelicae Sinensis extract

Peak No.	RRT	Acceptable Range
1 (ferulic acid)	0.32	±0.03
2	0.86	±0.03
3	0.97	±0.03
4 (marker, Z-ligustilide)	1.00	-

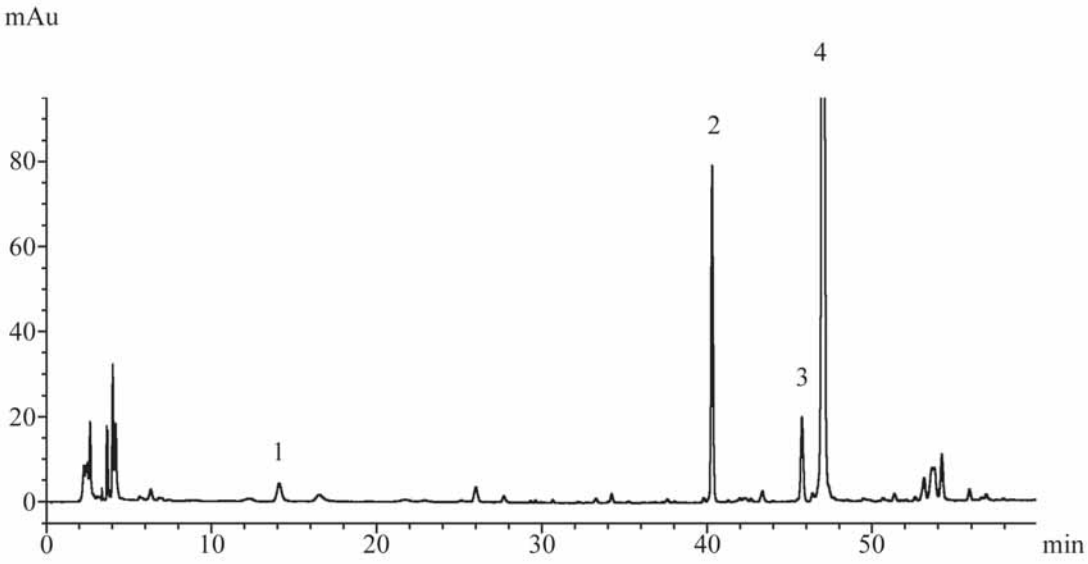

Figure 16 A reference fingerprint chromatogram of Radix Angelicae Sinensis extract

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. 16).

5. TESTS

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

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

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

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

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 48.0%.
Ethanol-soluble extractives (cold extraction method): not less than 55.0%.

7. ASSAY *(Appendix IV(B))*

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

Standard solution

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

Weigh accurately 25.0 mg of Z-ligustilide and dissolve in 25 mL of acetonitrile. Store at about -10 ˚C in the dark.

Z-ligustilide standard solution for assay, Std-AS

Measure accurately the volume of the Z-ligustilide Std-Stock, dilute with acetonitrile to produce a series of solutions of 10, 150, 250, 350, 500 mg/L for Z-ligustilide.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add accurately 25 mL of methanol and weigh. Sonicate the mixture for 100 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 540 x *g* for 5 min. Filter through a 0.2-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (350 nm) and a column (4.6 x 150 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 acetonitrile and water (6:4, v/v).

System suitability requirements

Perform at least five replicate injections each with 10 μL of Z-ligustilide Std-AS (250 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of Z-ligustilide should be not more than 3.0%; the RSD of the retention time of Z-ligustilide peak should be not more than 3.0%; the column efficiency determined from Z-ligustilide peak should be not less than 5,000 theoretical plates.

The *R* value between Z-ligustilide peak and the closest peak in the test solution should be not less than 1.5.

Calibration curve

Inject a series of Z-ligustilide Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of Z-ligustilide against the corresponding concentrations of Z-ligustilide 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 Z-ligustilide peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of Z-ligustilide Std-AS. The retention times of Z-ligustilide 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 Z-ligustilide in the test solution, and calculate the percentage content of Z-ligustilide in the powdered sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.60% of Z-ligustilide ($C_{12}H_{14}O_2$), calculated with reference to the dried substance.