Rhizoma Chuanxiong

Figure 1 A photograph of Rhizoma Chuanxiong

1. NAMES

Official Name: Rhizoma Chuanxiong

Chinese Name: 川芎

Chinese Phonetic Name: Chuanxiong

2. SOURCE

Rhizoma Chuanxiong is the dried rhizome of *Ligusticum chuanxiong* Hort. (Apiaceae/Umbelliferae). The rhizome of the cultivated plant is harvested in the summer of the second year of cultivation, when bulging nodes on the stem become prominent and slight purplish in colour. Dig up the whole plant, get the rhizome and removed the soil, then dried in a shaded area or by baking; once dried, the rootlets are removed to obtain Rhizoma Chuanxiong.

3. DESCRIPTION

In irregular knotty and fist-like masses, 2-7 cm in diameter. Externally dark brown, greyish-brown or yellowish-brown, rough and shrunken, with numerous parallel and raised annulations, showing dent and subround stem scars on the summit, and numerous warty rootlet scars beneath and at the annulations. Texture compact, not easily broken. When broken, the surface is yellowish-white or greyish-yellow, scattered with yellowish-brown oil cavities, the cambium forming an undulate ring. Odour, strongly fragrant; taste, bitter, acrid, slightly sweet afterwards, giving a slight numbing sensation (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The cork consists of over 10 rows of flat cells, the cortex narrow, scattered with root-trace vascular bundles. Phloem broad. Cambium undulate or irregularly polygonal. Xylem vessels mostly uniseriate or arranged in a V shape; xylem fibres in bundles are occasionally found. Pith broad. Oil cavities scattered in parenchyma, subround, ellipsoid or irregular, pale yellowish-brown, smaller

near the cambium, gradually becoming larger outwards. The parenchyma cells contain abundant starch grains (Fig. 2).

Powder

Colour pale yellowish-brown or greyish-brown. Starch grains abundant; simple grains ellipsoid, elongated-rounded, oval or kidney-shaped, 3-19 μm in diameter, hilum pointed, long cleft or V-shaped; compound grains few, consisting of 2-4 units, black cruciate-shaped as seen under the polarized microscope. Oil cavities mostly broken, secretory cells containing numerous droplets of oil. Cork cells closely arrayed, sub-polygonal in surface view, thin-walled, sinuately crooked, well-arranged in lateral view. Spiral vessels frequent, 14-50 μm in diameter, the thickened wall of some spiral vessels interconnected to form reticulated vessels. Clusters of calcium oxalate, in subround masses, rarely observed, intensely white in colour under the polarized microscope (Fig. 3).

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 (490 W) 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 under UV light (254 nm), a fluorescent blue spot is observed.

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

Standard solutions

Z-Ligustilide standard solution

Weigh 1.0 mg of Z-ligustilide CRS (Fig. 4) and dissolve in 1 mL of methanol.

E-Ferulic acid standard solution

Weigh 1.0 mg of E-ferulic acid CRS (Fig.4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane and diethyl ether (2:1, v/v).

Spray reagent

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

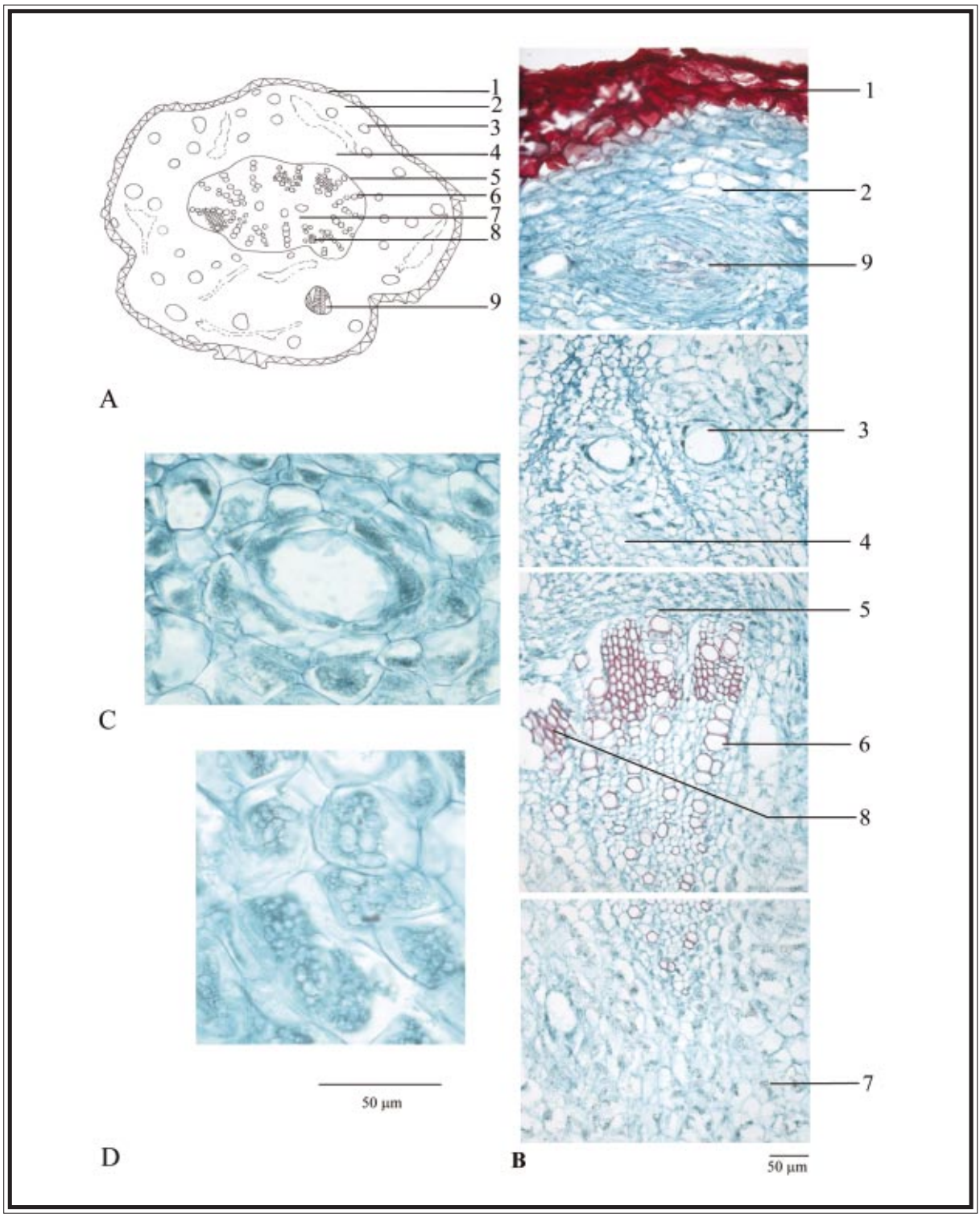


Figure 2 Microscopic features of transverse section of Rhizoma Chuanxiong

A. Sketch B. Section illustration C. Oil cavity D. Starch grains

1. Cork 2. Cortex 3. Oil cavities 4. Phloem 5. Cambium 6. Xylem 7. Pith 8. Xylem fibres 9. Root-trace vascular bundles

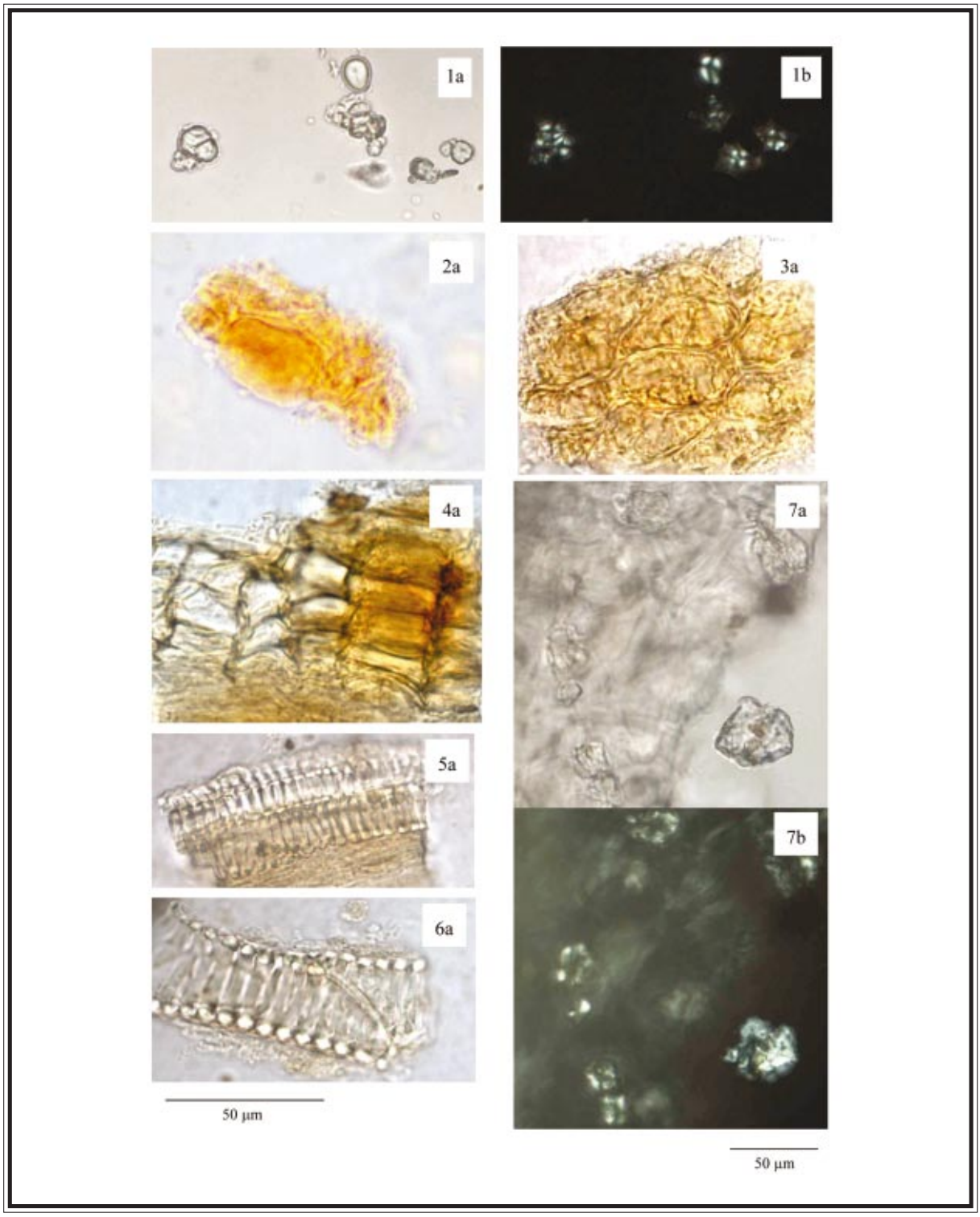


Figure 3 Microscopic features of powder of Rhizoma Chuanxiong

1. Starch grains 2. Fragments of oil cavities 3. Cork cells in surface view 4. Cork cells in lateral view
5. Spiral vessels 6. Reticulated vessel 7. Cluster crystals of calcium oxalate

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

Test solution

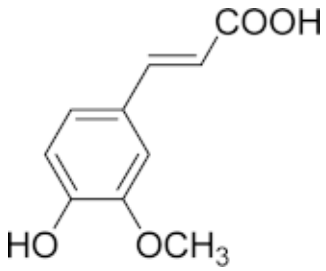
Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to another tube. Evaporate the solvent to dryness with a gentle stream of nitrogen. Dissolve the residue in 2 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. Apply separately Z-ligustilide standard solution, E-ferulic acid standard solution and the test solution (1 μL each) 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. Spray the plate evenly with the spray reagent, then heat at about 70°C until the spots or bands become visible (about 10 min). Examine the plate under UV light (366 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 Z-ligustilide and E-ferulic acid.

(i)



(ii)

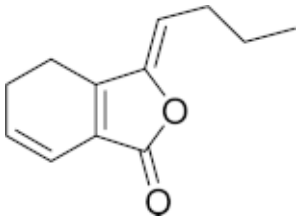


Figure 4 Chemical structures of (i) E-ferulic acid and (ii) Z-ligustilide

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

Standard solution

Z-ligustilide standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 5.0 mg of Z-ligustilide CRS and dissolve in 100 mL of methanol. Store at about -10°C in the dark.

Test solution

Weigh 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol. Sonicate (490 W) the mixture for 90 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask 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 detector (293 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 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	85 → 80	15 → 20	linear gradient
10 – 40	80 → 47	20 → 53	linear gradient
40 – 60	47 → 0	53 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of Z-ligustilide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of Z-ligustilide should not be more than 3.0%; the RSD of the retention time of Z-ligustilide peak should not be more than 2.0%; the column efficiency determined from Z-ligustilide peak should not be less than 150000 theoretical plates.

The *R* value between peak 7 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

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 the Z-ligustilide Std-FP and the retention times of the seven characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, 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-FP. The retention times of Z-ligustilide 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 Rhizoma Chuanxiong extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the seven characteristic peaks of Rhizoma Chuanxiong extract

Peak No.	RRT	Acceptable Range
1	0.23	±0.03
2 (E-ferulic acid)	0.36	±0.03
3	0.39	±0.03
4	0.46	±0.03
5	0.84	±0.03
6	0.91	±0.03
7 (marker, Z-ligustilide)	1.00	-

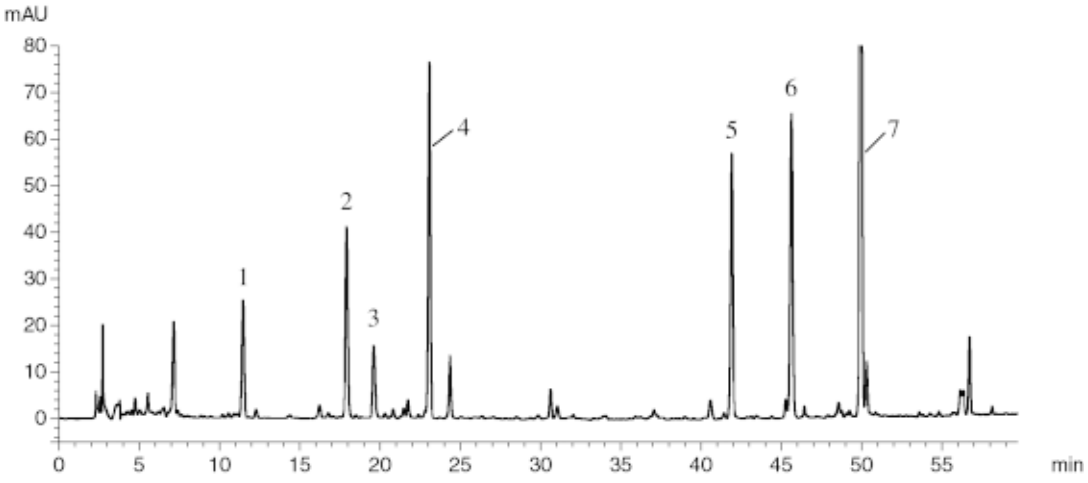


Figure 5 A reference fingerprint chromatogram of Rhizoma Chuanxiong extract

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. 5).

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 XVII)*: 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 2.0%.

5.7 Water Content *(Appendix X)*: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 27.0%.
Ethanol-soluble extractives (cold extraction method): not less than 23.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 10.0 mg of Z-ligustilide CRS and dissolve in 10 mL of methanol. 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 methanol to produce a series of solutions of 10, 25, 50, 100, 200 mg/L for Z-ligustilide.

Test solution

Weigh accurately 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction once. Wash the residue with 3 mL of methanol to the tube and centrifuge at about 1800 × *g* for 10 min. Combine the extracts and make up to the mark with methanol. Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (328 nm) and a column (4.6 × 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 0.1% phosphoric acid and methanol (32:68, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections each with 10 μL of Z-ligustilide Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of Z-ligustilide should not be more than 3.0%; the RSD of the retention time of Z-ligustilide peak should not be more than 3.0%; the column efficiency determined from Z-ligustilide peak should not be less than 3000 theoretical plates.

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

Calibration curve

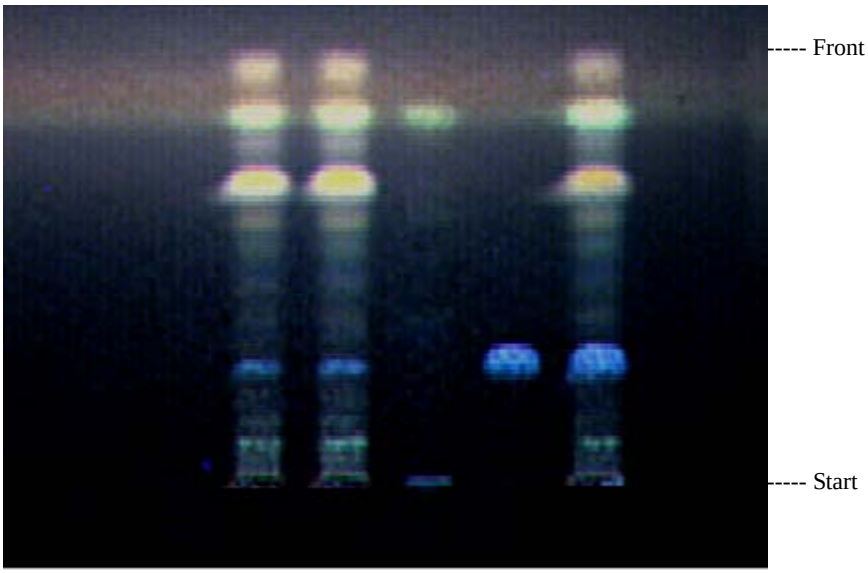
Inject a series of Z-ligustilide Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of Z-ligustilide against the corresponding concentrations of Z-ligustilide Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

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 2.0%. 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 sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.47% of Z-ligustilide ($C_{12}H_{14}O_2$), calculated with reference to the dried substance.



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (Rhizoma Chuanxiong)	Z-ligustilide and E-ferulic acid positive
3	Sample duplicate (Rhizoma Chuanxiong)	Z-ligustilide and E-ferulic acid positive
4	Standard (Z-ligustilide)	Z-ligustilide positive
5	Standard (E-ferulic acid)	E-ferulic acid positive
6	Spiked sample (Sample plus Z-ligustilide and E-ferulic acid)	Z-ligustilide and E-ferulic acid positive

Figure 1 TLC results of Rhizoma Chuanxiong extract observed under UV light (366 nm) after staining