Curculiginis Rhizoma

Figure 1 A photograph of Curculiginis Rhizoma

1. NAMES

Official Name: Curculiginis Rhizoma

Chinese Name: 仙茅

Chinese Phonetic Name: Xianmao

2. SOURCE

Curculiginis Rhizoma is the dried rhizome of *Curculigo orchioides* Gaertn. (Amaryllidaceae). The rhizome is collected in autumn or winter, removed the caudex and rootlets, washed clean, then dried under the sun to obtain Curculiginis Rhizoma.

3. DESCRIPTION

Cylindrical, slightly curved, 1.7-9.7 cm long, 2-12 mm in diameter. Externally brown to blackish-brown, coarse, with pitted scars of rootlets and transversal wrinkles. Texture hard and fragile, easily broken; fracture uneven, greyish-white to brown and blackish-brown in the centre. Odour slight and aromatic; taste bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex broad, with root-trace vascular bundles occasionally scattered. Mucilage cells numerous, scattered in parenchyma, subrounded, containing raphides of calcium oxalate. Endodermis distinct. Stele vascular bundles amphivasal or collateral, mostly scattered, near pericycle, gradually decreased inwards (Fig. 2).

Powder

Colour greyish-brown. Raphides of calcium oxalate, numerous, scattered or in bundles, 43-188 μm long; polychromatic under the polarized microscope. Simple starch granule subrounded, 1-90 μm in diameter, hilum indistinct, compound granules composed of 2-5 units; black and cruciate in shape under the polarized microscope. Mucilage cells numerous, subrounded or elliptic, 56-399 μm in diameter, filled with raphides of calcium oxalate; polychromatic under the polarized microscope. Cork cells subpolygonal, cell wall thickened (Fig. 3).

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

Standard solution

Curculigoside standard solution

Weigh 1.0 mg of curculigoside CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and formic acid (8:1:0.1, v/v).

Spray reagent

Solution A

Weigh 0.2 g of potassium ferricyanide and dissolve in 10 mL of water.

Solution B

Weigh 0.2 g of ferric chloride and dissolve in 10 mL of water.

Spray reagent

Mix 5 mL of Solution A and 5 mL of Solution B to a 50-mL conical flask.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately curculigoside standard solution and the test solution (5 µL each) 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. Spray the plate evenly with the spray reagent and dry it in air until the spots or bands become visible. 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 curculigoside.

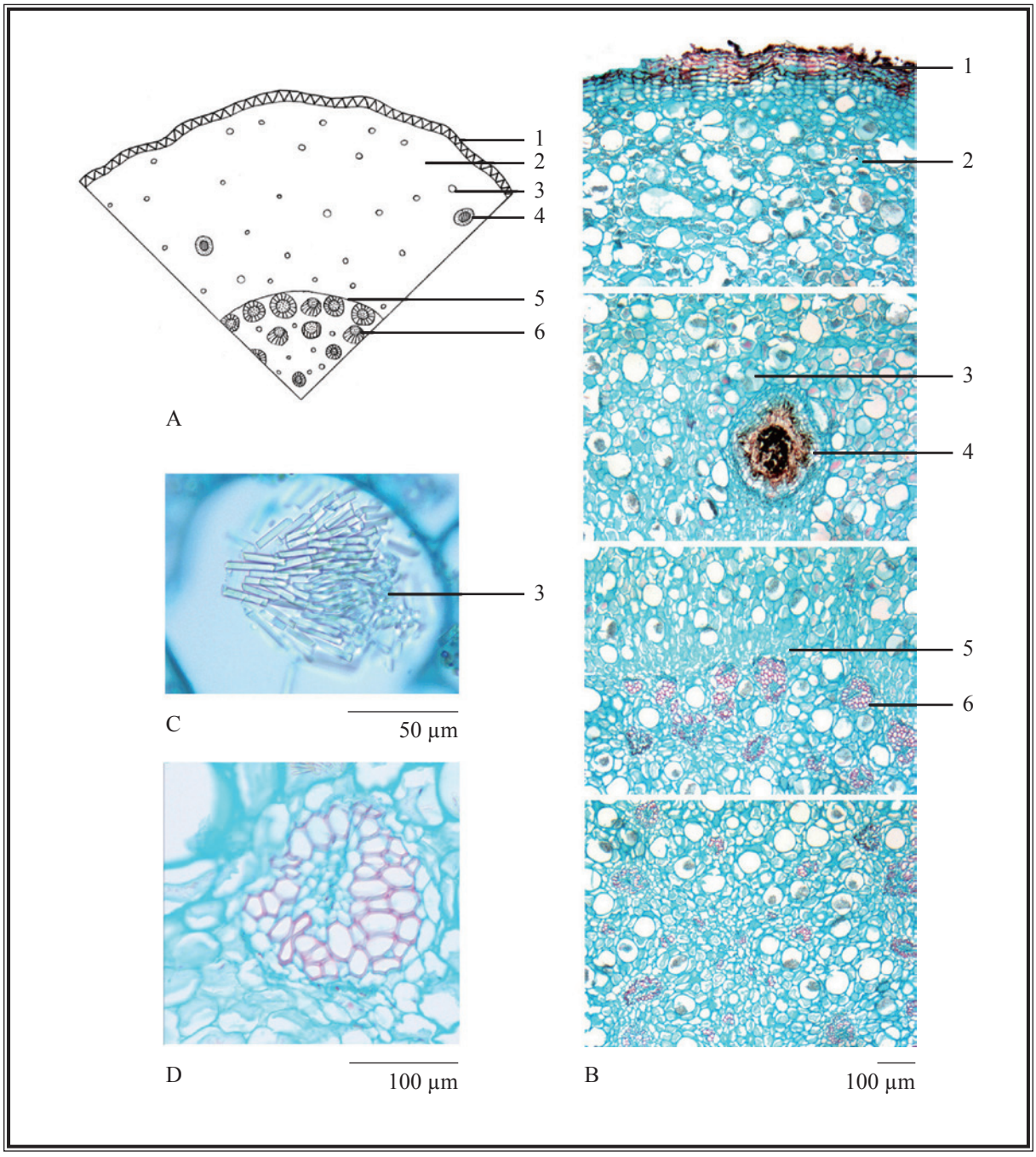


Figure 2 Microscopic features of transverse section of Curculiginis Rhizoma

A. Sketch B. Section illustration C. Mucilage cell containing raphides of calcium oxalate D. Vascular bundle

1. Cork 2. Cortex 3. Mucilage cell 4. Root-trace vascular bundle 5. Endodermis 6. Vascular bundle

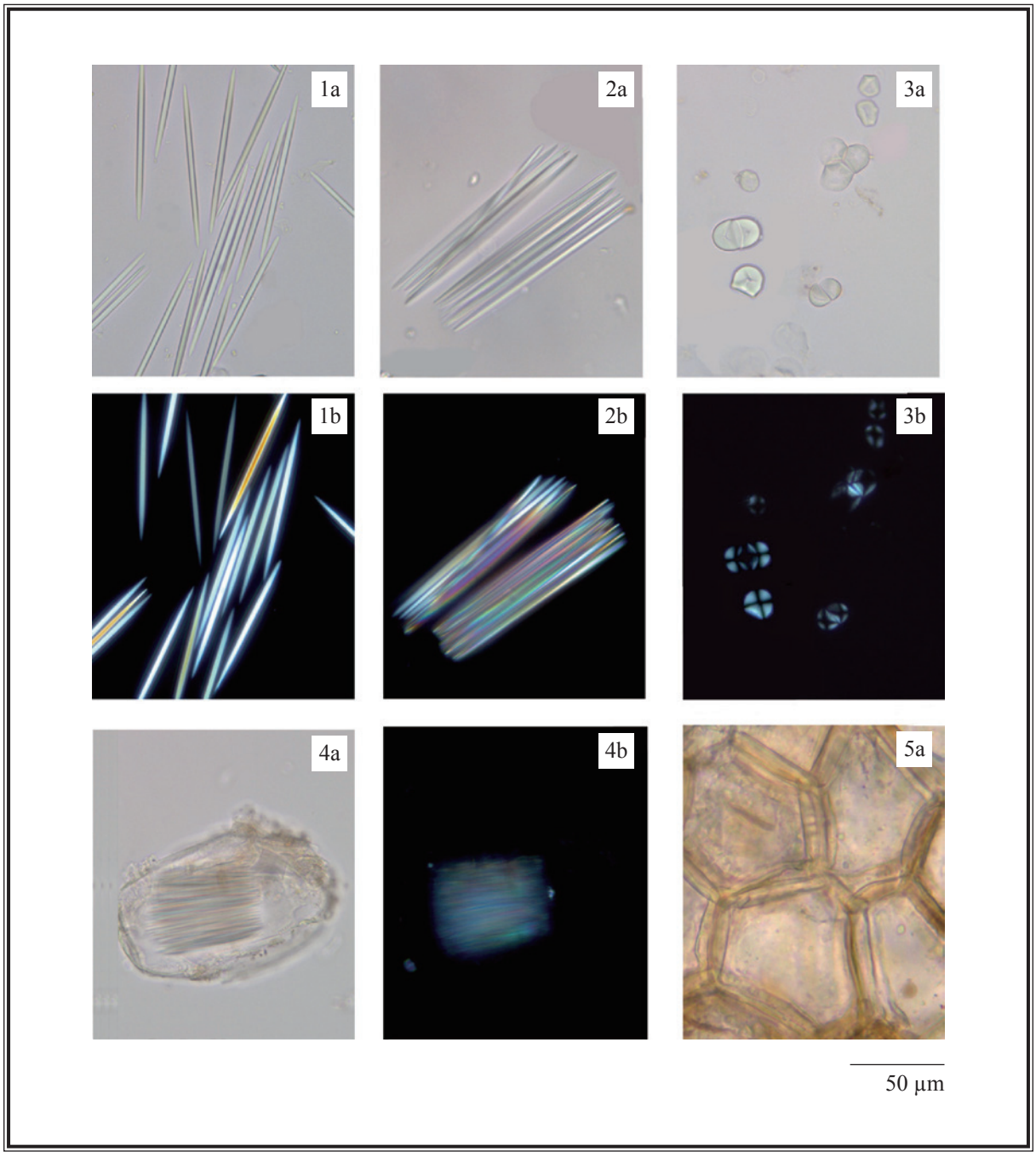


Figure 3 Microscopic features of powder of Curculiginis Rhizoma

1. Scattered raphides of calcium oxalate 2. Raphides of calcium oxalate in bundles 3. Starch granules
4. Mucilage cell 5. Cork cells

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

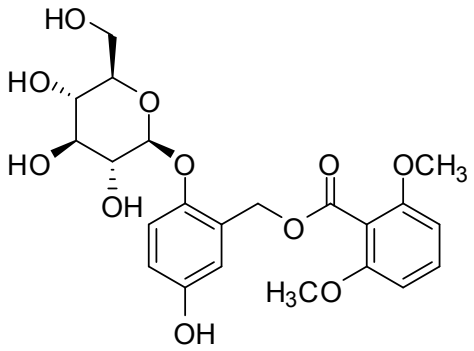


Figure 4 Chemical structure of curculigoside

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

Standard solution

Curculigoside standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of curculigoside CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 3.0 g of the powdered sample and place it in a 100-mL conical flask, then add 40 mL of methanol. Sonicate (150 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for two times each with 20 mL of methanol. Combine the solutions and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of water. Transfer the aqueous solution to a separating funnel. Extract for three times each with 20 mL of ethyl acetate. Combine the ethyl acetate extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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 (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.05% Trifluoroacetic acid (%, v/v)	Elution
0 – 20	4 → 13	96 → 87	linear gradient
20 – 30	13 → 17	87 → 83	linear gradient
30 – 45	17 → 20	83 → 80	linear gradient
45 – 60	20 → 30	80 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of curculigoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of curculigoside should not be more than 5.0%; the RSD of the retention time of curculigoside peak should not be more than 2.0%; the column efficiency determined from curculigoside peak should not be less than 100000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Curculiginis Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.31	± 0.03
2	0.55	± 0.03
3	0.68	± 0.03
4 (marker, curculigoside)	1.00	-

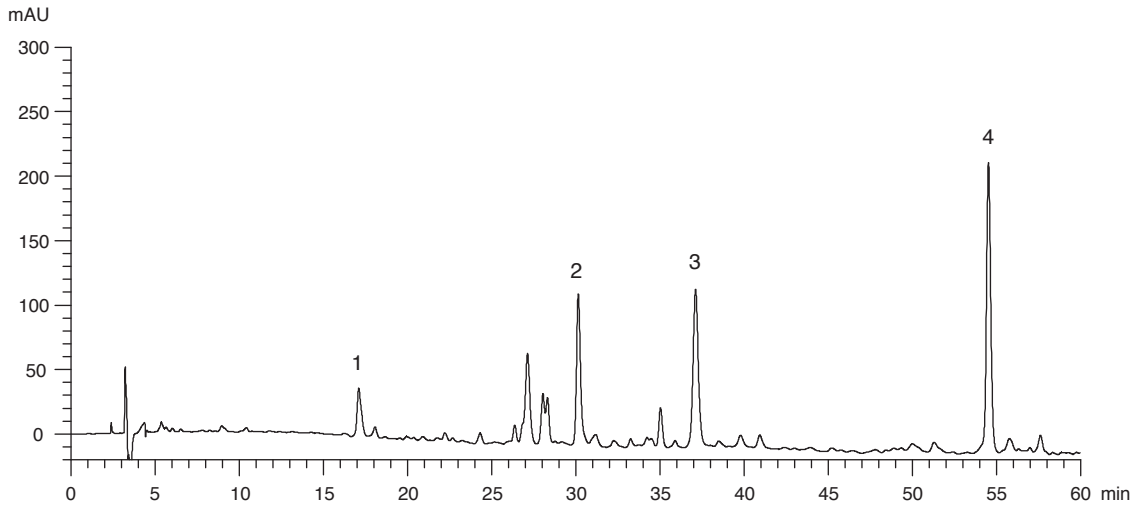


Figure 5 A reference fingerprint chromatogram of Curculiginis Rhizoma extract

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. 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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

Curculigoside standard stock solution, Std-Stock (400 mg/L)

Weigh accurately 4.0 mg of curculigoside CRS and dissolve in 10 mL of ethanol (70%).

Curculigoside standard solution for assay, Std-AS

Measure accurately the volume of the curculigoside Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 16, 24, 40, 60, 120 mg/L for curculigoside.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of ethanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Centrifuge at about 3000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with ethanol (70%). Centrifuge at about 3000 × *g* for 5 min. Filter and combine the filtrates. Make up to the mark with ethanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (285 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. The mobile phase is a mixture of water and acetonitrile (78:22, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of curculigoside Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of curculigoside should not be more than 5.0%; the RSD of the retention time of curculigoside peak should not be more than 2.0%; the column efficiency determined from curculigoside peak should not be less than 8000 theoretical plates.

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

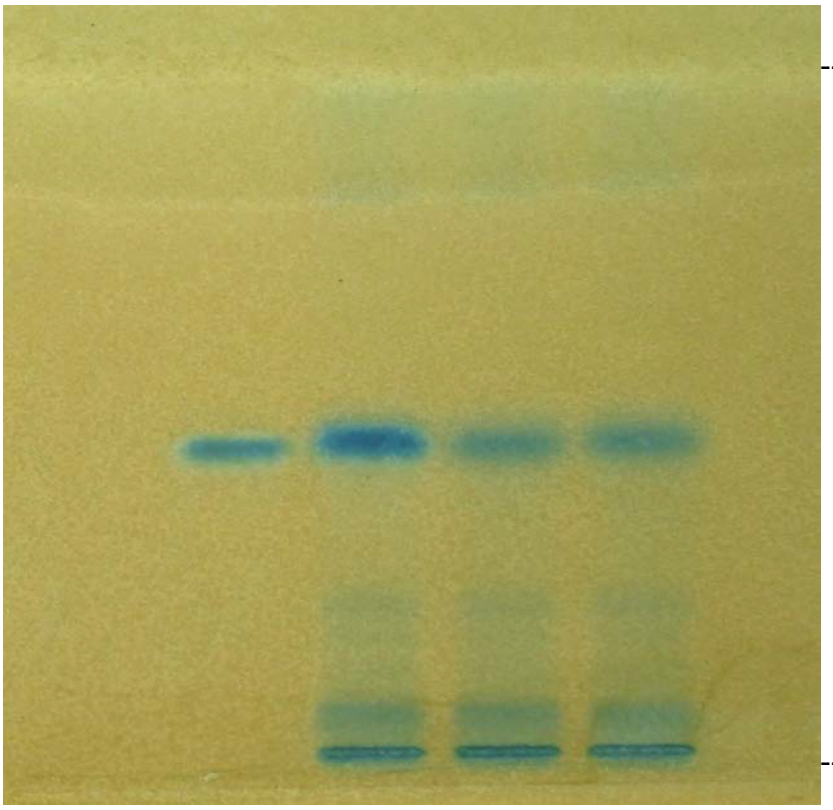
Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify curculigoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of curculigoside Std-AS. The retention times of curculigoside 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 curculigoside in the test solution, and calculate the percentage content of curculigoside in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.10% of curculigoside ($C_{22}H_{26}O_{11}$), calculated with reference to the dried substance.

Curculiginis Rhizoma　(仙茅)



Lane	Sample	Results
1	Blank (70% ethanol)	Negative
2	Standard (Curculigoside)	Curculigoside positive
3	Spiked sample (Sample plus curculigoside)	Curculigoside positive
4	Sample (Curculiginis Rhizoma)	Curculigoside positive
5	Sample duplicate (Curculiginis Rhizoma)	Curculigoside positive

Figure 1　TLC results of Curculiginis Rhizoma extract observed under visible light after staining