Curcumae Longae Rhizoma

Figure 1 A photograph of Curcumae Longae Rhizoma

1. NAMES

Official Name: Curcumae Longae Rhizoma

Chinese Name: 薑黃

Chinese Phonetic Name: Jianghuang

2. SOURCE

Curcumae Longae Rhizoma is the dried rhizome of *Curcuma longa* L. (Zingiberaceae). The rhizome is collected in winter when the aerial part withered, removed the rootlets, washed clean, boiled or steamed thoroughly, then dried under the sun to obtain Curcumae Longae Rhizoma.

3. DESCRIPTION

Irregularly ovoid, cylindrical or fusiform, curved, sometimes slightly branched into a Y-shape, 1.1-10.3 cm long, 5-30 mm in diameter. Externally dark yellow to yellowish-brown, rough, with wrinkled striations, distinct cyclic nodes, and rounded scars of root branches and rootlets. Texture hard, fractured brownish-yellow to golden yellow, cuticular, with waxy lustre, endodermis ring distinct, scattered with dotted vascular bundles. Odour characteristic and aromatic; taste slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells flattened and thin-walled. Cork composed of 6-8 layers of cells, flattened and thin-walled, regularly arranged. A few leaf-trace vascular bundles scattered in cortex broad. Endodermis distinct. Stele broad, collateral vascular bundles mostly scattered near pericycle, gradually decreased inwards (Fig. 2).

Powder

Yellowish-brown or reddish-brown. Starch gelatinous masses numerous, greyish-white, subround, polygonal or irregular in shape. Simple starch granules long elliptical or long ovate with angustate end or prominent beak, 5-65 μm long, hilum usually eccentric at the angustate

end; black, off-centre cruciate shape under the polarized microscope. Oil cells elliptical or rounded-ovate, relatively large, 15-131.5 μm in diameter, full of yellow or orange oily masses. Vessels mainly spiral and scalariform, 2.2-77.7 μm in diameter. Prisms of calcium oxalate in a small number, subsquare or rod-shaped; polychromatic under the polarized microscope. Non-glandular hairs yellow to dark yellow, unicellular; usually broken, with pointed tip. Cork cells pale yellow, wall thin, frequently overlapping (Fig. 3).

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

Standard solutions

Bisdesmethoxycurcumin standard solution

Weigh 1.0 mg of bisdesmethoxycurcumin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Curcumin standard solution

Weigh 2.0 mg of curcumin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Desmethoxycurcumin standard solution

Weigh 2.0 mg of desmethoxycurcumin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, ethanol and formic acid (15:0.4:0.3, v/v).

Test solution

Weigh 0.1 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 bisdesmethoxycurcumin standard solution (0.5 μL), curcumin standard solution (1 μL), desmethoxycurcumin standard solution (0.5 μL) and the test solution (3 μL) 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. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

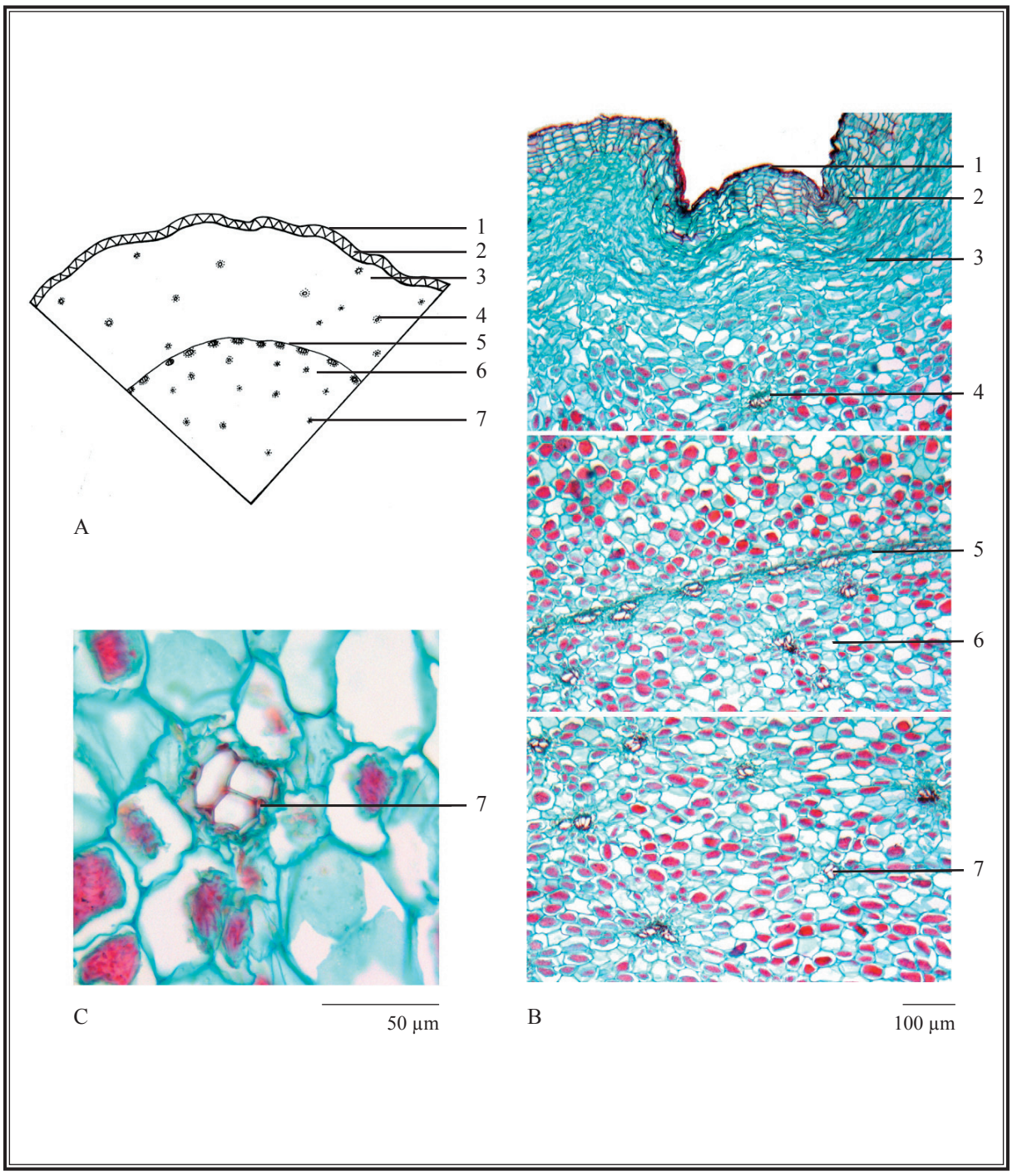


Figure 2 Microscopic features of transverse section of Curcumae Longae Rhizoma

A. Sketch B. Section illustration C. Vascular bundle

1. Remnants of epidermis 2. Cork 3. Cortex 4. Leaf-trace bundle 5. Endodermis 6. Stele 7. Vascular bundle

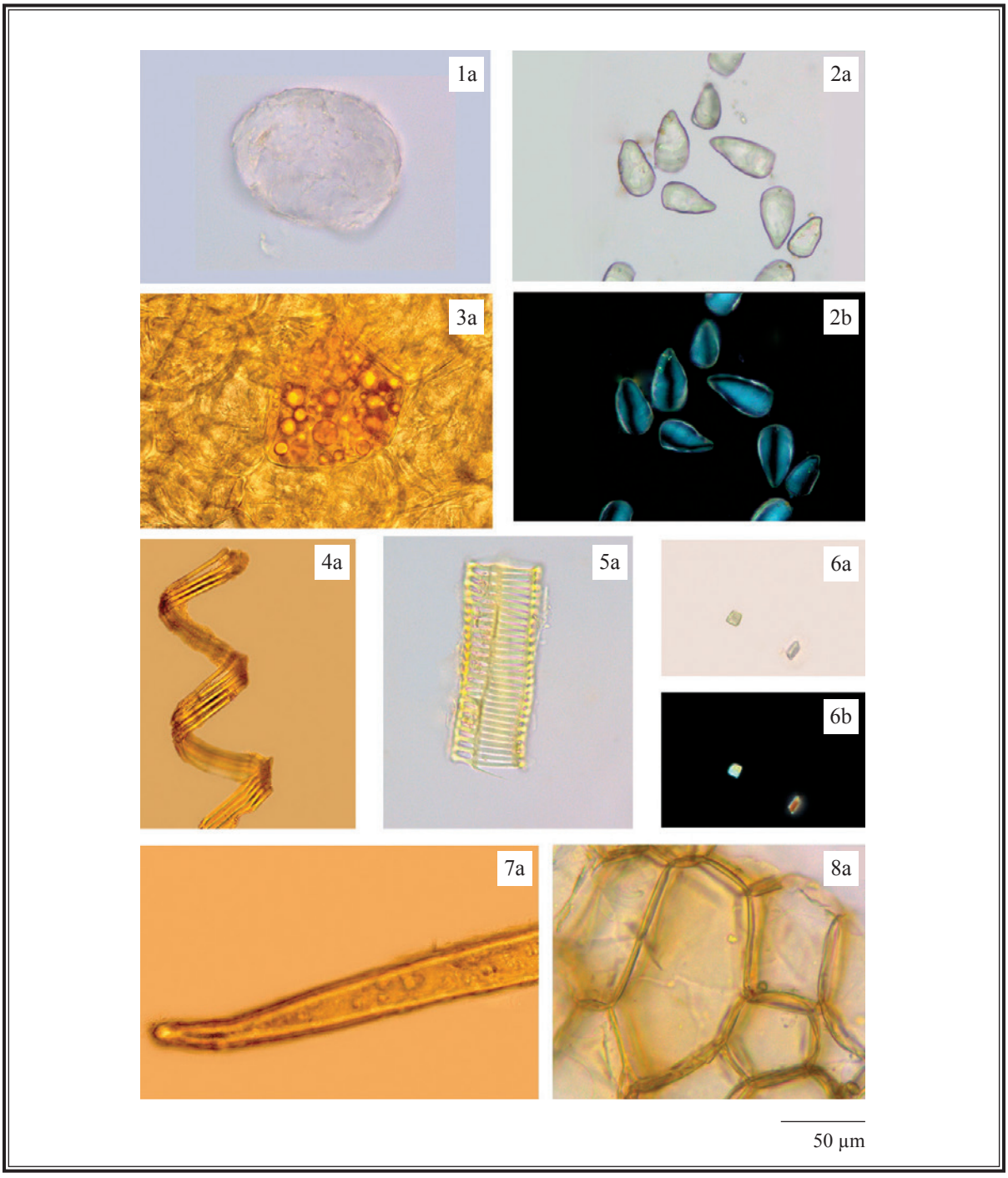


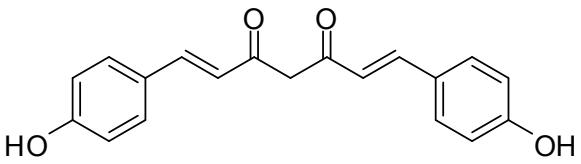
Figure 3 Microscopic features of powder of Curcumae Longae Rhizoma

1. Starch gelatinous masses 2. Starch granules 3. Oil cell 4. Spiral vessel 5. Scalariform vessel
6. Prisms of calcium oxalate 7. Non-glandular hair 8. Cork cells

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

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 bisdesmethoxycurcumin, curcumin and desmethoxycurcumin.

(i)



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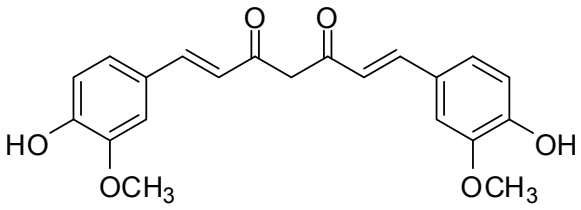

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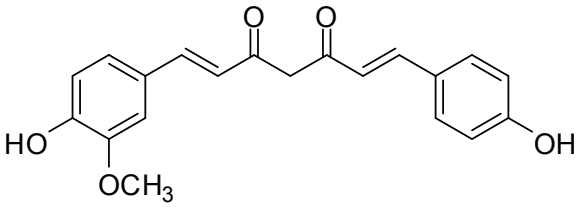


Figure 4 Chemical structures of (i) bisdesmethoxycurcumin (ii) curcumin and (iii) desmethoxycurcumin

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

Standard solutions

Bisdesmethoxycurcumin standard solution for fingerprinting, Std-FP (52 mg/L)

Weigh 1.3 mg of bisdesmethoxycurcumin CRS and dissolve in 25 mL of ethanol (70%).

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

Weigh 5.0 mg of curcumin CRS and dissolve in 25 mL of ethanol (70%).

Desmethoxycurcumin standard solution for fingerprinting, Std-FP (52 mg/L)

Weigh 1.3 mg of desmethoxycurcumin CRS and dissolve in 25 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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)	0.1% Trifluoroacetic acid (%, v/v)	Elution
0 – 35	44	56	isocratic
35 – 40	44 → 60	56 → 40	linear gradient
40 – 50	60 → 80	40 → 20	linear gradient
50 – 60	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of bisdesmethoxycurcumin Std-FP, curcumin Std-FP and desmethoxycurcumin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin should not be more than 5.0%; the RSD of the retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be more than 2.0%; the column efficiencies determined from bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be less than 11000 theoretical plates.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1 (bisdesmethoxycurcumin)	0.83	± 0.03
2 (desmethoxycurcumin)	0.91	± 0.03
3 (marker, curcumin)	1.00	-
4	1.43	± 0.06

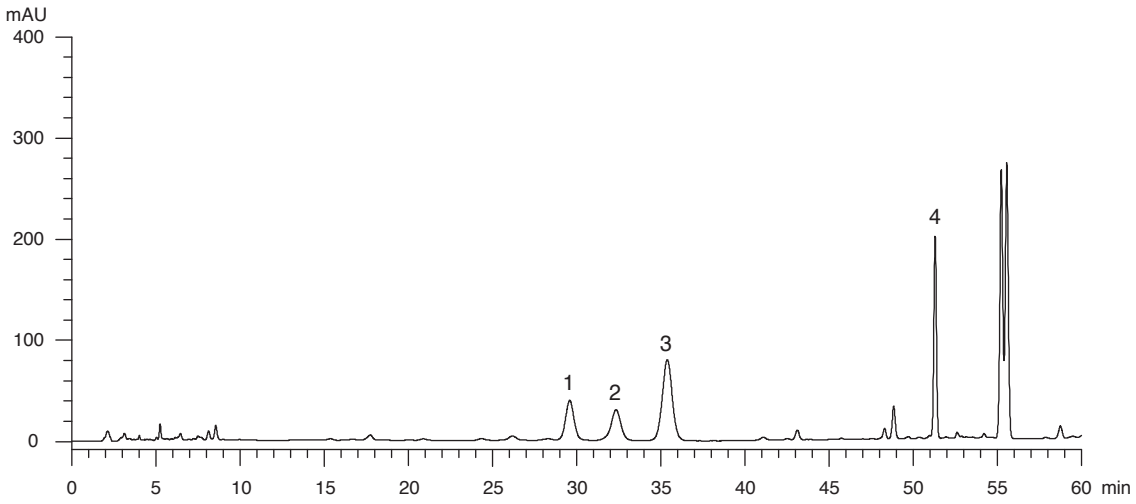


Figure 5 A reference fingerprint chromatogram of Curcumae Longae Rhizoma extract

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 6.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 13.0%.
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

Mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin standard stock solution, Std-Stock (50 mg/L for bisdesmethoxycurcumin, 200 mg/L for curcumin and 50 mg/L for desmethoxycurcumin)

Weigh accurately 2.5 mg of bisdesmethoxycurcumin CRS, 10.0 mg of curcumin CRS and 2.5 mg of desmethoxycurcumin CRS, and dissolve in 50 mL of ethanol (70%).

Mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin standard solution for assay, Std-AS

Measure accurately the volume of the mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 2, 5, 10, 25 mg/L for bisdesmethoxycurcumin, 4, 8, 20, 40, 100 mg/L for curcumin and 1, 2, 5, 10, 25 mg/L for desmethoxycurcumin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and 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 (430 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 0.1% trifluoroacetic acid and acetonitrile (52:48, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of the mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin Std-AS (5 mg/L for bisdesmethoxycurcumin, 20 mg/L for curcumin and 5 mg/L for desmethoxycurcumin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin should not be more than 5.0%; the RSD of the retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be more than 2.0%; the column efficiencies determined from bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks should not be less than 11000 theoretical plates.

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

Calibration curves

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

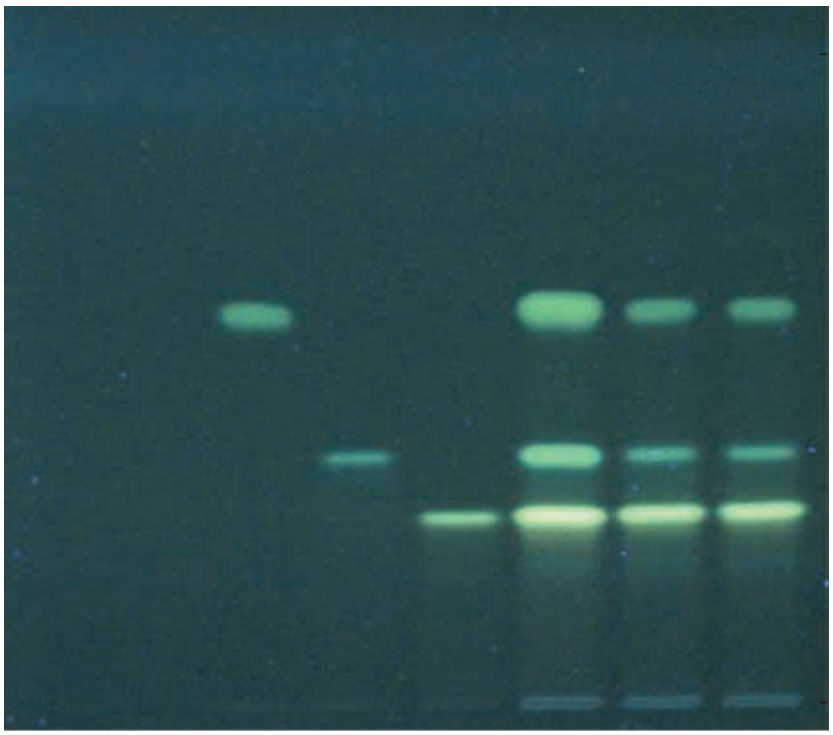
Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bisdesmethoxycurcumin, curcumin and desmethoxycurcumin Std-AS. The retention times of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin in the test solution, and calculate the percentage contents of bisdesmethoxycurcumin, curcumin and desmethoxycurcumin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 1.5% of the total content of bisdesmethoxycurcumin ($C_{19}H_{16}O_4$), curcumin ($C_{21}H_{20}O_6$) and desmethoxycurcumin ($C_{20}H_{18}O_5$), calculated with reference to the dried substance.

Curcumae Longae Rhizoma　(薑黃)



Lane	Sample	Results
1	Blank (70% ethanol)	Negative
2	Standard (Curcumin)	Curcumin positive
3	Standard (Desmethoxycurcumin)	Desmethoxycurcumin positive
4	Standard (Bisdesmethoxycurcumin)	Bisdesmethoxycurcumin positive
5	Spiked sample (Sample plus curcumin, desmethoxycurcumin and bisdesmethoxycurcumin)	Curcumin, desmethoxycurcumin and bisdesmethoxycurcumin positive
6	Sample (Curcumae Longae Rhizoma)	Curcumin, desmethoxycurcumin and bisdesmethoxycurcumin positive
7	Sample duplicate (Curcumae Longae Rhizoma)	Curcumin, desmethoxycurcumin and bisdesmethoxycurcumin

		positive

Figure 1 TLC results of Curcumae Longae Rhizoma extract observed under UV light (366 nm)