Wenyujin Rhizoma Concisum

Figure 1 A photograph of Wenyujin Rhizoma Concisum

1. NAMES

Official Name: Wenyujin Rhizoma Concisum

Chinese Name: 片薑黃

Chinese Phonetic Name: Pianjianghuang

2. SOURCE

Wenyujin Rhizoma Concisum is the dried rhizome of *Curcuma wenyujin* Y. H. Chen et C. Ling (Zingiberaceae). The rhizome is collected in winter after stem and leaf withered, washed clean, foreign matter removed, cut into thick longitudinal slices while fresh, then dried under the sun to obtain Wenyujin Rhizoma Concisum.

3. DESCRIPTION

Elliptic or irregular slices, 1.6-10.8 cm long, 0.9-4.5 cm wide, 1-9 mm thick. The outer bark greyish-yellow, rough and uneven with numerous annular nodes and rootlets scars, cut surface yellowish-white to brownish-yellow, with an annular ring and numerous dotted vascular bundles. Texture hard and fragile, fracture greyish-white to brownish-yellow, slightly starchy. Odour characteristic and aromatic; taste slightly bitter, pungent and cool (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of irregularly shaped cells, cell flattened, regularly arranged, cell walls suberized. Cortex broad, scattered with leaf-trace vascular bundles. Endodermis distinct, cells small. Oil cells scattered in cortex and stele. Vascular bundles collateral, tightly arranged, relatively small on the outer part of stele, xylem vessels few, sometimes 1-2 only. Starch granules numerous, gelatinized, elliptic to orbicular-ovate or short-rod-like in shape (Fig. 2).

Powder

Colour greyish-yellow. Oil cells elliptic to rounded-ovate, relatively large, 34-124 μm in diameter, full of yellow or orange oily masses. Starch granules abundant, orbicular-ovate, elliptic or short-rod-like in shape, 13-69 μm long, 5-52 μm in diameter, hilum usually eccentric, locates on the narrow end, striations visible; black and cruciate-shaped under the polarized microscope. Vessels mainly spiral, 10-94 μm in diameter. Non-glandular hair unicellular, usually broken, with pointed tip. Cork cells polygonal, with thickened wall (Fig. 3).

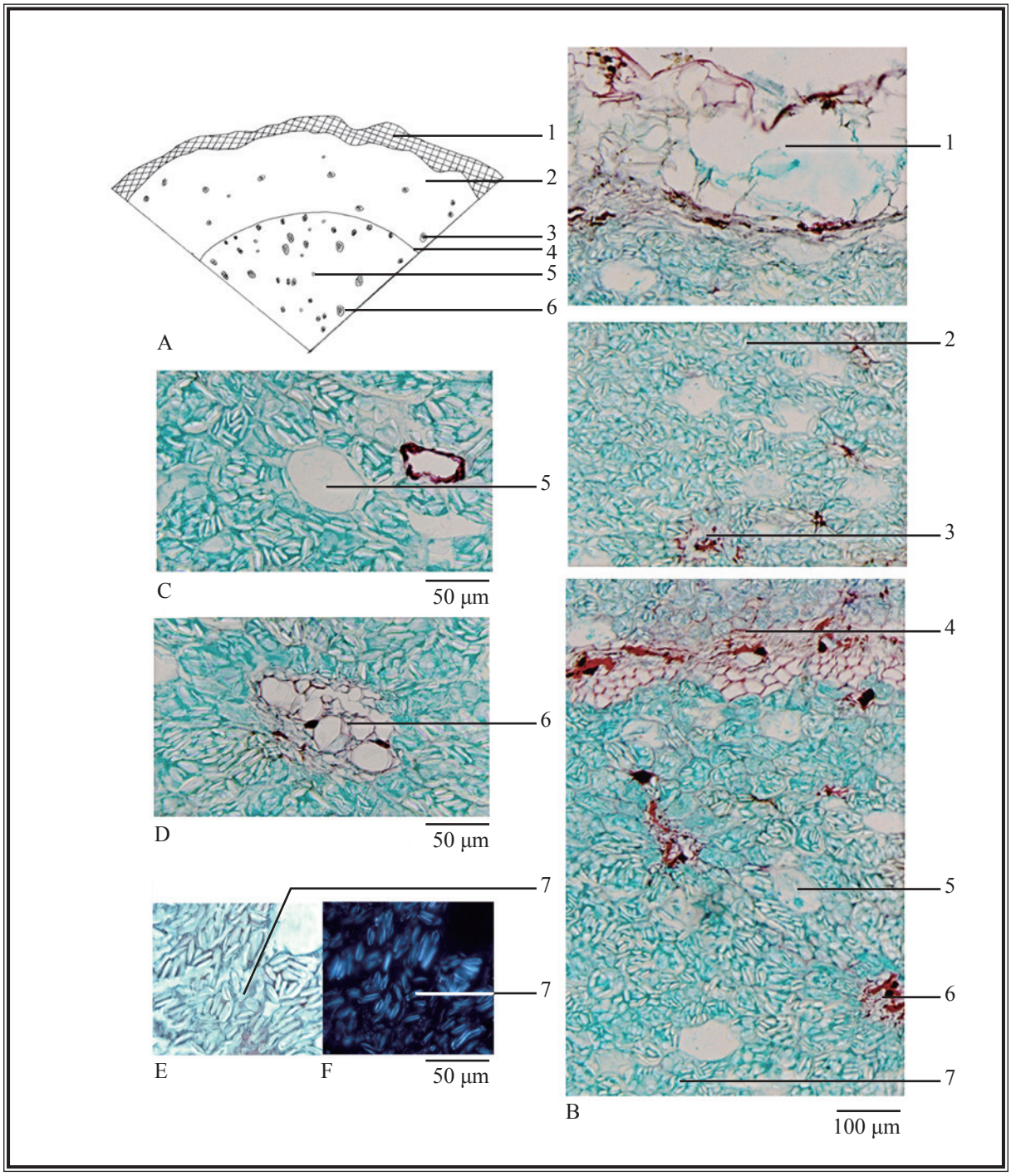

Figure 2 Microscopic features of transverse section of Wenyujin Rhizoma Concisum

A. Sketch B. Section illustration C. Oil cell D. Vascular bundle
E. Starch granules (under the light microscope) F. Starch granules (under the polarized microscope)

1. Cork 2. Cortex 3. Leaf-trace vascular bundle 4. Endodermis 5. Oil cell
6. Vascular bundle 7. Starch granule

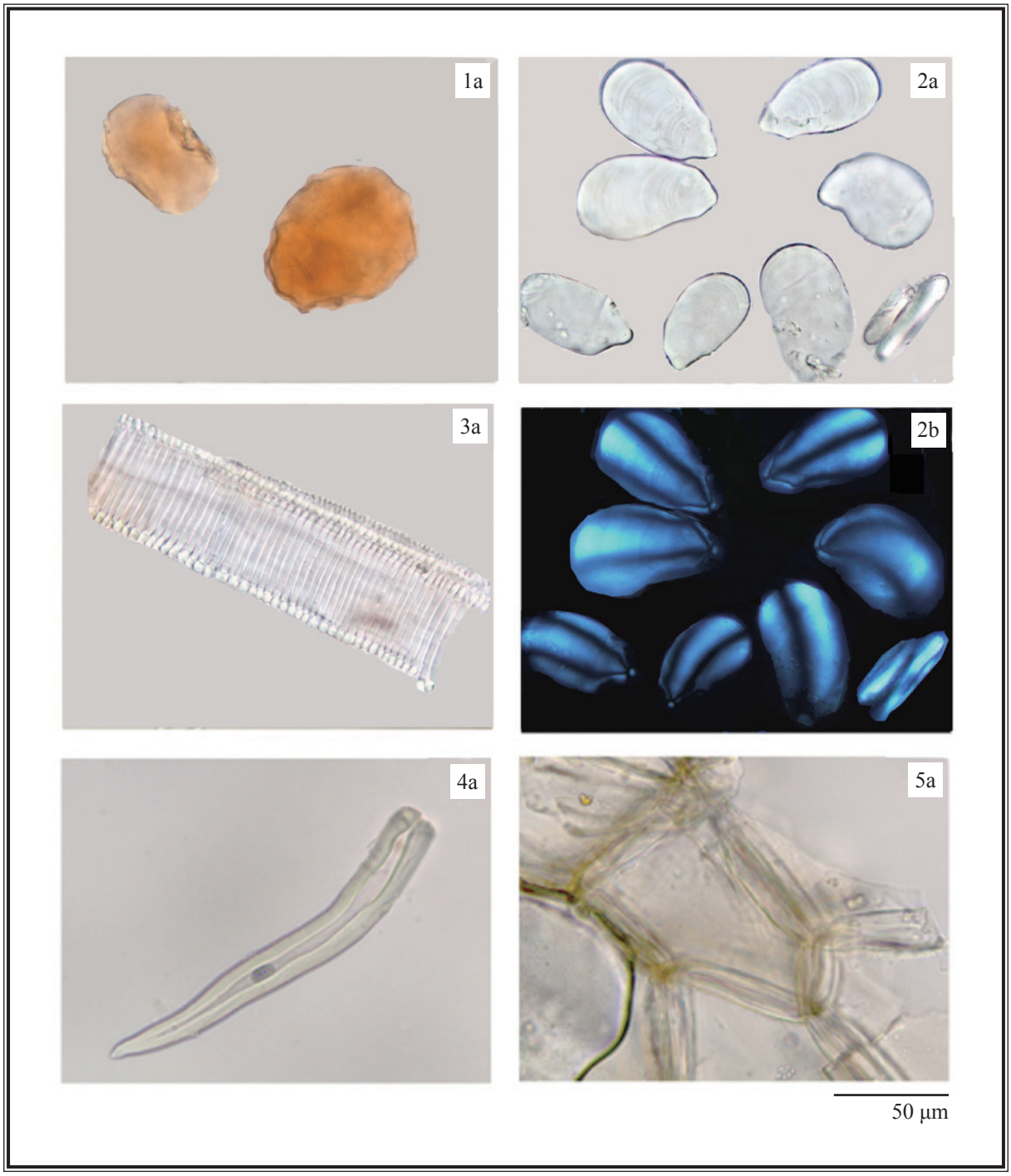

Figure 3 Microscopic features of powder of Wenyujin Rhizome Concisum

1. Oil cells 2. Starch granules 3. Spiral vessel 4. Non-glandular hair 5. Cork cells

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

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

Standard solution

Curcumenol standard solution

Weigh 2.0 mg of curcumenol CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and methanol (9:1:0.5, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of methanol. Sonicate (150 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately curcumenol standard solution (2 μL) and the test solution (10 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 (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

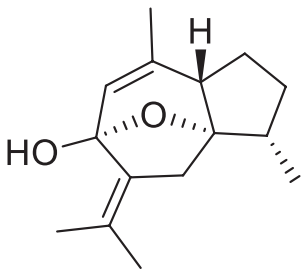

Figure 4 Chemical structure of curcumenol

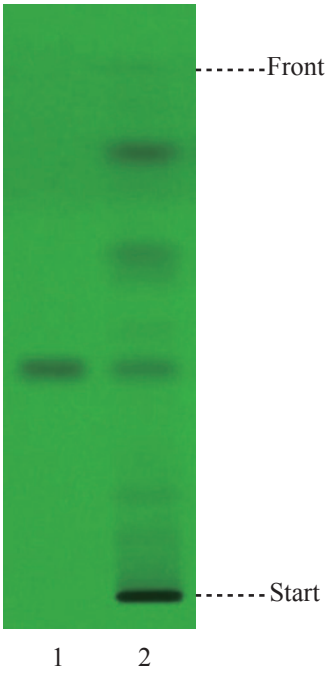

Figure 5 A reference HPTLC chromatogram of Wenyujin Rhizoma Concisum extract observed under UV light (254 nm)

1. Curcumenol standard solution 2. Test solution

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 curcumenol (Fig. 5).

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

Standard solution

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

Weigh 0.5 mg of curcumenol CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (150 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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)	0.04% Trifluoroacetic acid (%, v/v)	Trifluoroacetic acid : Acetonitrile (0.04:99.96, v/v) (%, v/v)	Elution
0 – 35	62	38	isocratic
35 – 45	62 → 47	38 → 53	linear gradient
45 – 60	47 → 38	53 → 62	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of curcumenol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of curcumenol should not be more than 5.0%; the RSD of the retention time of curcumenol peak should not be more than 2.0%; the column efficiency determined from curcumenol peak should not be less than 9000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject curcumenol Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of curcumenol peak in the chromatogram of curcumenol Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify curcumenol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of curcumenol Std-FP. The retention times of curcumenol 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 Wenyujin Rhizoma Concisum extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, curcumenol)	1.00	-
2	1.05	± 0.03
3	1.30	± 0.03
4	1.74	± 0.04

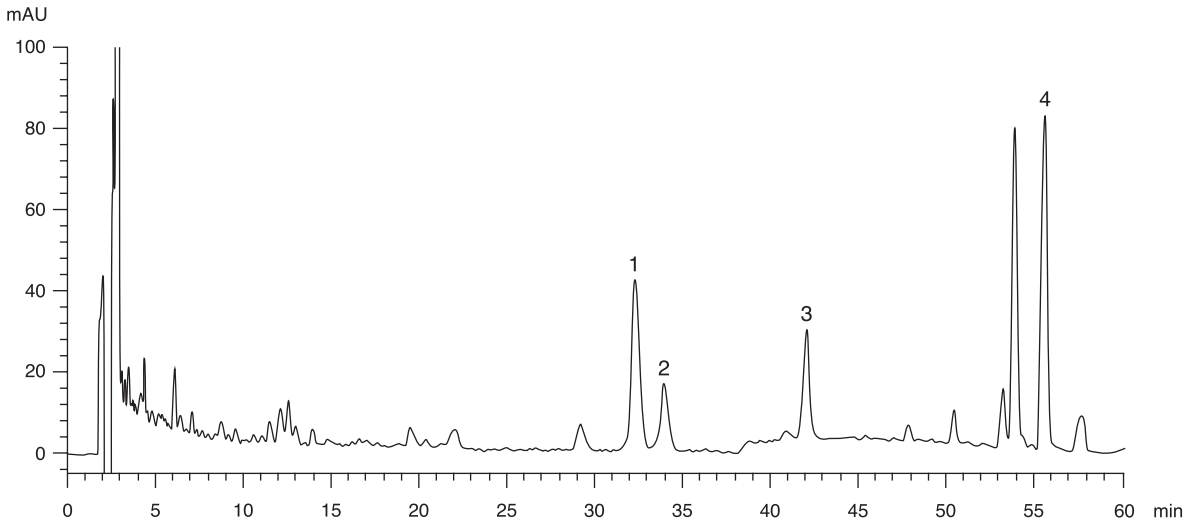

Figure 6 A reference fingerprint chromatogram of Wenyujin Rhizoma Concisum extract

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. 6).

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 XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 17.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

7.1 Assay of Curcumenol

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

Standard solution

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

Weigh accurately 10.0 mg of curcumenol CRS and dissolve in 10 mL of methanol.

Curcumenol standard solution for assay, Std-AS

Measure accurately the volume of the curcumenol Std-Stock, dilute with methanol to produce a series of solutions of 5, 30, 50, 70, 90 mg/L for curcumenol.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.04% Trifluoroacetic acid (%, v/v)	Trifluoroacetic acid : Acetonitrile (0.04:99.96, v/v) (%, v/v)	Elution
0 – 35	62	38	isocratic
35 – 45	62 → 47	38 → 53	linear gradient
45 – 60	47 → 38	53 → 62	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of curcumenol Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of curcumenol should not be more than 5.0%; the RSD of the retention time of curcumenol peak should not be more than 2.0%; the column efficiency determined from curcumenol peak should not be less than 9000 theoretical plates.

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

Calibration curve

Inject a series of curcumenol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of curcumenol against the corresponding concentrations of curcumenol 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 curcumenol peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of curcumenol Std-AS. The retention times of curcumenol 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 curcumenol in the test solution, and calculate the percentage content of curcumenol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.082% of curcumenol ($C_{15}H_{22}O_2$), calculated with reference to the dried substance.

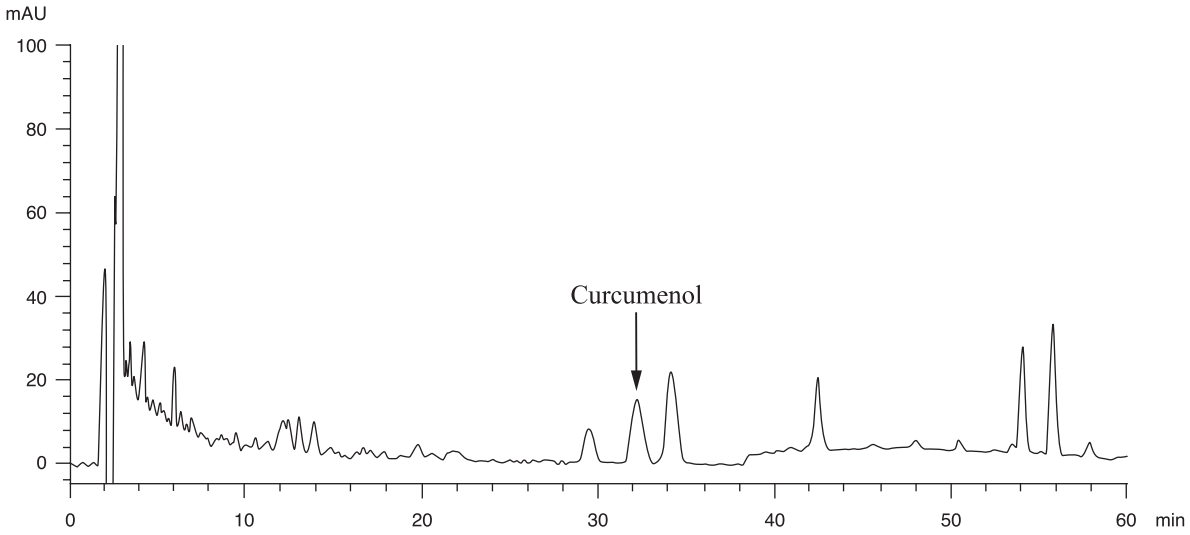

Figure 7 A reference assay chromatogram of Wenyujin Rhizoma Concisum extract

7.2 Assay of Volatile Oil

Weigh accurately 90 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 1.0% (v/w) of volatile oil.