

Anemones Raddeanae Rhizoma



Figure 1 A photograph of Anemones Raddeanae Rhizoma

A. Anemones Raddeanae Rhizoma

B. Magnified image of transverse section of rhizome

1. NAMES

Official Name: *Anemones Raddeanae Rhizoma*

Chinese Name: 兩頭尖

Chinese Phonetic Name: Liangtoujian

2. SOURCE

Anemones Raddeanae Rhizoma is the dried rhizome of *Anemone raddeana* Regel (Ranunculaceae). The rhizome is collected in summer, rootlets removed, washed clean, then dried under the sun to obtain *Anemones Raddeanae Rhizoma*.

3. DESCRIPTION

Long subfusiform, acute and pointed at both ends, both ends sometimes broken, slightly curved and one end somewhat expanded, 1-3 cm long, 2-7 mm in diameter. Externally brown to brownish-black, with fine longitudinal wrinkles, the expanded portion usually possessing 1-3 fin-like rootlet remnants, occasionally with 3-5 indistinct annulations. Texture hard and fragile, easily broken, fracture slightly even, whitish to greyish-brown, slightly horny. Odour slight; taste bland then slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Epidermis consists of 1 layer of cells, tangentially elongated, outer walls thickened. Cortex consists of more than 10 layers of subrounded parenchymatous cells. Vascular bundles collateral, 10 or more, arranged in an interrupted ring. Phloem cells shrunken. Xylem vessels 6-24. Rays broad. Pith relatively large, consisting of subrounded parenchymatous cells. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour greyish-brown. Starch granules numerous, simple starch granules subrounded to elliptic, 2-13 μm in diameter, hilum pointed or cleft-like, striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-4 units. Epidermal cells brownish-yellow to reddish-brown, outer walls suberized and thickened, frequently protruding into the cells to be ridge-like or tubercular. Vessels mostly reticulate, spiral or scalariform, bordered-pitted vessels few, 10-38 μm in diameter. Parenchymatous cells subrounded (Fig. 3).

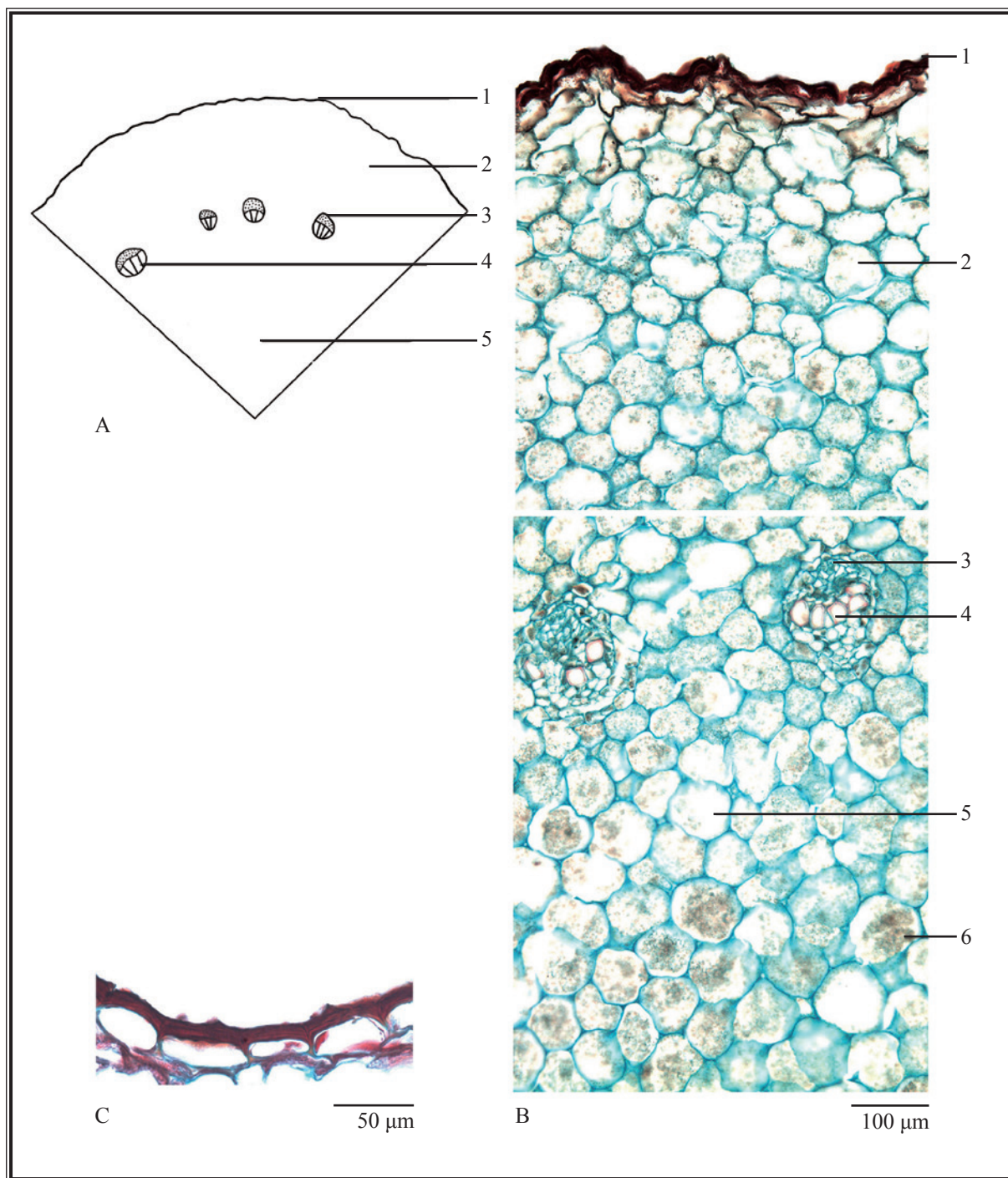


Figure 2 Microscopic features of transverse section of *Anemones Raddeanae* Rhizoma

A. Sketch B. Section illustration C. Epidermis

1. Epidermis 2. Cortex 3. Phloem 4. Xylem 5. Pith 6. Starch granules

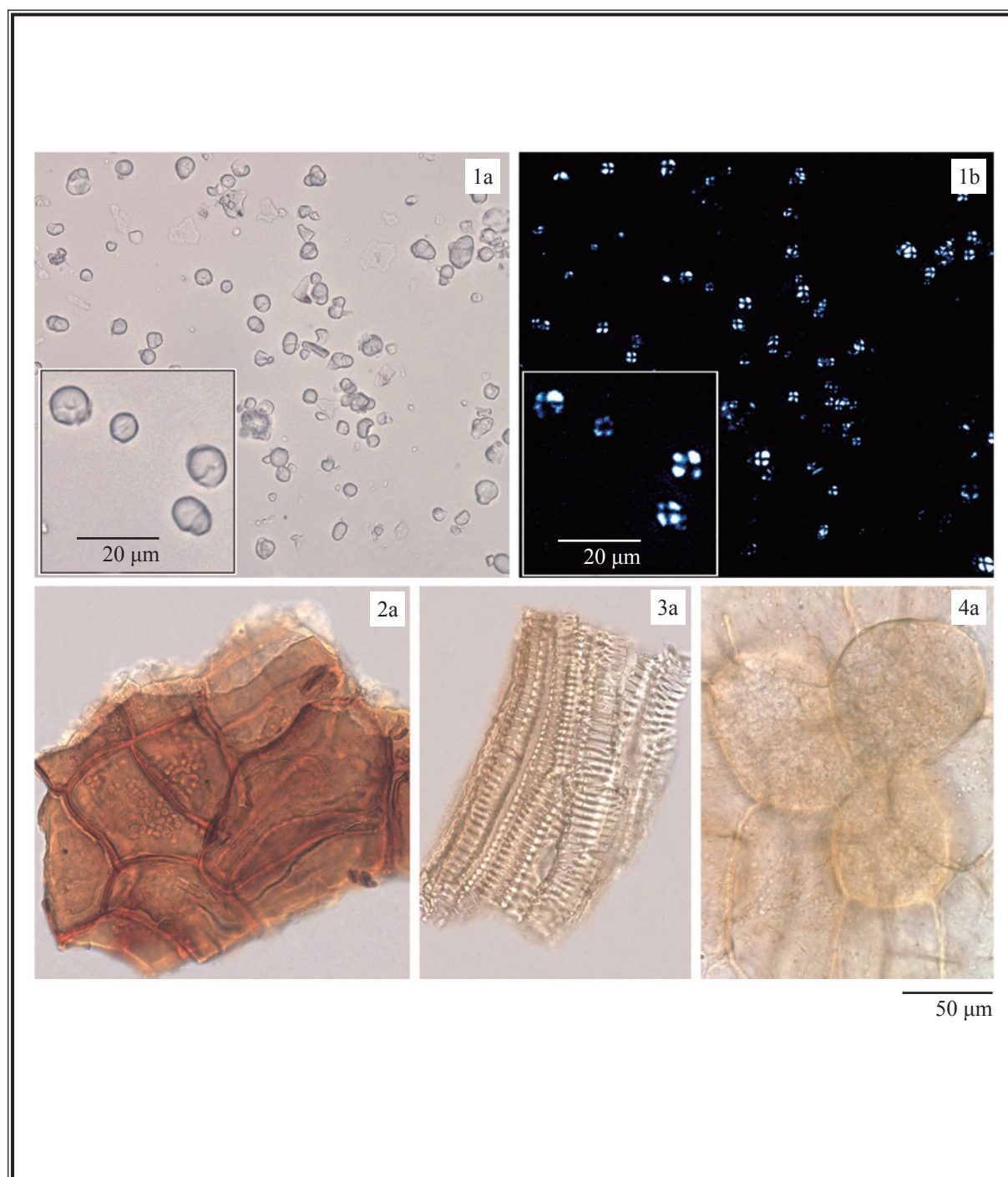


Figure 3 Microscopic features of powder of *Anemones Raddeanae Rhizoma*

1. Starch granules 2. Epidermal cells 3. Vessels 4. Parenchymatous cells

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

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

Standard solution

Raddeanin A standard solution

Weigh 1.0 mg of raddeanin A CRS (Fig. 4) and dissolve in 1 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of ethyl acetate, dichloromethane, methanol and water (4:3:2:0.5, v/v).

Spray reagent

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

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (95%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%).

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately raddeanin A standard solution and the test solution (1 µL each) to the plate. Develop over a path of about 6 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 heat at about 105°C (about 10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

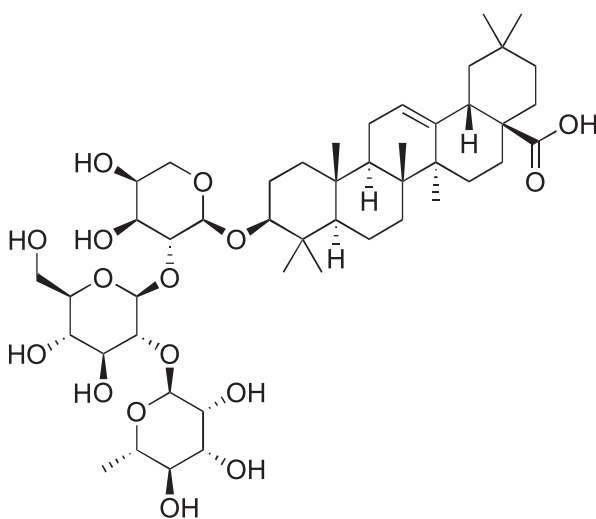


Figure 4 Chemical structure of raddeanin A

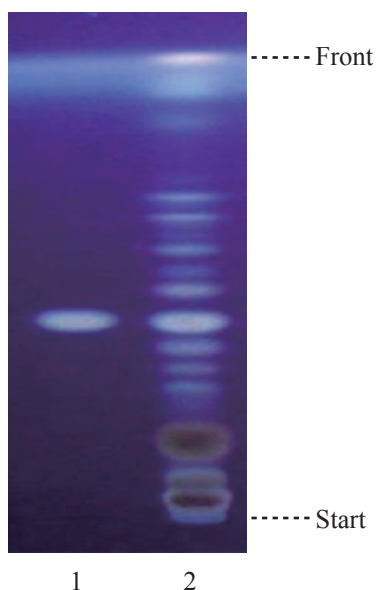


Figure 5 A reference HPTLC chromatogram of *Anemones Raddeanae Rhizoma* extract observed under UV light (366 nm) after staining

1. Raddeanin A 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 raddeanin A (Fig. 5).

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

Standard solution

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

Weigh 2.0 mg of raddeanin A CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (95%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $5000 \times g$ for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (95%). Filter through a 0.45- μ m RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (206 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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)	0.2% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 60	85 → 40	15 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of raddeanin A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of raddeanin A should not be more than 5.0%; the RSD of the retention time of raddeanin A peak should not be more than 2.0%; the column efficiency determined from raddeanin A peak should not be less than 150000 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. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of *Anemones Raddeanae* Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.26	± 0.04
2	0.53	± 0.03
3	0.61	± 0.03
4 (marker, raddeanin A)	1.00	-

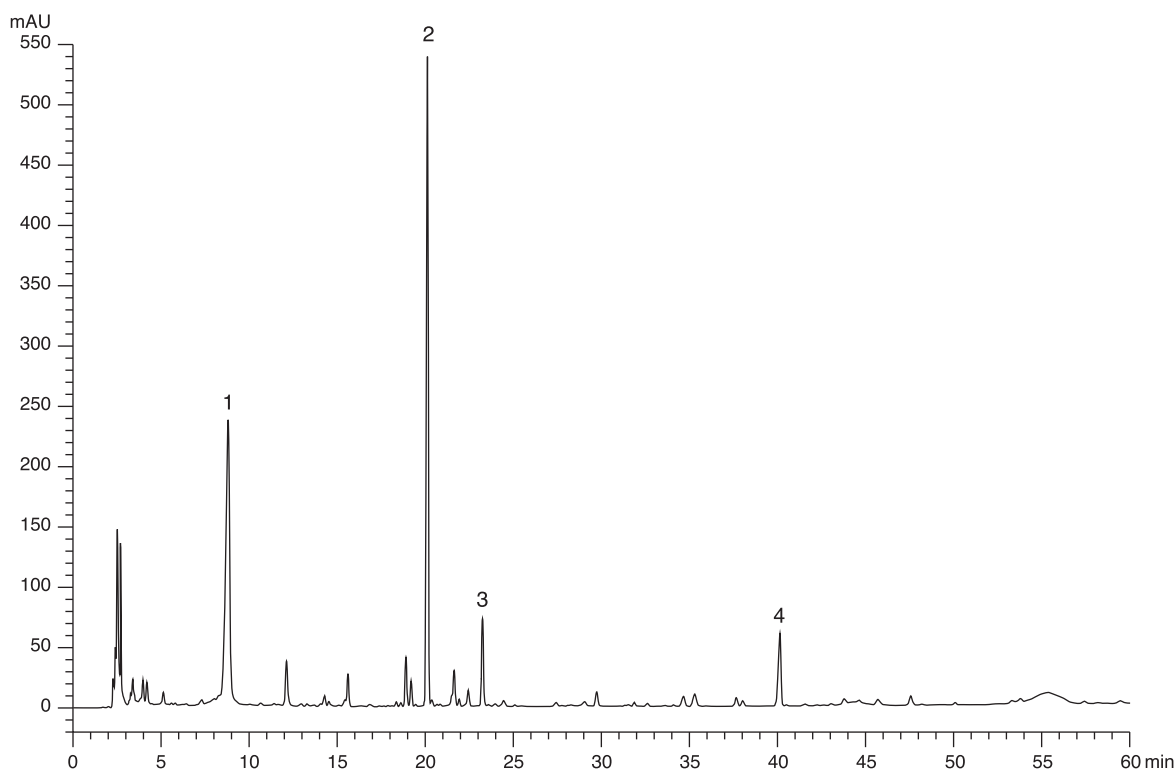


Figure 6 A reference fingerprint chromatogram of *Anemones Raddeanae Rhizoma* extract

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. 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 3.5%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*)

Oven dried method: not more than 12.0%.

6. EXTRACTIVES (Appendix XI)

Ethanol-soluble extractives (cold extraction method): not less than 16.0%.
Note: no water-soluble extractives is proposed due to the high viscosity of water-soluble extracts.

7. ASSAY

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

Standard solution

Raddeanin A standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of raddeanin A CRS and dissolve in 5 mL of ethanol.
Raddeanin A standard solution for assay, Std-AS
Measure accurately the volume of the raddeanin A Std-Stock, dilute with ethanol to produce a series of solutions of 5, 100, 200, 300, 400 mg/L for raddeanin A.

Test solution

Weigh accurately 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (95%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × g for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (95%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (206 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)	0.2% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 60	85 → 40	15 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of raddeanin A Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of raddeanin A should not be more than 5.0%; the RSD of the retention time of raddeanin A peak should not be more than 2.0%; the column efficiency determined from raddeanin A peak should not be less than 150000 theoretical plates.

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

Limits

The sample contains not less than 0.11% of raddeanin A (C₄₇H₇₆O₁₆), calculated with reference to the dried substance.

Anemones Raddeanae Rhizoma (兩頭尖)

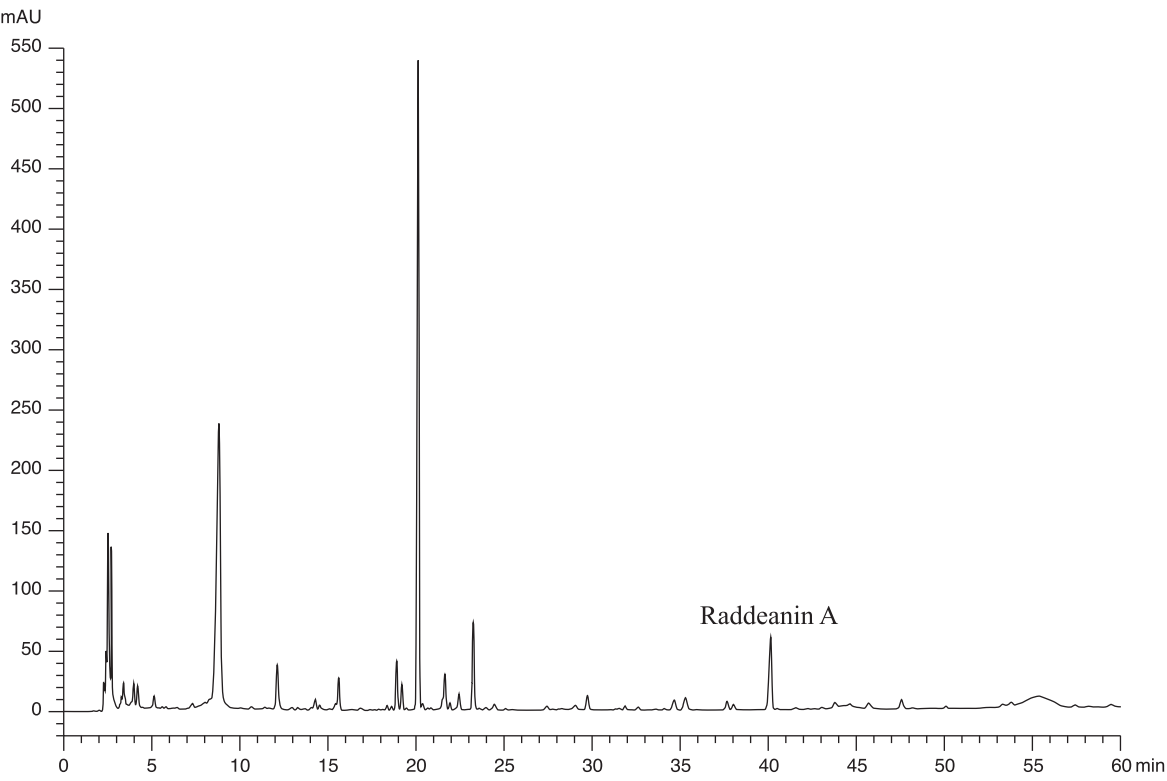


Figure 1 A reference assay chromatogram of *Anemones Raddeanae* Rhizoma extract