Ranunculi Ternati Radix

Figure 1 A photograph of Ranunculi Ternati Radix

A. Ranunculi Ternati Radix

B. A single root tuber (a piece of claw)

C. Magnified image of cut surface of Ranunculi Ternati Radix

1. NAMES

Official name: Ranunculi Ternati Radix

Chinese name: 貓爪草

Chinese phonetic name: Maozhaocao

2. SOURCE

Ranunculi Ternati Radix is the dried root tuber of *Ranunculus ternatus* Thunb. (Ranunculaceae). The root tuber is collected in spring and autumn, foreign matter removed, then dried under the sun to obtain Ranunculi Ternati Radix.

3. DESCRIPTION

Single root tubers fusiform, 2.7–10.4 mm long, 1.3–5.9 mm in diameter, several to dozens of fusiform root tubers fascicle like a cat's claw, apex with yellowish-brown remains of stem or stem scars. Externally brown to greyish-brown, gradually darken on long term storage, with slight longitudinal wrinkles and some dotted rootlets scars or residual rootlets. Texture compact, cut surface whitish to yellowish-white, mostly solid, rarely hollow at the centre, powderly. Odour slight; taste slightly sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal hairs slightly curved, slender, unicellular, sometimes broken, walls relatively thick, lumen clear. Epidermis consists of 1 layer of cells, tangentially elongated, yellowish-brown, some differentiated into epidermal hairs, slightly lignified. Cortex consists of 20-30 layers of parenchymatous cells, subpolygonal or irregular in shape, walls slightly thickened. Endodermis indistinct, containing 2-3 strands each of xylem and phloem. Xylem and phloem arranged alternatively. Starch granules numerously found in parenchymatous cells of cortex (Fig. 2).

Powder

Colour brown. Epidermal cells brownish-yellow to brown; subsquare, subpolygonal or irregular in shape on the surface view; walls relatively thick, cells contain subrounded or elliptical nucleus-like masses. Epidermal hairs slender, slightly curved, unicellular, 8-32 μm in diameter; walls relatively thick and lumen clear. Starch granules numerous; simple starch granules subrounded, subtriangular or irregular in shape, 4-20 μm in diameter; hilum unclear; black and cruciate shaped under the polarized microscope; compound starch granules composed of 2-3 units. Vessels mainly scalariform and spiral vessels, 7-37 μm in diameter. Parenchymatous cells subpolygonal or irregular in shape, slightly thickened, containing numerous starch granules (Fig. 3).

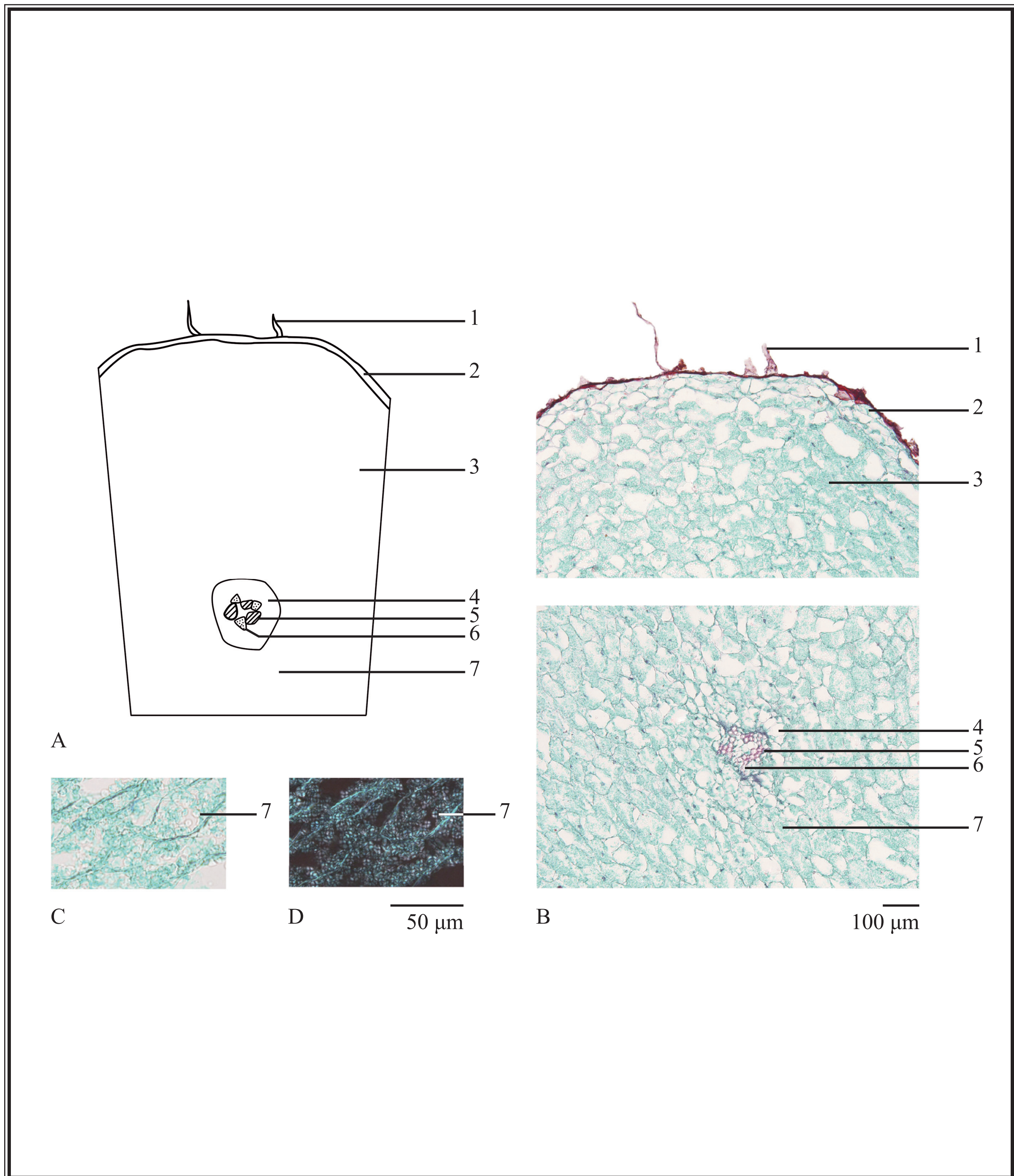

Figure 2 Microscopic features of transverse section of Ranunculi Ternati Radix

A. Sketch B. Section illustration
C. Starch granules in parenchymatous cells (under the light microscope)
D. Starch granules in parenchymatous cells (under the polarized microscope)

1. Epidermal hair 2. Epidermis 3. Cortex 4. Endodermis
5. Xylem 6. Phloem 7. Starch granules

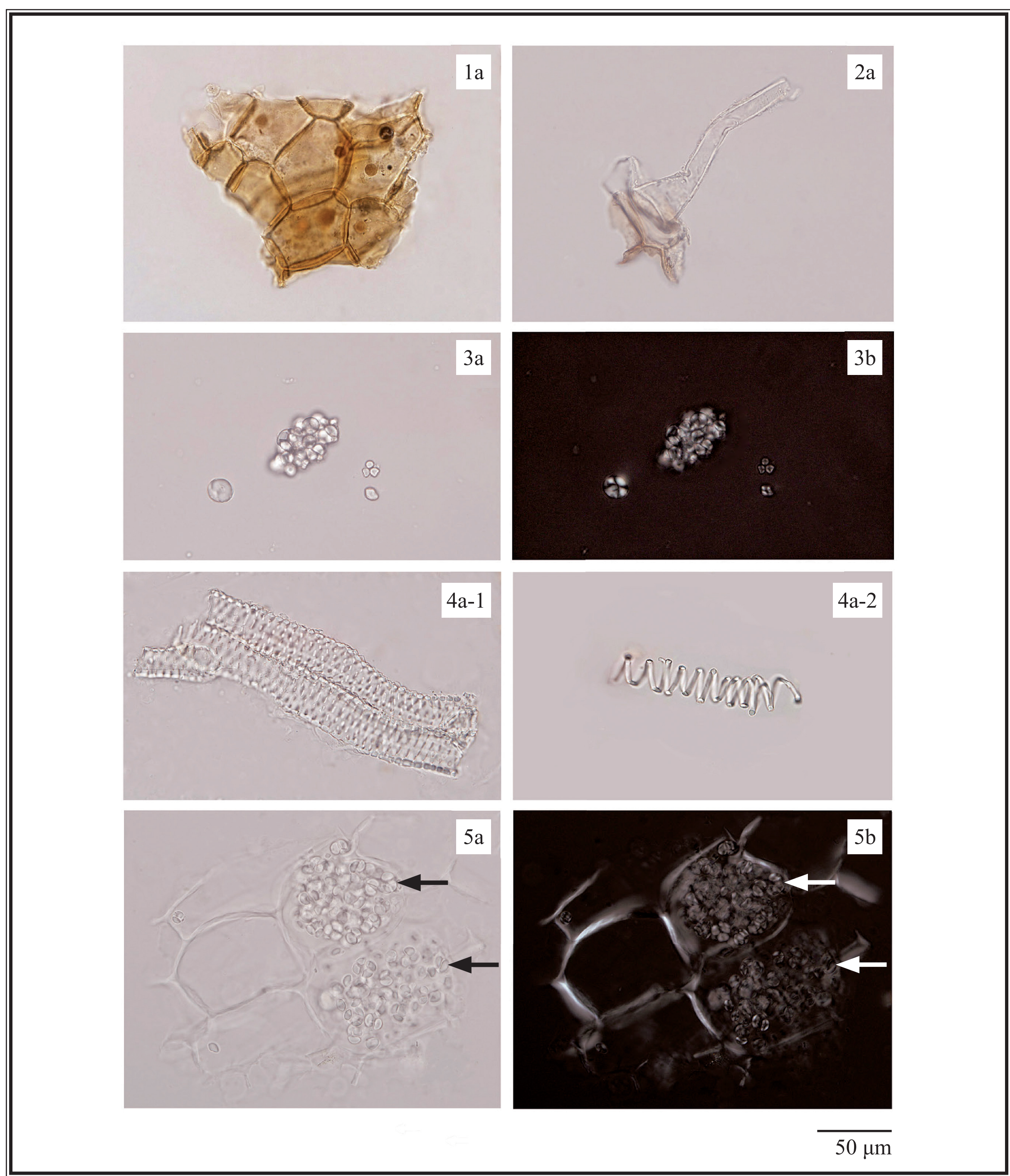

Figure 3 Microscopic features of powder of Ranunculi Ternati Radix

1. Epidermal cells 2. Epidermal hair (attached to epidermal cells)
3. Starch granules 4. Vessels (4-1 scalariform vessels, 4-2 spiral vessel)
5. Parenchymatous cells containing starch granules (→)

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

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

Standard solutions

Linoleic acid standard solution

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

Palmitic acid standard solution

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

Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate, acetonitrile and glacial acetic acid (6:2.5:0.25:0.1, v/v).

Spray reagent

Weigh 0.1 g of 2’,7’-dichlorofluorescein and dissolve in 50 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of *n*-hexane. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC plate of silica gel F_{254} with 15% silver nitrate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately linoleic acid standard solution (10 µL), palmitic acid standard solution (10 µL) and the test solution (4 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. Spray the plate evenly with the spray reagent and heat at about 105ºC (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).

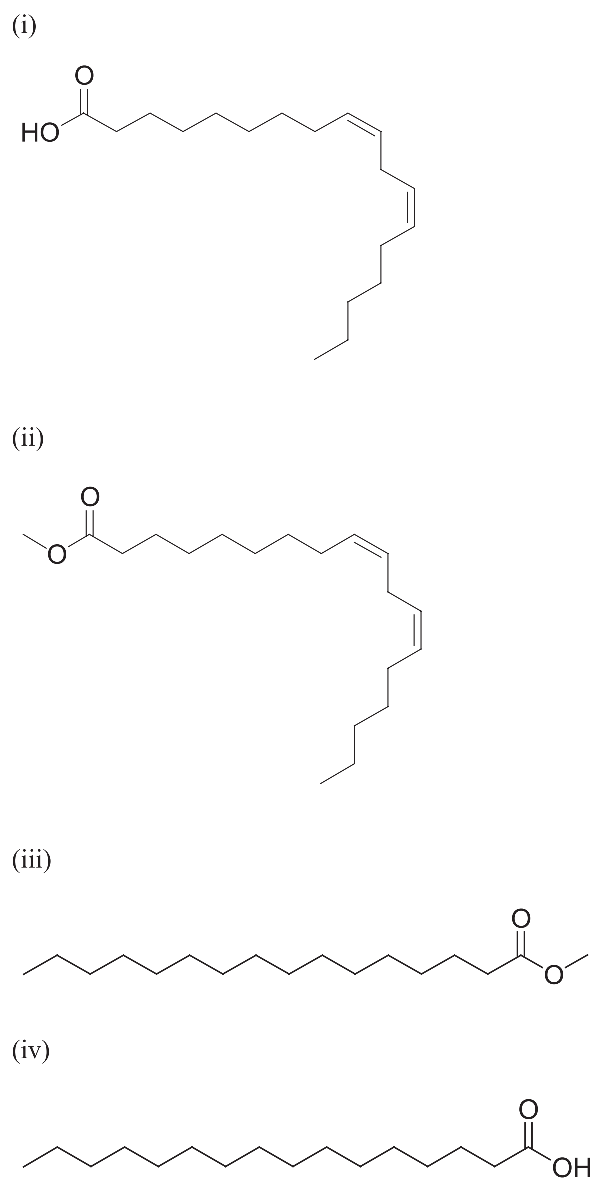

Figure 4 Chemical structures of (i) linoleic acid (ii) methyl linoleate (iii) methyl palmitate and (iv) palmitic acid

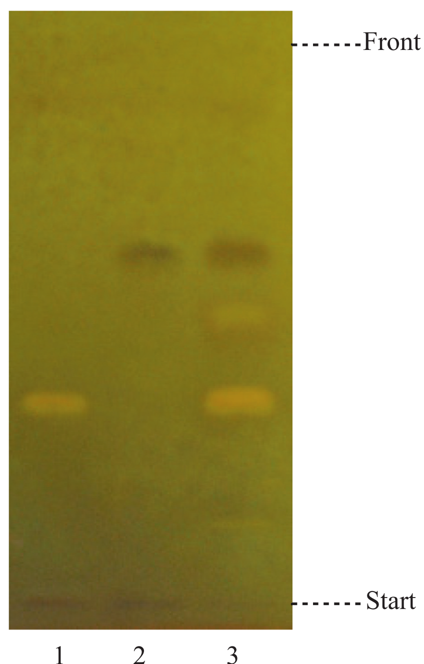

Figure 5 A reference HPTLC chromatogram of Ranunculi Ternati Radix extract observed under UV light (366 nm) after staining

1. Linoleic acid standard solution 2. Palmitic acid standard solution
3. Test solution

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 linoleic acid and palmitic acid (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Methyl linoleate standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 0.1 mg of methyl linoleate CRS (Fig. 4) and dissolve in 1 mL of *n*-hexane.

Methyl palmitate standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 0.1 mg of methyl palmitate CRS (Fig. 4) and dissolve in 1 mL of *n*-hexane.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of methanol and 1 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with methanol. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of *n*-hexane. Transfer the extract to a 250-mL separating funnel. Extract with 10 mL of water. Collect the *n*-hexane extract. Extract the aqueous layer for four times each with 20 mL of *n*-hexane. Combine the *n*-hexane extracts and add 1.0 g of anhydrous sodium sulphate. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with *n*-hexane. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in *n*-hexane. Transfer the extract to a 10-mL volumetric flask and make up to the mark with *n*-hexane. Pipette 1 mL of the extract to a 10-mL volumetric flask and make up to the mark with *n*-hexane. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-WAX, 0.25 mm × 30 m) of which the internal wall is covered with polyethylene glycol in a layer about 0.25 µm thick. Nitrogen is used as carrier gas at 1.0 mL/min. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 1) -

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 10	110 → 120	1
10 – 20	120 → 160	4
20 – 60	160 → 180	0.5

System suitability requirements

Perform at least five replicate injections, each using 1 µL of methyl linoleate Std-FP and methyl palmitate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of methyl linoleate and methyl palmitate should not be more than 5.0%; the RSD of the retention times of methyl linoleate and methyl palmitate peaks should not be more than 2.0%; the column efficiencies determined from methyl linoleate and methyl palmitate peaks should not be less than 100000 and 150000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject methyl linoleate Std-FP, methyl palmitate Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention times of methyl linoleate and methyl palmitate peaks in the chromatograms of methyl linoleate Std-FP, methyl palmitate Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify methyl linoleate and methyl palmitate peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of methyl linoleate Std-FP and methyl palmitate Std-FP. The retention times of methyl linoleate and methyl palmitate 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 six characteristic peaks of Ranunculi Ternati Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Ranunculi Ternati Radix extract

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2 (methyl palmitate)	0.64	± 0.03
3 (methyl stearate)	0.91	± 0.03
4 (methyl oleate)	0.93	± 0.03
5 (marker, methyl linoleate)	1.00	-
6 (methyl linolenate)	1.11	± 0.03

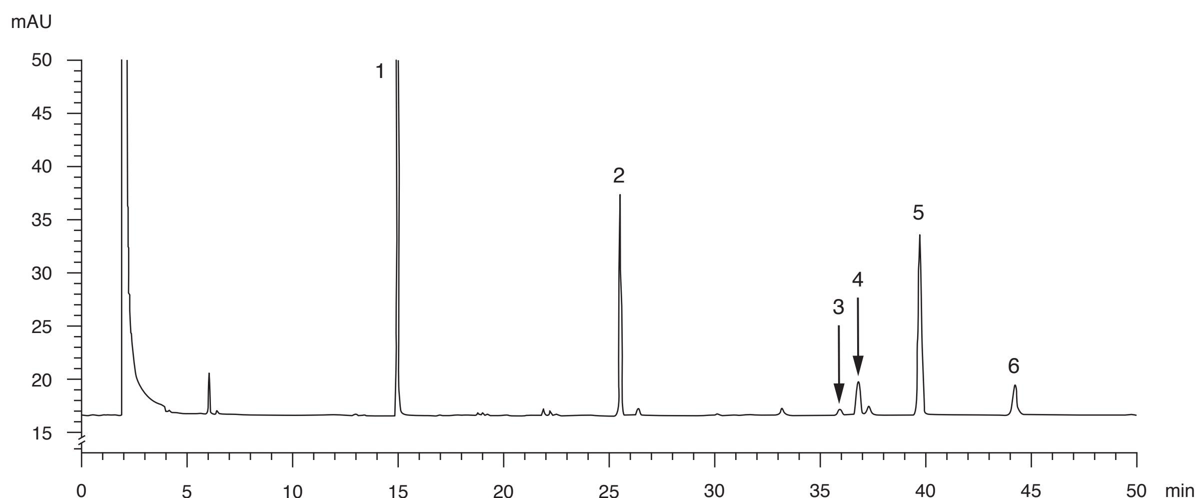

Figure 6 A reference GC fingerprint chromatogram of Ranunculi Ternati Radix extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference GC 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 *(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 7.5%.
Acid-insoluble ash: not more than 4.0%.

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 38.0%.
Ethanol-soluble extractives (cold extraction method): not less than 34.0%.

7. ASSAY

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

Standard solution

Mixed methyl linoleate and methyl palmitate standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of methyl linoleate CRS and 5.0 mg of methyl palmitate CRS, and dissolve in 10 mL of *n*-hexane.

Mixed methyl linoleate and methyl palmitate standard solution for assay, Std-AS

Measure accurately the volume of the mixed methyl linoleate and methyl palmitate Std-Stock, dilute with *n*-hexane to produce a series of solutions of 25, 50, 100, 250, 500 mg/L for methyl linoleate and 10, 25, 50, 100, 250 mg/L for methyl palmitate.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of methanol and 1 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with methanol. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of *n*-hexane. Transfer the extract to a 250-mL separating funnel. Extract with 10 mL of water. Collect the *n*-hexane extract. Extract the aqueous layer for four times each with 20 mL of *n*-hexane. Combine the *n*-hexane extracts and add 1.0 g of anhydrous sodium sulphate. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with *n*-hexane. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in *n*-hexane. Transfer the extract to a 10-mL volumetric flask and make up to the mark with *n*-hexane. Pipette 1 mL of the extract to a 10-mL volumetric flask and make up to the mark with *n*-hexane. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-WAX, 0.25 mm × 30 m) of which the internal wall is covered with polyethylene glycol in a layer about 0.25 µm thick. Nitrogen is used as carrier gas at 1.0 mL/min. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 3) -

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 10	110 → 120	1
10 – 20	120 → 160	4
20 – 60	160 → 180	0.5

System suitability requirements

Perform at least five replicate injections, each using 1 µL of the mixed methyl linoleate and methyl palmitate Std-AS (100 mg/L for methyl linoleate and 50 mg/L for methyl palmitate). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of methyl linoleate and methyl palmitate should not be more than 5.0%; the RSD of the retention times of methyl linoleate and methyl palmitate peaks should not be more than 2.0%; the column efficiencies determined from methyl linoleate and methyl palmitate peaks should not be less than 100000 and 150000 theoretical plates respectively.

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

Calibration curves

Inject a series of the mixed methyl linoleate and methyl palmitate Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of methyl linoleate and methyl palmitate against the corresponding concentrations of the mixed methyl linoleate and methyl palmitate Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify methyl linoleate and methyl palmitate peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed methyl linoleate and methyl palmitate Std-AS. The retention times of methyl linoleate and methyl palmitate 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 methyl linoleate and methyl palmitate in the test solution, and calculate the percentage contents of linoleic acid (the percentage content of methyl linoleate × 0.95, where 0.95 is the molar mass ratio of linoleic acid and methyl linoleate) and palmitic acid (the percentage content of methyl palmitate × 0.95, where 0.95 is the molar mass ratio of palmitic acid and methyl palmitate) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.52% of the total content of linoleic acid ($C_{18}H_{32}O_2$) and palmitic acid ($C_{16}H_{32}O_2$), calculated with reference to the dried substance.

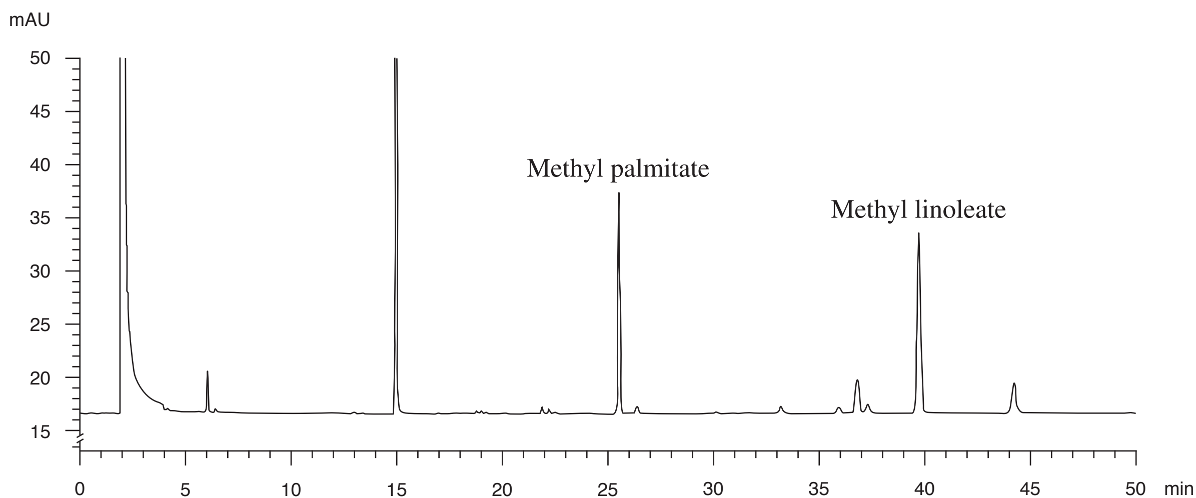

Figure 7 A reference GC assay chromatogram of Ranunculi Ternati Radix extract