Ginseng Folium

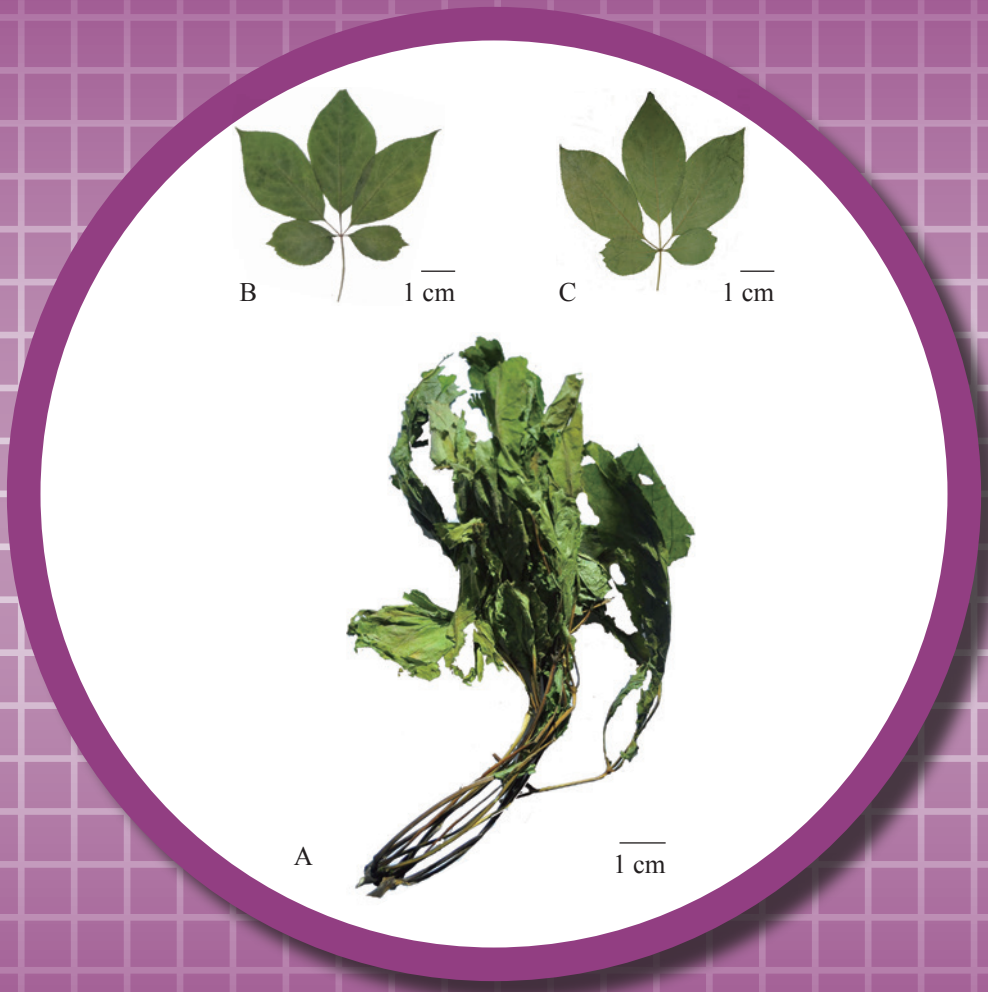


Figure 1 A photograph of Ginseng Folium

A. Ginseng Folium B. Upper surface of palmately compound leaf
C. Lower surface of palmately compound leaf

1. NAMES

Official Name: Ginseng Folium

Chinese Name: 人參葉

Chinese Phonetic Name: Renshenye

2. SOURCE

Ginseng Folium is the dried leaf of *Panax ginseng* C. A. Mey. (Araliaceae). The leaf is collected in autumn, then dried under the sun or dried in a shaded area to obtain Ginseng Folium.

3. DESCRIPTION

Leaves palmately compound, dark green to yellowish-green, petiolate, 10-36 cm long. Leaflets usually 5, occasionally 7 or 9, ovate or obovate, leaflet 2-16 cm long, 1-7 cm wide; base cuneate, apex acuminate, margin serrulate with setae. Upper surface with some setae; lower surface with protuberant veins. Texture papery and fragile. Odour slightly fragrant; taste predominantly bitter with slightly sweet aftertaste (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermis consists of 1 layer of subsquare to rectangular cells. Palisade tissue absent, mesophyll cells rounded or subrounded, with clusters of calcium oxalate visible. Collenchyma consists of 1-3 layers of cells, underneath the upper and lower epidermis of the midrib. Clusters of calcium oxalate occasionally found in the midrib parenchymatous cells. Vascular bundles collateral. Xylem vessels radially arranged. Lower epidermis consists of 1 layer of irregularly shaped cells (Fig. 2).

Powder

Colour greyish-green. Anticlinal walls of lower epidermal cells undulated and curved; stomata anomocytic, with 4-5 subsidiary cells, 3 subsidiary cells occasionally visible, surface of guard cells contain chloroplasts. Anticlinal walls of upper epidermal cells slightly curved,

anomocytic stomata occasionally visible. Clusters of calcium oxalate numerous, 12-42 μm in diameter; polychromatic under the polarized microscope. Scalariform and spiral vessels visible, reticulate vessels occasionally visible, 6-40 μm in diameter. Mesophyll cells rounded to subrounded (Fig. 3).

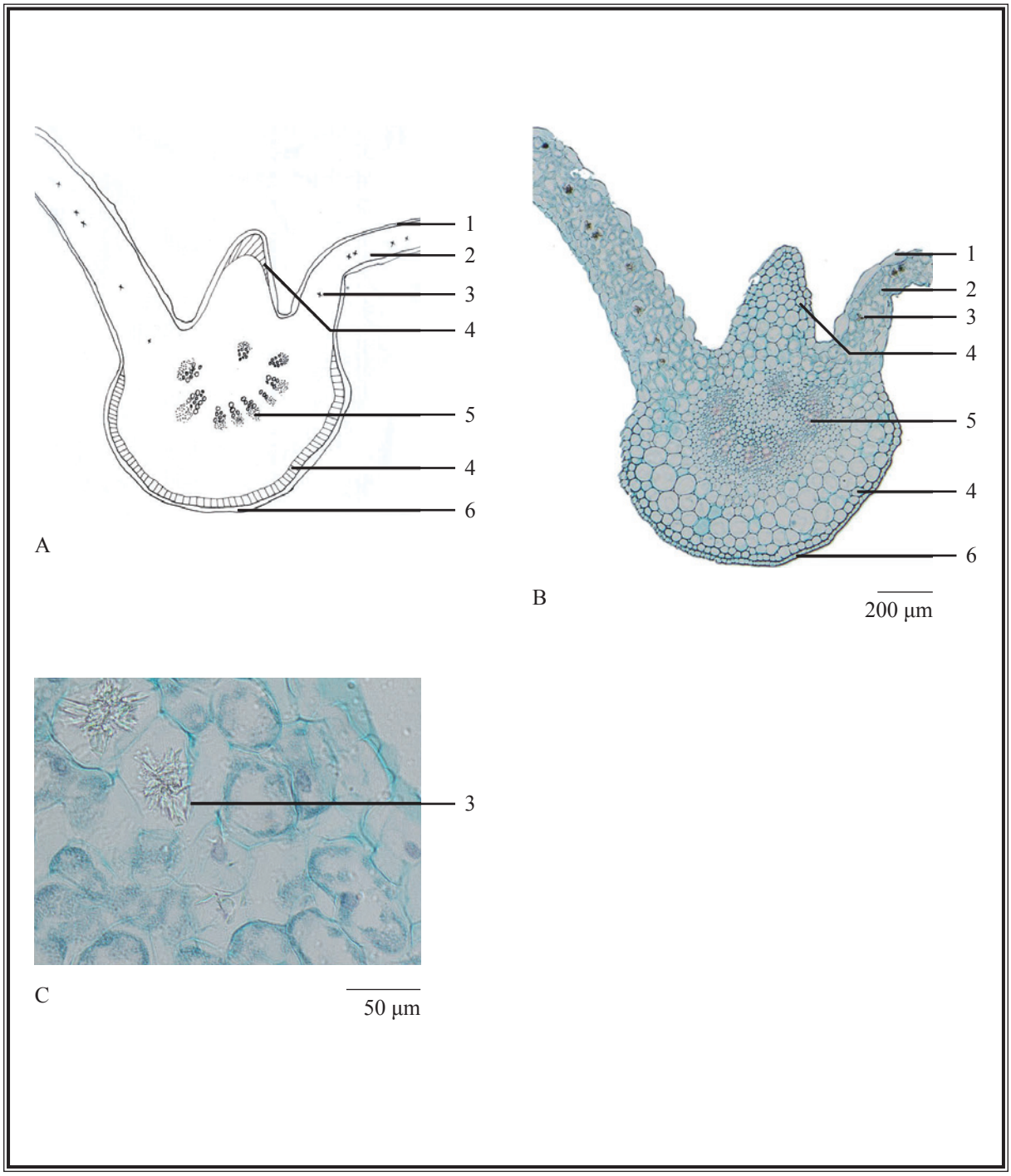


Figure 2 Microscopic features of transverse section of Ginseng Folium

A. Sketch B. Section illustration C. Clusters of calcium oxalate

1. Upper epidermis 2. Mesophyll 3. Clusters of calcium oxalate 4. Collenchyma 5. Vascular bundles 6. Lower epidermis

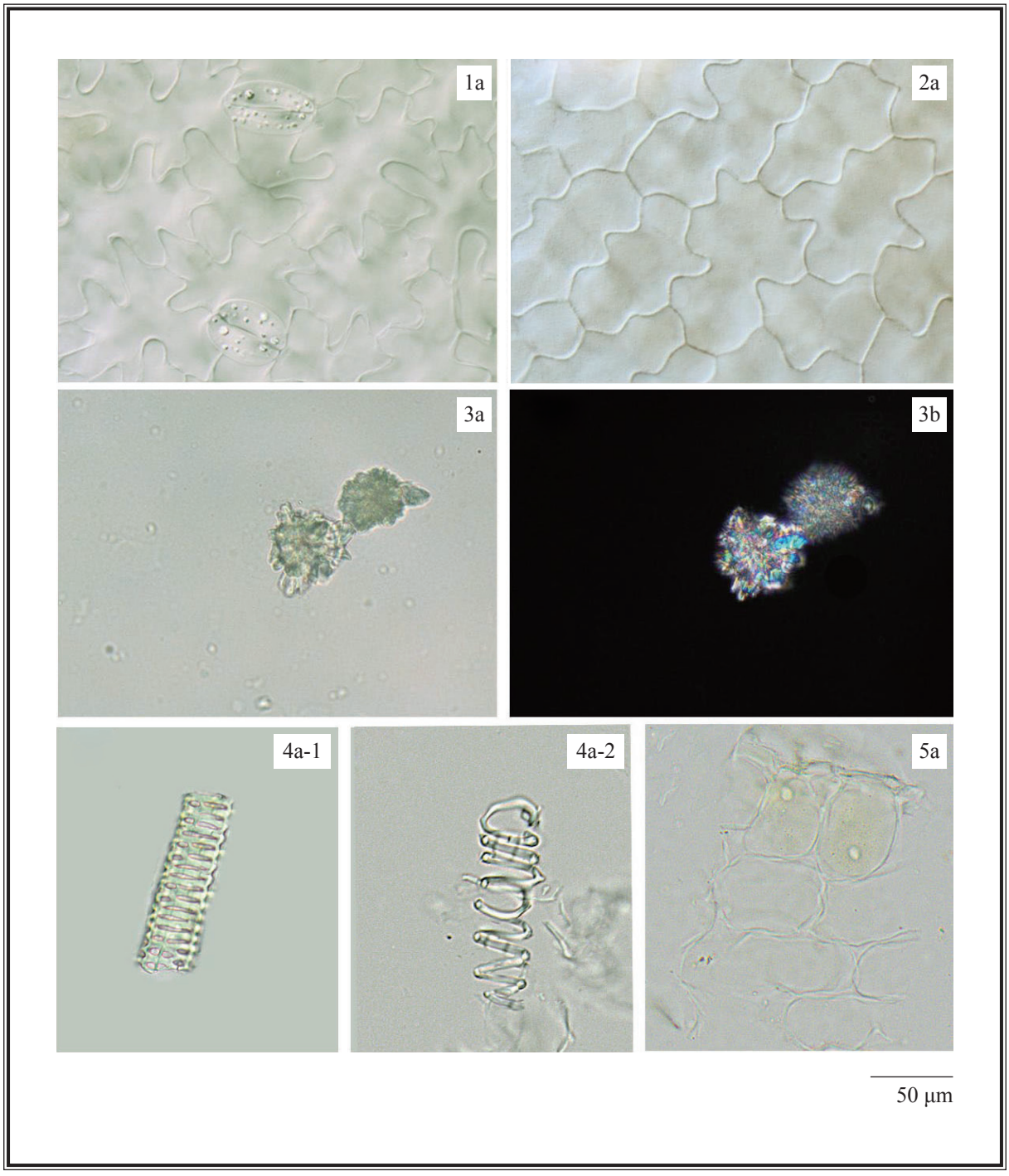


Figure 3 Microscopic features of powder of Ginseng Folium

1. Lower epidermal cells with stomata 2. Upper epidermal cells 3. Clusters of calcium oxalate
4. Vessel (4-1 scalariform vessel, 4-2 spiral vessel) 5. Mesophyll cells

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

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

Standard solutions

Ginsenoside Re standard solution

Weigh 1.5 mg of ginsenoside Re CRS (Fig. 4) and dissolve in 1 mL of methanol.

Ginsenoside Rg_1 standard solution

Weigh 1.4 mg of ginsenoside Rg_1 CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of water, *n*-butanol and ethyl acetate (5:4:1, v/v). Use the upper layer.

Spray reagent

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

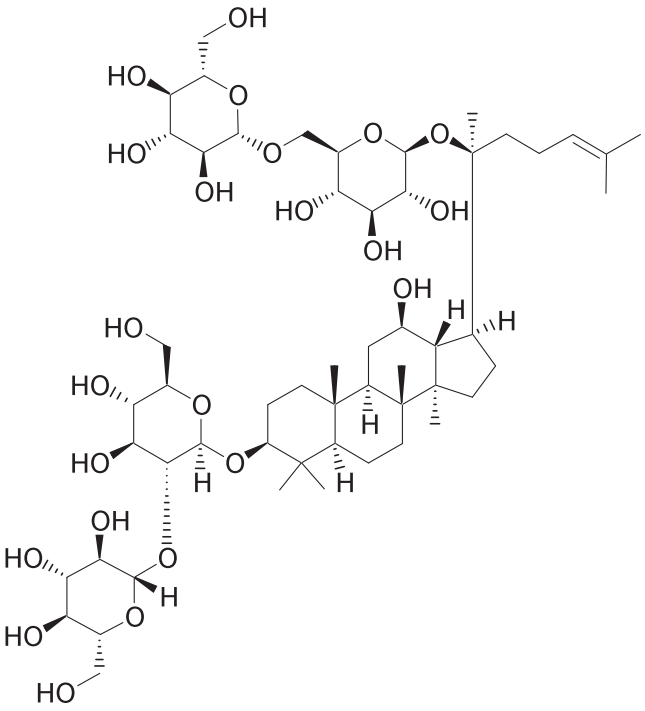
Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (350 W) the mixture for 30 min. Filter through a 0.45-μm nylon filter.

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 ginsenoside Re standard solution (1.5 μL), ginsenoside Rg_1 standard solution (1.5 μL) and the test solution (2 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 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 105ºC (about 3 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



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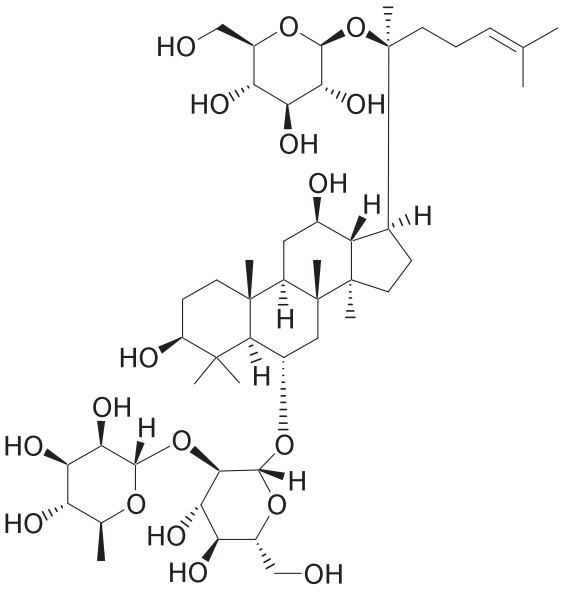

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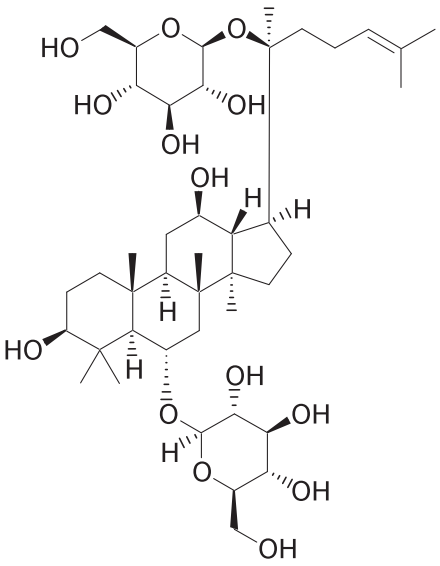

Figure 4 Chemical structures of (i) ginsenoside Rb_1 (ii) ginsenoside Re and (iii) ginsenoside Rg_1

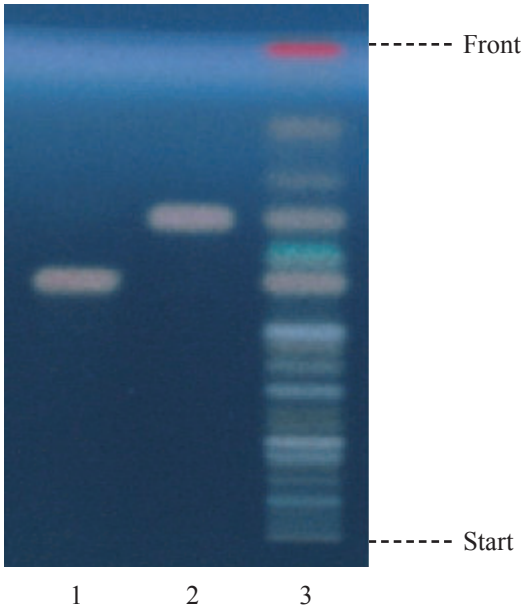

Figure 5 A reference HPTLC chromatogram of Ginseng Folium extract observed under UV light (366 nm) after staining

1. Ginsenoside Re standard solution 2. Ginsenoside Rg_1 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 ginsenoside Re and ginsenoside Rg_1 (Fig. 5).

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

Standard solutions

Ginsenoside Re standard solution for fingerprinting, Std-FP (170 mg/L)

Weigh 1.7 mg of ginsenoside Re CRS and dissolve in 10 mL of methanol (70%).

Ginsenoside Rg_1 standard solution for fingerprinting, Std-FP (70 mg/L)

Weigh 0.7 mg of ginsenoside Rg_1 CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of petroleum ether (60-80°C). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Discard the petroleum ether layer. Repeat the extraction for two more times. Extract the residue in 20 mL of methanol (70%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	79	21	isocratic
10 – 30	79 → 70	21 → 30	linear gradient
30 – 60	70 → 68	30 → 32	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ginsenoside Re Std-FP and ginsenoside Rg_1 Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ginsenoside Re and ginsenoside Rg_1 should not be more than 5.0%; the RSD of the retention times of ginsenoside Re and ginsenoside Rg_1 peaks should not be more than 2.0%; the column efficiencies determined from ginsenoside Re and ginsenoside Rg_1 peaks should not be less than 15000 and 12000 theoretical plates respectively.

The *R* value between peak 1 and peak 2 in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject ginsenoside Re Std-FP, ginsenoside Rg_1 Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of ginsenoside Re and ginsenoside Rg_1 peaks in the chromatograms of ginsenoside Re Std-FP, ginsenoside Rg_1 Std-FP and the retention times of the nine characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify ginsenoside Re and ginsenoside Rg_1 peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of ginsenoside Re Std-FP and ginsenoside Rg_1 Std-FP. The retention times of ginsenoside Re and ginsenoside Rg_1 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 nine characteristic peaks of Ginseng Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the nine characteristic peaks of Ginseng Folium extract

Peak No.	RRT	Acceptable Range
1 (marker, ginsenoside Rg_1)	1.00	-
2 (ginsenoside Re)	1.08	± 0.03
3	2.31	± 0.06
4	2.39	± 0.06
5 (ginsenoside Rb_1)	2.57	± 0.05
6 (ginsenoside Rc)	2.75	± 0.04
7	2.86	± 0.06
8 (ginsenoside Rb_2)	2.94	± 0.04
9 (ginsenoside Rd)	3.39	± 0.04

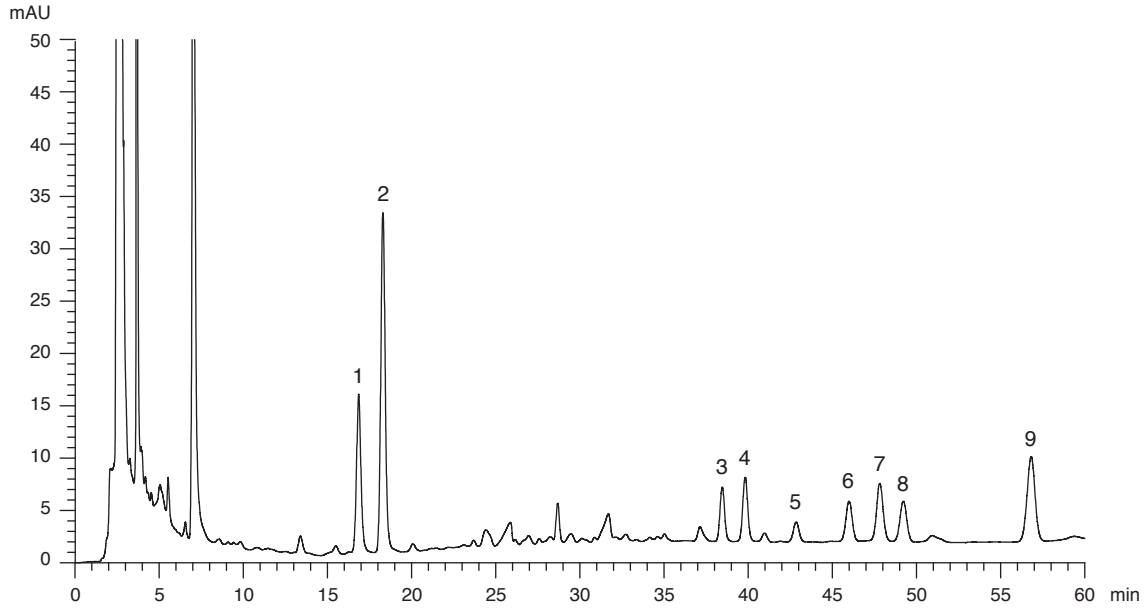

Figure 6 A reference fingerprint chromatogram of Ginseng Folium extract

For positive identification, the sample must give the above nine 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 XVII)*: 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 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 33.0%.
Ethanol-soluble extractives (hot extraction method): not less than 28.0%.

7. ASSAY

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

Standard solution

Mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 standard stock solution, Std-Stock (100 mg/L for ginsenoside Rb_1, 500 mg/L for ginsenoside Re and 200 mg/L for ginsenoside Rg_1)

Weigh accurately 1.0 mg of ginsenoside Rb_1 CRS (Fig. 4), 5.0 mg of ginsenoside Re CRS and 2.0 mg of ginsenoside Rg_1 CRS, and dissolve in 10 mL of methanol (70%).

Mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 standard solution for assay, Std-AS

Measure accurately the volume of the mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 Std-Stock, dilute with methanol (70%) to produce a series of solutions of 0.5, 2.5, 5, 25, 100 mg/L for ginsenoside Rb_1, 12.5, 25, 90, 125, 250 mg/L for ginsenoside Re and 5, 10, 36, 50, 100 mg/L for ginsenoside Rg_1.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of petroleum ether (60-80°C). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Discard the petroleum ether layer. Repeat the extraction for two more times. Extract the residue in 20 mL of methanol (70%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min and transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Combine the solutions and make up the mark with methanol (70%). Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	79	21	isocratic
10 – 30	79 → 70	21 → 30	linear gradient
30 – 60	70 → 68	30 → 32	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of the mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 Std-AS (5 mg/L for ginsenoside Rb_1, 90 mg/L for ginsenoside Re and 36 mg/L for ginsenoside Rg_1). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 should not be more than 5.0%; the RSD of the retention times of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 peaks should not be more than 2.0%; the column efficiencies determined from ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 peaks should not be less than 60000, 15000 and 12000 theoretical plates respectively.

The *R* value between ginsenoside Rb_1 peak and the closest peak; and the *R* value between ginsenoside Re peak and ginsenoside Rg_1 peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 against the corresponding concentrations of the mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 Std-AS. The retention times of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 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 ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 in the test solution, and calculate the percentage contents of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.28% of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$) and not less than 5.3% of the total content of ginsenoside Re ($C_{48}H_{82}O_{18}$) and ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$), calculated with reference to the dried substance.