

Ginseng Radix et Rhizoma Rubra



Figure 1 A photograph of Ginseng Radix et Rhizoma Rubra

- A. Ginseng Radix et Rhizoma Rubra (flat square-columnar root) (rhizome →)
- B. Ginseng Radix et Rhizoma Rubra (fusiform root) (rhizome →)
- C. Ginseng Radix et Rhizoma Rubra (cylindrical root) (rhizome →)
- D. Magnified image of cut surface of root
- E. Magnified image of upper part of root

1. NAMES

Official Name: Ginseng Radix et Rhizoma Rubra

Chinese Name: 紅參

Chinese Phonetic Name: Hongshen

2. SOURCE

Ginseng Radix et Rhizoma Rubra is the steamed dried root and rhizome of the cultivar of *Panax ginseng* C.A. Mey. (Araliaceae). The root and rhizome are harvested in autumn, washed clean, steamed at 98°C, then air-dried to obtain Ginseng Radix et Rhizoma Rubra.

3. DESCRIPTION

Main roots fusiform, cylindrical or flat square-columnar, 2-12 cm long, 10-20 mm in diameter. Externally reddish-brown to yellowish-brown, occasionally exhibiting a few dark yellowish-brown patches, with longitudinal fissures, wrinkles and rootlet scars; the upper part sometimes exhibiting interrupted indistinct annulations, the lower part sometimes bearing 2-3 twisted and intersected branch roots and curved rootlets or just showing remnants of rootlets. Rhizomes 1-2 cm long, showing several impressed-circular stem scars, some bearing 1-2 entire or broken adventitious roots. Texture hard and fragile, fracture flat, horny. Odour delicately aromatic; taste sweet at first, then slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork consists of several layers of flat cells. Cortex narrow. Phloem showing clefts in the outer part, parenchymatous cells in the inner part densely arranged; resin canals scattered. Cambium in a ring. Xylem rays broad. Vessels singly scattered or in groups, with interrupted radial arrangement. Parenchymatous cells contain clusters of calcium oxalate, the crystals in rosette aggregate (Fig. 2).

Powder

Colour yellowish-white. Fragments of resin canals contain yellow or yellowish-brown secretions. Cluster of calcium oxalate numerous, in rosette aggregate, 14-69 µm in diameter; polychromatic under the polarized microscope. Reticulate vessels and scalariform vessels visible, 15-71 µm in diameter. Gelatinization Starch granules irregular; outline indistinct. Cork cells polygonal in surface view, narrow-rectangular in lateral view, walls thickened (Fig. 3).

Ginseng Radix et Rhizoma Rubra

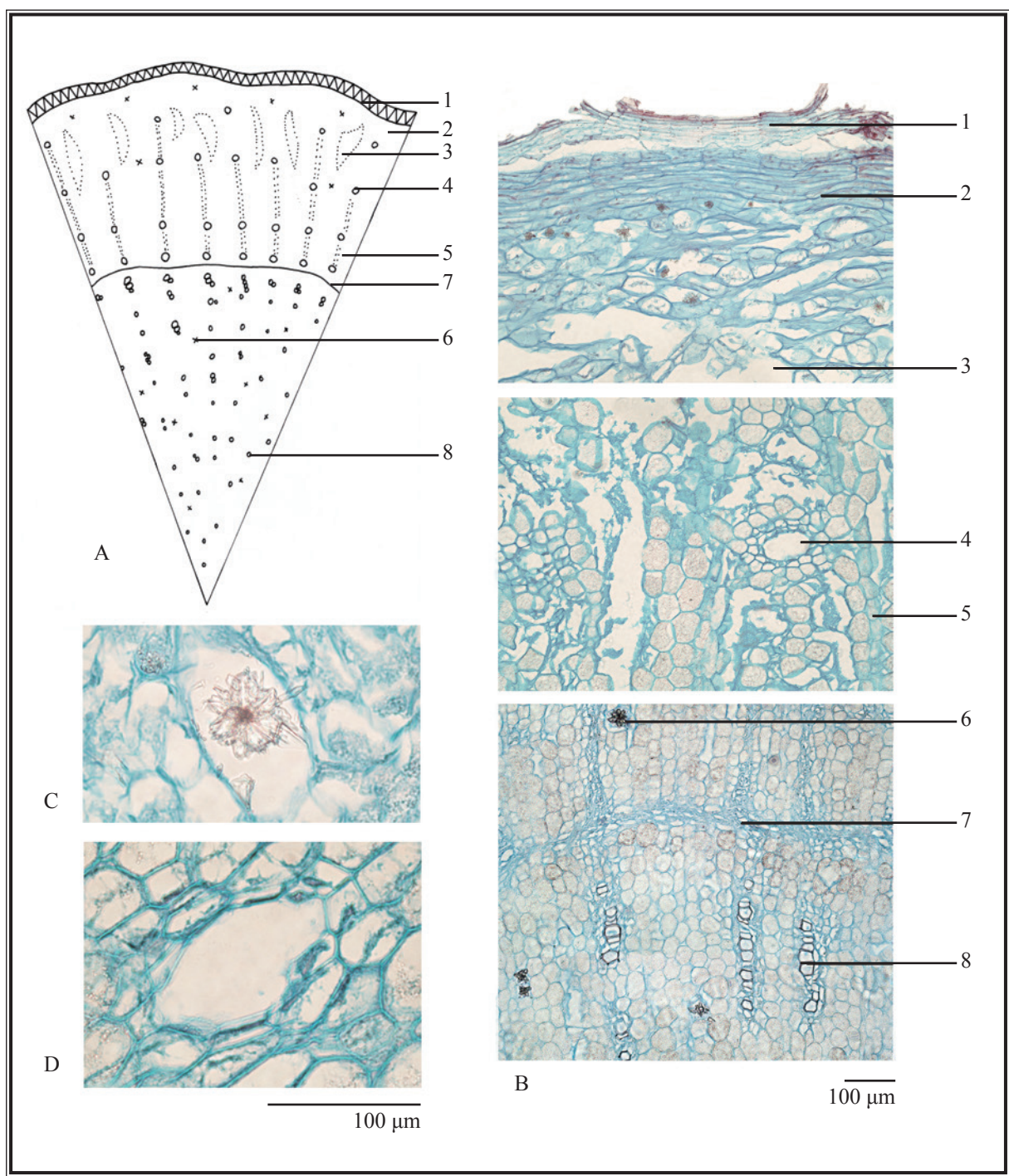


Figure 2 Microscopic features of transverse section of root of Ginseng Radix et Rhizoma Rubra

- A. Sketch B. Section illustration C. Cluster of calcium oxalate D. Resin canal
1. Cork 2. Cortex 3. Cleft 4. Resin canal 5. Phloem 6. Cluster of calcium oxalate
7. Cambium 8. Xylem

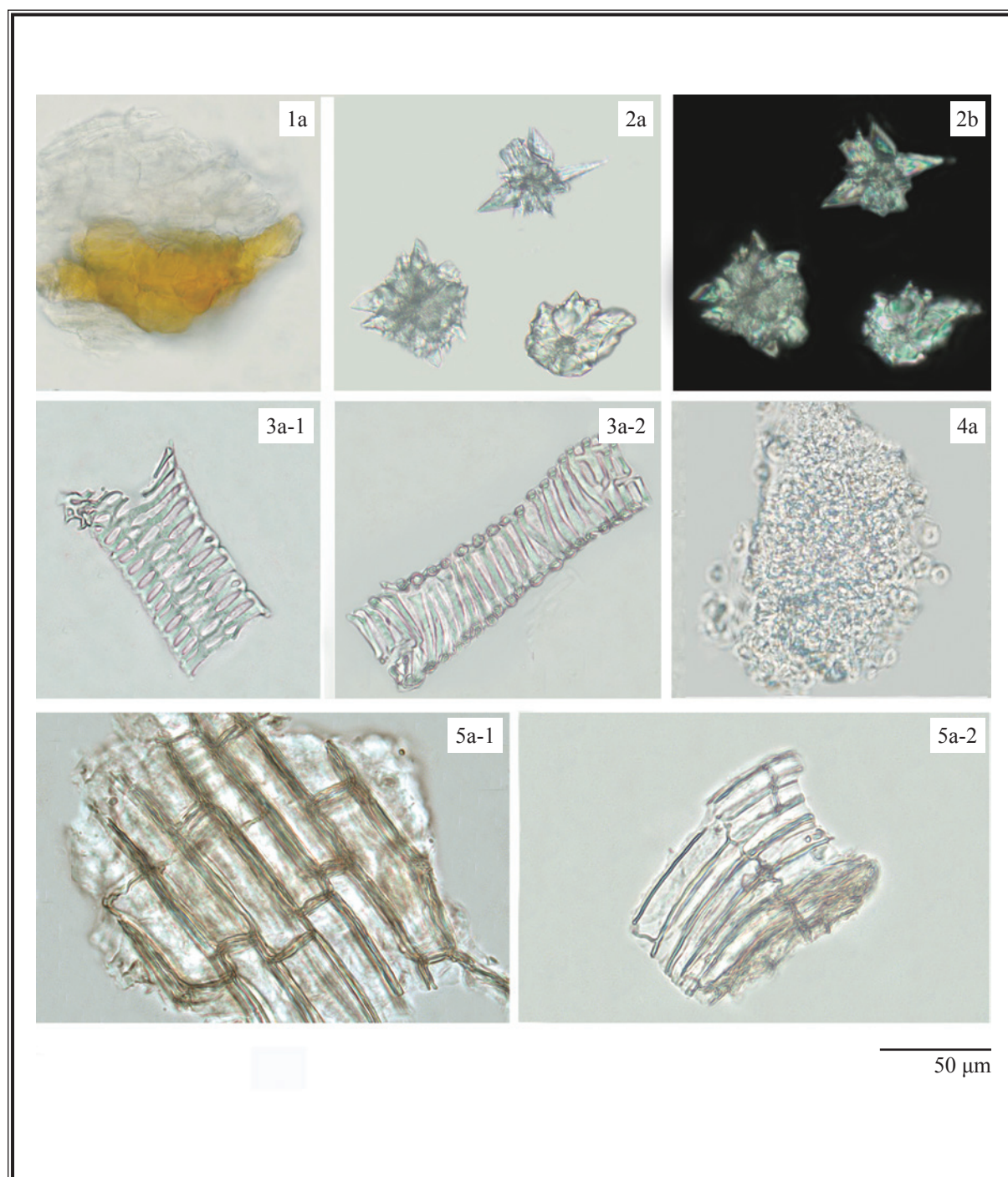


Figure 3 Microscopic features of powder of Ginseng Radix et Rhizoma Rubra

1. Resin canal 2. Clusters of calcium oxalate
3. Vessels (3-1 reticulate vessel, 3-2 scalariform vessel) 4. Gelatinized starch granules
5. Cork cells (5-1 surface view, 5-2 lateral view)

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

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

Standard solutions

Ginsenoside Rb₁ standard solution

Weigh 1.0 mg of ginsenoside Rb₁ CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Ginsenoside Re standard solution

Weigh 1.0 mg of ginsenoside Re CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Ginsenoside Rf standard solution

Weigh 1.0 mg of ginsenoside Rf CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Ginsenoside Rg₁ standard solution

Weigh 1.0 mg of ginsenoside Rg₁ CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Developing solvent system

Prepare a mixture of water, *n*-butanol, ethyl acetate and methanol (5:4:1:1, 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 5 mL of methanol (70%). 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 F₂₅₄ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately ginsenoside Rb₁ standard solution (1 μL), ginsenoside Re standard solution (1 μL), ginsenoside Rf standard solution (1 μL), ginsenoside Rg₁ standard solution (1 μL) and the test solution (1.5 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 7 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 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).

Amomi Fructus
砂仁

苦地丁
Corydalis Bungeanae Herba

Ginseng Radix et Rhizoma Rubra
紅參

Garcinia Resina (unprocessed)
藤黃(生)

千年健
Homalomenae Rhizoma

天冬
Asparagi Radix

Bletillae Rhizoma
白及

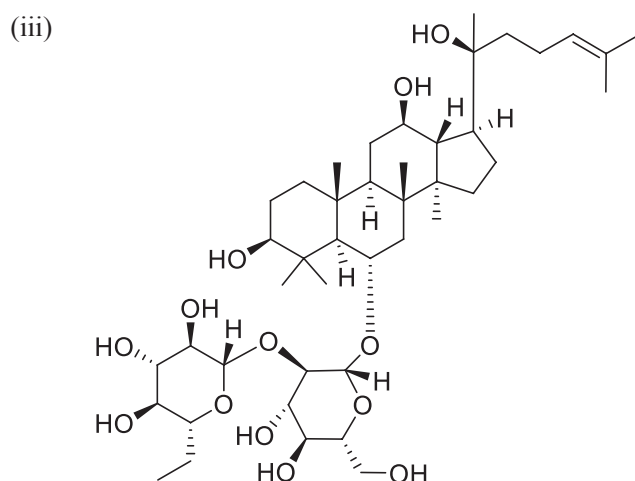
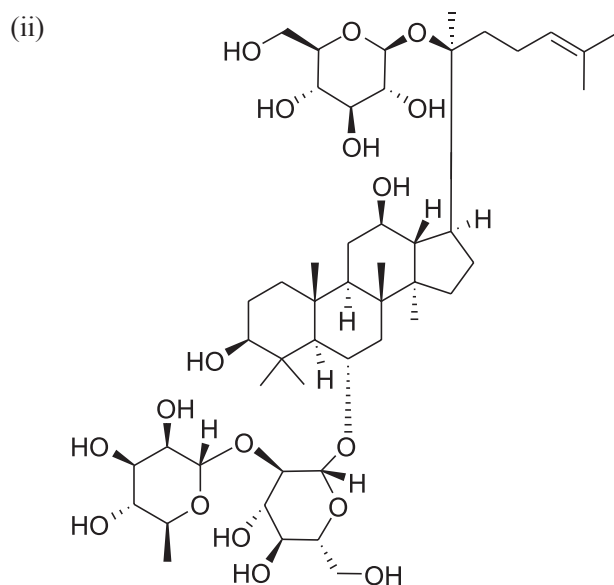
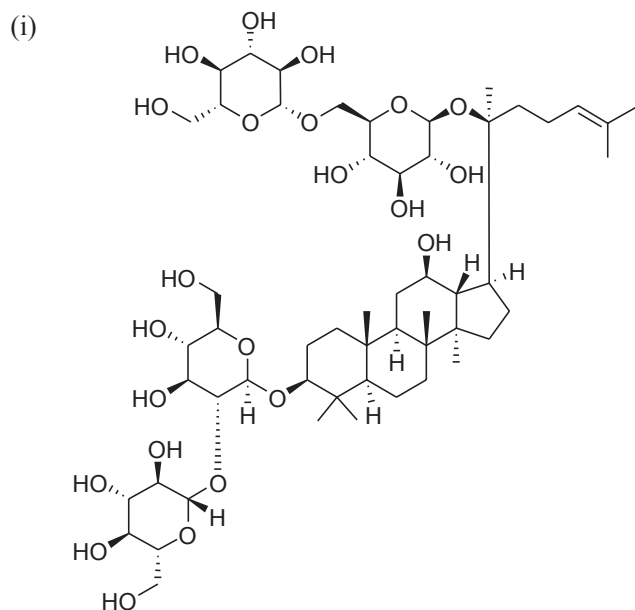
毛冬青
Ilicis Pubescentis Radix et Caulis

Elephantopi Herba
地膽草

Glechomae Herba
連錢草

Hoveniae Semen
枳椇子

Ginseng Radix et Rhizoma Rubra



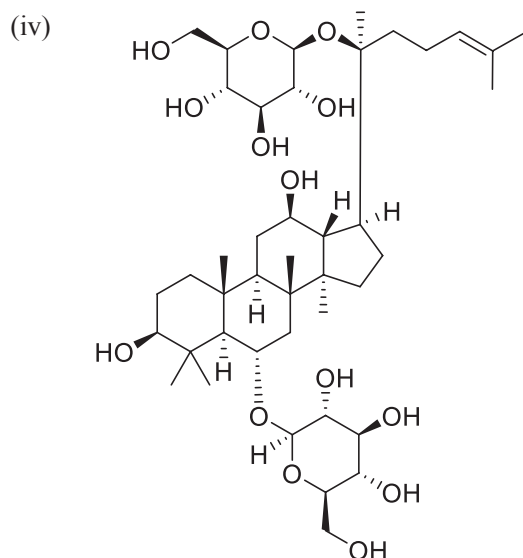


Figure 4 Chemical structures of (i) ginsenoside Rb₁ (ii) ginsenoside Re (iii) ginsenoside Rf and (iv) ginsenoside Rg₁

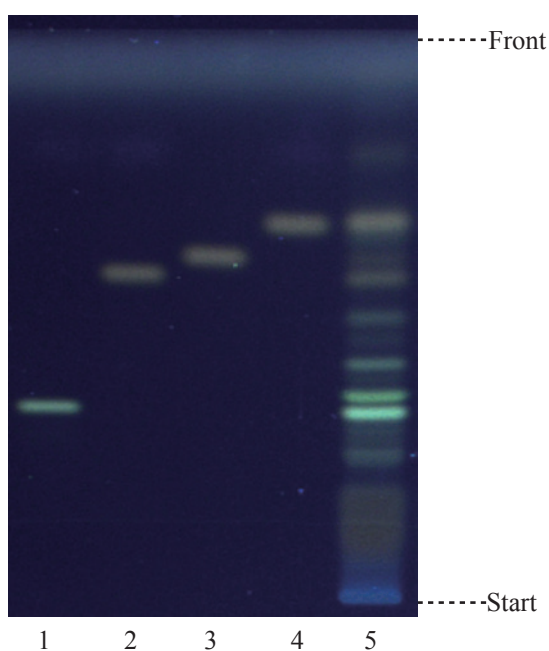


Figure 5 A reference HPTLC chromatogram of Ginseng Radix et Rhizoma Rubra extract observed under UV light (366 nm) after staining

1. Ginsenoside Rb₁ standard solution
2. Ginsenoside Re standard solution
3. Ginsenoside Rf standard solution
4. Ginsenoside Rg₁ standard solution
5. 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 Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

Ginsenoside Rb₁ standard solution for fingerprinting, Std-FP (350 mg/L)

Weigh 3.5 mg of ginsenoside Rb₁ CRS and dissolve in 10 mL of methanol (70%).

Ginsenoside Re standard solution for fingerprinting, Std-FP (150 mg/L)

Weigh 1.5 mg of ginsenoside Re CRS and dissolve in 10 mL of methanol (70%).

Ginsenoside Rg₁ standard solution for fingerprinting, Std-FP (350 mg/L)

Weigh 3.5 mg of ginsenoside Rg₁ CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol (70%). Sonicate (350 W) the mixture for 15 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 column temperature is maintained at 40°C during the separation. 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 – 20	81	19	isocratic
20 – 30	81 → 69	19 → 31	linear gradient
30 – 40	69	31	isocratic
40 – 60	69 → 60	31 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ginsenoside Rb₁ Std-FP, ginsenoside Re Std-FP and ginsenoside Rg₁ Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ should not be more than 5.0%; the RSD of the retention times of ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ peaks should not be more than 2.0%; the column efficiencies determined from ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ peaks should not be less than 8000, 15000 and 8000 theoretical plates respectively.

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 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject ginsenoside Rb₁ Std-FP, ginsenoside Re Std-FP, ginsenoside Rg₁ Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ peaks in the chromatograms of ginsenoside Rb₁ Std-FP, ginsenoside Re Std-FP, ginsenoside Rg₁ Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of ginsenoside Rb₁ Std-FP, ginsenoside Re Std-FP and ginsenoside Rg₁ Std-FP. The retention times of ginsenoside Rb₁, ginsenoside Re and ginsenoside Rg₁ 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 seven characteristic peaks of Ginseng Radix et Rhizoma Rubra extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Ginseng Radix et Rhizoma Rubra extract

Peak No.	RRT	Acceptable Range
1 (ginsenoside Rg ₁)	0.62	± 0.03
2 (ginsenoside Re)	0.63	± 0.03
3 (ginsenoside Rf)	0.78	± 0.03
4 (marker, ginsenoside Rb ₁)	1.00	-
5 (ginsenoside Rc)	1.05	± 0.03
6 (ginsenoside Rb ₂)	1.09	± 0.03
7 (ginsenoside Rd)	1.18	± 0.03

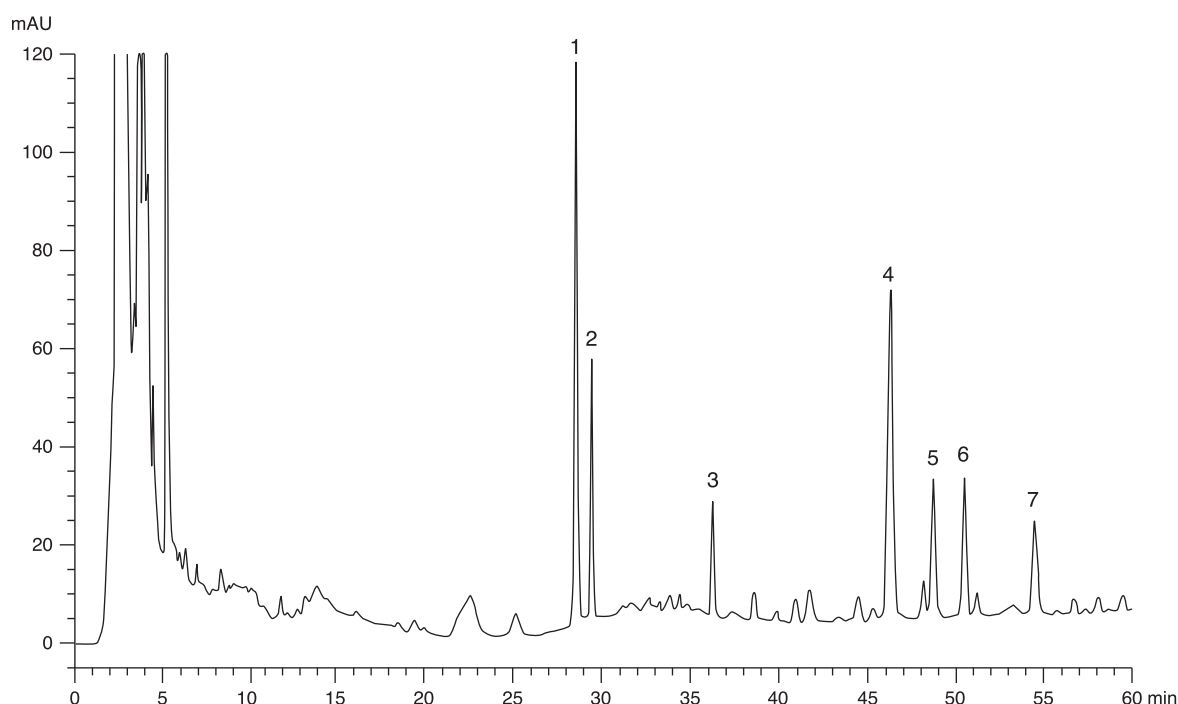


Figure 6 A reference fingerprint chromatogram of Ginseng Radix et Rhizoma Rubra extract

For positive identification, the sample must give the above seven 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 4.0%.

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)

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

Ethanol-soluble extractives (cold extraction method): not less than 28.0%.

7. ASSAY

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

Standard solution

Mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ standard stock solution, Std-Stock (400 mg/L for ginsenoside Rb₁, 200 mg/L for ginsenoside Re, 100 mg/L for ginsenoside Rf and 420 mg/L for ginsenoside Rg₁)

Weigh accurately 4.0 mg of ginsenoside Rb₁ CRS, 2.0 mg of ginsenoside Re CRS, 1.0 mg of ginsenoside Rf CRS and 4.2 mg of ginsenoside Rg₁ CRS, and dissolve in 10 mL of methanol (70%).

Mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ standard solution for assay, Std-AS

Measure accurately the volume of the mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ Std-Stock, dilute with methanol (70%) to produce a series of solutions of 6, 25, 50, 100, 200 mg/L for ginsenoside Rb₁, 3, 12.5, 25, 50, 100 mg/L for ginsenoside Re, 1.5, 6.3, 12.5, 25, 50 mg/L for ginsenoside Rf and 6.3, 26.3, 52.5, 105, 210 mg/L for ginsenoside Rg₁.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol (70%). Sonicate (120 W) the mixture for 15 min. Centrifuge at about 4000 × g for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to 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 column temperature is maintained at 40°C during the separation. 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 – 20	81	19	isocratic
20 – 30	81 → 69	19 → 31	linear gradient
30 – 40	69	31	isocratic
40 – 60	69 → 60	31 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of the mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ Std-AS (50 mg/L for ginsenoside Rb₁, 25 mg/L for ginsenoside Re, 12.5 mg/L for ginsenoside Rf and 52.5 mg/L for ginsenoside Rg₁). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ should not be more than 5.0%; the RSD of the retention times of ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ peaks should not be more than 2.0%; the column efficiencies determined from ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ peaks should not be less than 80000, 150000, 150000 and 65000 theoretical plates respectively.

The *R* value between ginsenoside Rb₁ peak and the closest peak; the *R* value between ginsenoside Re peak and the closest peak; the *R* value between ginsenoside Rf peak and the closest peak; and the *R* value between ginsenoside Rg₁ 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 ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ against the corresponding concentrations of the mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ Std-AS. Obtain the slopes, y-intercepts and the *r*² values from the corresponding 5-point calibration curves.

Procedure

Inject 20 μ L of the test solution into the HPLC system and record the chromatogram. Identify ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ Std-AS. The retention times of ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ 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 Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ in the test solution, and calculate the percentage contents of ginsenoside Rb₁, ginsenoside Re, ginsenoside Rf and ginsenoside Rg₁ in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.39% ginsenoside Rb₁ (C₅₄H₉₂O₂₃); and not less than 0.049% ginsenoside Rf (C₄₂H₇₂O₁₄); and not less than 0.34% of the total content of ginsenoside Re (C₄₈H₈₂O₁₈) and ginsenoside Rg₁ (C₄₂H₇₂O₁₄), calculated with reference to the dried substance.

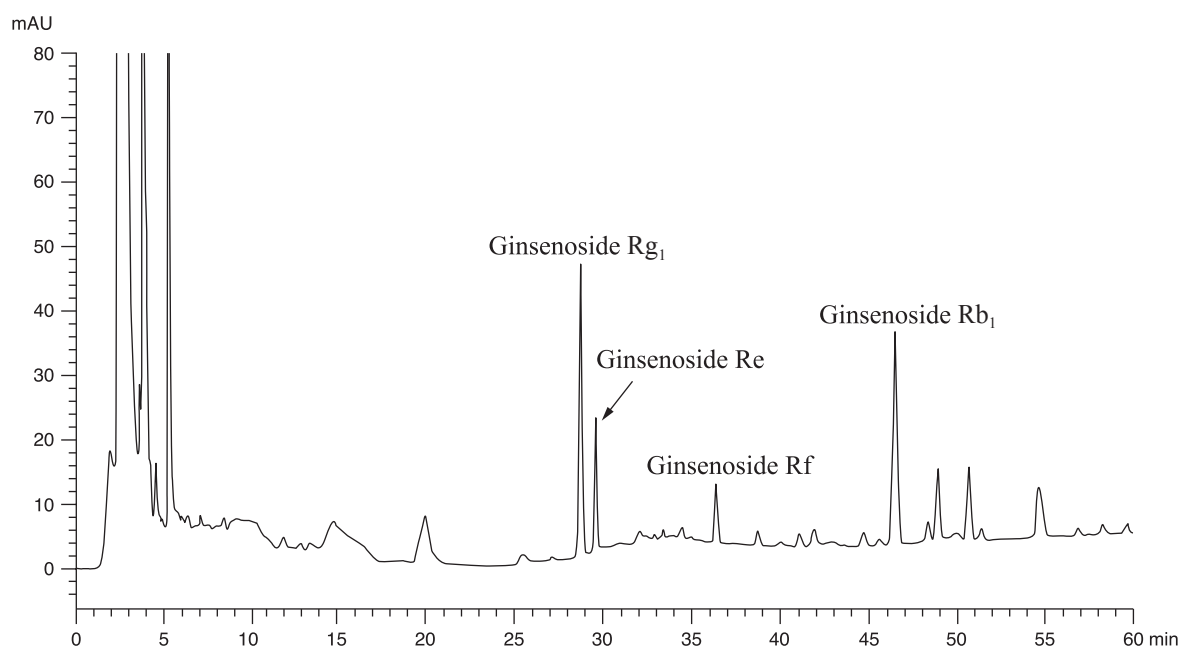


Figure 7 A reference assay chromatogram of Ginseng Radix et Rhizoma Rubra extract