Radix Ginseng

Figure 21　A photograph of Radix Ginseng

1. Rhizome　2. Stem scar　3. Main root　4. Adventitious root　5. Branch root

1. NAMES

Official Name: Radix Ginseng

Chinese Name: 人參

Chinese Phonetic Name: Renshen

2. SOURCE

Radix Ginseng is the dried root of *Panax ginseng* C. A. Mey. (Fam. Araliaceae). The root is collected in autumn after 4 to 6 years of plantation. The rootlets removed and washed clean, dried in the sun or over a fire to produce ‘Shengshaishen’ (Sun-Dried Ginseng); or dried in the sun without removing rootlets to produce ‘Quanxu Shengshaishen’ (Sun-Dried Whole Ginseng).

3. DESCRIPTION

The main root of "Sun-Dried Ginseng" is fusiform or cylindrical. Externally greyish-yellow; upper part or entire root exhibiting sparse, shallow, interrupted and coarse transverse striations, and distinct longitudinal wrinkles; lower part bearing 2–3 branching lateral roots. Rhizome 1–4 cm in length by 3–15 mm in diameter, mostly constricted and curved, bearing adventitious roots and showing sparse dented stem scars. Texture relatively hard, fracture pale yellowish-white, starchy. Cambium ring brownish-yellow. Bark exhibiting yellowish-brown dotted resin ducts and radial clefts. Odour, slightly fragrant and distinctive; taste, slightly bitter and sweet.

The "Sun-Dried Whole Ginseng" shows similar features as "Sun-Dried Ginseng", except that lower part bears 2–3 branch roots and numerous slender rootlets with inconspicuous minute tubercles. (Fig. 21)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

The transverse section shows cork consisting of several rows of flat cells. Cortex narrow. Phloem showing clefts in the outer part, and parenchymatous cells in the inner part densely arranged and

occasionally scattered with resin canals containing pale yellow or yellowish-brown secretions in the inner part. Cambium in a ring. Xylem rays broad (2–26 rows). Vessels singly scattered or in group, with interrupted radial arrangement. Parenchymatous cells containing clusters of calcium oxalate. (Fig. 22)

Powder

Yellowish-white. Surface view of cork cells sub-square, sub-rectangular or polygonal, with thin and sinuous walls, array closed; lateral view of cork cells flattened. Fragments of resin canals containing yellow or yellowish-brown secretions. Reticulate and scalariform vessels, 19–100 µm in diameter, pit of reticulate vessels larger compared to scalariform vessels. Starch granules fairly abundant; simple granules subspheroidal, semi-circular or irregular polygonal, with dotted, V-shaped, or slit-shaped hilum; compound granules of 2–11 units; showing a black, cross-shape under a polarizing microscope. Clusters of calcium oxalate, 19–173 µm in diameter, with acute angles. (Fig. 23)

4.2 Physicochemical Identification

Procedure

Weigh 2.0 g of the powdered sample and put into a test tube, then add 6 mL of dichloromethane. Sonicate the mixture for 30 min. Filter and transfer 1 mL of the filtrate to a test tube. Cautiously add about 1 mL of sulphuric acid along the inner wall of the tube. A red or brown layer is observed in the interface of the two solvent layers.

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

Standard solutions

Ginsenoside Rb_1 standard solution

Weigh 0.5 mg of ginsenoside Rb_1 and dissolve in 1 mL of methanol.

Ginsenoside Rc standard solution

Weigh 0.5 mg of ginsenoside Rc and dissolve in 1 mL of methanol.

Ginsenoside Rf standard solution

Weigh 0.5 mg of ginsenoside Rf and dissolve in 1 mL of methanol.

Ginsenoside Rg_1 standard solution

Weigh 0.5 mg of ginsenoside Rg_1 and dissolve in 1 mL of methanol.

Pseudoginsenoside F_{11} standard solution

Weigh 0.5 mg of pseudoginsenoside F_{11} and dissolve in 1 mL of methanol.

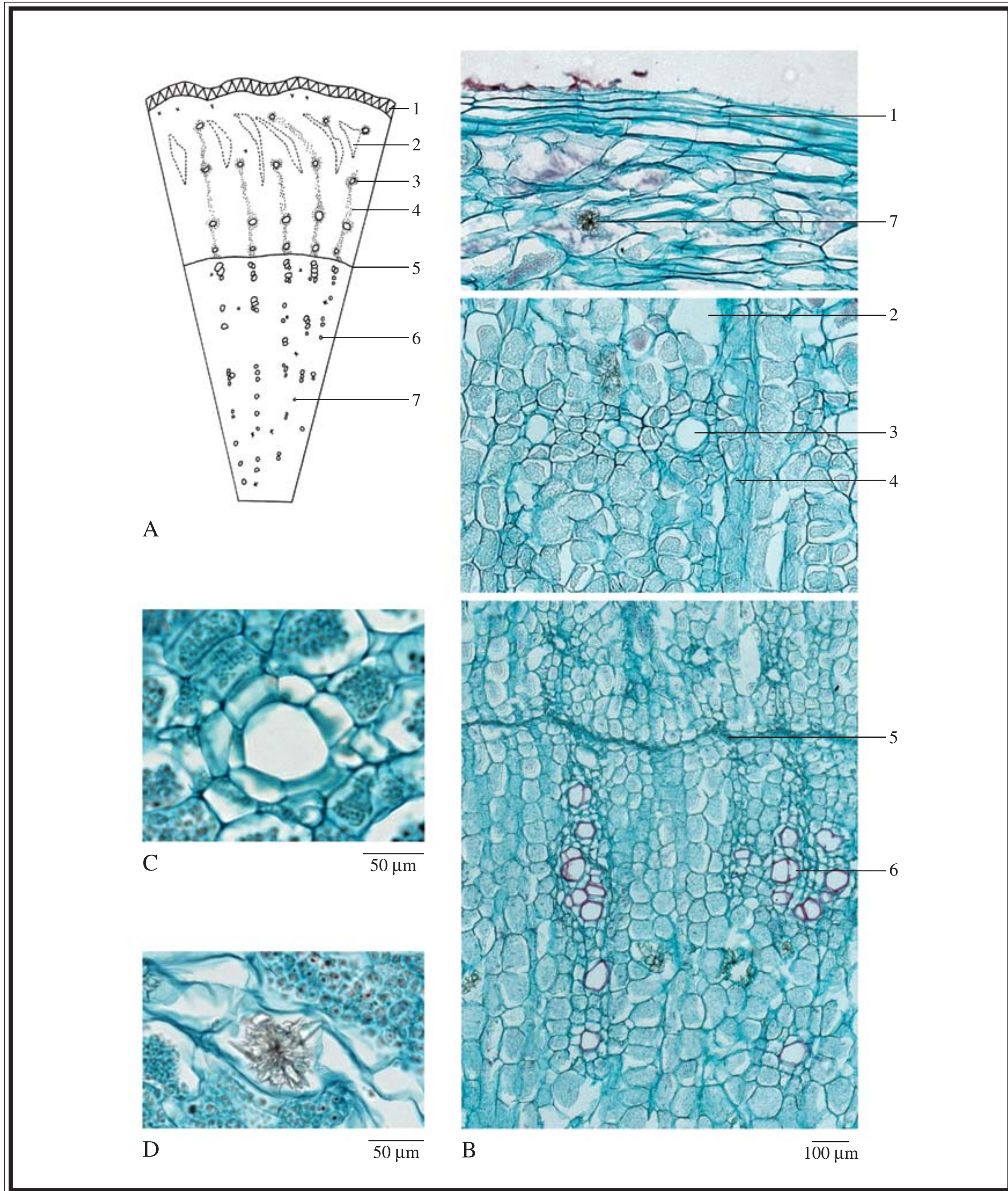

Figure 22 Microscopic features of transverse section of Radix Ginseng

A. Sketch B. Section illustration C. Resin canal D. Cluster of calcium oxalate

1. Cork 2. Clefts 3. Resin canal 4. Phloem 5. Cambium 6. Xylem 7. Clusters of calcium oxalate

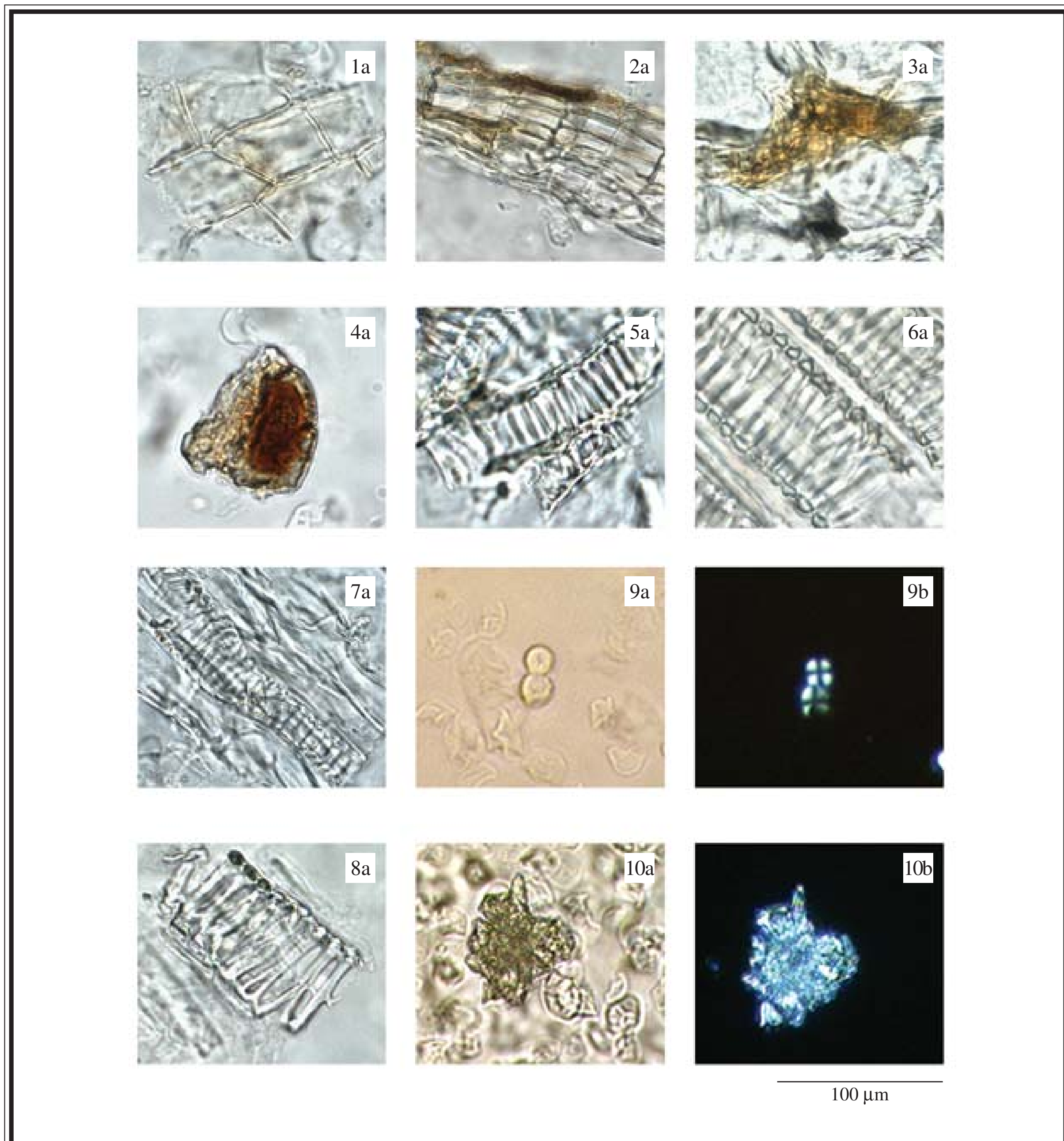

Figure 23 Microscopic features of powder of Radix Ginseng

1. Cork cells (surface view) 2. Cork cells (lateral view) 3. Resin canal
4. Resin canal with yellowish-brown secretion 5. Single scalariform vessel
6. Reticulated vessels in a group 7. Single scalariform vessel with parenchymatous cells
8. Single reticulated vessel 9. Starch grains 10. Cluster of calcium oxalate

a. Features under a light microscope b. Features under a polarizing microscope

牡丹皮 Cortex Moutan 關黃柏 Cortex Phellodendri Amurensis Cortex Phellodendri Chinensis 川黃柏 當歸 Radix Angelicae Sinensis Radix As

Developing solvent system

Prepare a mixture of chloroform, methanol and water (13:7:2, v/v). Keep in a refrigerator at a temperature below 6 °C for at least 10 h. Use the lower layer.

Spray reagent

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

Test solution

Weigh 0.2 g of the powdered sample and put into a 10-mL centrifugal tube, then add 5 mL of methanol. Sonicate the mixture for 30 min. Shake and centrifuge at about 1200 x *g* for 10 min. Collect the supernatant.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Develop over a path of about 8 cm. Apply separately ginsenoside Rb_1 standard solution, ginsenoside Rc standard solution, ginsenoside Rf standard solution, ginsenoside Rg_1 standard solution and pseudoginsenoside F_{11} standard solution (1 μL each) and the test solution (3 μL) to the plate. After the development, remove the plate from the chamber, mark the solvent front and dry. Spray the plate evenly with the spray reagent and heat at above 95 °C until the spots or bands become visible. Examine the plate in UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV(A).

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 Rb_1, ginsenoside Rc, ginsenoside Rf and ginsenoside Rg_1, but no spots or bands corresponding to pseudoginsenoside F_{11}.

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

Standard solutions

Ginsenoside Rb_1 standard stock solution, Std-Stock (500 mg/L)

Weigh 2.5 mg of ginsenoside Rb_1 and dissolve in 5 mL of methanol. Store at about -10 °C in the dark.

Ginsenoside Rb_1 standard solution for fingerprinting, Std-FP (100 mg/L)

Pipette 1.0 mL of ginsenoside Rb_1 Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

Ginsenoside Rg_1 standard stock solution, Std-Stock (500 mg/L)

Weigh 2.5 mg of ginsenoside Rg_1 and dissolve in 5 mL of methanol. Store at about -10 °C in the dark.

Ginsenoside Rg_1 standard solution for fingerprinting, Std-FP (100 mg/L)

Pipette 1.0 mL of ginsenoside Rg_1 Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol and weigh. Sonicate the mixture for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 540 x *g* for 5 min. Filter through a 0.2-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (203 nm) and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 25 °C during the separation. The flow rate is about 1.6 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	80	20	isocratic
20–60	80 ➔ 58	20 ➔ 42	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of ginsenoside Rb_1 Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ginsenoside Rb_1 should be not more than 3.0%; the RSD of the retention time of ginsenoside Rb_1 peak should be not more than 2.0%; the column efficiency determined from ginsenoside Rb_1 peak should be not less than 150,000 theoretical plates.

The *R* value between ginsenoside Rg_1 peak and ginsenoside Re peak (Fig. 24) in the test solution should be not less than 1.0.

Procedure

Separately inject ginsenoside Rb_1 Std-FP, ginsenoside Rg_1 Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of ginsenoside Rb_1 peak and ginsenoside Rg_1 peak in the chromatograms of the corresponding Std-FP and the retention times of the seven characteristic peaks (Fig. 24) in the chromatogram of the test solution. Under the same HPLC conditions, identify ginsenoside Rb_1 peak and ginsenoside

牡丹皮 Cortex Moutan 關黃柏 Cortex Phellodendri Amurensis 川黃柏 Cortex Phellodendri Chinensis 當歸 Radix Angelicae Sinensis Radix As

Rg_1 peak in the chromatogram of the test solution by comparing their retention times with those in the chromatograms of the corresponding Std-FP. The retention times of ginsenoside Rb_1 peaks and ginsenoside Rg_1 peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ from their counterparts by more than 3.0%. 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 Radix Ginseng extract are listed in Table 8.

Table 8 The RRTs and acceptable ranges of the seven characteristic peaks of Radix Ginseng extract

Peak No.	RRT	Acceptable Range
1 (marker 1, ginsenoside Rg_1)	1.00	-
2 (ginsenoside Re)	1.06 (vs Rg_1)	±0.03
3 (ginsenoside Rf)	0.89 (vs Rb_1)	±0.03
4 (marker 2, ginsenoside Rb_1)	1.00	-
5 (ginsenoside Rc)	1.03 (vs Rb_1)	±0.03
6 (ginsenoside Rb_2)	1.06 (vs Rb_1)	±0.03
7 (ginsenoside Rd)	1.13 (vs Rb_1)	±0.03

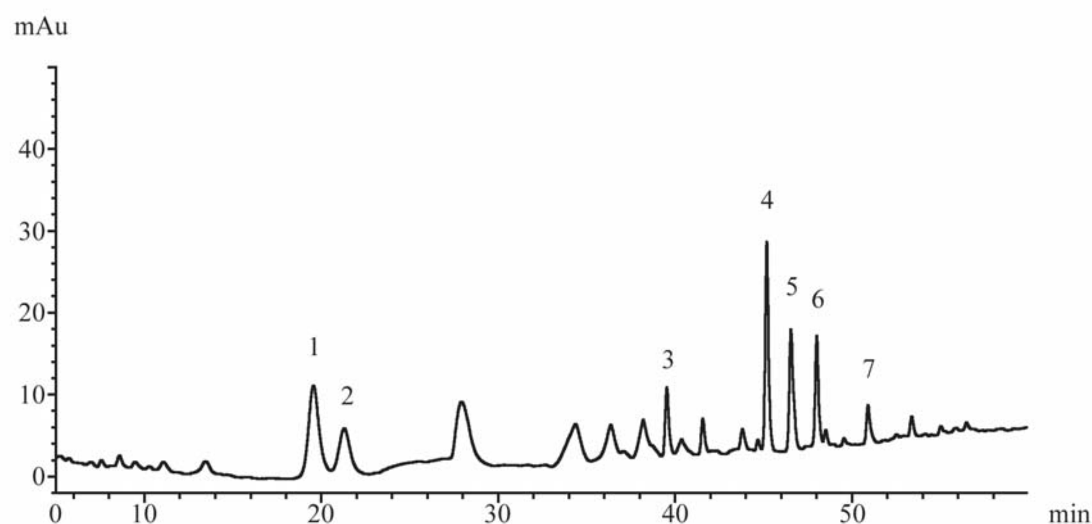

Figure 24 A reference fingerprint chromatogram of Radix Ginseng extract

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. 24).

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 XIV)*: 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)*: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 27.0%.
Ethanol-soluble extractives (cold extraction method): not less than 22.0%.

7. ASSAY

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

Standard solution

Mixed ginsenosides Rb_1, Re and Rg_1 standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 10.0 mg of ginsenoside Rb_1, 10.0 mg of ginsenoside Re and 10.0 mg of ginsenoside Rg_1 and dissolve in 10 mL of methanol. Store at about -10 ˚C in the dark.

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

Measure accurately the volume of the mixed ginsenosides Rb_1, Re and Rg_1 Std-Stock, dilute with methanol to produce a series of solutions of 25, 50, 100, 200, 400 mg/L for ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate the mixture for 30 min. Centrifuge at about 540 x *g* for 5 min and transfer the supernatant to a 100-mL round-bottomed flask. Repeat the extraction twice. Wash the residues by adding 5 mL of methanol to the tube and centrifuge at about 540 x *g* for 5 min. Transfer the supernatant to the same 100-mL round-bottomed flask. Repeat the washing twice. Mix all the extracts and the washings, and then evaporate to dryness at reduced pressure in a rotary evaporator. Add 2 mL of methanol to the residues and transfer to a 10-mL volumetric flask. Repeat three more times each with 2 mL of methanol and make up to the mark with methanol. Mix and filter through a 0.2-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (203 nm) and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 25 ˚C during the separation. The flow rate is about 1.6 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	80	20	isocratic
20–60	80 ➔ 58	20 ➔ 42	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 µL of ginsenoside Rb_1 Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ginsenoside Rb_1 should be not more than 3.0%; the RSD of the retention time of ginsenoside Rb_1 peak should be not more than 2.0%; the column efficiency determined from ginsenoside Rb_1 peak should be not less than 150,000 theoretical plates.

The *R* value between ginsenoside Rg_1 peak and ginsenoside Re peak in the test solution should be not less than 1.0.

Calibration curves

Inject a series of the mixed ginsenosides Rb_1, Re and Rg_1 Std-AS (5 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 ginsenosides Rb_1, Re and Rg_1 Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify the peaks of ginsenoside Rb_1, ginsenoside Re and ginsenoside Rg_1 in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ginsenosides Rb_1, Re and Rg_1 Std-AS. The retention times of the peaks of ginsenoside Rg_1, ginsenoside Re and ginsenoside Rb_1 in both chromatograms should not differ from their counterparts 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 indicated in Appendix IV(B).

Limits

The sample contains not less than 0.20% of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$); and not less than 0.19% of combined 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.