Radix Panacis Quinquefolii

Figure 1(i) A photograph of Radix Panacis Quinquefolii (from Ji Lin, China)

Figure 1(ii) A photograph of Radix Panacis Quinquefolii (from Ontario, Canada)

Figure 1(iii) A photograph of Radix Panacis Quinquefolii (from British Columbia, Canada)

Figure 1(iv) A photograph of Radix Panacis Quinquefolii (from Wisconsin, USA)

1. NAMES

Official Name: Radix Panacis Quinquefolii

Chinese Name: 西洋參

Chinese Phonetic Name: Xiyangshen

2. SOURCE

Radix Panacis Quinquefolii is the dried root of *Panax quinquefolium* L. (Araliaceae). All the commercial supplies are obtained from cultivated plants. The root is collected in autumn, washed clean and dried under the sun or at a temperature below 40ºC to obtain Radix Panacis Quinquefolii.

3. DESCRIPTION

Fusiform, cylindrical or conical, 3-20 cm (occasionally up to 24 cm) long, 4-28 mm (occasionally up to 34 mm) in diameter. Externally yellowish-brown, yellowish-white, pale yellowish-brown or pale yellowish-white, exhibiting transverse annulations and linear lenticels, and showing shallow, fine and dense longitudinal wrinkles, and scars of rootlets. The middle and lower part of the main root, with 1 to several lateral roots, are mostly broken off. In some cases, the upper end presents remains of a rhizome (Lutou), with prominent annular nodes. Stem scars (Luwan) rounded or semirounded, with adventitious roots (Ding) remaining or already broken off. Texture heavy and hard, uneasily broken; fracture even, yellowish-white, horny or slightly starchy, with the bark exhibiting yellowish-brown to reddish-brown dotted resin canals, a brownish-yellow cambium ring, and wood arranged roughly in a radial pattern. Odour slight but characteristic; taste slightly bitter, gradually becoming sweet [Fig. 1(i), (ii), (iii) and (iv)].

Radix Panacis Quinquefolii from the four production areas show some differences from one another, as follows –

Samples from China (Ji Lin): pale yellowish-brown to pale yellowish-white, plump, with apparent longitudinal wrinkles. They are relatively pale, plump and light in texture than those from USA and Canada.

Samples from Canada (Ontario and British Columbia): pale yellowish-white, larger, with distinct longitudinal wrinkles. Their textures are heavier than that from China.

Samples from USA (Wisconsin): yellowish-brown, smaller, with distinct transverse striations. Their textures are the heaviest among samples from the four areas.

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 6-8 rows of elongated tangential cells, with several layers often peeled off at the outer side. Cortex consists of more than 10 rows of parenchyma cells, some containing cluster of calcium oxalate. Resin canals scattered, surrounded by 5-11 secretory cells. In the phloem, resin canals are frequent and usually arranged in 1-3 concentric rings, the rays in the outer part with clefts. Cambium ring distinct. Vessels scattered singly or in groups of 2-10, with interrupted radial arrangement, and lignified or slightly lignified. Rays consist of 1-4 rows of cells wide. Parenchyma cells contain starch granules (Fig. 2).

Powder

Colour pale brown or pale yellowish-white. Resin canals in longitudinal view appearing pipeline-shaped, containing a large quantity of yellow oil secretion in the shape of a drop, as well as a jacinth secretion in the shape of a strip. Cluster of calcium oxalate numerous, 8-91 μm in diameter, bright polychromatic observed under a polarized microscope. Cork cells colourless, pale yellow to pale yellowish-brown, subpolygonal or subsquare, anticlinal wall thin and undulately curved. Vessels mainly reticulate and scalariform, but annular and spiral vessels can also be observed. Simple starch granules subglobose to ovoid, 2-28 μm in diameter, hilum V-shaped, dotted or slit-shaped, the striations distinct; compound granules few, composed of 2-9 components; black, cruciate shape is observed under a polarized microscope (Fig. 3).

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

Standard solutions

Ginsenoside Rb_1 standard solution

Weigh 0.5 mg of ginsenoside Rb_1 CRS (Fig. 4) and dissolve in 1 mL of methanol.

Ginsenoside Rc standard solution

Weigh 0.5 mg of ginsenoside Rc CRS (Fig. 4) and dissolve in 1 mL of methanol.

Ginsenoside Rg_1 standard solution

Weigh 0.5 mg of ginsenoside Rg_1 CRS (Fig. 4) and dissolve in 1 mL of methanol.

24-(R)-pseudoginsenoside F_{11} standard solution

Weigh 0.5 mg of 24-(R)-pseudoginsenoside F_{11} CRS (Fig. 4) and dissolve in 1 mL of methanol.

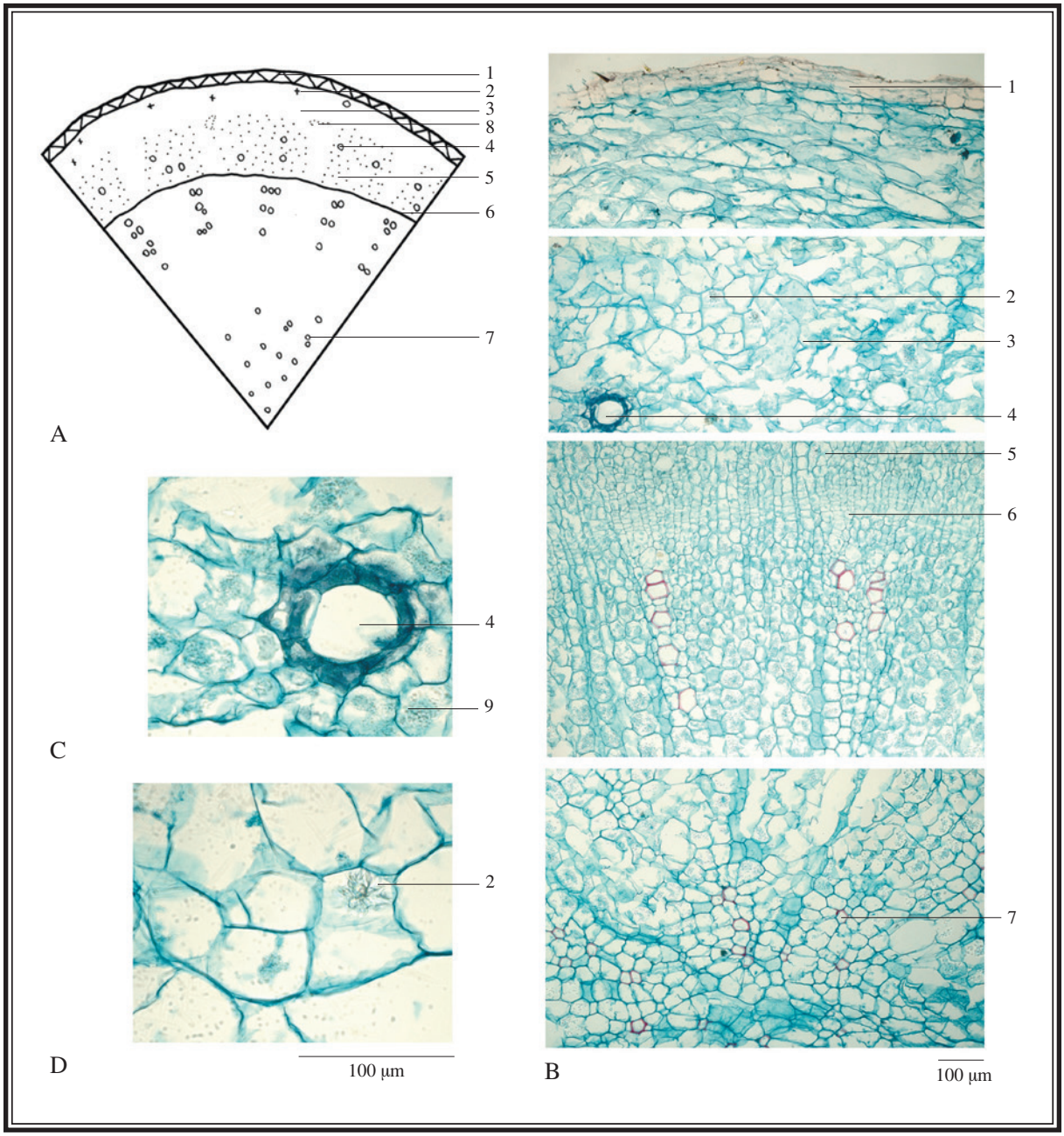


Figure 2 Microscopic features of transverse section of Radix Panacis Quinquefolii

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

1. Cork 2. Cluster of calcium oxalate 3. Cortex 4. Resin canal
5. Phloem 6. Cambium 7. Xylem 8. Cleft 9. Starch granules

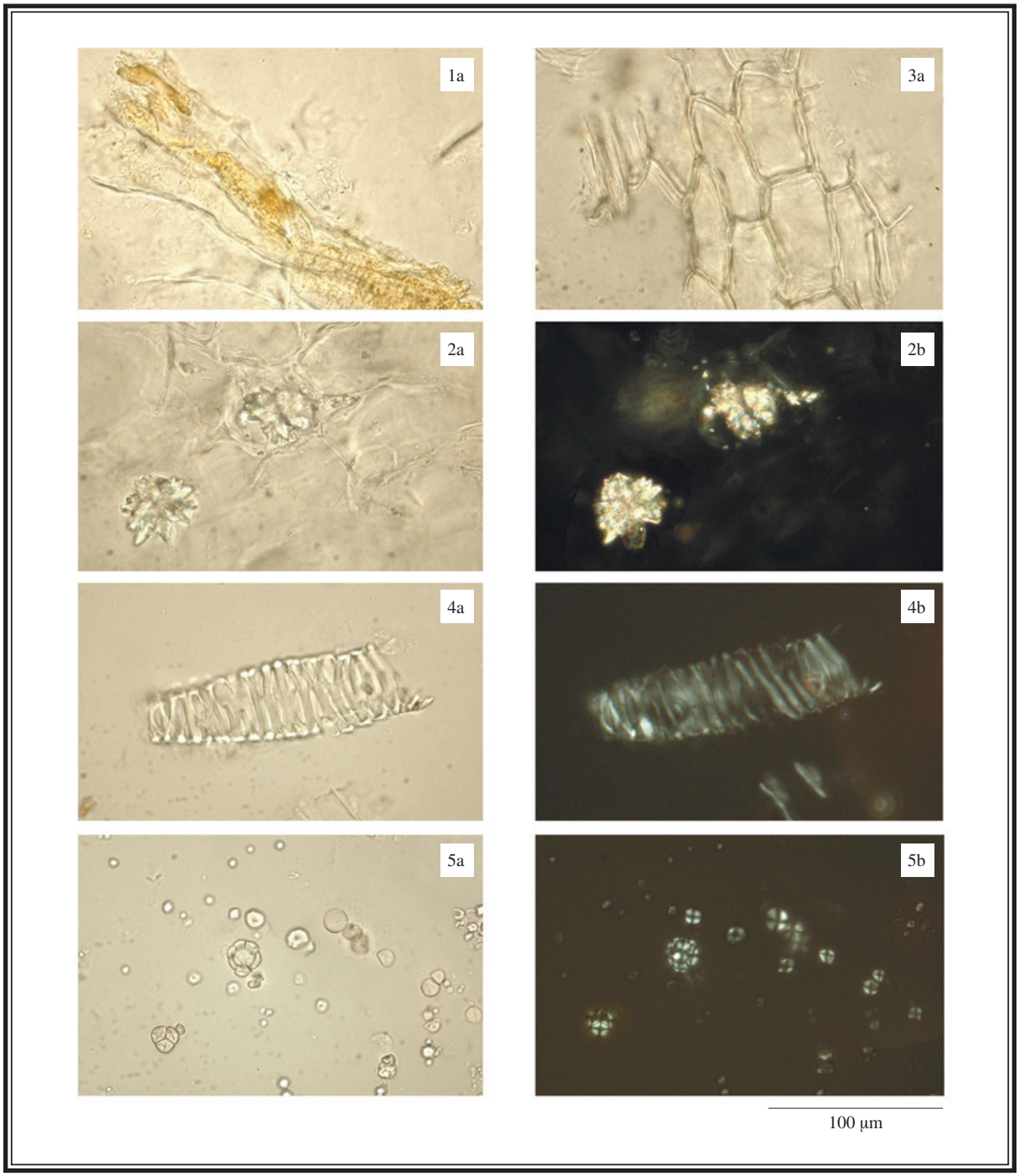


Figure 3 Microscopic features of powder of Radix Panacis Quinquefolii

1. Resin canal 2. Clusters of calcium oxalate
3. Cork cells 4. Vessel 5. Starch granules

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

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (13:7:2, v/v). Keep in a refrigerator at a temperature below 6ºC 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 place it in a 10-mL centrifuge tube, then add 5 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min and then filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately ginsenoside Rb_1 standard solution, ginsenoside Rc standard solution, ginsenoside Rg_1 standard solution and 24-(R)-pseudoginsenoside F_{11} standard solution (1 μL each) and the test solution (3 μL) to the plate. 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 120ºC until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 Rg_1 and 24-(R)-pseudoginsenoside F_{11}.

(i)

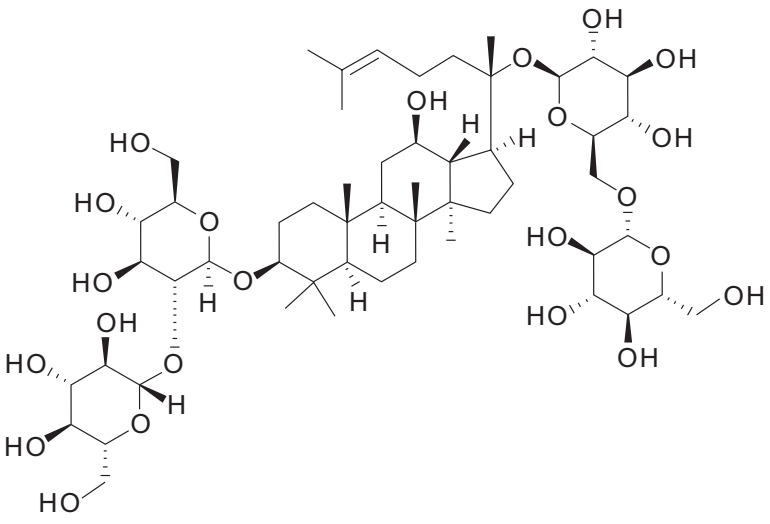
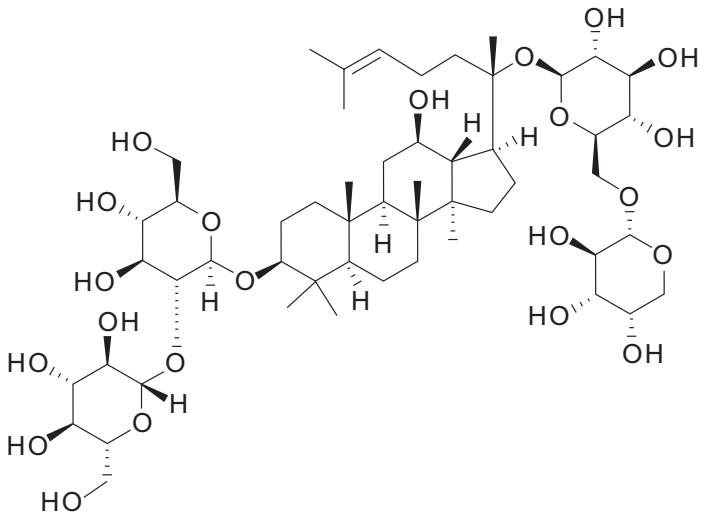
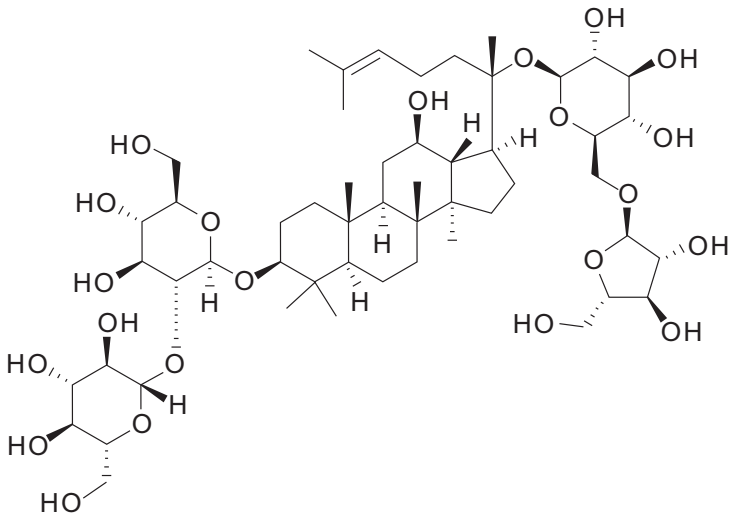


Figure 4 Chemical structure of (i) ginsenoside Rb_1

(ii)



(iii)



(iv)

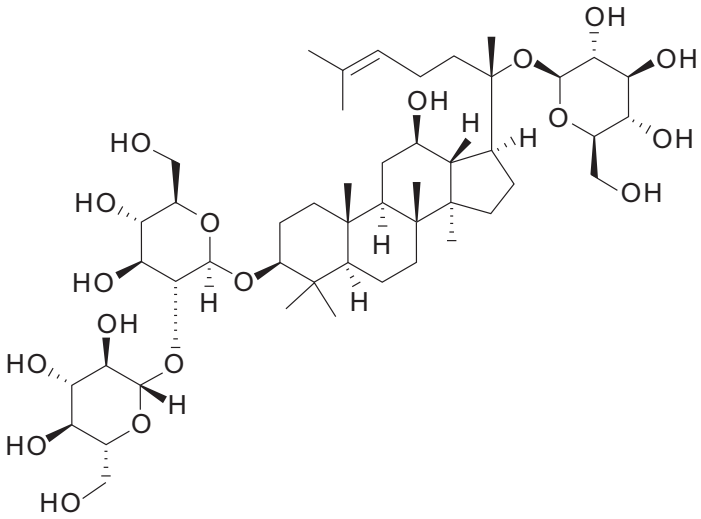
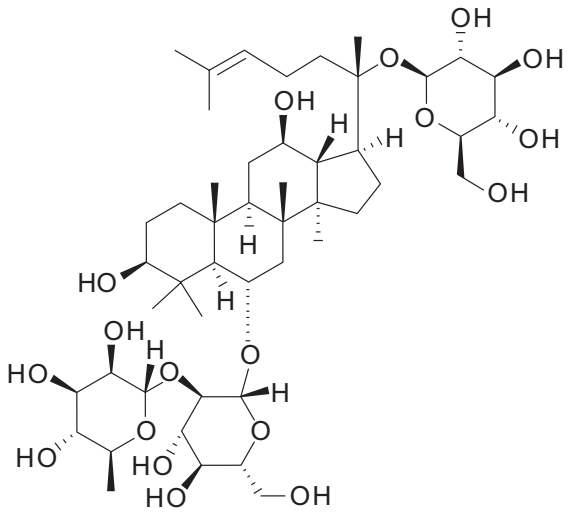
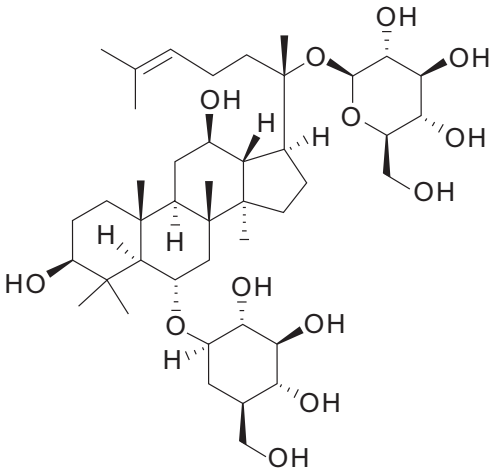


Figure 4 Chemical structures of (ii) ginsenoside Rb_2 (iii) ginsenoside Rc (iv) ginsenoside Rd

(v)



(vi)



(vii)

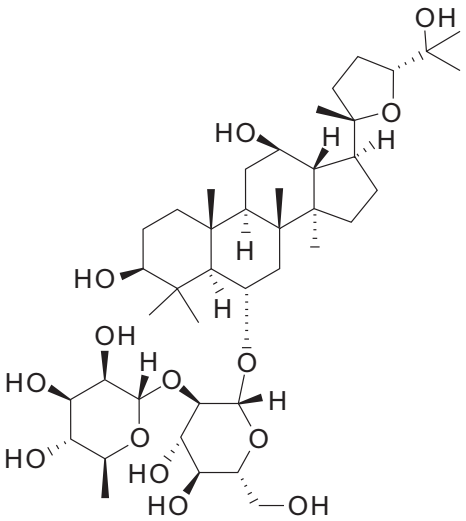


Figure 4 Chemical structures of (v) ginsenoside Re (vi) ginsenoside Rg_1 (vii) 24-(R)-pseudoginsenoside F_{11}

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

(I) High performance liquid chromatography with evaporative light scattering detector (HPLC/ELSD)

Standard solutions

Ginsenoside Rc standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 1.0 mg of ginsenoside Rc CRS and dissolve in 5 mL of methanol (70%).

24-(R)-pseudoginsenoside F_{11} standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 1.0 mg of 24-(R)-pseudoginsenoside F_{11} CRS and dissolve in 5 mL of methanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Wash the residue with 20 mL of methanol (70%). Centrifuge at about 1800 × *g* for 5 min. Combine the extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 105ºC; nebulizer gas (N_2) flow: 2.4 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–15	68	32	isocratic
15–50	68→57	32→43	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of 24-(R)-pseudoginsenoside F_{11} Std-FP and ginsenoside Rc Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 24-(R)-pseudoginsenoside F_{11} and ginsenoside Rc should not be more than 3.0%; the RSD of the retention times of 24-(R)-pseudoginsenoside F_{11} peak and ginsenoside Rc peak should not be more than 2.0%; the column efficiencies determined from 24-(R)-pseudoginsenoside F_{11} and ginsenoside Rc peaks should not be less than 15000 theoretical plates.

The *R* value between peak 1 and the closest peak and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(i), (ii), (iii) or (iv)].

Procedure

Separately inject 24-(R)-pseudoginsenoside F_{11} Std-FP, ginsenoside Rc Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of 24-(R)-pseudoginsenoside F_{11} and ginsenoside Rc peaks in the chromatogram of 24-(R)-pseudoginsenoside F_{11} Std-FP, ginsenoside Rc Std-FP and the retention times of the six characteristic peaks [Fig. 5(i), (ii), (iii) or (iv)] in the chromatogram of the test solution. Identify 24-(R)-pseudoginsenoside F_{11} and ginsenoside Rc peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 24-(R)-pseudoginsenoside F_{11} Std-FP and ginsenoside Rc Std-FP. The retention times of 24-(R)-pseudoginsenoside F_{11} and ginsenoside Rc 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 six characteristic peaks of Radix Panacis Quinquefolii extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Radix Panacis Quinquefolii extract

Peak No.	RRT	Acceptable Range
1 [marker 1, 24-(R)-pseudoginsenoside F_{11}]	1.00	-
2 (marker 2, ginsenoside Rc)	1.00	-
3 (ginsenoside Rb_2)	1.12 (vs peak 2)	±0.03
4	1.17 (vs peak 2)	±0.06
5 (ginsenoside Rd)	1.41 (vs peak 2)	±0.07
6	1.57 (vs peak 2)	±0.09

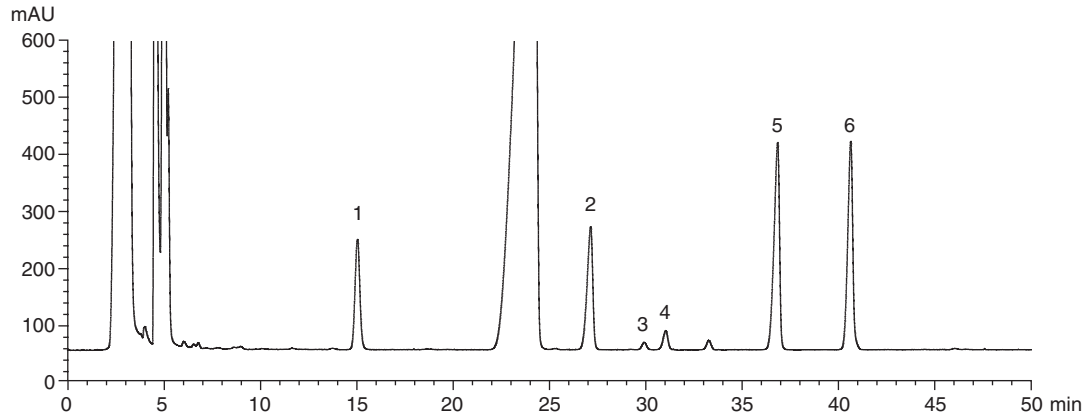


Figure 5(i) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Shandong, China)

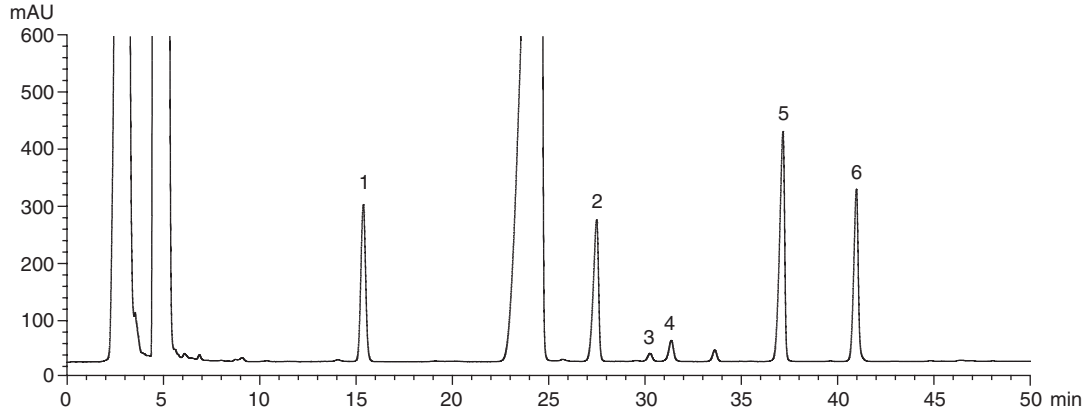


Figure 5(ii) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Ontario, Canada)

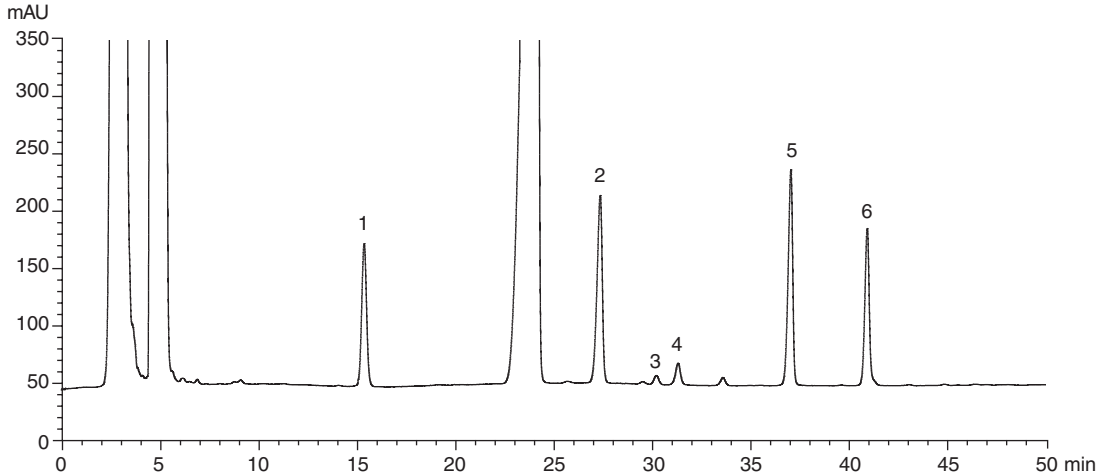


Figure 5(iii) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from British Columbia, Canada)

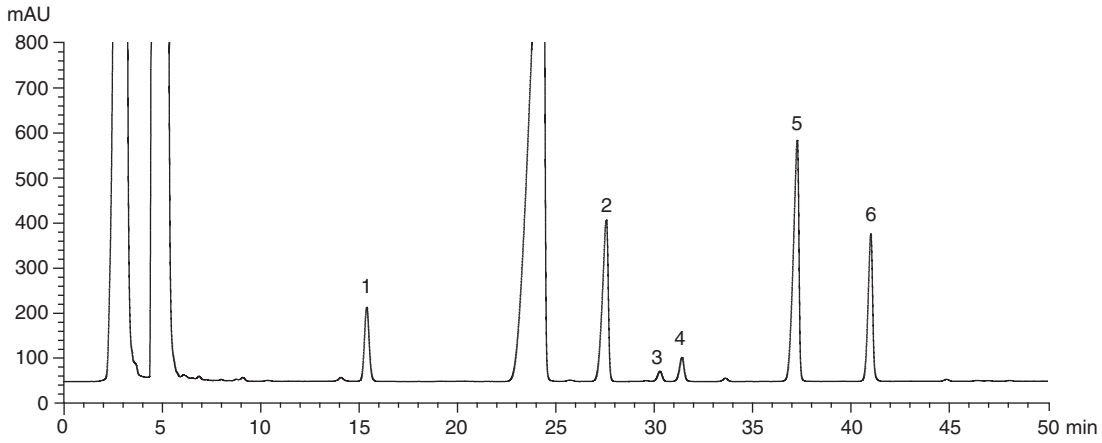


Figure 5(iv) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Wisconsin, USA)

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 fingerprint chromatogram [Fig. 5(i), (ii), (iii) or (iv)].

(II) High performance liquid chromatography with diode array detection (HPLC/DAD)

Standard solution

Ginsenoside Re standard solution for fingerprinting, Std-FP (250 mg/L)

Weigh 2.5 mg of ginsenoside Re CRS (Fig. 4) and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (270 W) the mixture for 20 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for three more times. Wash the residue with 10 mL of methanol (70%). Centrifuge at about 1800 × *g* for 5 min. Combine the extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE 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 22ºC 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)	0.002M Potassium dihydrogen phosphate (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	79	21	isocratic
15 – 69	79 → 62	21 → 38	linear gradient
69 – 75	62	38	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of ginsenoside Re Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ginsenoside Re should not be more than 3.0%; the RSD of the retention time of ginsenoside Re peak should not be more than 2.0%; the column efficiency determined from ginsenoside Re peak should not be less than 20000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 6(i), (ii), (iii) or (iv)].

Procedure

Separately inject ginsenoside Re Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ginsenoside Re peak in the chromatogram of ginsenoside Re Std-FP and the retention times of the six characteristic peaks [Fig. 6(i), (ii), (iii) or (iv)] in the chromatogram of the test solution. Under the same HPLC conditions, identify ginsenoside Re peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ginsenoside Re Std-FP. The retention times of ginsenoside Re 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 six characteristic peaks of Radix Panacis Quinquefolii extract are listed in Table 4.

Table 4 The RRTs and acceptable ranges of the six characteristic peaks of Radix Panacis Quinquefolii extract

Peak No.	RRT	Acceptable Range
1 (ginsenoside Rg_1)	0.94	±0.04
2 (marker, ginsenoside Re)	1.00	-
3 (ginsenoside Rb_1)	1.85	±0.07
4 (ginsenoside Rc)	1.93	±0.06
5 (ginsenoside Rb_2)	2.00	±0.09
6 (ginsenoside Rd)	2.15	±0.08

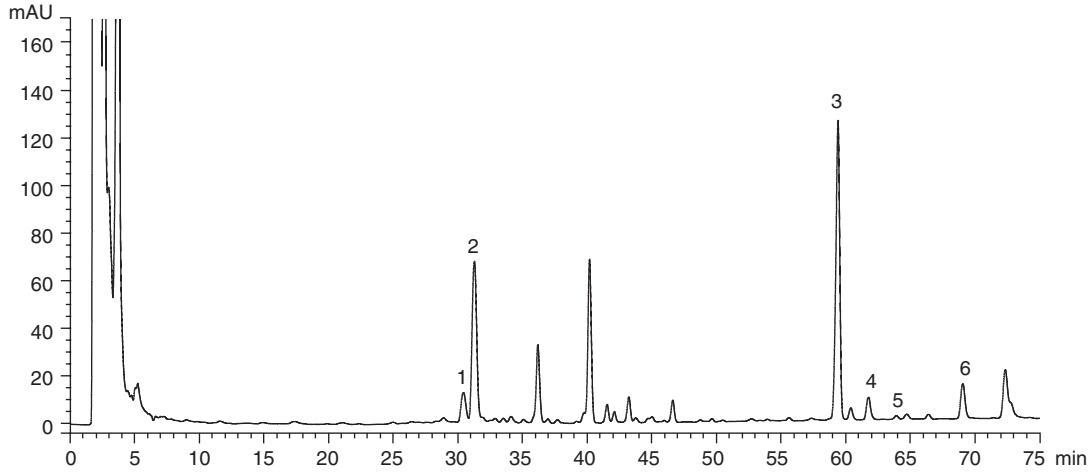


Figure 6(i) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Ji Lin, China)

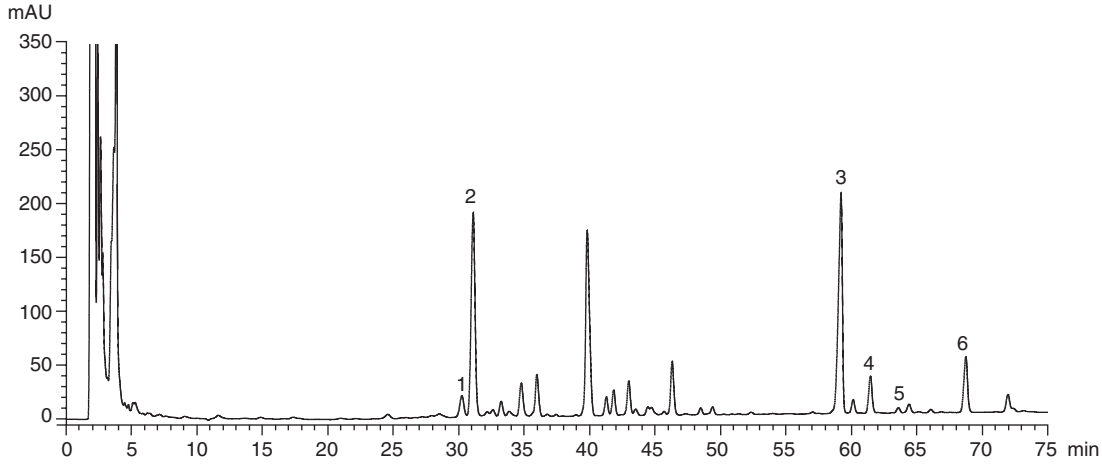


Figure 6(ii) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Ontario, Canada)

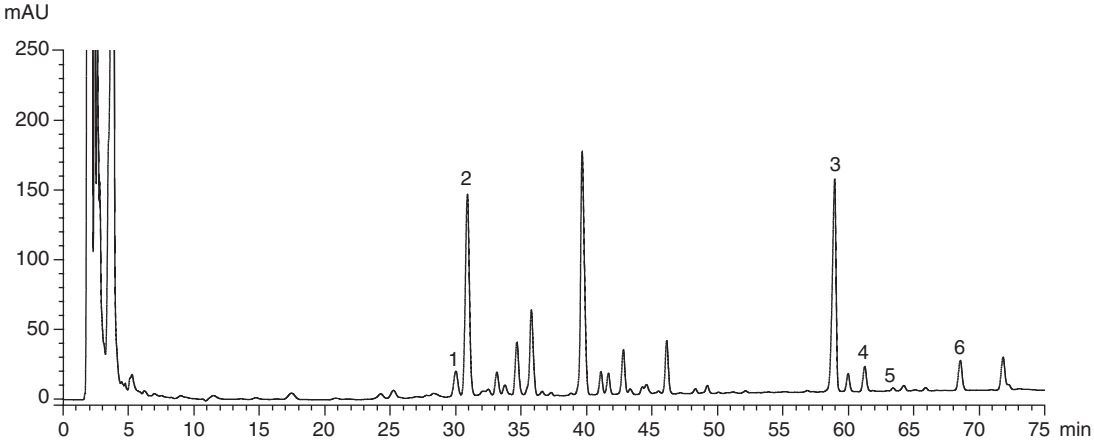


Figure 6(iii) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from British Columbia, Canada)

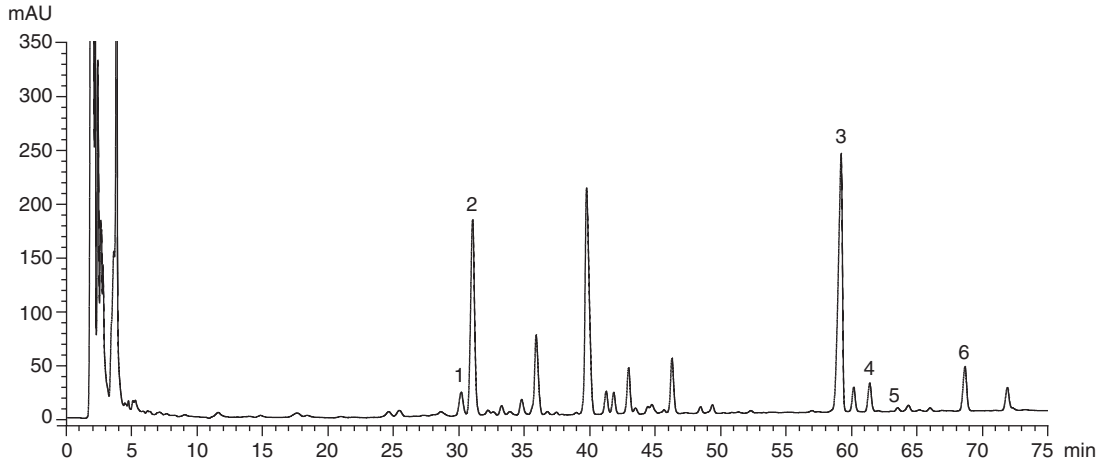


Figure 6(iv) A reference fingerprint chromatogram of Radix Panacis Quinquefolii extract (from Wisconsin, USA)

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 fingerprint chromatogram [Fig. 6(i), (ii), (iii) or (iv)].

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 XV)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 50.0%.
Ethanol-soluble extractives (cold extraction method): not less than 24.0%.

7. ASSAY

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

Standard solution

Mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 standard stock solution, Std-Stock (ginsenoside Rb_1 6000 mg/L, ginsenoside Rb_2 150 mg/L, ginsenoside Rc 600 mg/L, ginsenoside Rd 1500 mg/L, ginsenoside Re 3000 mg/L, ginsenoside Rg_1 1000 mg/L)

Weigh accurately 30.0 mg of ginsenoside Rb_1 CRS, 0.75 mg of ginsenoside Rb_2 CRS, 3.0 mg of ginsenoside Rc CRS, 7.5 mg ginsenoside Rd CRS (Fig. 4), 15.0 mg of ginsenoside Re CRS, 5.0 mg of ginsenoside Rg_1 CRS and dissolve in 5 mL of methanol (70%). Store at about −10ºC.

Mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 standard solution for assay, Std-AS

Measure accurately the volume of the mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 Std-Stock, dilute with methanol (70%) to produce a series of solutions of 150, 300, 500, 1000, 3000 mg/L for ginsenoside Rb_1; 3.75, 7.5, 12.5, 25, 75 mg/L for ginsenoside Rb_2; 15, 30, 50, 100, 300 mg/L for ginsenoside Rc; 37.5, 75, 125, 250, 750 mg/L for ginsenoside Rd; 75, 150, 250, 500, 1500 mg/L for ginsenoside Re; 25, 50, 83.3, 166.67, 500 mg/L for ginsenoside Rg_1.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (270 W) the mixture for 20 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for three more times. Wash the residue with 10 mL of methanol (70%). Centrifuge at about 1800 × *g* for 5 min. Combine the extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE 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 22ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 5) –

Table 5 Chromatographic system conditions

Time (min)	0.002M Potassium dihydrogen phosphate (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	79	21	isocratic
15 – 69	79 → 62	21 → 38	linear gradient
69 – 75	62	38	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 Std-AS (500 mg/L for ginsenoside Rb_1; 12.5 mg/L for ginsenoside Rb_2; 50 mg/L for ginsenoside Rc; 125 mg/L for ginsenoside Rd; 250 mg/L for ginsenoside Re; 83.3 mg/L for ginsenoside Rg_1). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 should not be more than 3.0%; the RSD of the retention times of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 peak should not be more than 2.0%; the column efficiencies determined from ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 peak should not be less than 20000 theoretical plates.

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

Calibration curve

Inject a series of mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 against the corresponding concentrations of mixed ginsenosides Rb_1, Rb_2, Rc, Rd, 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 20 μL of the test solution into the HPLC system and record the chromatogram. Identify ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of mixed ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 Std-AS. The retention times of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 peaks from the two chromatograms should not differ from their counterparts by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 in the test solution, and calculate the percentage contents of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 in the sample by using the equations indicated in Appendix IV (B).

Limits

The sample contains not less than 2.2% of the total content of ginsenosides [Rb_1 ($C_{54}H_{92}O_{23}$), Rb_2 ($C_{53}H_{90}O_{22}$), Rc ($C_{53}H_{90}O_{22}$), Rd ($C_{48}H_{82}O_{18}$), Re ($C_{48}H_{82}O_{18}$) and Rg_1 ($C_{42}H_{72}O_{14}$)], calculated with reference to the dried substance.