

# Radix Notoginseng



Figure 25 Photographs of Radix Notoginseng

- A. Radix Notoginseng (Sanqi) used in Mainland China
- B. Radix Notoginseng (Sanqi) used in Hong Kong

## 1. NAMES

Official Name: Radix Notoginseng

Chinese Name: 三七

Chinese Phonetic Name: Sanqi

## 2. SOURCE

Radix Notoginseng is the dried root of *Panax notoginseng* (Burk.) F. H. Chen (Fam. Araliaceae). The root is collected in autumn after 3 to 4 years of plantation before efflorescence. Branch roots and rhizome removed, washed clean and then dried in the sun to obtain Radix Notoginseng.

## 3. DESCRIPTION

The main root is subconical or cylindrical, 1–6 cm in length and 10–40 mm in diameter. Externally dull greyish-brown or greyish-yellow with interrupted longitudinal wrinkles and branch root scars; stem scars at the apex surrounded by warty protruberances. Texture heavy, hard and compact, fracture greyish-green, yellowish-green or greyish-white, bark and wood often separable from each other when crumbled. Bark dotted with small brownish resin canals. Wood slightly radially arranged. Odour, slight; taste, bitter but sweetish afterwards.

Note: The Radix Notoginseng available in the market in Hong Kong have been coloured with coal smoke and polished with bee's wax. (Fig. 25)

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (Appendix III)

#### Transverse section

The transverse section shows cork consisting of several rows of flat cells. Clusters of calcium oxalate are rare and mostly distributed in parenchyma close to cork. Phloem parenchyma scattered with resin canals containing yellow masses of secretion. Cambium in a ring, sometimes strongly undulant. Xylem rays broad. Vessels 1–2 rows in groups, arranged radially. (Fig. 26)

### Powder

Greyish-yellow. Starch grains fairly abundant; simple granules spherical, hemispherical or round-polygonal, 6–53 µm in diameter; compound granules of 2–9 units; showing a black, cross-shape when examined under a polarizing microscope. Fragments of resin canals containing yellowish-brown secretions. Scalariform, reticulated and spiral vessels, 20–106 µm in diameter. Clusters of calcium oxalate infrequent, 37–125 µm in diameter; showing a light white and blue colour under a polarizing microscope. (Fig. 27)

## 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<sub>1</sub> standard solution*

Weigh 0.5 mg of ginsenoside Rb<sub>1</sub> and dissolve in 1 mL of methanol.

*Ginsenoside Rg<sub>1</sub> standard solution*

Weigh 0.5 mg of ginsenoside Rg<sub>1</sub> and dissolve in 1 mL of methanol.

*Notoginsenoside R<sub>1</sub> standard solution*

Weigh 0.5 mg of notoginsenoside R<sub>1</sub> and dissolve in 1 mL methanol.

### 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.



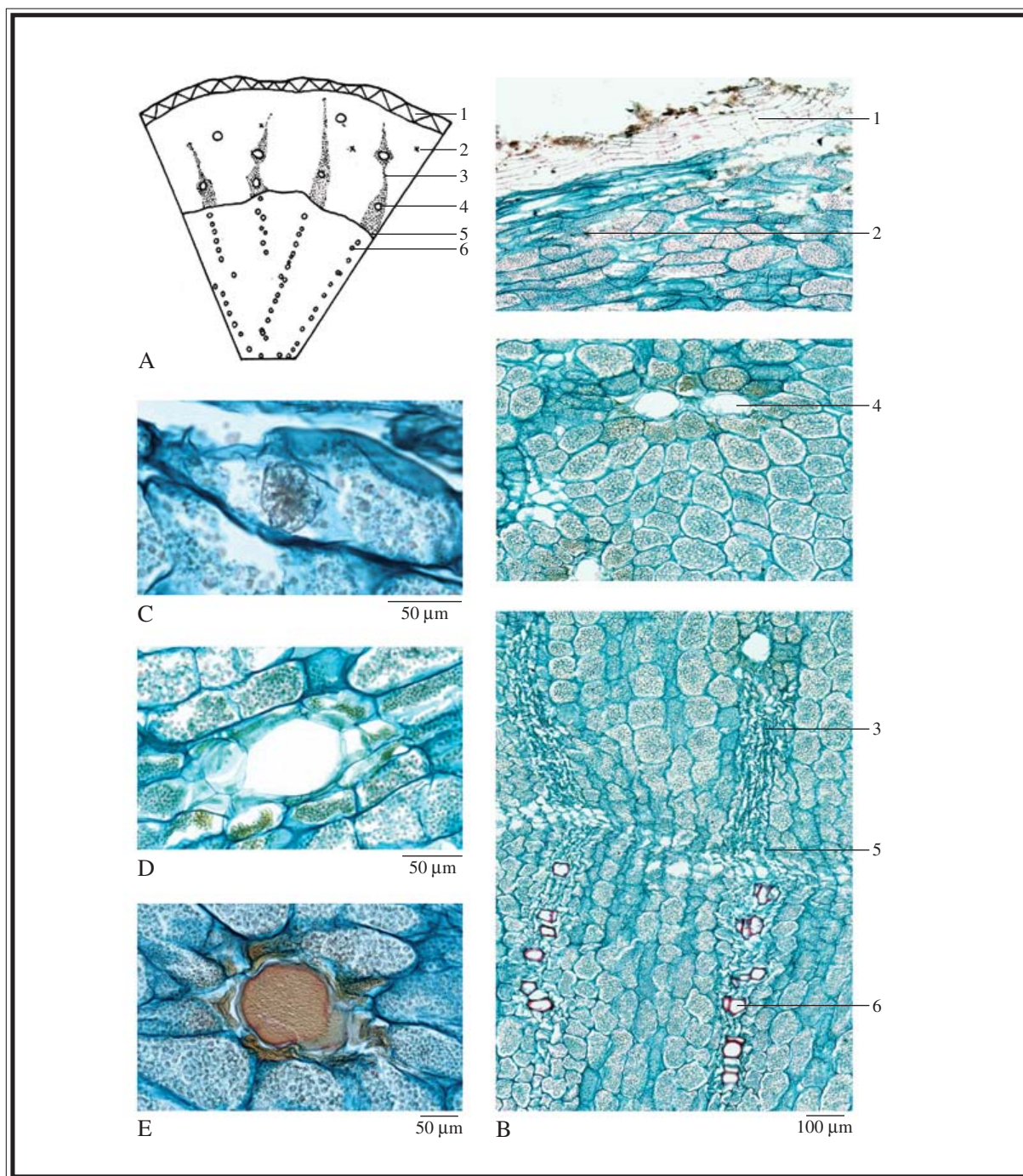


Figure 26 Microscopic features of transverse section of Radix Notoginseng

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

E. Resin canal with yellowish-brown secretion

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

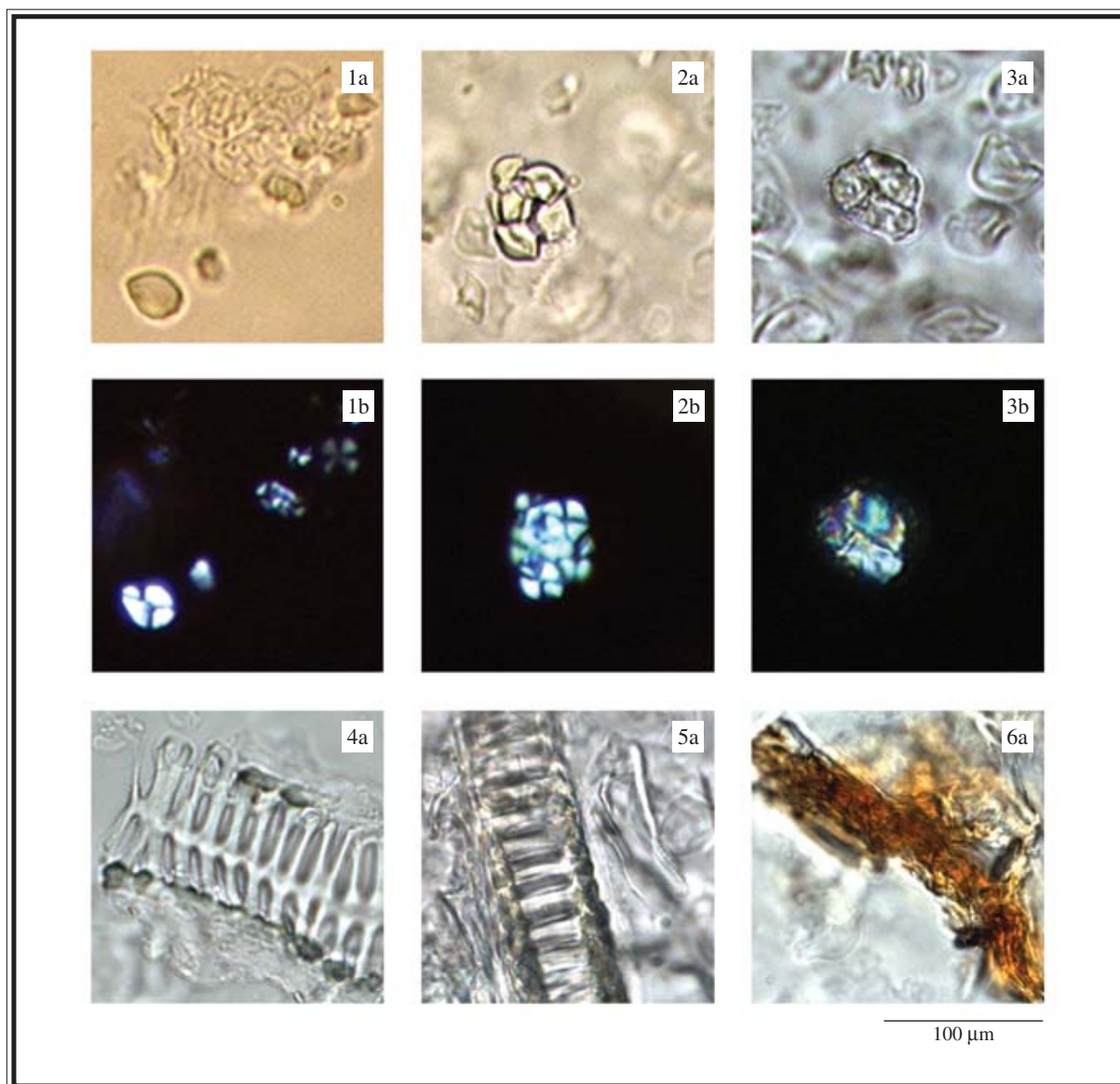


Figure 27 Microscopic features of powder of Radix Notoginseng

1. Simple starch granules   2. Compound starch granules   3. Cluster of calcium oxalate
4. Reticulated vessel   5. Scalariform vessel   6. Resin canal

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

**Procedure**

Carry out the method by using a HPTLC silica gel F<sub>254</sub> plate and a freshly prepared developing solvent system as described above. Develop over a path of about 8 cm. Apply separately ginsenoside Rb<sub>1</sub> standard solution, ginsenoside Rg<sub>1</sub> standard solution, notoginsenoside R<sub>1</sub> standard solution and the test solution (1 µL each) to the plate. After the development, remove the plate from the chamber, mark the solvent front and allow to dry in air. 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<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub>.

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

*Ginsenoside Rb<sub>1</sub> standard stock solution, Std-Stock (500 mg/L)*

Weigh 2.5 mg of ginsenoside Rb<sub>1</sub> and dissolve in 5 mL of methanol. Store at about -10 °C in the dark.

*Ginsenoside Rb<sub>1</sub> standard solution for fingerprinting, Std-FP (100 mg/L)*

Pipette 1.0 mL of ginsenoside Rb<sub>1</sub> Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

*Ginsenoside Rg<sub>1</sub> standard stock solution, Std-Stock (500 mg/L)*

Weigh 2.5 mg of ginsenoside Rg<sub>1</sub> and dissolve in 5 mL of methanol. Store at about -10 °C in the dark.

*Ginsenoside Rg<sub>1</sub> standard solution for fingerprinting, Std-FP (100 mg/L)*

Pipette 1.0 mL of ginsenoside Rg<sub>1</sub> Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

*Notoginsenoside R<sub>1</sub> standard stock solution, Std-Stock (500 mg/L)*

Weigh 2.5 mg of notoginsenoside R<sub>1</sub> and dissolve in 5 mL of methanol. Store at about -10 °C in the dark.

*Notoginsenoside R<sub>1</sub> standard solution for fingerprinting, Std-FP (100 mg/L)*

Pipette 1.0 mL of notoginsenoside R<sub>1</sub> Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

**Test solution**

Weigh 0.2 g of the powdered sample into a 10-mL centrifugal tube, then add 5 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- $\mu$ m 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  $\mu$ m 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  $\mu$ L of ginsenoside Rb<sub>1</sub> Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ginsenoside Rb<sub>1</sub> should be not more than 3.0%; the RSD of the retention time of ginsenoside Rb<sub>1</sub> peak should be not more than 2.0%; the column efficiency determined from ginsenoside Rb<sub>1</sub> peak should be not less than 150,000 theoretical plates.

The *R* value between ginsenoside Rg<sub>1</sub> peak and ginsenoside Re peak (Fig. 28) in the test solution should be not less than 1.0.

### Procedure

Separately inject ginsenoside Rb<sub>1</sub> Std-FP, ginsenoside Rg<sub>1</sub> Std-FP, notoginsenoside R<sub>1</sub> Std-FP and the test solution (20  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention times of the peaks of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> in the chromatograms of the corresponding Std-FP and the retention times of the six characteristic peaks in the chromatogram of the test solution (Fig. 28). Under the same HPLC conditions, identify the peaks of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> 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 the peaks of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> in the chromatograms of the test solution and the corresponding Std-FP should not differ from their counterparts 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 Notoginseng extract are listed in Table 9.

Table 9 The RRTs and acceptable ranges of the six characteristic peaks of Radix Notoginseng extract

Peak No.	RRT	Acceptable Range
1 (marker 1, notoginsenoside R <sub>1</sub> )	1.00	-
2 (marker 2, ginsenoside R <sub>g1</sub> )	1.00	-
3 (ginsenoside Re)	1.06 (vs R <sub>g1</sub> )	±0.04
4 (marker 3, ginsenoside R <sub>b1</sub> )	1.00	-
5 (ginsenoside Rd)	1.12 (vs R <sub>b1</sub> )	±0.03
6	1.17 (vs R <sub>b1</sub> )	±0.03

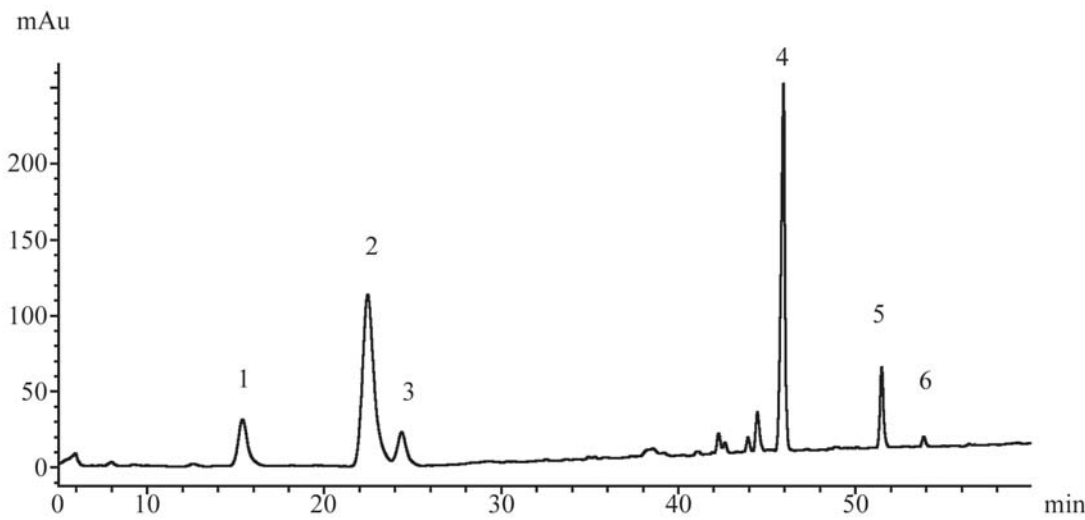


Figure 28 A reference fingerprint chromatogram of Radix Notoginseng extract

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 fingerprint chromatogram (Fig. 28).

### 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 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 1.0%.

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

## 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 26.0%.

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 ginsenoside R<sub>b1</sub>, ginsenoside R<sub>g1</sub> and notoginsenoside R<sub>1</sub> standard stock solution, Std-Stock*  
(2000 mg/L for ginsenoside R<sub>b1</sub> and ginsenoside R<sub>g1</sub> and 1000 mg/L for notoginsenoside R<sub>1</sub>)

Weigh accurately 20.0 mg of ginsenoside R<sub>b1</sub>, 20.0 mg of ginsenoside R<sub>g1</sub> and 10.0 mg of notoginsenoside R<sub>1</sub> and dissolve in 10 mL of methanol. Store at about -10 °C in the dark.

*Mixed ginsenoside R<sub>b1</sub>, ginsenoside R<sub>g1</sub> and notoginsenoside R<sub>1</sub> standard solution for assay, Std-AS*

Measure accurately the volume of the mixed ginsenoside R<sub>b1</sub>, ginsenoside R<sub>g1</sub> and notoginsenoside R<sub>1</sub> Std-Stock, dilute with methanol to produce a series of solutions of 20, 50, 100, 200, 500 mg/L for both ginsenoside R<sub>b1</sub> and ginsenoside R<sub>g1</sub>, and 10, 25, 50, 100, 250 mg/L for notoginsenoside R<sub>1</sub>.

### Test solution

Weigh accurately 0.25 g of the powdered sample into a 10-mL centrifugal tube. Add 5 mL of methanol to the tube and mix. Sonicate the mixture for 30 min. Centrifuge at about 540 x g for 5 min and transfer the supernatant to a 25-mL volumetric flask. Repeat the above extraction for three more times. Combine the extracts. 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<sub>1</sub> Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ginsenoside Rb<sub>1</sub> should be not more than 3.0%; the RSD of the retention time of ginsenoside Rb<sub>1</sub> peak should be not more than 2.0%; the column efficiency determined from the ginsenoside Rb<sub>1</sub> peak should be not less than 150,000 theoretical plates.

The *R* value between ginsenoside Rg<sub>1</sub> peak and ginsenoside Re peak in the test solution should be not less than 1.0.

Calibration curves

Inject a series of the mixed ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> against the corresponding concentrations of the mixed ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> Std-AS. Obtain the slopes, y-intercepts and the *r*<sup>2</sup> 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<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> Std-AS. The retention times of the peaks of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> 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<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> in the test solution, and calculate the percentage contents of ginsenoside Rb<sub>1</sub>, ginsenoside Rg<sub>1</sub> and notoginsenoside R<sub>1</sub> in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 1.7% of ginsenoside Rb<sub>1</sub> (C<sub>54</sub>H<sub>92</sub>O<sub>23</sub>); not less than 2.0% of ginsenoside Rg<sub>1</sub> (C<sub>42</sub>H<sub>72</sub>O<sub>14</sub>); and not less than 0.49% of notoginsenoside R<sub>1</sub> (C<sub>47</sub>H<sub>80</sub>O<sub>18</sub>), calculated with reference to the dried substance.