Sparganii Rhizoma

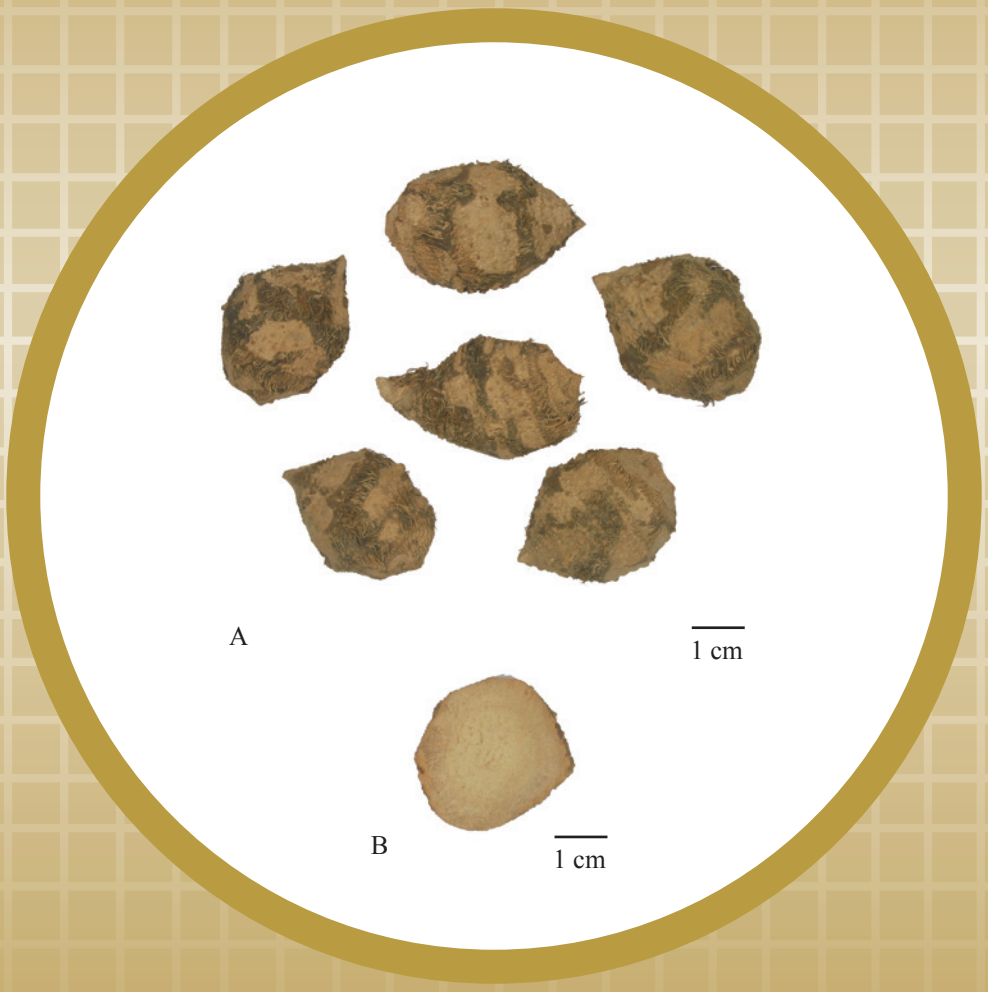

Figure 1 A photograph of Sparganii Rhizoma

A. Sparganii Rhizoma

B. Cut surface of rhizome

1. NAMES

Official Name: Sparganii Rhizoma

Chinese Name: 三棱

Chinese Phonetic Name: Sanleng

2. SOURCE

Sparganii Rhizoma is the dried rhizome of *Sparganium stoloniferum* Buch.-Ham. (Typhaceae). The rhizome is collected from winter to the next spring, washed clean, partly peeled, then dried under the sun to obtain Sparganii Rhizoma.

3. DESCRIPTION

Conical or obovate, slightly flattened, 2-8 cm long, 20-45 mm in diameter. Externally yellowish-white to greyish-yellow, with marks pared with a knife, fibrous root scars small, densely spotted, slightly annulated arranged transversely. Texture hard, compact and heavy in weight. Fracture greyish-yellow to pale brown, slightly flattened, with numerous scattered spots. Odour slight; taste bland, slightly numb and pungent on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cortex broken, the remnants of the cortex consists of irregular parenchymatous cells, intercellular spaces relatively large. Endodermis consists of 1 layer of tangentially elongated cells, densely arranged, inner and lateral walls of some cells thickened. Parenchymatous cells of stele subrounded or subpolygonal, walls slightly thickened, containing starch granules. Secretory cells scattered in cortex and stele, containing brownish-red contents. Collateral and amphivasal vascular bundles scattered (Fig. 2).

Powder

Colour yellowish-white. Starch granules numerous, simple starch granules subrounded, subpolygonal or elliptic, 2-10 μm in diameter, dotted or slit-shaped hilum slightly visible in bigger granules; black and cruciate-shaped under the polarized microscope; compound starch granules mostly composed of 2-3 units. Secretory cells contain reddish-brown contents. Parenchymatous cells subrectangular, long elliptical or irregular in shape, walls beaded, slightly lignified. Fibres usually in bundles, walls relatively thick, slightly lignified or lignified, with sparse and oblique pits. Asteroidal parenchymatous cells occasionally visible, usually connected with sclerenchymatous cells, irregular in shape (Fig. 3).

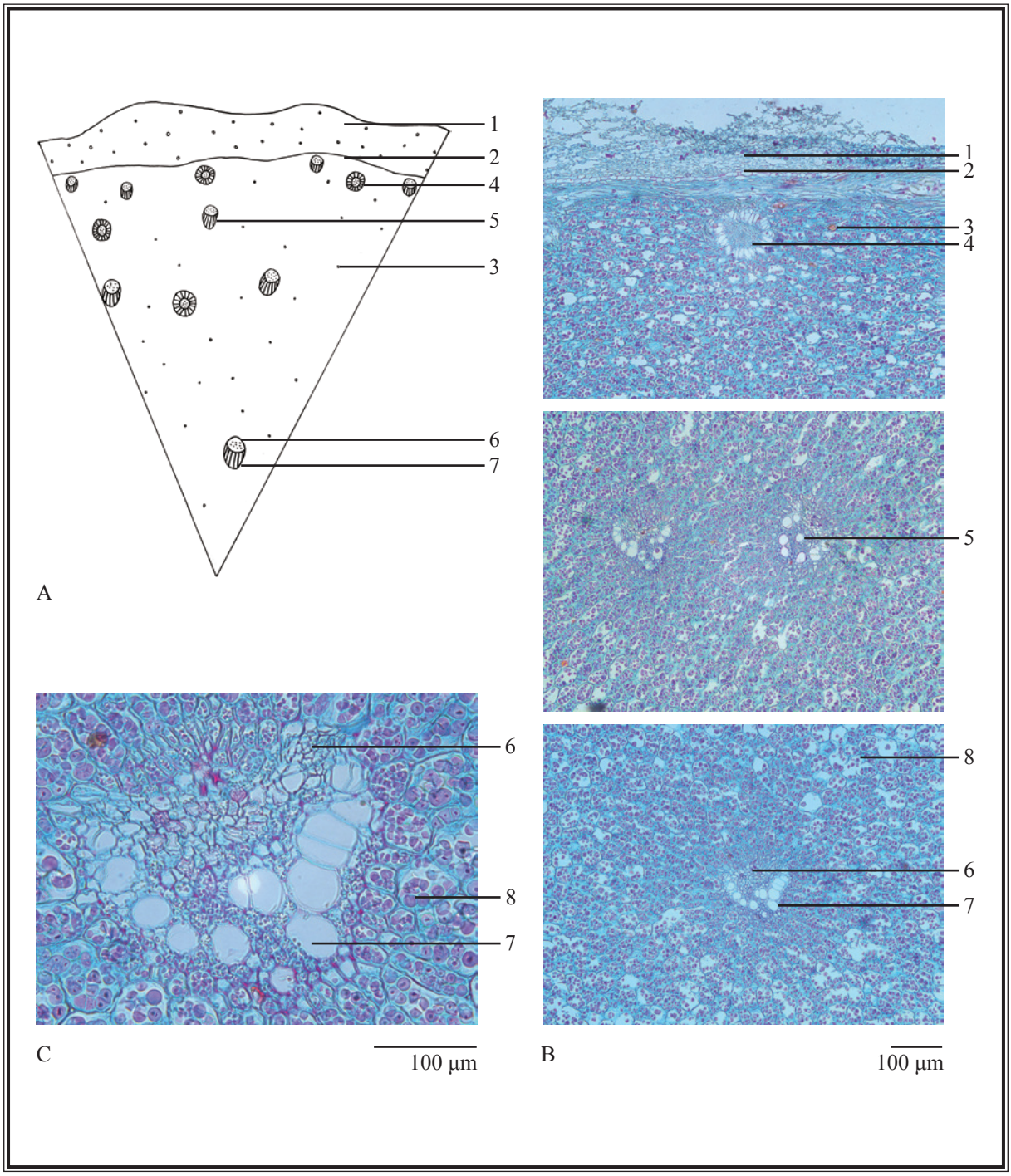

Figure 2 Microscopic features of transverse section of Sparganii Rhizoma

A. Sketch B. Section illustration C. Vascular bundle (collateral type)

1. Remnants of cortex 2. Endodermis 3. Secretory cell 4. Vascular bundle (amphivasal type)
5. Vascular bundle (collateral type) 6. Phloem 7. Xylem 8. Starch granule

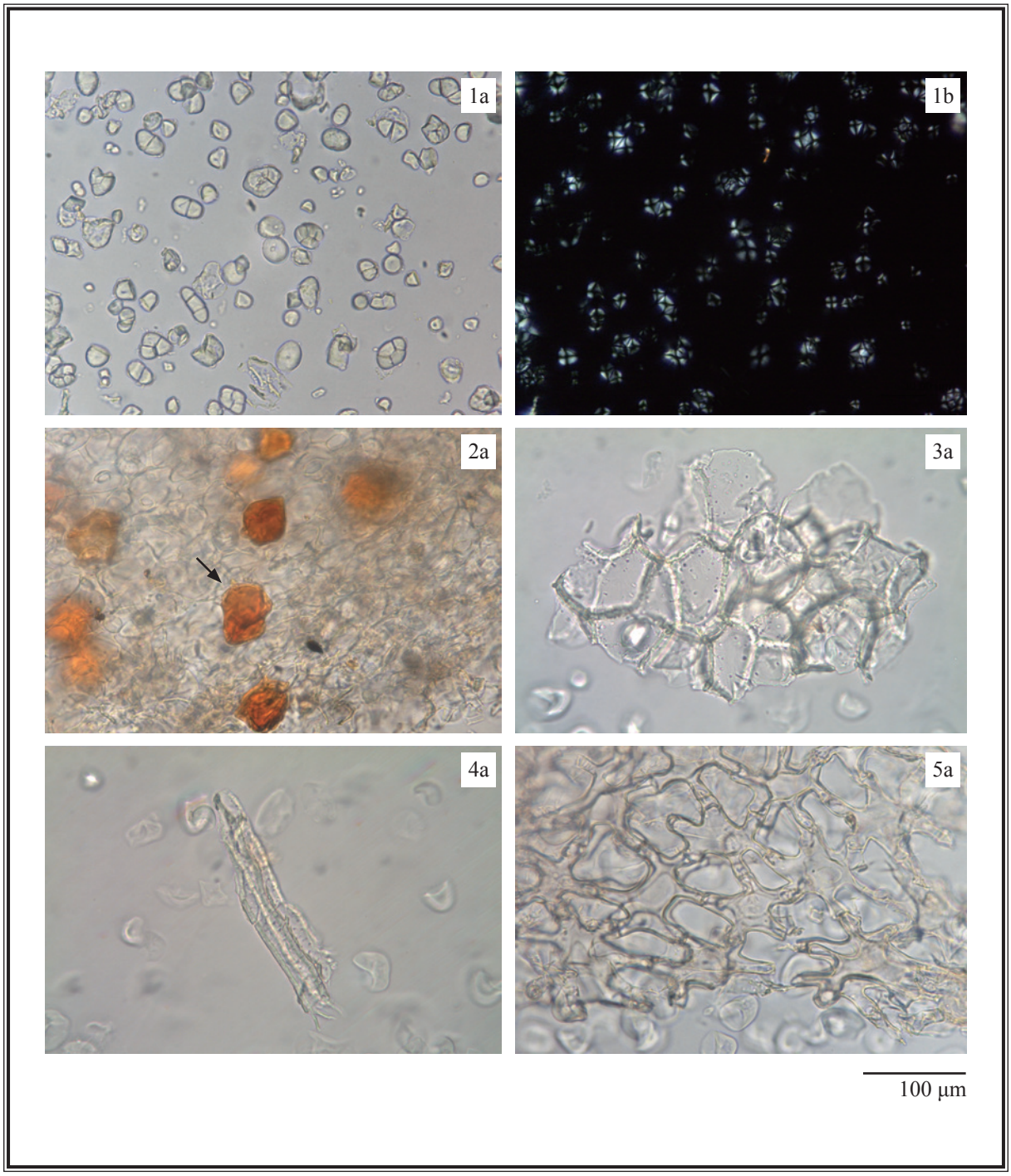

Figure 3 Microscopic features of powder of Sparganii Rhizoma

1. Starch granules 2. Secretory cells (→) 3. Lignified parenchymatous cells 4. Fibres 5. Asteroidal parenchymatous cells

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

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

Standard solutions

β-Sitosterol standard solution

Weigh 4.5 mg of β-sitosterol CRS (Fig. 4) and dissolve in 25 mL of methanol. Keep at about 4°C.

β-Sitosterol glucoside standard solution

Weigh 4.5 mg of β-sitosterol glucoside CRS (Fig. 4) and dissolve in 25 mL of methanol. Keep at about 4°C.

Developing solvent system

Prepare a mixture of dichloromethane, petroleum ether (60-80°C) and methanol (5:3:1, v/v).

Spray reagent

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

Test solution

Weigh 5.0 g of the powdered sample and place it in a 100-mL conical flask, then add 25 mL of methanol. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate and a freshly prepared developing solvent system as described above. Apply separately β-sitosterol standard solution (2 μL), β-sitosterol glucoside standard solution (2 μL) and the test solution (5 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 105°C (about 10 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

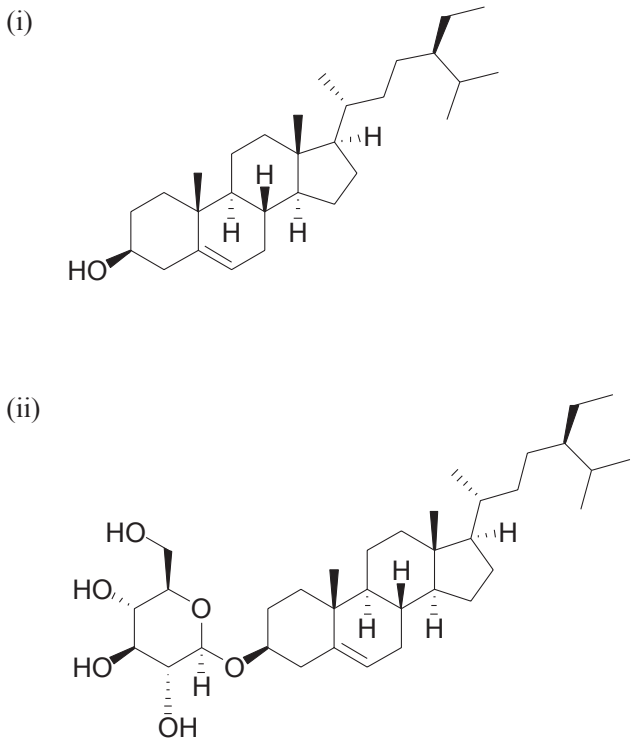

Figure 4 Chemical structures of (i) β-sitosterol and (ii) β-sitosterol glucoside

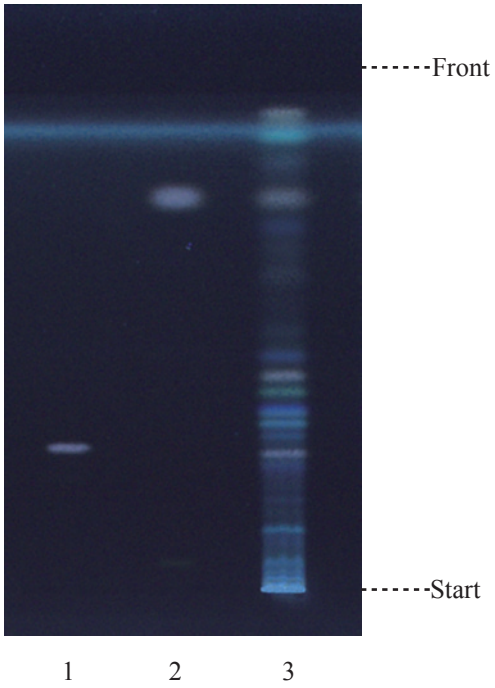

Figure 5 A reference HPTLC chromatogram of Sparganii Rhizoma extract observed under UV light (366 nm) after staining

1. β-Sitosterol glucoside standard solution 2. β-Sitosterol 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 β-sitosterol and β-sitosterol glucoside (Fig. 5).

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

Standard solutions

β-Sitosterol standard solution for fingerprinting, Std-FP (130 mg/L)

Weigh 1.3 mg of β-sitosterol CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

β-Sitosterol glucoside standard solution for fingerprinting, Std-FP (110 mg/L)

Weigh 1.1 mg of β-sitosterol glucoside CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 100-mL conical flask, then add 25 mL of methanol. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with methanol. Combine the solutions. Repeat the extraction for one more time. Combine the solutions. Evaporate the solvent to dryness below 60°C at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 40°C; nebulizer gas (N_2) pressure: 3.5 bar] and a column (4.6 × 250 mm) packed with OS 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 50	85 → 100	15 → 0	linear gradient
50 – 60	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of β-sitosterol Std-FP and β-sitosterol glucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of β-sitosterol and β-sitosterol glucoside should not be more than 5.0%; the RSD of the retention times of β-sitosterol and β-sitosterol glucoside peaks should not be more than 2.0%; the column efficiencies determined from β-sitosterol and β-sitosterol glucoside peaks should not be less than 30000 and 15000 theoretical plates respectively.

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 β-sitosterol Std-FP, β-sitosterol glucoside Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of β-sitosterol and β-sitosterol glucoside peaks in the chromatograms of β-sitosterol Std-FP, β-sitosterol glucoside Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify β-sitosterol and β-sitosterol glucoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of β-sitosterol Std-FP and β-sitosterol glucoside Std-FP. The retention times of β-sitosterol and β-sitosterol glucoside 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 five characteristic peaks of Sparganii Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Sparganii Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.68	± 0.03
2 (β-sitosterol glucoside)	0.73	± 0.03
3	0.94	± 0.03
4 (marker, β-sitosterol)	1.00	-
5	1.49	± 0.05

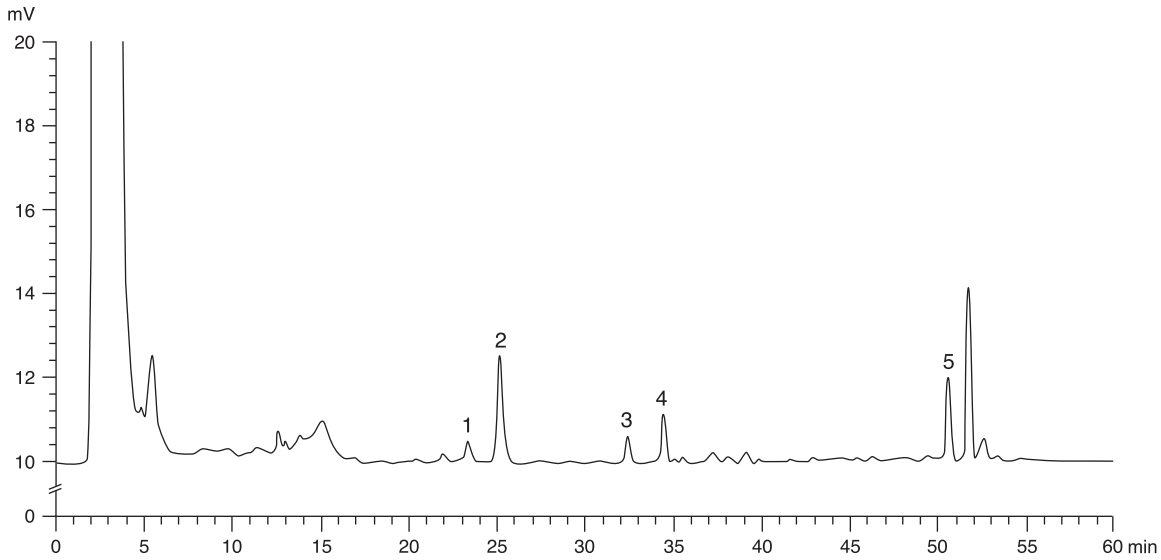

Figure 6 A reference fingerprint chromatogram of Sparganii Rhizoma extract

For positive identification, the sample must give the above five 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 3.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Mixed β-sitosterol and β-sitosterol glucoside standard stock solution, Std-Stock (380 mg/L for β-sitosterol and 320 mg/L for β-sitosterol glucoside)

Weigh accurately 3.8 mg of β-sitosterol CRS and 3.2 mg of β-sitosterol glucoside CRS, and dissolve in 10 mL of methanol. Keep at about 4°C.

Mixed β-sitosterol and β-sitosterol glucoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed β-sitosterol and β-sitosterol glucoside Std-Stock, dilute with methanol to produce a series of solutions of 19, 38, 76, 133, 190 mg/L for β-sitosterol and 16, 32, 64, 160, 256 mg/L for β-sitosterol glucoside. Keep at about 4°C.

Test solution

Weigh accurately 5.0 g of the powdered sample and place it in a 100-mL conical flask, then add 25 mL of methanol. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with methanol. Combine the solutions. Repeat the extraction for one more time. Combine the solutions. Evaporate the solvent to dryness below 60°C at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 40°C; nebulizer gas (N_2) pressure: 3.5 bar] and a column (4.6 × 250 mm) packed with OS 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 50	85 → 100	15 → 0	linear gradient
50 – 60	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed β-sitosterol and β-sitosterol glucoside Std-AS (76 mg/L for β-sitosterol and 64 mg/L for β-sitosterol glucoside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of β-sitosterol and β-sitosterol glucoside should not be more than 5.0%; the RSD of the retention times of β-sitosterol and β-sitosterol glucoside peaks should not be more than 2.0%; the column efficiencies determined from β-sitosterol and β-sitosterol glucoside peaks should not be less than 30000 and 15000 theoretical plates respectively.

The *R* value between β-sitosterol peak and the closest peak; and the *R* value between β-sitosterol glucoside 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 β-sitosterol and β-sitosterol glucoside Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of β-sitosterol and β-sitosterol glucoside against the natural logarithm of the corresponding concentrations of the mixed β-sitosterol and β-sitosterol glucoside 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 β-sitosterol and β-sitosterol glucoside peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed β-sitosterol and β-sitosterol glucoside Std-AS. The retention times of β-sitosterol and β-sitosterol glucoside 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 β-sitosterol and β-sitosterol glucoside in the test solution, and calculate the percentage contents of β-sitosterol and β-sitosterol glucoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.010% of the total content of β-sitosterol ($C_{29}H_{50}O$) and β-sitosterol glucoside ($C_{35}H_{60}O_6$), calculated with reference to the dried substance.

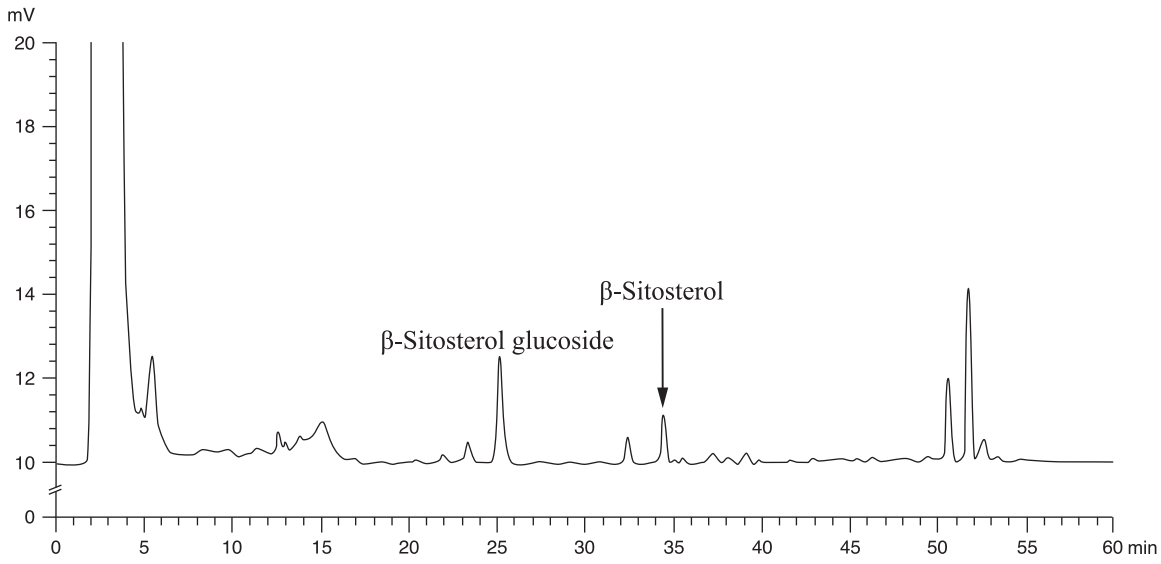

Figure 7 A reference assay chromatogram of Sparganii Rhizoma extract