

Dioscoreae Nipponicae Rhizoma

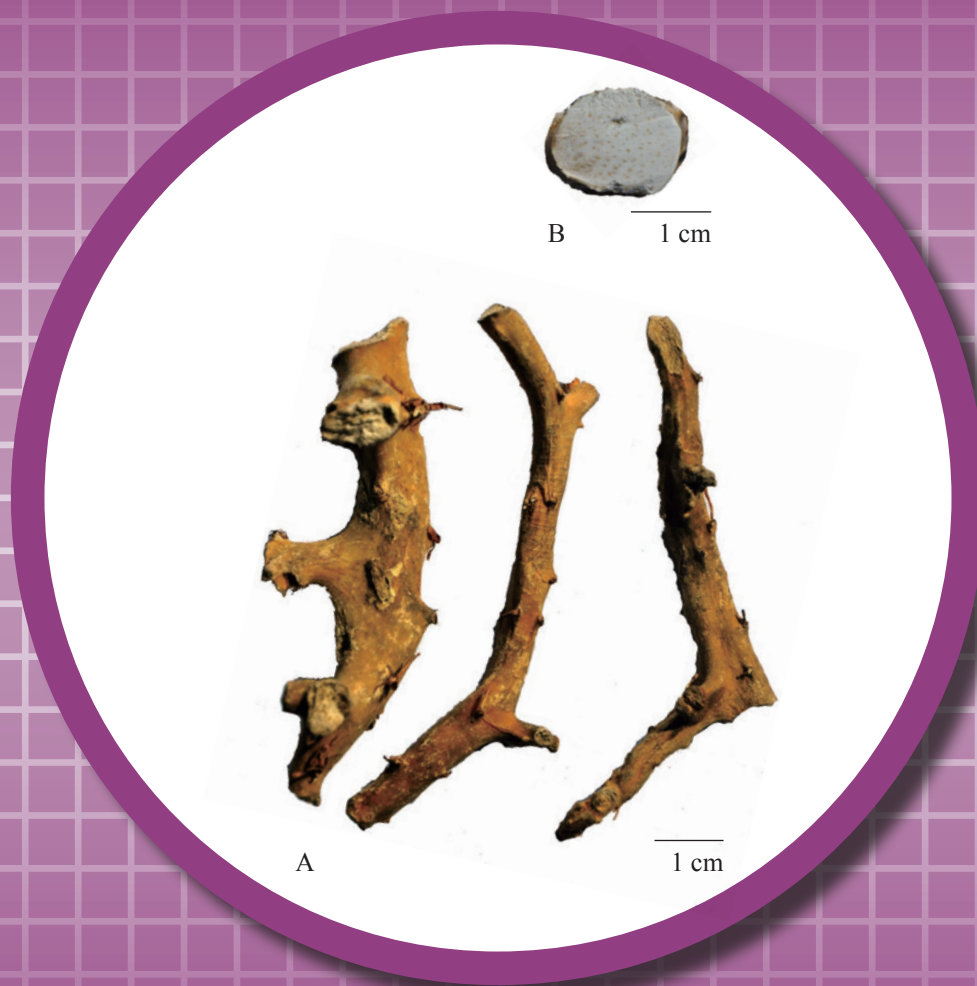


Figure 1 A photograph of Dioscoreae Nipponicae Rhizoma

A. Dioscoreae Nipponicae Rhizoma

B. Magnified image of transverse section of rhizome

1. NAMES

Official Name: Dioscoreae Nipponicae Rhizoma

Chinese Name: 穿山龍

Chinese Phonetic Name: Chuanshanlong

2. SOURCE

Dioscoreae Nipponicae Rhizoma is the dried rhizome of *Dioscorea nipponica* Makino (Dioscoreaceae). The rhizome is collected in spring and autumn. Rootlets and foreign matter removed, washed clean, then dried under the sun to obtain Dioscoreae Nipponicae Rhizoma.

3. DESCRIPTION

Cylindrical, slightly curved, 3-30 cm long, 4-18 mm in diameter. Externally yellowish-white to brown, with irregular longitudinal furrows, dotted root scars and protuberant stem scars on one side. Residual rootlets occasionally present. Texture hard. Fracture even, white to yellowish-white, with small pale yellowish-brown vascular bundles. Odour slight; taste bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork consists of several layers of cells, sometimes fallen off, the cells rectangular, tangentially extended and closely arranged. Cortex relatively narrow, mucous cells subrounded, containing raphides of calcium oxalate. Stele broad, scattered with collateral vascular bundles (Fig. 2).

Powder

Colour pale yellow. Starch granules fairly abundant, broadly ovoid, elongated-ellipsoid or irregular in shape, 3-18 µm in diameter, hilum slit-shaped, striations distinct; black and cruciate-shaped under the polarized microscope; compound starch granules rare, composed of 2-3 units. Raphides of calcium

oxalate scattered or in bundles, 31-101 μm long; polychromatic under the polarized microscope. Vessels mainly bordered-pitted, 8-61 μm in diameter. Cork cells brown, polygonal to subsquare. Lignified parenchymatous cells pale yellow or yellow, elongated elliptical, rectangular or rhombic, with small and sparse pits. Stone cells usually scattered singly, 42-276 μm long, 30-80 μm in diameter, walls 9-38 μm thick, with several striated layers, distinct pits and pit canals (Fig. 3).

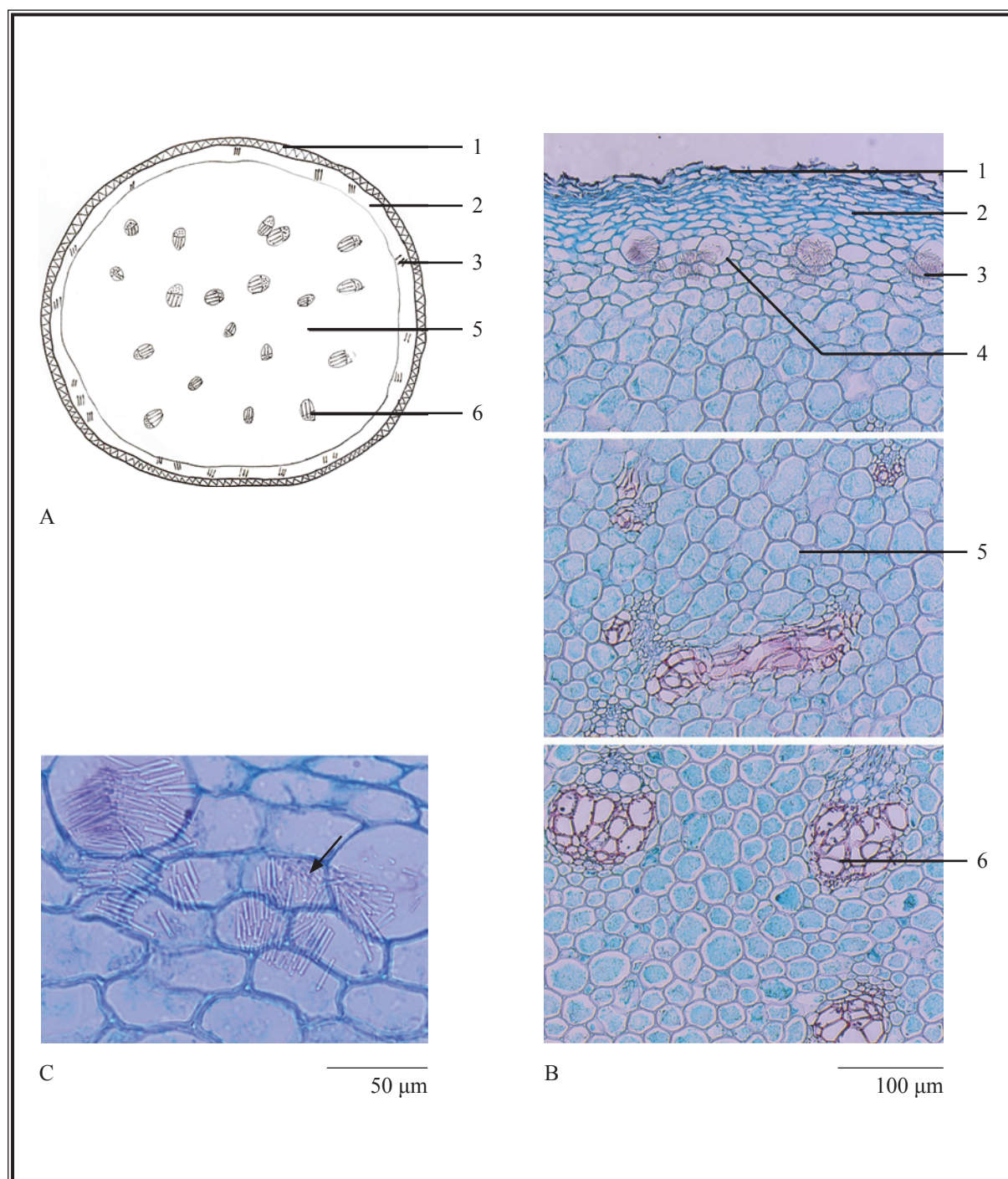


Figure 2 Microscopic features of transverse section of *Dioscoreae Nipponicae Rhizoma*

A. Sketch B. Section illustration C. Raphides of calcium oxalate

1. Cork 2. Cortex 3. Raphides of calcium oxalate 4. mucous cells 5. Stele 6. Vascular bundles

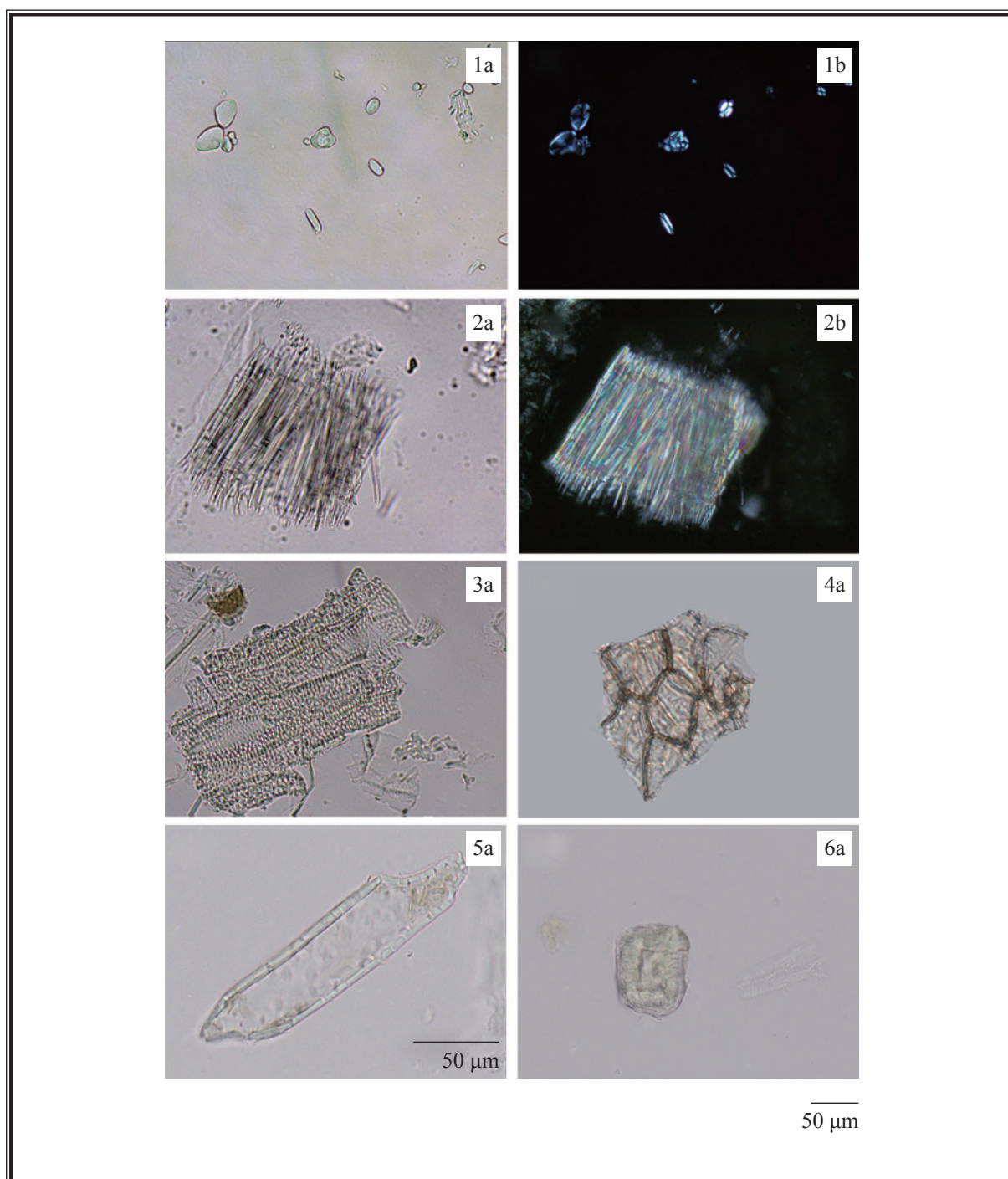


Figure 3 Microscopic features of powder of *Dioscoreae Nipponicae Rhizoma*

1. Starch granules 2. Raphides of calcium oxalate 3. Bordered-pitted vessels 4. Cork cells
5. Lignified parenchymatous cell 6. Stone cell

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

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

Standard solution

Dioscin standard solution

Weigh 0.8 mg of dioscin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of water, *n*-butanol and ethyl acetate (5:4:1, v/v). Use the upper layer.

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 centrifuge tube, then add 20 mL of ethanol (65%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about $4500 \times g$ for 10 min. Filter through a 0.45- μ m 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 dioscin standard solution (1.5 μ L) and the test solution (2 μ L) 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 6 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 value by using the equation as indicated in Appendix IV (A).

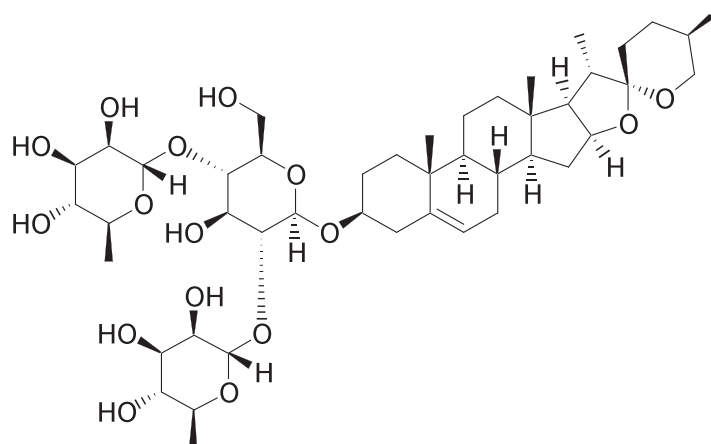


Figure 4 Chemical structure of dioscin

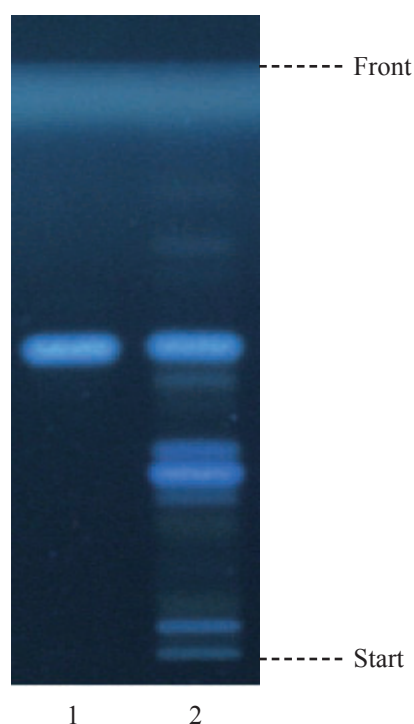


Figure 5 A reference HPTLC chromatogram of *Dioscoreae Nipponicae Rhizoma* extract observed under UV light (366 nm) after staining

1. Dioscin standard solution 2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of dioscin (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Dioscin standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 0.5 mg of dioscin CRS and dissolve in 1 mL of ethanol (65%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (65%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4500 × 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 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 – 10	50	50	isocratic
10 – 50	50 → 0	50 → 100	linear gradient
50 – 60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of dioscin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dioscin should not be more than 5.0%; the RSD of the retention time of dioscin peak should not be more than 2.0%; the column efficiency determined from dioscin peak should not be less than 10000 theoretical plates.

The R value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject dioscin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of dioscin peak in the chromatogram of dioscin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify dioscin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dioscin Std-FP. The

retention times of dioscin 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 three characteristic peaks of Dioscoreae Nipponicae Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Dioscoreae Nipponicae Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (marker, dioscin)	1.00	-
2	2.87	± 0.08
3	3.19	± 0.07

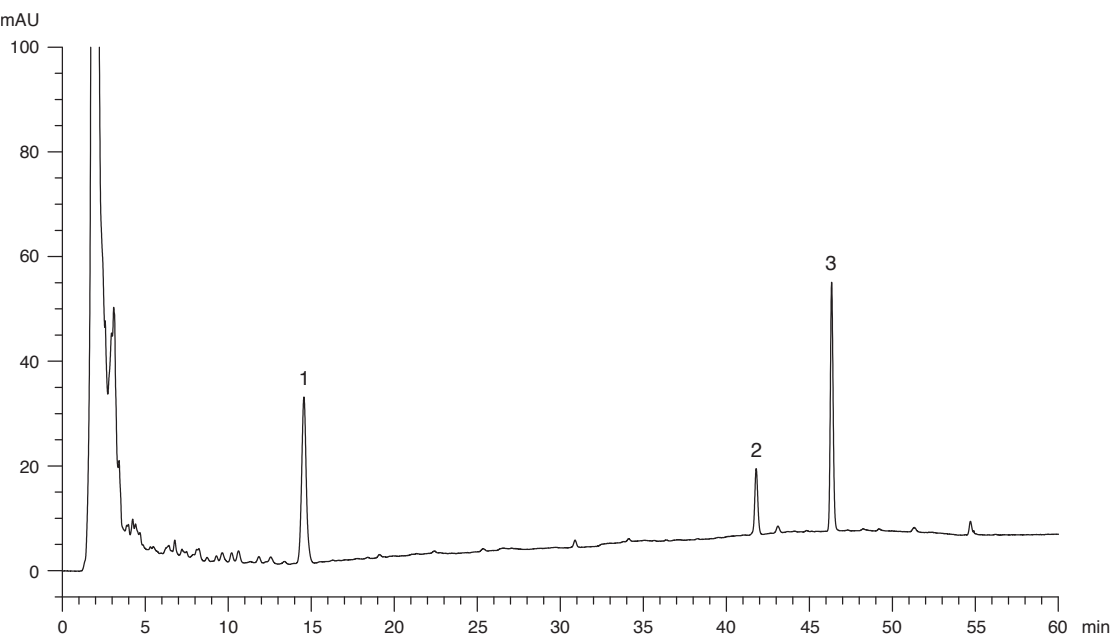


Figure 6 A reference fingerprint chromatogram of Dioscoreae Nipponicae Rhizoma extract

For positive identification, the sample must give the above three 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 XVII*): meet the requirements.

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

5.6 Ash (*Appendix IX*)

Total ash: not more than 4.5%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (*Appendix X*)

Oven dried method: not more than 11.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 32.0%.

Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Dioscin standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 2.0 mg of dioscin CRS and dissolve in 2 mL of ethanol (65%).

Dioscin standard solution for assay, Std-AS

Measure accurately the volume of the dioscin Std-Stock, dilute with ethanol (65%) to produce a series of solutions of 12.5, 62.5, 180, 250, 500 mg/L for dioscin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of ethanol (65%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about $4000 \times g$ for 10 min. Transfer the supernatant to a 10-mL volumetric flask and make up to the mark with ethanol (65%). Filter through a 0.45- μ m 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 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 – 10	50	50	isocratic
10 – 30	50 → 25	50 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of dioscin Std-AS (180 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dioscin should not be more than 5.0%; the RSD of the retention time of dioscin peak should not be more than 2.0%; the column efficiency determined from dioscin peak should not be less than 8000 theoretical plates.

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

Calibration curve

Inject a series of dioscin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dioscin against the corresponding concentrations of dioscin Std-AS. Obtain the slope, y-intercept and the *r*² value from the 5-point calibration curve.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify dioscin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dioscin Std-AS. The retention times of dioscin peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of dioscin in the test solution, and calculate the percentage content of dioscin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.3% of dioscin (C₄₅H₇₂O₁₆), calculated with reference to the dried substance.