

Morindae Officinalis Radix

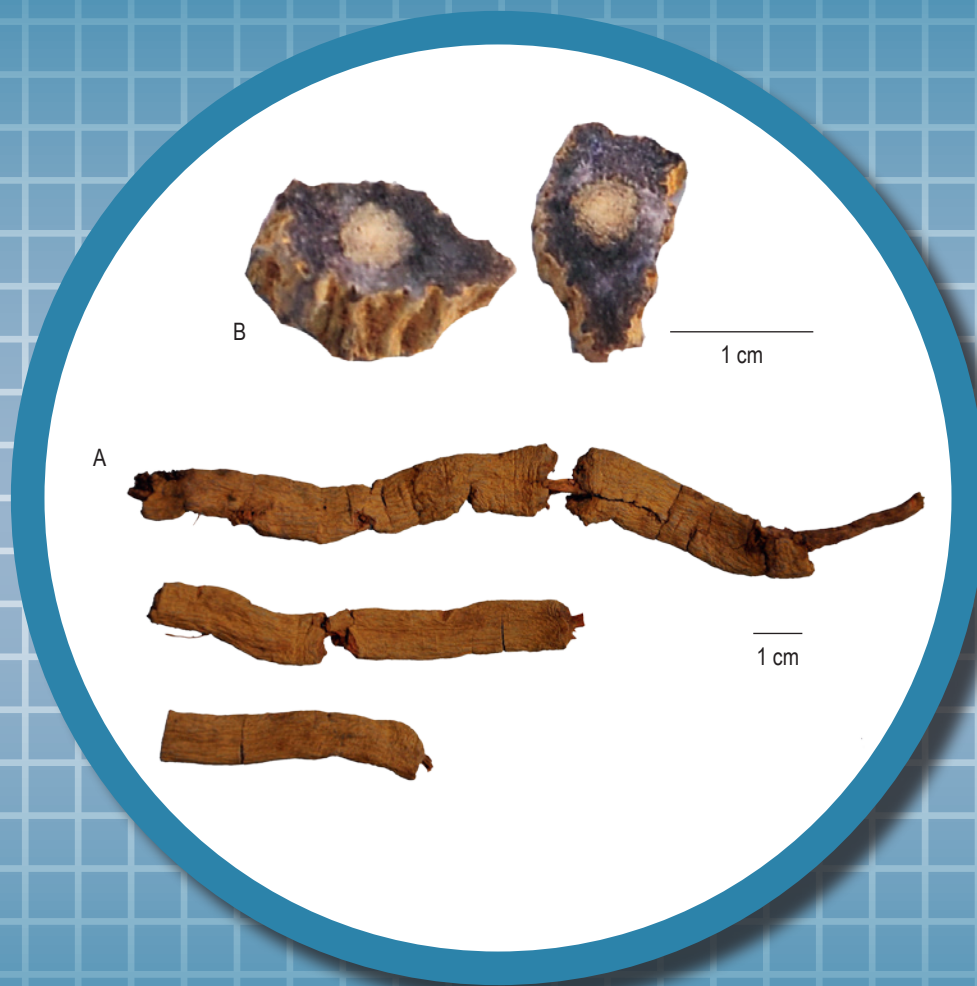


Figure 1 A photograph of Morindae Officinalis Radix

A. Morindae Officinalis Radix B. Transverse section

1. NAMES

Official Name: Morindae Officinalis Radix

Chinese Name: 巴戟天

Chinese Phonetic Name: Bajitian

2. SOURCE

Morindae Officinalis Radix is the dried root of *Morinda officinalis* F. C. How (Rubiaceae). The root is collected throughout the year, the rootlets removed, washed, then semi-dried, followed by gentle beating with a cudgel to compress the root tissues, then dried under the sun to obtain Morindae Officinalis Radix.

3. DESCRIPTION

Compressed-cylindrical, slightly curved, varying in length, 4-20 mm in diameter. Externally greyish-yellow to dark grey, with longitudinal wrinkles and transverse cracks, some bark transversely broken and wood exposed. Texture tough. Fracture with thicken bark, purple to dark brown, easily separated from wood; wood hard, 1-6 mm in diameter, yellowish-brown to yellowish-white. Odour slight; taste sweet and slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork consists of several layers of suberized cells. Cortex narrow; stone cells present singly or in groups in the outer part of cortex, arranged in an interrupted ring; parenchymatous cells contain raphides of calcium oxalate. Phloem relatively broad; parenchymatous cells in the inner part contains raphides of calcium oxalate. Cambium distinct. Xylem vessels scattered singly or 2-3 in groups, arranged radially; xylem rays 1-3 rows of cells wide (Fig. 2).

Powder

Colour greyish-brown to dark brown. Raphides of calcium oxalate scattered in the parenchymatous cells, mostly in bundles, 19-132 μm long; polychromatic under the polarized microscope. Stone cells scattered singly or in groups, subrounded, subsquare, strip-shaped or irregular, 18-81 μm in diameter, wall thick, pit distinct; bright orangish-red and polychromatic under the polarized microscope. Fibres mostly in bundles, 6-72 μm in diameter, long-fusiform, with relatively large bordered pits and pit canals, pit apertures obliquely slit-shaped, V-shaped and cross-shaped; polychromatic under the polarized microscope. Cork cells subsquare or subpolygonal. Vessels mainly bordered-pitted, 13-65 μm in diameter, pits arranged closely (Fig. 3).

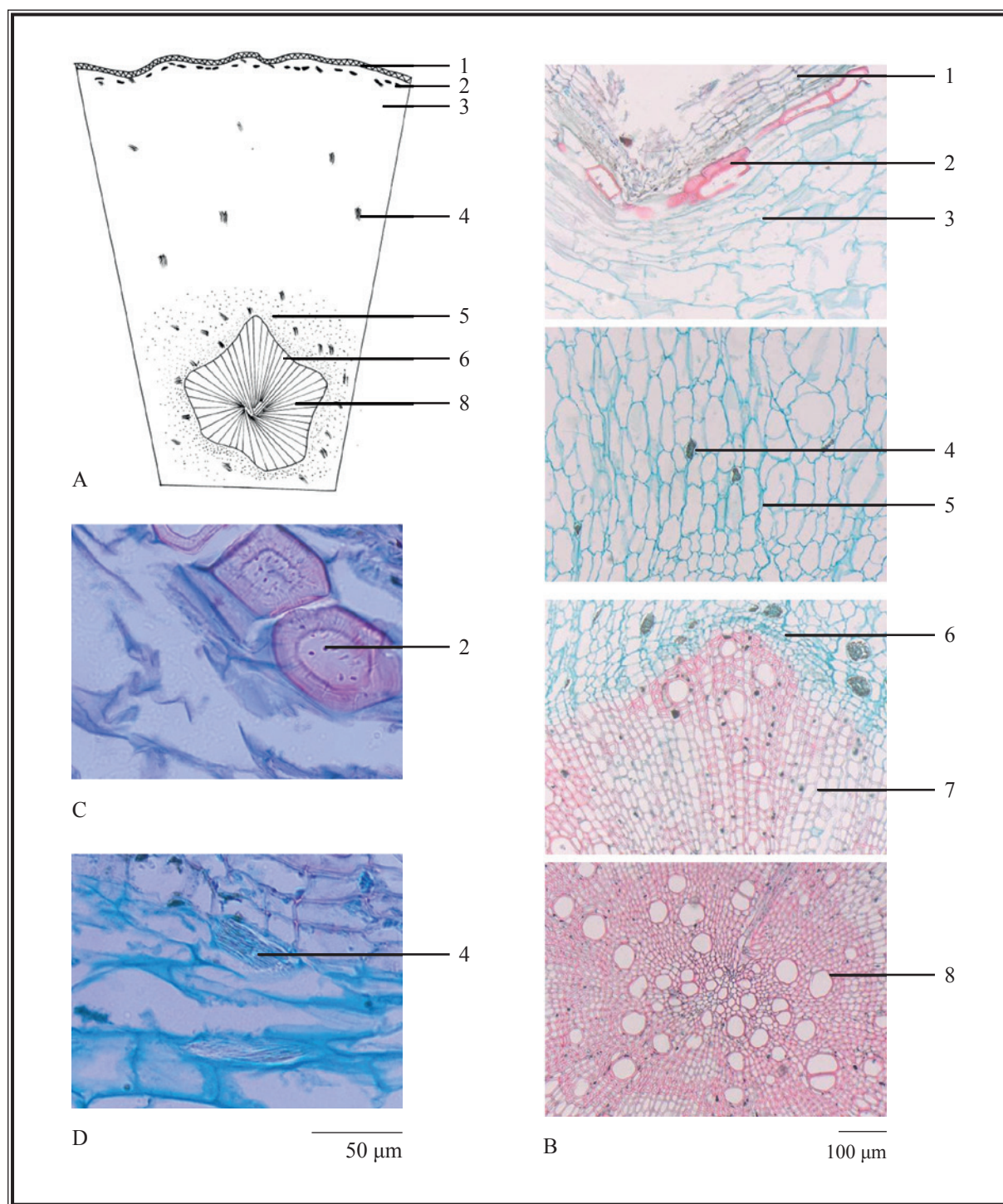


Figure 2 Microscopic features of transverse section of *Morindae Officinalis Radix*

A. Sketch B. Section illustration C. Stone cells

D. Raphides of calcium oxalate

1. Cork 2. Stone cells 3. Cortex 4. Raphides of calcium oxalate 5. Phloem

6. Cambium 7. Xylem ray 8. Xylem

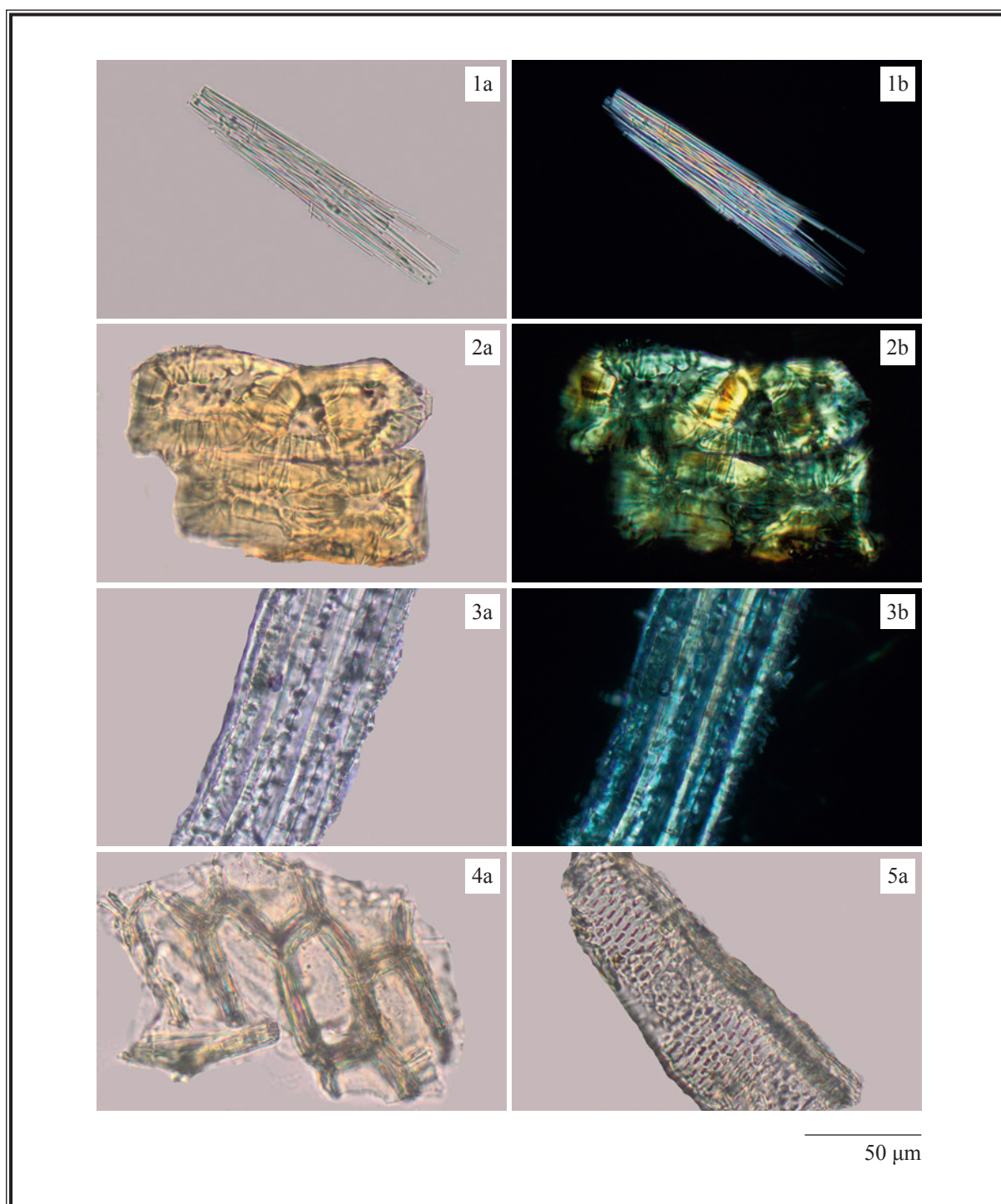


Figure 3 Microscopic features of powder of *Morindae Officinalis Radix*

1. Raphides of calcium oxalate
2. Stone cells
3. Fibres
4. Cork cells
5. Bordered-pitted vessels

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

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

Standard solution

Nystose standard solution

Weigh 0.5 mg of nystose CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, water, formic acid and glacial acetic acid (6:3:2:2, v/v).

Spray reagent

Add slowly 11 mL of sulphuric acid to 89 mL of ethanol. Add 2.75 g of α -naphthol and 7 mL of water. Freshly prepare the reagent.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (200 W) the mixture for 15 min. Filter the mixture.

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 nystose standard solution (15 μ L) and the test solution (1.5 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 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 100°C until the spots or bands become visible (about 3 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 nystose.

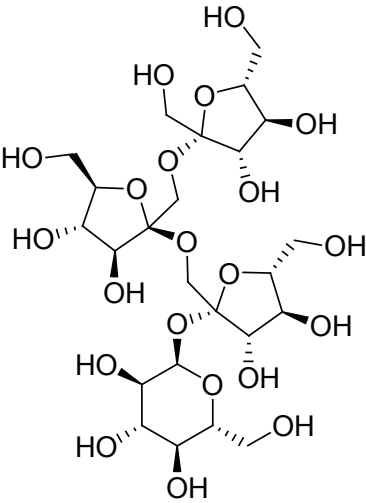


Figure 4 Chemical structure of nystose

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Nystose standard solution for fingerprinting, Std-FP (300 mg/L)
Weigh 0.6 mg of nystose CRS and dissolve in 2 mL of ethanol (60%).

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 (60%). Sonicate (200 W) the mixture for 15 min. Centrifuge at about 4000 × g for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 92°C; nebulizer gas (N₂) flow: 1.2 L/min] and a Hydrophilic Interaction Chromatography (HILIC) column (4.6 × 250 mm), 5 μm particle size. The internal diameter of inlet column tubing is about 0.5 mm. 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 – 18	15 → 28	85 → 72	linear gradient
18 – 40	28 → 40	72 → 60	linear gradient

System suitability requirements

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

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject nystose Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of nystose peak in the chromatogram of nystose Std-FP and the retention times of the twelve characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify nystose peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nystose Std-FP. The retention times of nystose 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 twelve characteristic peaks of *Morindae Officinalis Radix* extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the twelve characteristic peaks of *Morindae Officinalis Radix* extract

Peak No.	RRT	Acceptable Range
1	0.67	± 0.03
2	0.86	± 0.03
3 (marker, nystose)	1.00	-
4	1.13	± 0.03
5	1.26	± 0.03
6	1.38	± 0.03
7	1.49	± 0.03
8	1.59	± 0.03
9	1.67	± 0.03
10	1.75	± 0.03
11	1.83	± 0.03
12	1.89	± 0.03

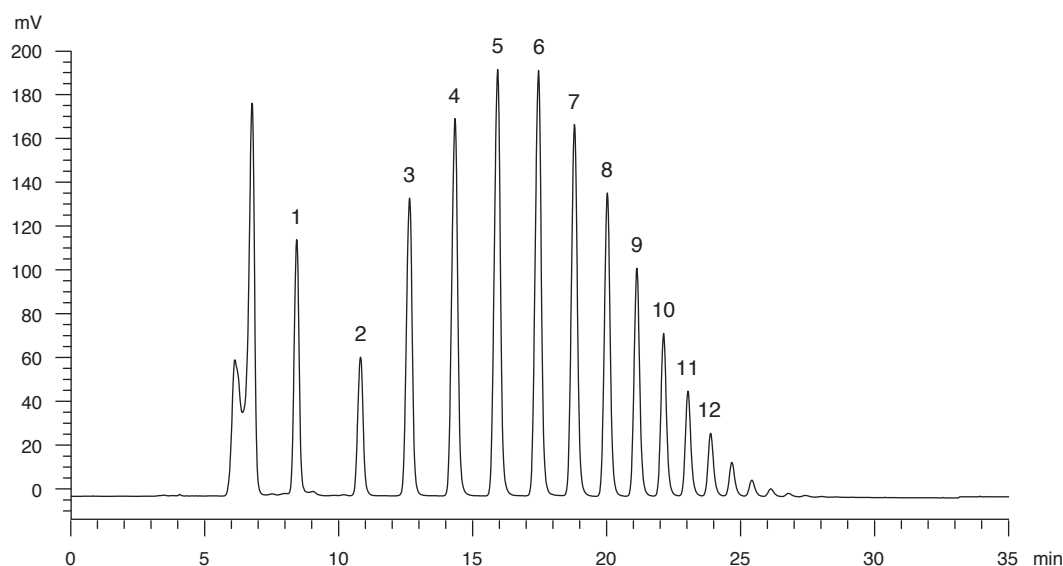


Figure 5 A reference fingerprint chromatogram of *Morindae Officinalis Radix* extract

For positive identification, the sample must give the above twelve characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

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 5.5%.

Acid-insoluble ash: not more than 1.5%.

5.7 Water Content (Appendix X)

Oven dried method: not more than 13.0%.

6. EXTRACTIVES (Appendix XI)

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

Ethanol-soluble extractives (hot extraction method): not less than 60.0%.

7. ASSAY

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

Standard solution

Nystose standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 4.0 mg of nystose CRS and dissolve in 2 mL of ethanol (60%).

Nystose standard solution for assay, Std-AS

Measure accurately the volume of the nystose Std-Stock, dilute with ethanol (60%) to produce a series of solutions of 160, 200, 320, 600, 720 mg/L for nystose.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (60%). Sonicate (200 W) the mixture for 15 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (60%). Combine the solutions and make up to the mark with ethanol (60%). Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 92°C; nebulizer gas (N₂) flow: 1.2 L/min] and a Hydrophilic Interaction Chromatography (HILIC) column (4.6 × 250 mm), 5 µm particle size. The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Lophatheri Herba

淡竹葉

Hyperici Japonici Herba

田基黃

Rhizoma

續斷

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 18	15 → 28	85 → 72	linear gradient
18 – 40	28 → 40	72 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of nystose Std-AS (320 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of nystose should not be more than 5.0%; the RSD of the retention time of nystose peak should not be more than 2.0%; the column efficiency determined from nystose peak should not be less than 10000 theoretical plates.

The *R* value between nystose 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 nystose Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of nystose against the natural logarithm of the corresponding concentrations of nystose 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 nystose peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nystose Std-AS. The retention times of nystose peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of nystose in the test solution by using the following equation –

Concentration of nystose in the test solution = e ^{[Ln (A)-I]/m}

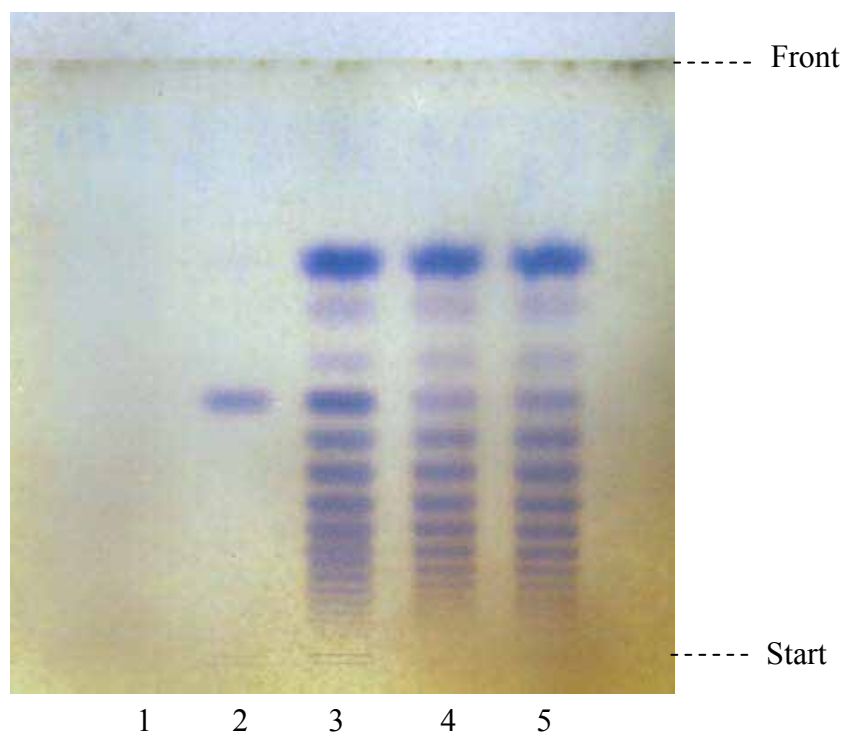
Where A = the peak area of nystose in the test solution,
 I = the y-intercept of the 5-point calibration curve of nystose,
 m = the slope of the 5-point calibration curve of nystose.

Calculate the percentage content of nystose in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 2.3% of nystose (C₂₄H₄₂O₂₁), calculated with reference to the dried substance.

Morindae Officinalis Radix (巴戟天)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Nystose)	Nystose positive
3	Spiked sample (Sample plus nystose)	Nystose positive
4	Sample (Morindae Officinalis Radix)	Nystose positive
5	Sample duplicate (Morindae Officinalis Radix)	Nystose positive

Figure 1 TLC results of Morindae Officinalis Radix extract observed under visible light after staining