Magnoliae Officinalis Flos

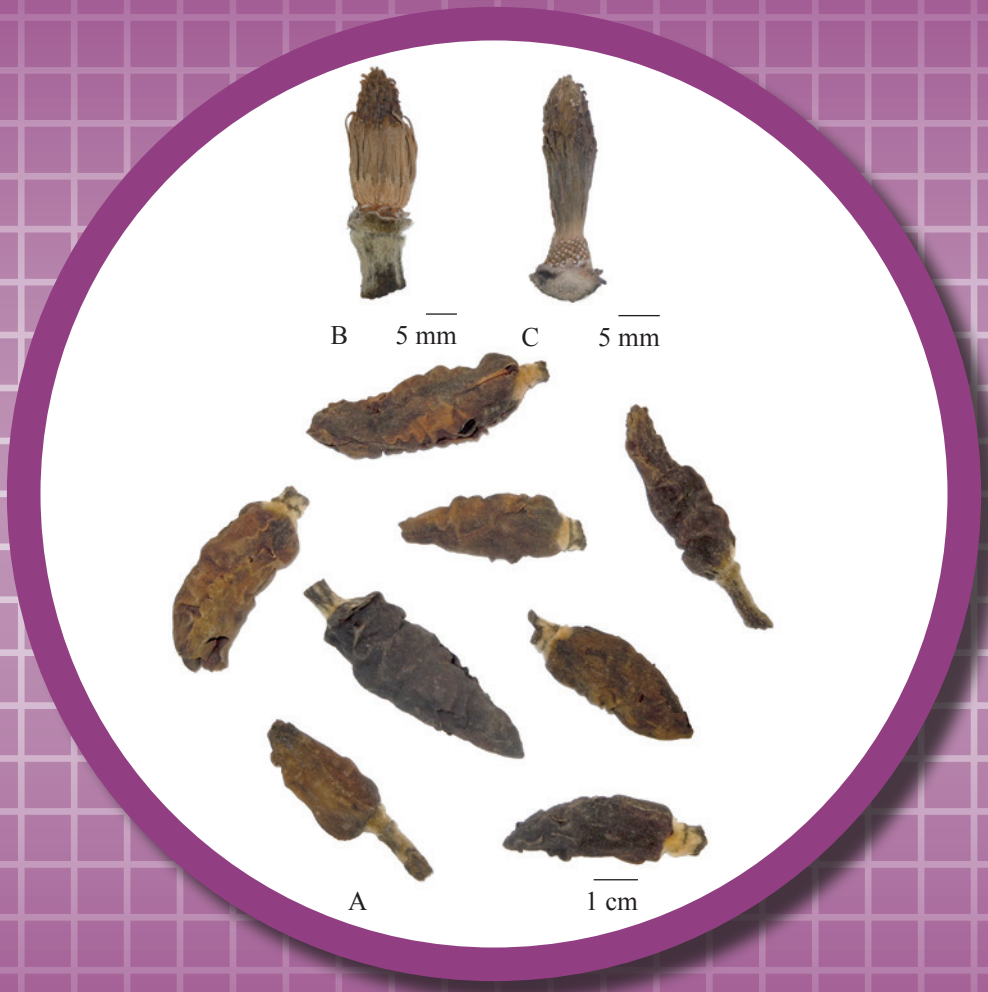

Figure 1 A photograph of Magnoliae Officinalis Flos

A. Magnoliae Officinalis Flos
B. Magnified image of carpels, stamens and pedicel
C. Magnified image of carpels and receptacle

1. NAMES

Official Name: Magnoliae Officinalis Flos

Chinese Name: 厚朴花

Chinese Phonetic Name: Houpohua

2. SOURCE

Magnoliae Officinalis Flos is the dried flower bud of *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae). The flower bud is collected in spring before flowering, steamed briefly, then dried under the sun to obtain Magnoliae Officinalis Flos.

3. DESCRIPTION

Flower buds long-conical or oblong, 3.0-6.8 cm long, 1.1-3.2 cm in diameter. Tepals fleshy, surface scabrous, outermost whorl of tepals relatively large and thin, reddish-brown to blackish-brown; inner whorls relatively small and thick, reddish-brown to dark brown. Stamens numerous, spirally arranged on elongated receptacles, anthers flat linear, filaments short and board; carpels numerous, separated, spirally arranged on elongated receptacles. Pedicels long, densely covered with yellowish-white or greyish-white villi. Texture fragile, easily broken. Odour fragrant; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pedicel: Epidermis consists of 1 layer of cells, covered with cuticle, with non-glandular hairs. Cortex consists of parenchymatous cells, scattered with collateral or amphicribral vascular bundles, varying in size. Inner vascular bundles fusiform or oblong, collateral, arranged in an interrupted ring. Pith consists of parenchymatous cells. Oil cells and groups of stone cells scattered in parenchymatous cells (Fig. 2).

Powder

Colour brown. Oil cells numerous, subrounded to subelliptic, 24-96 μm in diameter, walls slightly thickened, filling with yellowish-orange to brownish-yellow substances. Non-glandular hairs very long, 1- to 5-celled, 10-42 μm in diameter, mostly broken, walls thick, basal cells relatively short; bright yellowish-white or polychromatic under the polarized microscope. Stone cells always in groups, colourless to pale yellow, irregularly branched, walls slightly thickened, lumens large, some with pits; yellowish-white under the polarized microscope. Pollen grains ellipsoid to subglobular, 38-72 μm in diameter, exine thin, nearly smooth or slightly scabrous, one anacolpus barely visible. Epidermal cells of tepals yellowish-brown, polygonal to subpolygonal in surface view, anticlinal walls slightly beaded-thickened or straight; walls of some cells relatively thin, colourless to pale yellow, surface with cuticular striations; stomata occasionally found. Cells of endothecium pale yellow, subpolygonal in surface view, walls beaded-thickened. Crystals of calcium oxalate present in parenchymatous cells, small clusters numerous, prisms few; bright white or polychromatic under the polarized microscope. Vessels mainly spiral, 4-51 μm in diameter (Fig. 3).

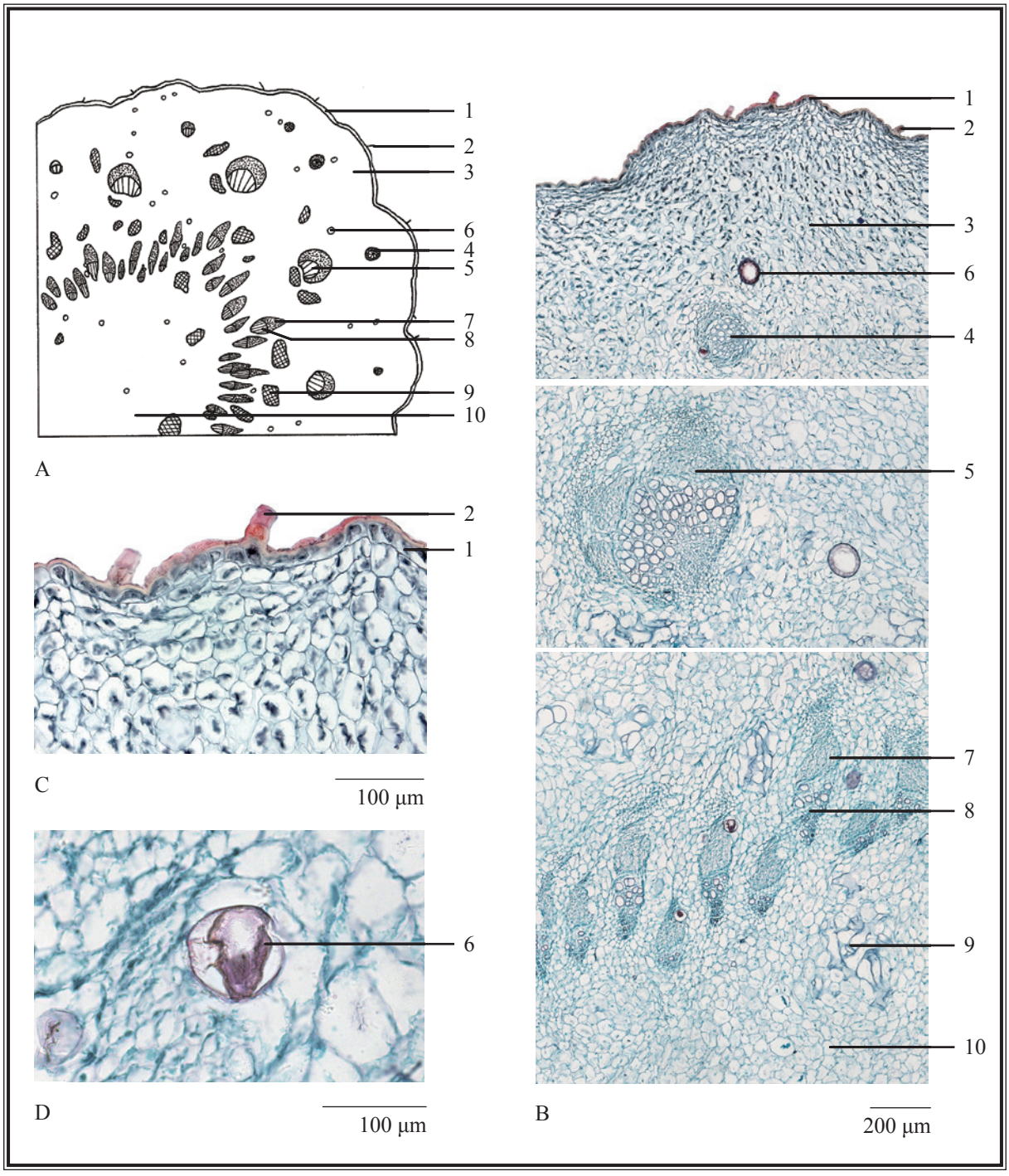

Figure 2 Microscopic features of transverse section of pedicel of Magnoliae Officinalis Flos

A. Sketch B. Section illustration C. Epidermis and non-glandular hairs D. Oil cell

1. Epidermis 2. Non-glandular hair 3. Cortex 4. Amphicribral vascular bundle
5. Collateral vascular bundle 6. Oil cell 7. Phloem 8. Xylem 9. Stone cells 10. Pith

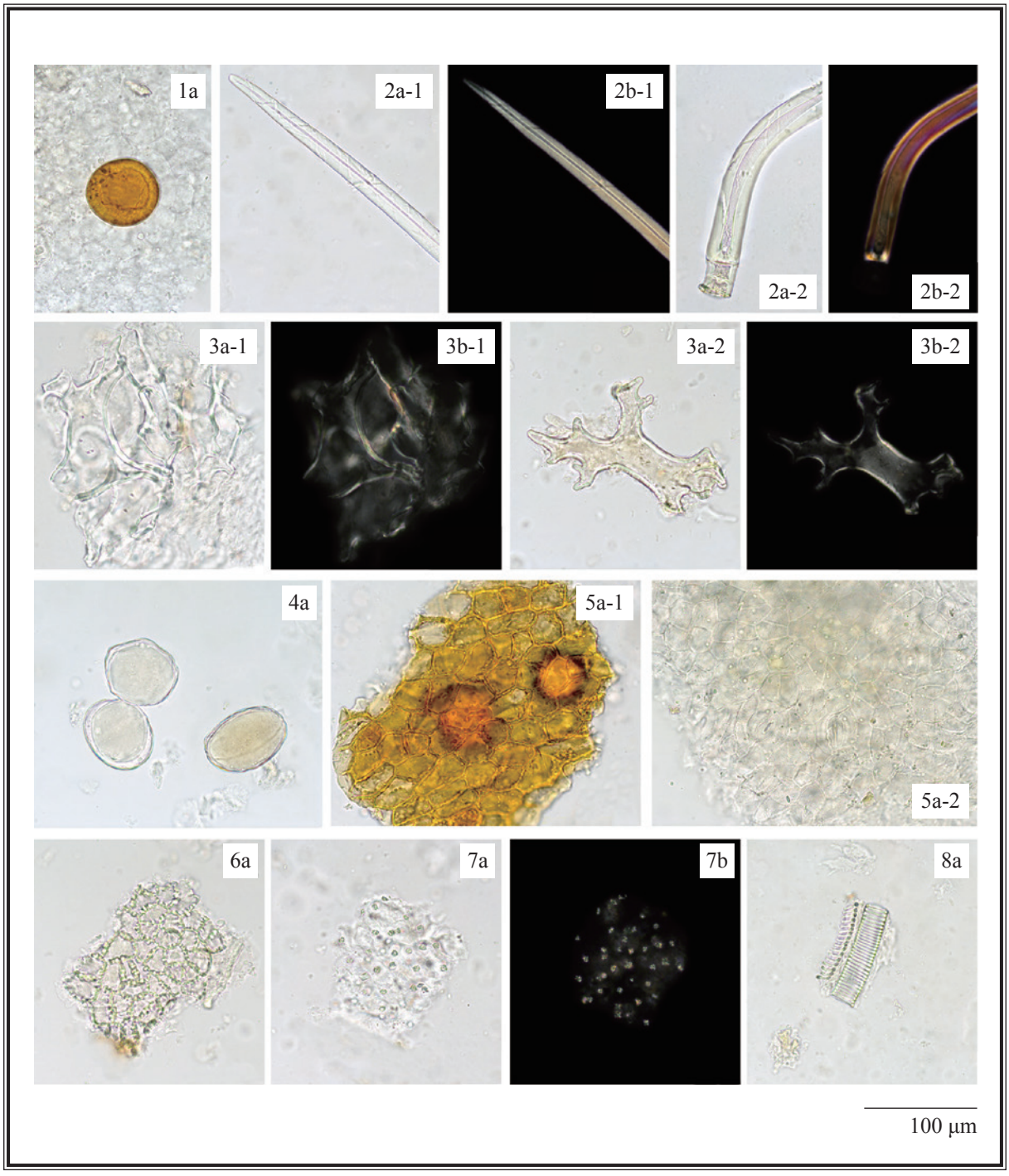

Figure 3 Microscopic features of powder of Magnoliae Officinalis Flos

1. Oil cells 2. Non-glandular hair (2-1 apex, 2-2 base) 3. Stone cells 4. Pollen grains
5. Epidermal cells of tepals 6. Cells of endothecium 7. Crystals of calcium oxalate 8. Vessels

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

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

Standard solutions

Honokiol standard solution

Weigh 1.0 mg of honokiol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Magnolol standard solution

Weigh 1.0 mg of magnolol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (7:3, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 5 g of vanillin. Freshly prepare the reagent.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately honokiol standard solution (2 μL), magnolol standard solution (2 μL) and the test solution (15 μL) 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 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

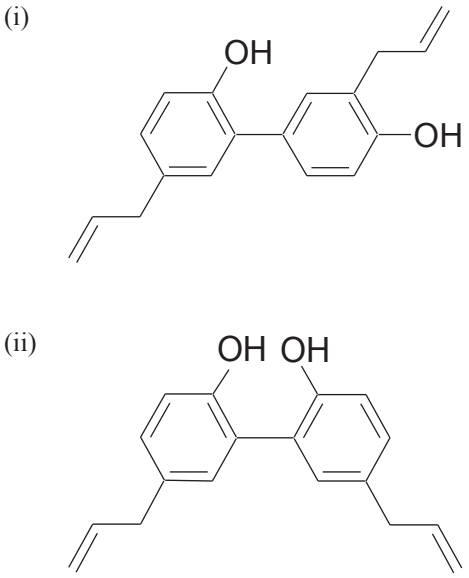

Figure 4 Chemical structures of (i) honokiol and (ii) magnolol

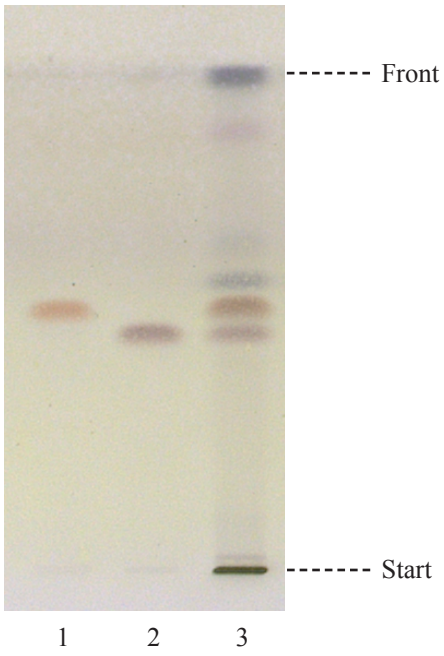

Figure 5 A reference HPTLC chromatogram of Magnoliae Officinalis Flos extract observed under visible light after staining

1. Magnolol standard solution 2. Honokiol 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 honokiol and magnolol (Fig. 5).

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

Standard solutions

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

Weigh 0.2 mg of honokiol CRS and dissolve in 1 mL of methanol (70%).

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

Weigh 0.2 mg of magnolol CRS and dissolve in 1 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 40 mL of methanol (70%). Sonicate (270 W) the mixture for 1 h. Centrifuge at about 2800 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 25-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 (320 nm) 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)	0.4% Formic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 5	50	50	isocratic
5 – 40	50 → 0	50 → 100	linear gradient
40 – 45	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 μL of honokiol Std-FP and magnolol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of honokiol and magnolol should not be more than 5.0%; the RSD of the retention times of honokiol and magnolol peaks should not be more than 2.0%; the column efficiencies determined from honokiol and magnolol peaks should not be less than 50000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject honokiol Std-FP, magnolol Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of honokiol and magnolol peaks in the chromatograms of honokiol Std-FP, magnolol Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify honokiol and magnolol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of honokiol Std-FP and magnolol Std-FP. The retention times of honokiol and magnolol 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 seven characteristic peaks of Magnoliae Officinalis Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Magnoliae Officinalis Flos extract

Peak No.	RRT	Acceptable Range
1	0.40	± 0.03
2 (honokiol)	0.92	± 0.03
3 (marker, magnolol)	1.00	-
4	1.03	± 0.03
5	1.07	± 0.03
6	1.12	± 0.03
7	1.14	± 0.03

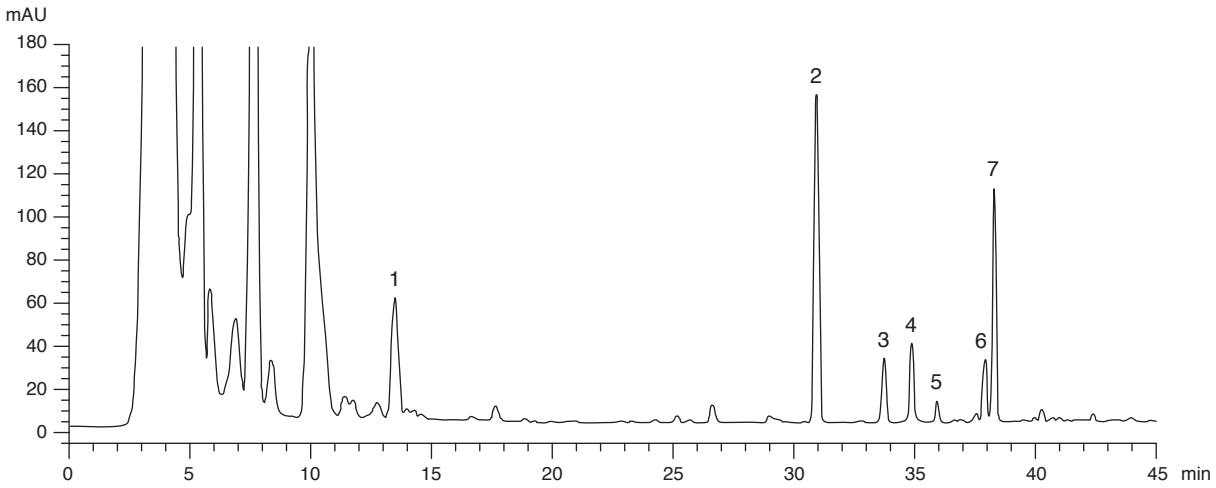

Figure 6 A reference fingerprint chromatogram of Magnoliae Officinalis Flos extract

For positive identification, the sample must give the above seven 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 *(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 8.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 24.0%.
Ethanol-soluble extractives (hot extraction method): not less than 25.0%.

7. ASSAY

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

Standard solution

Mixed honokiol and magnolol standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 1.0 mg of honokiol CRS and 1.0 mg of magnolol CRS, and dissolve in 1 mL of methanol (70%).

Mixed honokiol and magnolol standard solution for assay, Std-AS

Measure accurately the volume of the mixed honokiol and magnolol Std-Stock, dilute with methanol (70%) to produce a series of solutions of 10, 25, 50, 100, 125 mg/L for both honokiol and magnolol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Centrifuge at about 2800 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (294 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. The mobile phase is a mixture of 0.4% formic acid and acetonitrile (35:65, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed honokiol and magnolol Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of honokiol and magnolol should not be more than 5.0%; the RSD of the retention times of honokiol and magnolol peaks should not be more than 2.0%; the column efficiencies determined from honokiol and magnolol peaks should not be less than 8000 theoretical plates.

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

Calibration curves

Inject a series of the mixed honokiol and magnolol Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of honokiol and magnolol against the corresponding concentrations of the mixed honokiol and magnolol Std-AS. Obtain the slopes, y-intercepts and the r^2 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 honokiol and magnolol peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed honokiol and magnolol Std-AS. The retention times of honokiol and magnolol 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 honokiol and magnolol in the test solution, and calculate the percentage contents of honokiol and magnolol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of the total content of honokiol ($C_{18}H_{18}O_2$) and magnolol ($C_{18}H_{18}O_2$), calculated with reference to the dried substance.