

Hedysari Radix



Figure 1 A photograph of Hedysari Radix

- A. Hedysari Radix
- B. Magnified image of root
- C. Magnified image of transverse section of root
- D. Magnified image of fracture of root

1. NAMES

Official Name: Hedysari Radix

Chinese Name: 紅芪

Chinese Phonetic Name: Hongqi

2. SOURCE

Hedysari Radix is the dried root of *Hedysarum polybotrys* Hand.-Mazz. (Fabaceae). The root is collected in spring and autumn, rootlets and top part removed, then dried under the sun to obtain Hedysari Radix.

3. DESCRIPTION

Long cylindrical, straight, rarely branched, 3-24 mm in diameter. Externally dull reddish-brown, with longitudinal wrinkles, scanty rootlet scars and transversely elongated lenticel-like protrusions. Outer bark easily exfoliated, exposing yellowish inner layer. Texture hard and tough, uneasily broken, fracture fibrous and starchy, bark yellowish-white, wood pale yellowish-brown, cambium ring brown, with radial striations. Odour slight; taste slightly sweet, with slight soybean-like flavour when chewed (Fig.1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse Section

Cork consists of several layers of flat cells, sometimes fallen off. Phelloderm narrow, some cells containing prisms of calcium oxalate. Phloem broad, fibre bundles abundant, surrounded by cells containing prisms of calcium oxalate, forming crystal fibres, rays distinct, usually with clefts on the outer side. Cambium in a ring. Xylem consists of several vessels singly scattered or in groups, always accompanied by crystal fibres, rays distinct, sometimes scattered with clefts on the inner side. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour yellowish-brown. Fibres numerous, mainly in bundles, 4-28 μm in diameter, usually surrounded by cells contain prisms of calcium oxalate, forming crystal fibres, walls of crystal cells unevenly thickened; bright polychromatic under the polarized microscope. Prisms of calcium oxalates abundant, scattered or present in crystal cells, double-conical, polygonal, subsquare or rectangular, 2-21 μm in diameter, 4-37 μm long; polychromatic or yellowish-white under the polarized microscope. Starch granules numerous, simple starch granules subrounded or elliptical, 1-23 μm in diameter, hilum dotted, slit-shaped or stellate; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-9 units. Cork cells colourless to yellowish-brown, rectangular, subsquare or polygonal in surface view. Vessels mainly reticulate and bordered-pitted, 3-148 μm in diameter (Fig. 3).

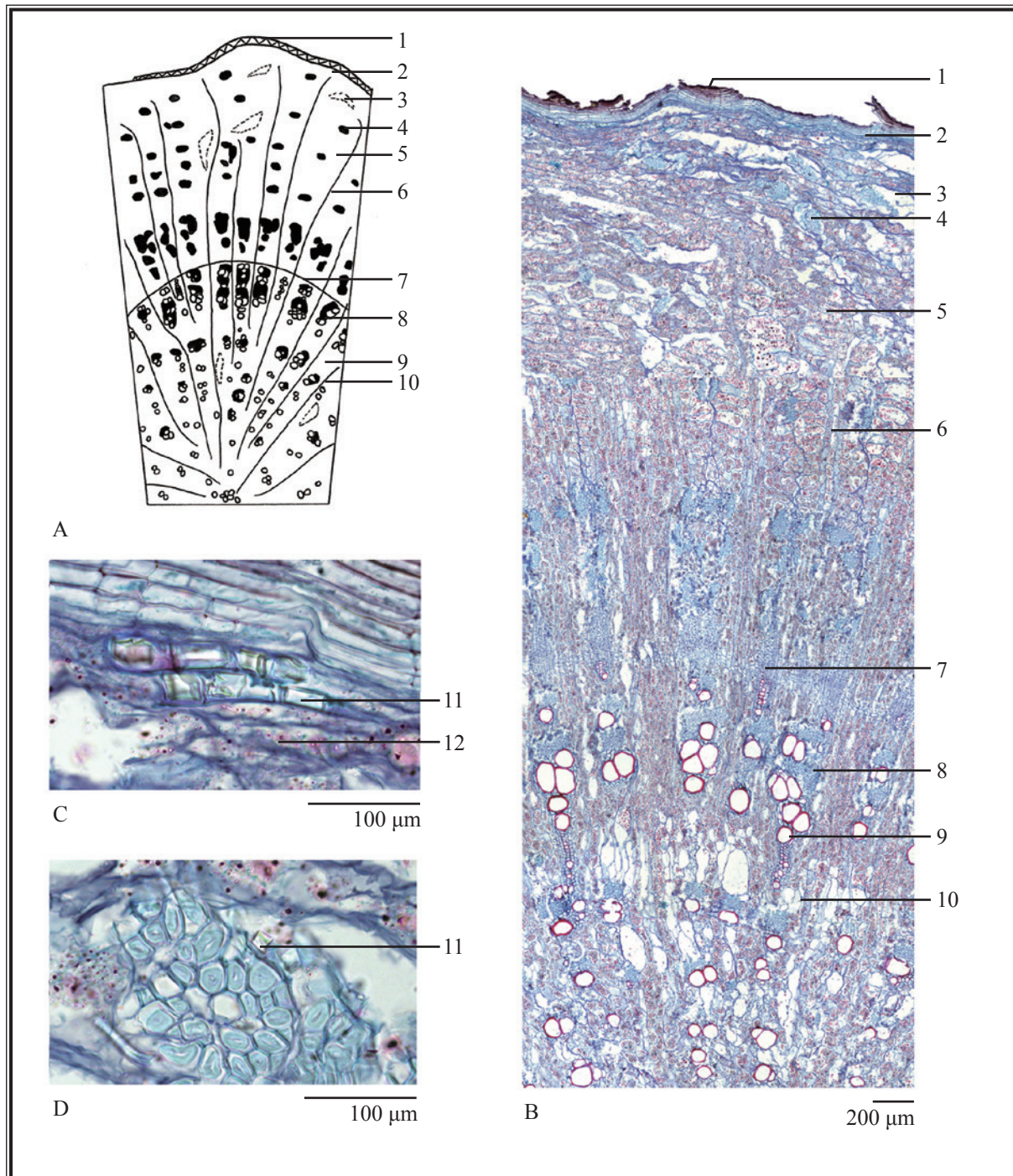


Figure 2 Microscopic features of transverse section of Hedysari Radix

A. Sketch B. Section illustration C. Magnified image of phelloderm
 D. Magnified image of crystal fibre

1. Cork 2. Phelloderm 3. Cleft 4. Phloem fibre 5. Phloem
 6. Phloem ray 7. Cambium 8. Xylem fibre 9. Xylem
 10. Xylem ray 11. Prism of calcium oxalate 12. Starch granule

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Loniceræ Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

杠板歸

北豆根
Menispermī Rhizoma

山銀花

Plumbaginis Zeylanicae Radix

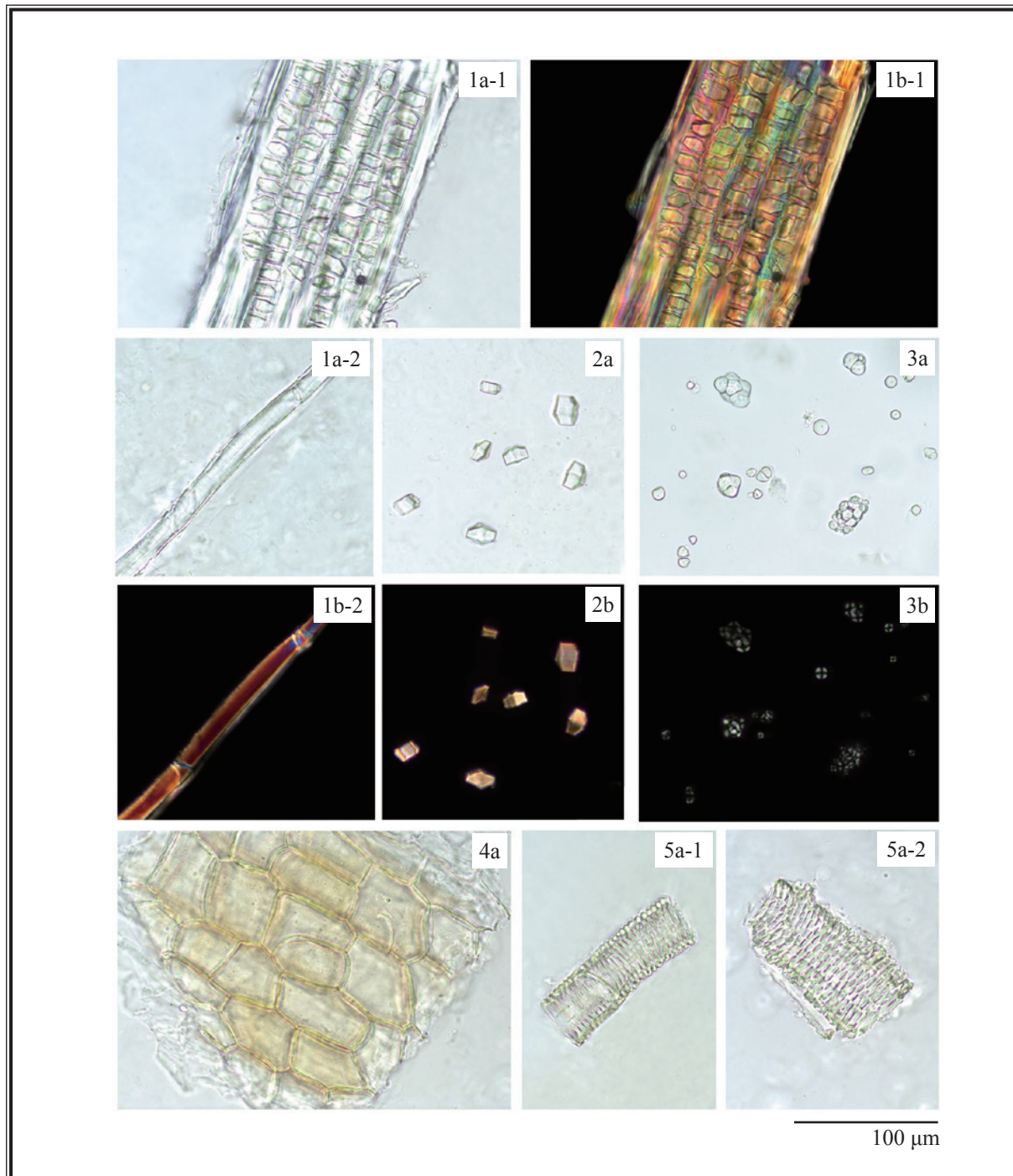
Hedysari Radix

Figure 3 Microscopic features of powder of *Hedysari Radix*

1. Crystal fibres (1-1 crystal fibres, 1-2 singly scattered fibre)
2. Prisms of calcium oxalate
3. Starch granules
4. Cork cells
5. Vessels (5-1 reticulate vessel, 5-2 bordered-pitted vessel)

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

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

Standard solutions

Formononetin standard solution

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

Ononin standard solution

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

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and methanol (4:4:1.3, v/v).

Spray reagent

Add slowly 3 mL of sulphuric acid to 97 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 4 mL of methanol. Filter through a 0.45- μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately formononetin standard solution (0.2 μL), ononin standard solution (0.3 μL) and the test solution (5 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 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 3-5 min). Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

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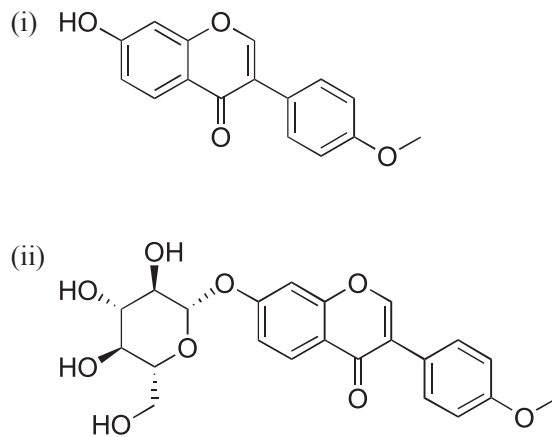
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Figure 4 Chemical structures of (i) formononetin and (ii) ononin

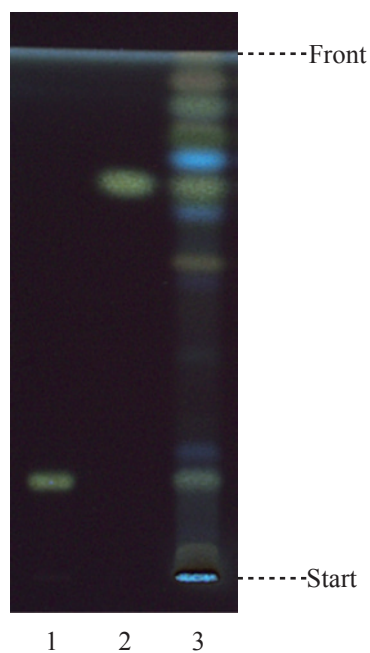


Figure 5 A reference HPTLC chromatogram of *Hedysari Radix* extract observed under UV light (254 nm) after staining

1. Ononin standard solution 2. Formononetin 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 formononetin and ononin (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

Formononetin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of formononetin CRS and dissolve in 10 mL of methanol.

Ononin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of ononin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (270 W) the mixture in a water bath at 60°C for 30 min. Centrifuge at about 3500 × g for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Wash the residue with methanol. Centrifuge at about 3500 × g for 10 min. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (230 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30°C during the separation. 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 – 20	92 → 80	8 → 20	linear gradient
20 – 35	80 → 55	20 → 45	linear gradient
35 – 50	55 → 40	45 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of formononetin Std-FP and ononin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of formononetin and ononin should not be more than 5.0%; the RSD of the retention times of formononetin and ononin peaks should not be more than 2.0%; the column efficiencies determined from formononetin and ononin peaks should not be less than 200000 theoretical plates.

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The *R* value between peak 3 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 formononetin Std-FP, ononin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of formononetin and ononin peaks in the chromatograms of formononetin Std-FP, ononin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify formononetin and ononin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of formononetin Std-FP and ononin Std-FP. The retention times of formononetin and ononin 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 Hedysari Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Hedysari Radix extract

Peak No.	RRT	Acceptable Range
1	0.29	± 0.03
2	0.45	± 0.03
3 (marker, ononin)	1.00	-
4 (formononetin)	1.31	± 0.03
5	1.41	± 0.03

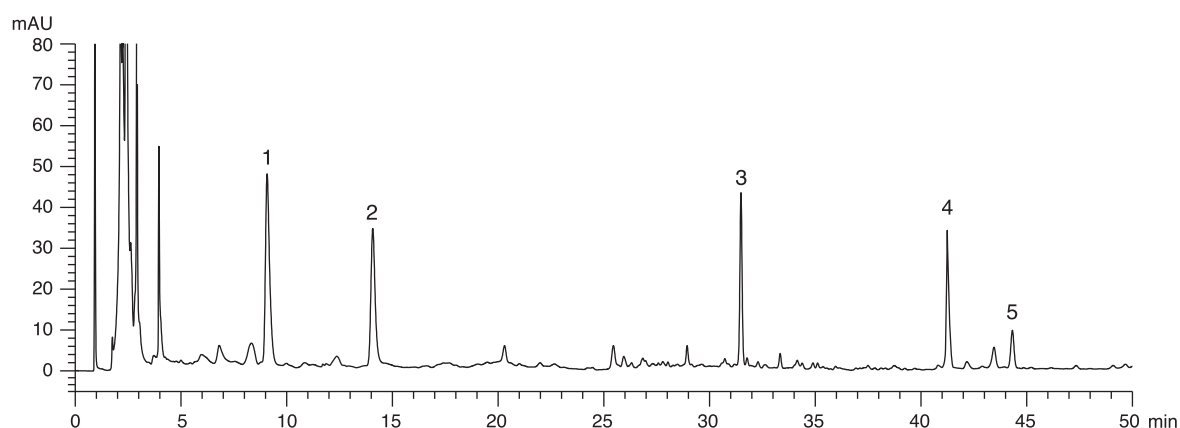


Figure 6 A reference fingerprint chromatogram of Hedysari Radix extract

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 (*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 4.5%.

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 32.0%.

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

7. ASSAY

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

Standard solution

Mixed formononetin and ononin standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of formononetin CRS and 5.0 mg of ononin CRS, and dissolve in 10 mL of methanol.

Mixed formononetin and ononin standard solution for assay, Std-AS

Measure accurately the volume of the mixed formononetin and ononin Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 10, 30, 50 mg/L for both formononetin and ononin.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (270 W) the mixture in a water bath at 60°C for 30 min. Centrifuge at about $3500 \times g$ for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Wash the residue with methanol. Centrifuge at about $3500 \times g$ for 10 min. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. 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 – 20	92 \rightarrow 80	8 \rightarrow 20	linear gradient
20 – 35	80 \rightarrow 55	20 \rightarrow 45	linear gradient
35 – 50	55 \rightarrow 40	45 \rightarrow 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed formononetin and ononin Std-AS (10 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of formononetin and ononin should not be more than 5.0%; the RSD of the retention times of formononetin and ononin peaks should not be more than 2.0%; the column efficiencies determined from formononetin and ononin peaks should not be less than 200000 theoretical plates.

The *R* value between formononetin peak and the closest peak; and the *R* value between ononin 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 formononetin and ononin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of formononetin and ononin against the corresponding concentrations of the mixed formononetin and ononin 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 formononetin and ononin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed formononetin and ononin Std-AS. The retention times of formononetin and ononin 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 formononetin and ononin in the test solution, and calculate the percentage contents of formononetin and ononin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.022% of the total content of formononetin ($C_{16}H_{12}O_4$) and ononin ($C_{22}H_{22}O_9$), calculated with reference to the dried substance.