Hoveniae Semen

Figure 1 A photograph of Hoveniae Semen

A. Hoveniae Semen

B. Magnified image of seeds (1 in lateral view, 2 in surface view)

1. NAMES

Official Name: Hoveniae Semen

Chinese Name: 枳椇子

Chinese Phonetic Name: Zhijuzi

2. SOURCE

Hoveniae Semen is the dried ripe seed of *Hovenia acerba* Lindl. (Rhamnaceae). The ripe fruit with fleshy stalk is collected in autumn, dried under the sun, pericarp removed; the seeds gathered and then dried again under the sun to obtain Hoveniae Semen.

3. DESCRIPTION

Subrounded and slightly flattened, 2.8-5.7 mm long, 2.2-5 mm wide, 0.9-2.6 mm thick. Externally reddish-brown, yellowish-brown or blackish-brown, lustrous, scattered with dented dots; dorsal side slightly protuberant, ventral side flattened, with a longitudinal convex raphe. One dented edge with hilum, apex with a slightly convex chalaza. Texture hard, uneasily broken. Odour slight; taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer epidermis of testa consists of 1 layer of palisade cells, outer part of the layer bearing a light line, covered with cuticle, elongated strip-shaped. Pigment layer consists of several layers of cells, subovate or polygonal, containing reddish-brown contents; underneath the pigment layer is several layers of parenchymatous cells, without pigments. Inner epidermis of testa consists of 1 layer of parenchymatous cells. Perisperm cells decadent, occasionally contain prisms of calcium oxalate. Endosperm consists of cells with relatively thickened walls, containing aleurone grains. Cotyledons consists of cells with thin walls, filled with aleurone grains; fine and minute spheroidal crystals arranged along the margins of the inner side of cotyledons; raphe vascular bundle visible (Fig. 2).

Powder

Colour yellowish-brown to reddish-brown. Palisade cells of testa polygonal in surface view, walls thickened, lumen small; elongated strip-shaped in lateral view, 114-245 μm long, densely arranged, covered with cuticle, with a distinct light line on the outer side; polychromatic under the polarized microscope. Pigment cells subovate or polygonal, containing reddish-brown contents. Spheroidal crystals fine and minute, 2-8 μm in diameter; bright white under the polarized microscope. Cotyledon cells subrectangular or elongated-ovate, walls thin, containing fine and minute spheroidal crystals, and oil droplets. Prisms of calcium oxalate 2-11 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

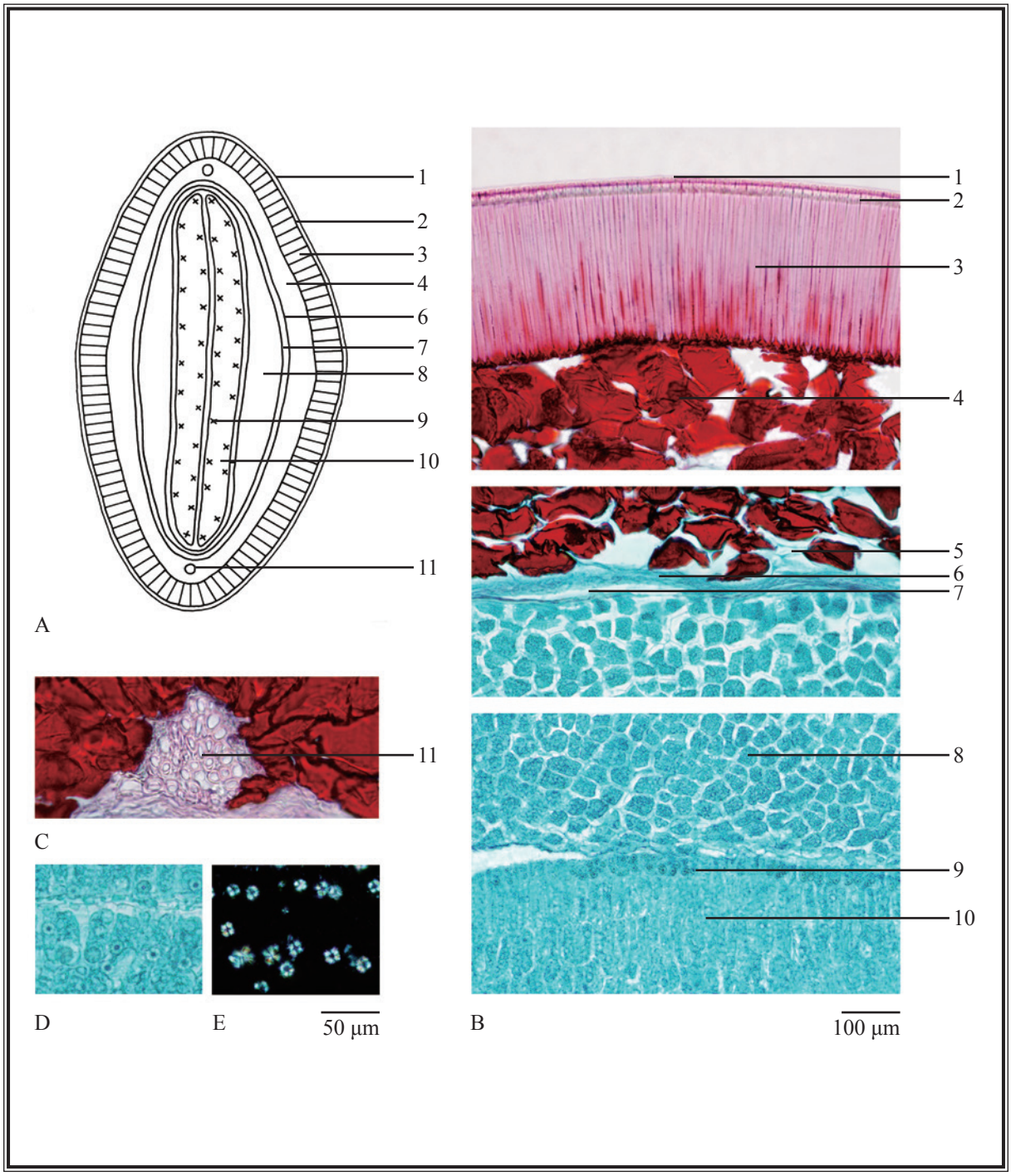

Figure 2 Microscopic features of transverse section of Hoveniae Semen

A. Sketch B. Section illustration C. Raphe vascular bundle
D. Spheroidal crystals (under the light microscope) E. Spheroidal crystals (under the polarized microscope)

1. Cuticle 2. Light line 3. Outer epidermis of testa (palisade tissue) 4. Pigment layer
5. Parenchymatous cells 6. Inner epidermis of testa 7. Perisperm 8. Endosperm
9. Spheroidal crystals 10. Cotyledon 11. Raphe vascular bundle

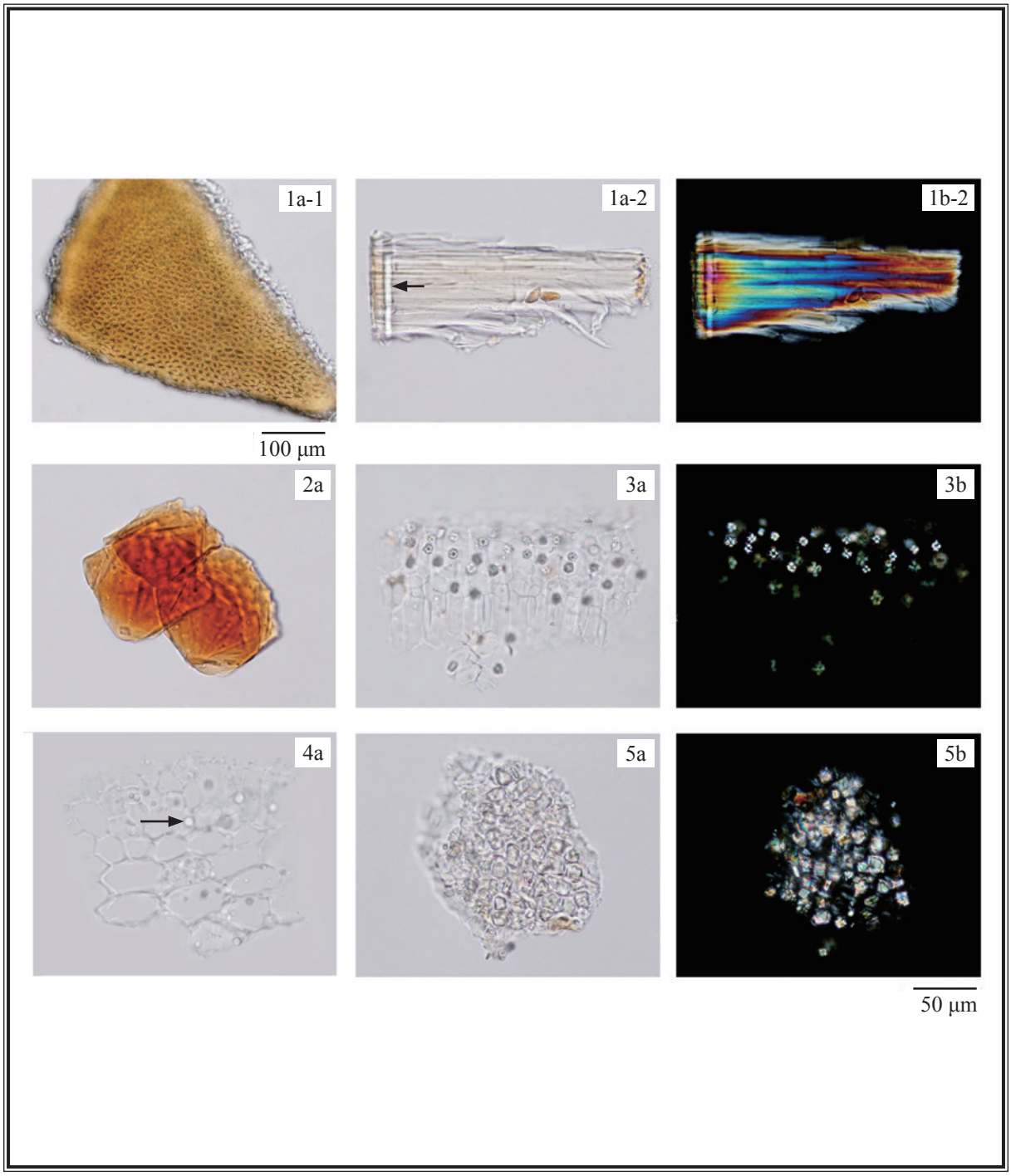

Figure 3 Microscopic features of powder of Hoveniae Semen

1. Palisade cells of testa (1-1 in surface view, 1-2 in lateral view (light line ⟶)
2. Pigment cells 3. Spheroidal crystals 4. Cotyledon cells (oil droplet ⟶)
5. Prisms of calcium oxalate

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

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

Standard solution

Dihydromyricetin standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate and formic acid (10:8:5, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Heat the mixture in a water bath at about 60ºC for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. 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 dihydromyricetin standard solution and the test solution (2 μL each) 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. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

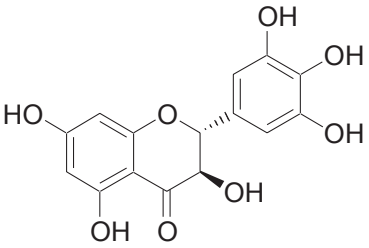

Figure 4 Chemical structure of dihydromyricetin

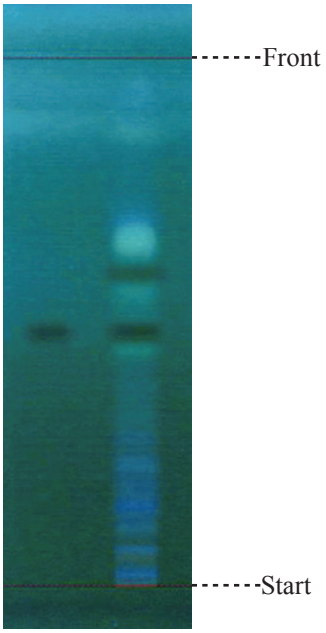

Figure 5 A reference HPTLC chromatogram of Hoveniae Semen extract observed under UV light (366 nm)

1. Dihydromyricetin 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 dihydromyricetin (Fig. 5).

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

Standard solution

Dihydromyricetin standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of dihydromyricetin CRS and dissolve in 10 mL of a mixture of methanol (50%) and formic acid (99.9:0.1, v/v).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	88 → 80	12 → 20	linear gradient
35 – 45	80 → 35	20 → 65	linear gradient
45 – 50	35 → 5	65 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of dihydromyricetin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dihydromyricetin should not be more than 5.0%; the RSD of the retention time of dihydromyricetin peak should not be more than 2.0%; the column efficiency determined from dihydromyricetin peak should not be less than 18000 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 dihydromyricetin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of dihydromyricetin peak in the chromatogram of dihydromyricetin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify dihydromyricetin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dihydromyricetin Std-FP. The retention times of dihydromyricetin 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 Hoveniae Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Hoveniae Semen extract

Peak No.	RRT	Acceptable Range
1 (marker, dihydromyricetin)	1.00	-
2	1.16	± 0.03
3	1.79	± 0.03

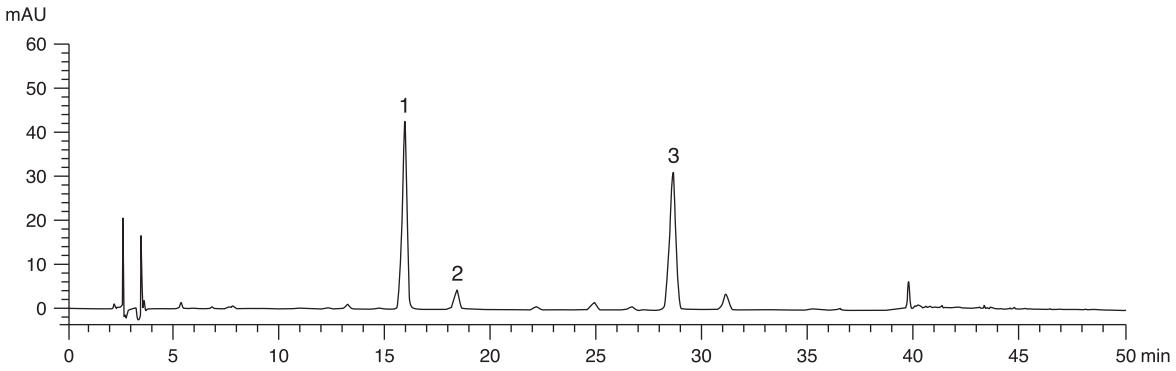

Figure 6 A reference fingerprint chromatogram of Hoveniae Semen extract

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Dihydromyricetin standard stock solution, Std-Stock (400 mg/L)

Weigh accurately 4.0 mg of dihydromyricetin CRS and dissolve in 10 mL of a mixture of methanol (50%) and formic acid (99.9:0.1, v/v).

Dihydromyricetin standard solution for assay, Std-AS

Measure accurately the volume of the dihydromyricetin Std-Stock, dilute with a mixture of methanol (50%) and formic acid (99.9:0.1, v/v) to produce a series of solutions of 7.5, 15, 30, 60, 120 mg/L for dihydromyricetin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	88 → 80	12 → 20	linear gradient
35 – 45	80 → 35	20 → 65	linear gradient
45 – 50	35 → 5	65 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of dihydromyricetin Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dihydromyricetin should not be more than 5.0%; the RSD of the retention time of dihydromyricetin peak should not be more than 2.0%; the column efficiency determined from dihydromyricetin peak should not be less than 18000 theoretical plates.

The *R* value between dihydromyricetin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of dihydromyricetin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dihydromyricetin against the corresponding concentrations of dihydromyricetin 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 dihydromyricetin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dihydromyricetin Std-AS. The retention times of dihydromyricetin 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 dihydromyricetin in the test solution, and calculate the percentage content of dihydromyricetin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.42% of dihydromyricetin ($C_{15}H_{12}O_8$), calculated with reference to the dried substance.

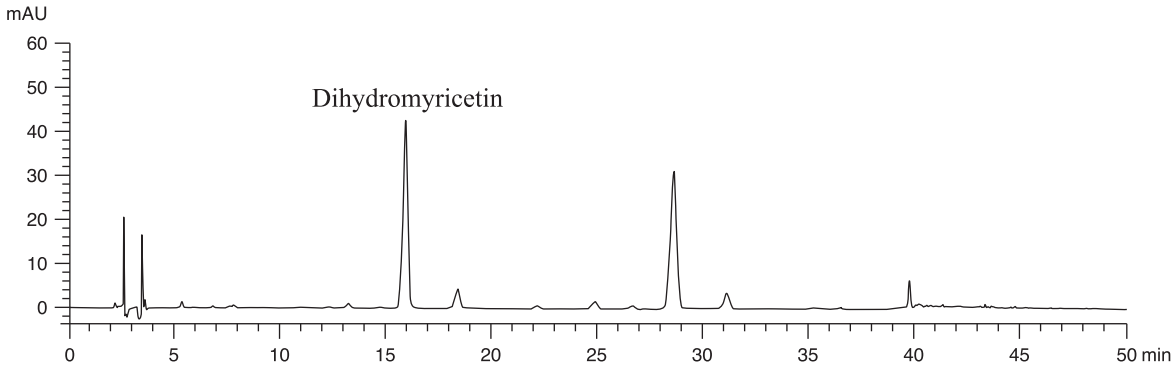

Figure 7 A reference assay chromatogram of Hoveniae Semen extract