Nigellae Semen

Figure 1 A photograph of Nigellae Semen

A. Nigellae Semen B. Magnified image of seeds
C. Magnified image of cut surface of seeds

1. NAMES

Official name: Nigellae Semen

Chinese name: 黑種草子

Chinese phonetic name: Heizhongcaozi

2. SOURCE

Nigellae Semen is the dried ripe seed of *Nigella glandulifera* Freyn et Sint. (Ranunculaceae). The seed is collected in autumn, foreign matter removed, then dried under the sun to obtain Nigellae Semen.

3. DESCRIPTION

Triangular-ovoid, 2.2-3.5 mm long, 1.2-2.2 mm wide. Externally black and rough, apex somewhat narrow and sharp; base relatively obtuse, with irregular protrudings. Texture hard; cut surface greyish-white, oily. Odour slightly aromatic; taste pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer epidermis of testa consists of 1 layer of cells, varying in size, subrectangular or irregularly oblong, frequently elongated tangentially, outer walls mostly with papillate or non-glandular hair-like excrescences, walls slightly thickened, dark brown before stained colour; cuticle somewhat thin, finely and densely granulate striations indistinctly visible. Parenchyma of testa consists of 3-4 layers of cells, rectangular or irregular in shape, somewhat elongated tangentially. Inner epidermis of testa consists of 1 layer of cells, flattened and brown. Perisperm consists of 1 layer of rectangular cells, elongated radially, sometimes dilapidated. Endosperm cells polygonal, filled with aleurone grains. Cotyledons 2, cells polygonal or subrounded, the outer layer of cells slightly elongated radially, filled with aleurone grains. Vascular bundle small, in the middle of outer layers of endosperm (Fig. 2).

Powder

Colour greyish-black. Outer epidermal cells of testa dark brown, polygonal in surface view, varying in size, the outer walls papillate in lateral view. Inner epidermal cells of testa brown, rectangular, subsquare or subpolygonal in surface view, anticlinal walls beaded, periclinal walls finely and densely reticulate-striated. Endosperm cells polygonal, containing oil droplets and aleurone grains. Oil droplets numerous, rounded, colourless, light red after stained with Sudan III. Aleurone grains plenty, colourless, reddish-brown after stained with iodine. Cotyledon cells rectangular or subrectangular. Vessels rare, small, mainly spiral (Fig. 3).

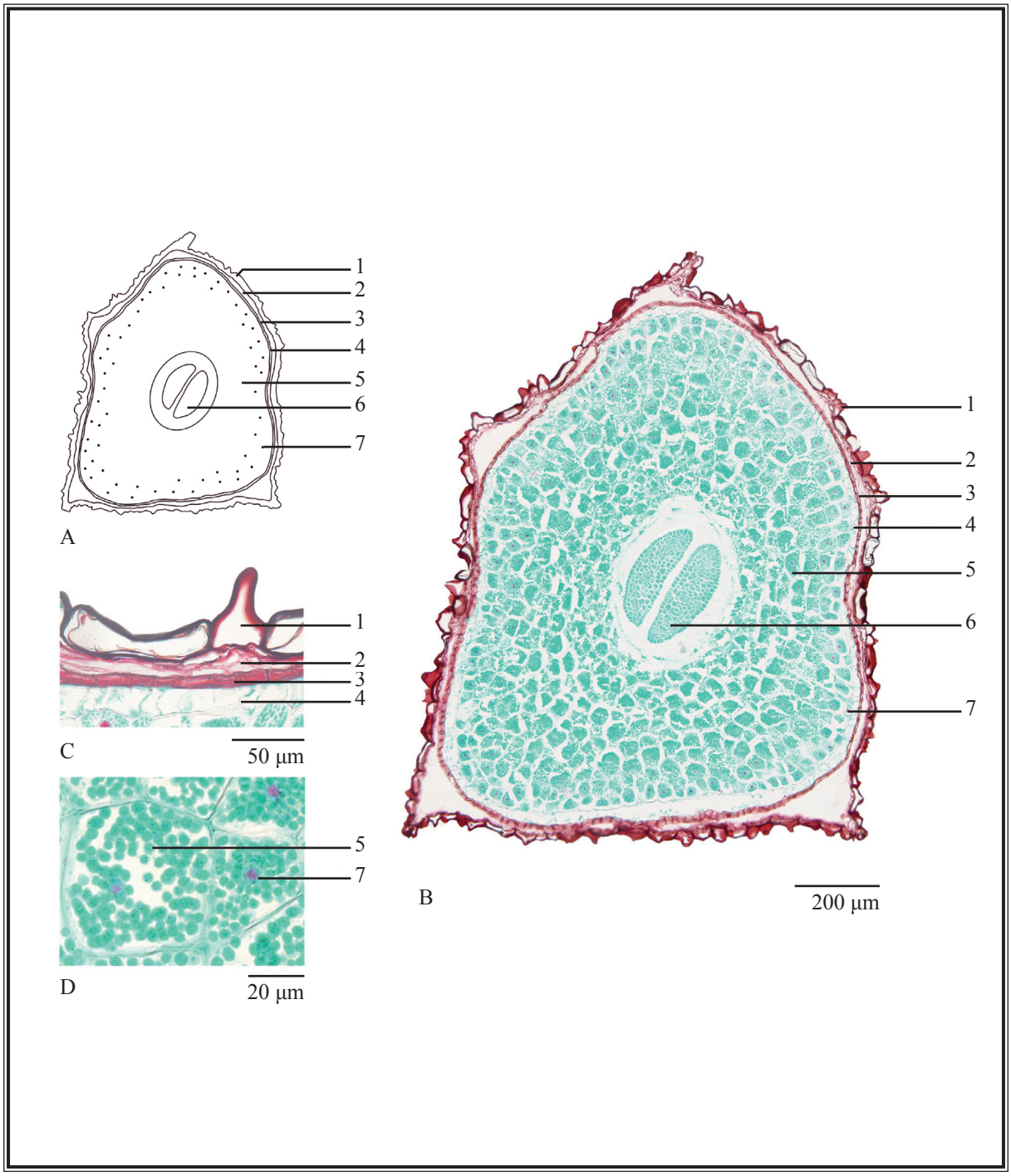

Figure 2 Microscopic features of transverse section of Nigellae Semen

A. Sketch B. Section illustration C-D. Section magnified

1. Outer epidermis of testa 2. Parenchyma of testa 3. Inner epidermis of testa
4. Perisperm 5. Endosperm 6. Cotyledon 7. Vascular bundle

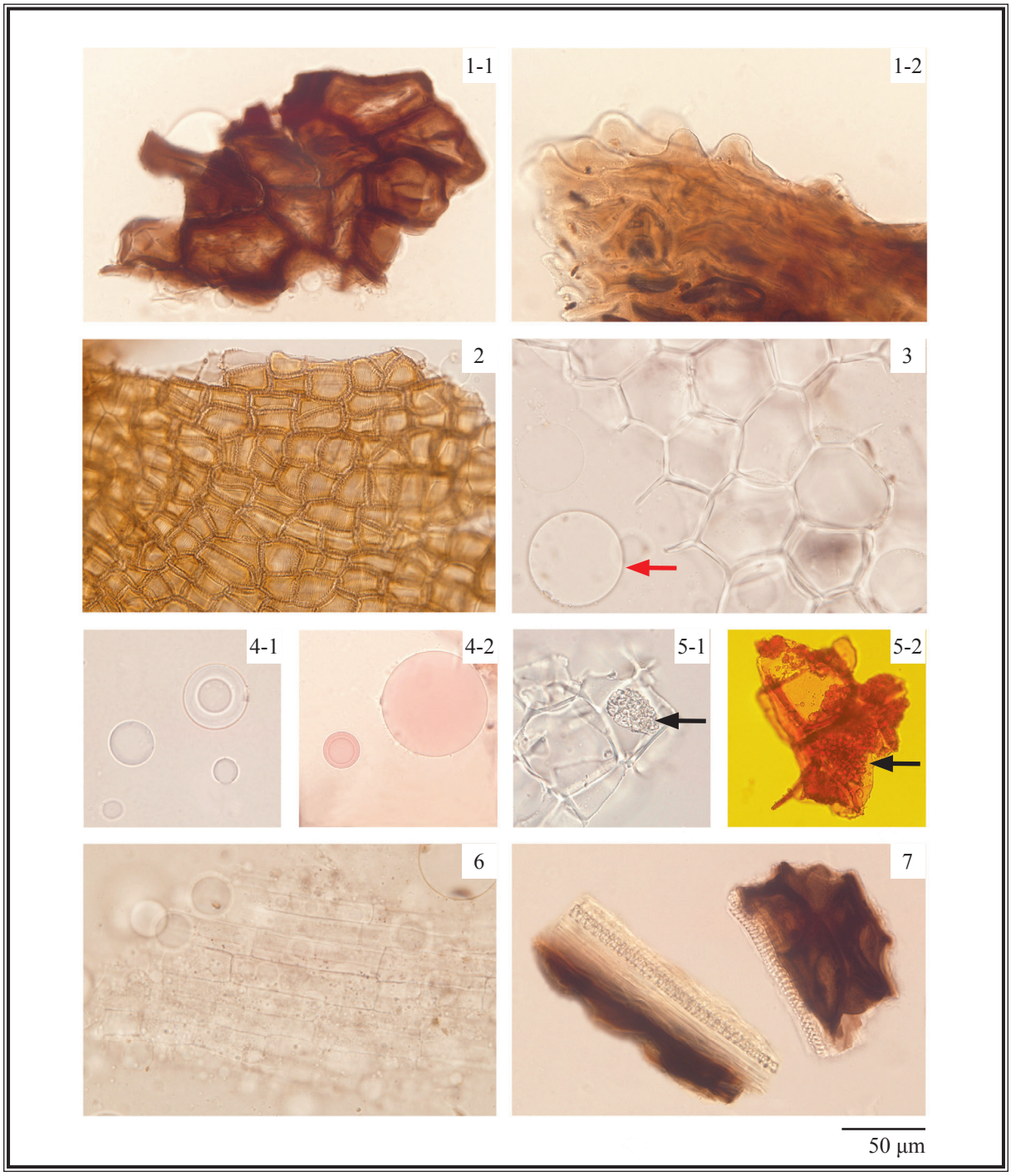

Figure 3 Microscopic features of powder of Nigellae Semen (under the light microscope)

1. Outer epidermal cells of testa (1-1 in surface view, 1-2 in lateral view)
2. Inner epidermal cells of testa 3. Endosperm cells with oil droplets (→)
4. Oil droplets (4-1 without staining, 4-2 stained with Sudan III)
5. Aleurone grains (5-1 without staining, 5-2 stained with iodine)
6. Cotyledon cells 7. Spiral vessels

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

Standard solution

Hederagenin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and formic acid (5:3:0.1, v/v).

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 100-mL round-bottomed flask, then add 25 mL of methanol and 2.5 mL of hydrochloric acid. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Wash the residue with methanol. Combine the extract and make up to the mark with methanol. Filter through a 0.45-μm PTFE 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 hederagenin standard solution (2 μL) and the test solution (10 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 105ºC until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

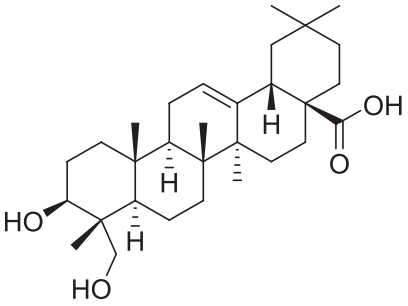

Figure 4 Chemical structure of hederagenin

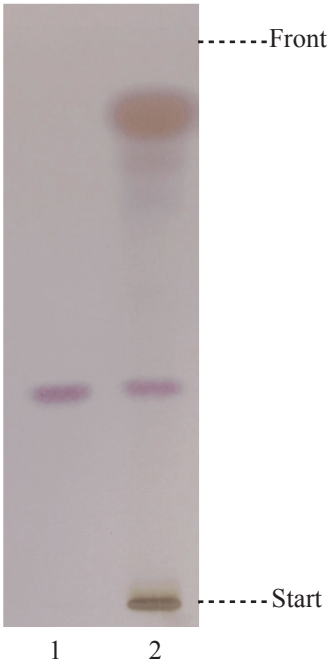

Figure 5 A reference HPTLC chromatogram of Nigellae Semen extract observed under visible light after staining

1. Hederagenin 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 hederagenin (Fig. 5).

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

Standard solution

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

Weigh 0.2 mg of hederagenin CRS and dissolve in 1 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of methanol and 2.5 mL of hydrochloric acid. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Wash the residue with methanol. Combine the extract 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 (210 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	95 → 60	5 → 40	linear gradient
30 – 40	60 → 30	40 → 70	linear gradient
40 – 60	30 → 20	70 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hederagenin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of hederagenin should not be more than 5.0%; the RSD of the retention time of hederagenin peak should not be more than 2.0%; the column efficiency determined from hederagenin peak should not be less than 160000 theoretical plates.

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

Procedure

Separately inject hederagenin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of hederagenin peak in the chromatogram of hederagenin Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify hederagenin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hederagenin Std-FP. The retention times of hederagenin 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 seven characteristic peaks of Nigellae Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Nigellae Semen extract

Peak No.	RRT	Acceptable Range
1	0.35	± 0.03
2	0.37	± 0.03
3	0.38	± 0.03
4	0.58	± 0.03
5	0.74	± 0.03
6 (marker, hederagenin)	1.00	-
7	1.05	± 0.03

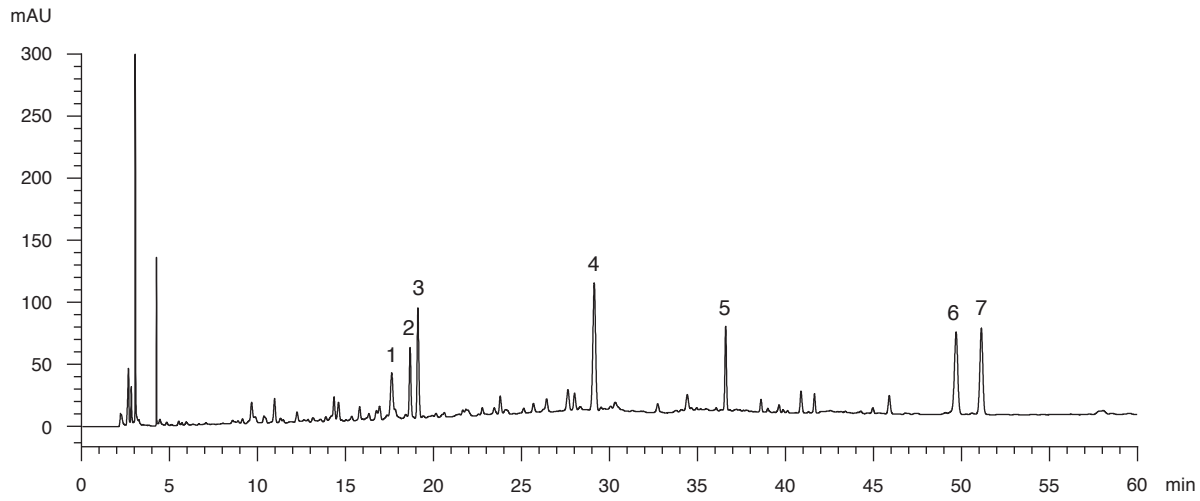

Figure 6 A reference fingerprint chromatogram of Nigellae Semen extract

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 7.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 13.0%.
Ethanol-soluble extractives (cold extraction method): not less than 27.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of hederagenin CRS, and dissolve in 5 mL of methanol.

Hederagenin standard solution for assay, Std-AS

Measure accurately the volume of the hederagenin Std-Stock, dilute with methanol to produce a series of solutions of 50, 100, 200, 250, 300 mg/L for hederagenin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of methanol and 2.5 mL of hydrochloric acid. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Wash the residue with methanol. Combine the extract 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 (210 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	95 → 60	5 → 40	linear gradient
30 – 40	60 → 30	40 → 70	linear gradient
40 – 60	30 → 20	70 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hederagenin Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of hederagenin should not be more than 5.0%; the RSD of the retention time of hederagenin peak should not be more than 2.0%; the column efficiency determined from hederagenin peak should not be less than 160000 theoretical plates.

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

Limits

The sample contains not less than 0.75% of hederagenin ($C_{30}H_{48}O_4$), calculated with reference to the dried substance.

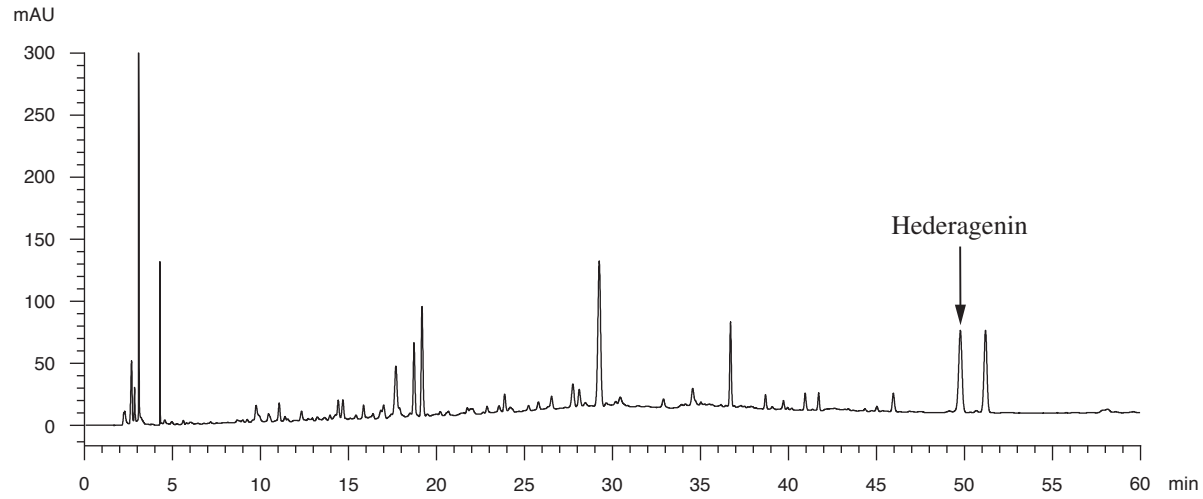

Figure 7 A reference assay chromatogram of Nigellae Semen extract