Kochiae Fructus

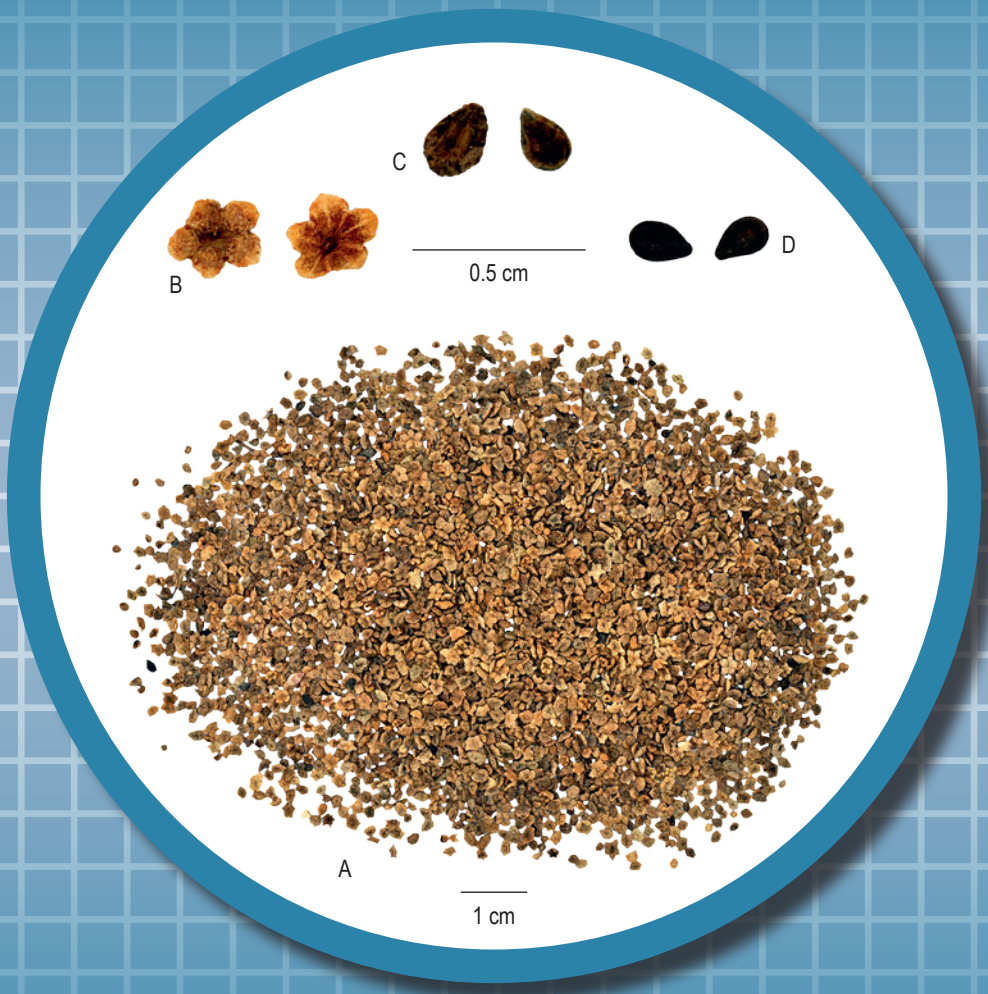

Figure 1 A photograph of Kochiae Fructus

A. Kochiae Fructus B. Magnified fruits with persistent perianth
C. Magnified fruits without persistent perianth D. Magnified seeds

1. NAMES

Official Name: Kochiae Fructus

Chinese Name: 地膚子

Chinese Phonetic Name: Difuzi

2. SOURCE

Kochiae Fructus is the dried ripe fruit of *Kochia scoparia* (L.) Schrad. (Chenopodiaceae). The ripe fruit is collected in autumn, dried under the sun, then foreign matter removed to obtain Kochiae Fructus.

3. DESCRIPTION

Flattened, spherical, and five-pointed star in shape, 2-4 mm in diameter, covered with persistent perianth. Externally pale brown to greyish-brown, with 5 membranous winglets; the centre of dorsal surface has a slightly prominent, dotted fruit stalk scar and 5-11 radial veins. After taking off the perianth, a membranous and translucent pericarp visible. Seeds flattened and ovoid, 1.5-2.5 mm long, brownish-black to black. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Persistent perianth consists of 1 layer of parenchymatous cells, stone cells present occasionally. Pericarp consists of 1 layer of U-shaped sclerenchymatous cells, containing numerous small prisms of calcium oxalate. Testa consists of 1 layer of cells, yellowish-brown. Perisperm consists of polygonal parenchymatous cells, filled with minute starch granules. Radicle relatively small, consisting of parenchymatous cells, filled with aleurone grains. Cotyledons relatively large, the cells filled with aleurone grains and oil droplets. Epicotyl located at the centre of the cotyledons (Fig. 2).

Powder

Colour yellowish-brown to brown. Epidermal cells of persistent perianth polygonal, wall slightly thickened. Stomata of persistent perianth anomocytic, 19-35 μm long, 15-30 μm wide. Pollen grains from persistent perianth yellowish-green, spherical, 15-40 μm in diameter. Stone cells occasionally found, short fibre-like, wall thick and slightly lignified. Testa cells brown, rectangular to irregular. Perisperm cells consist of polygonal parenchymatous cells, filled with minute starch granules. Non-glandular hairs consist of 2-3 cells, 4-18 μm in diameter. Clusters of calcium oxalate present in persistent perianth, 8-53 μm in diameter; polychromatic under the polarized microscope. Pericarp cells rectangular to subsquare, wall thickened and sinuous, containing numerous prisms of calcium oxalate, 2-10 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

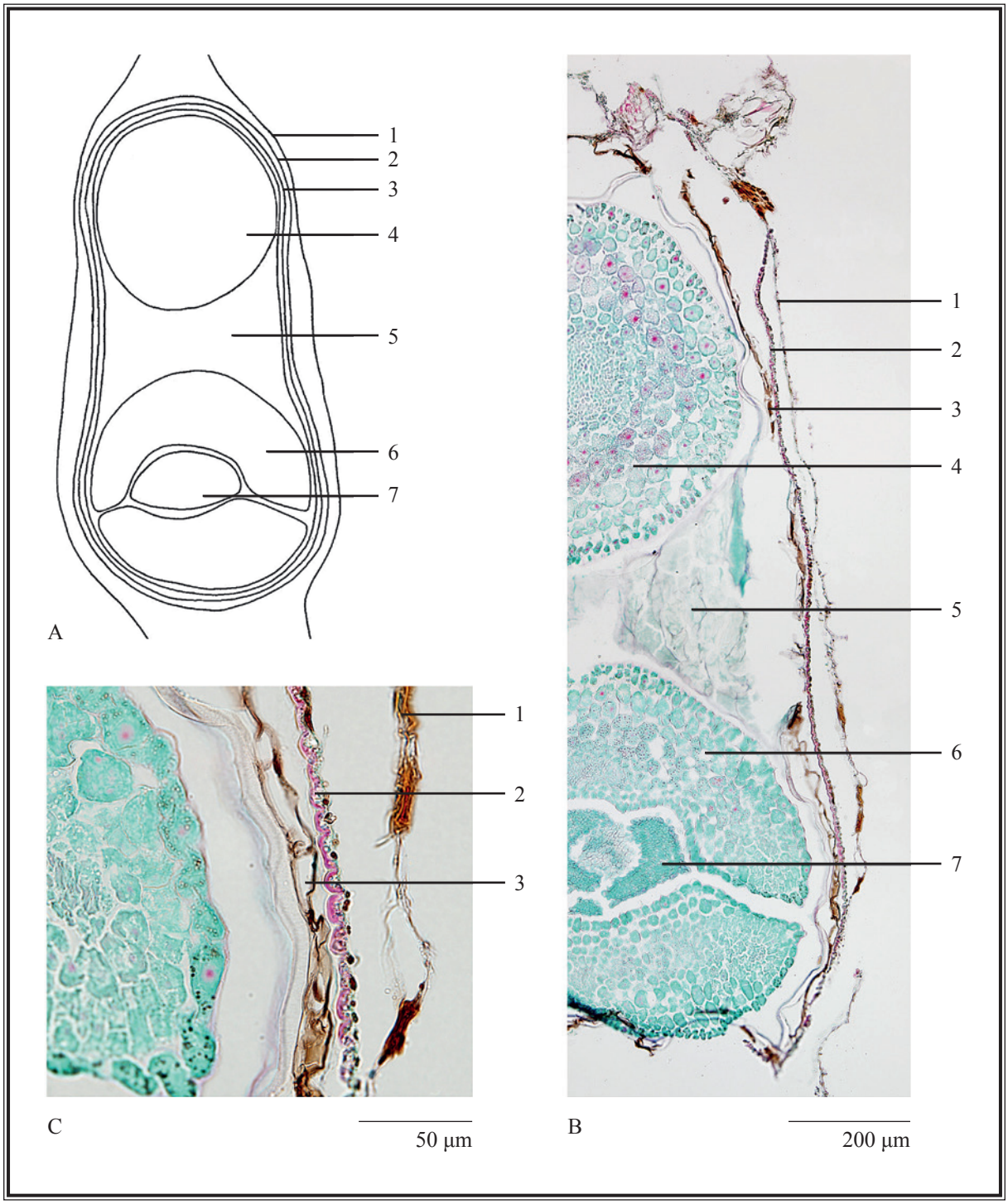

Figure 2 Microscopic features of transverse section of Kochiae Fructus

A. Sketch B. Section illustration C. Section magnified

1. Persistent perianth 2. Pericarp 3. Testa 4. Radicle
5. Perisperm 6. Cotyledon 7. Epicotyl

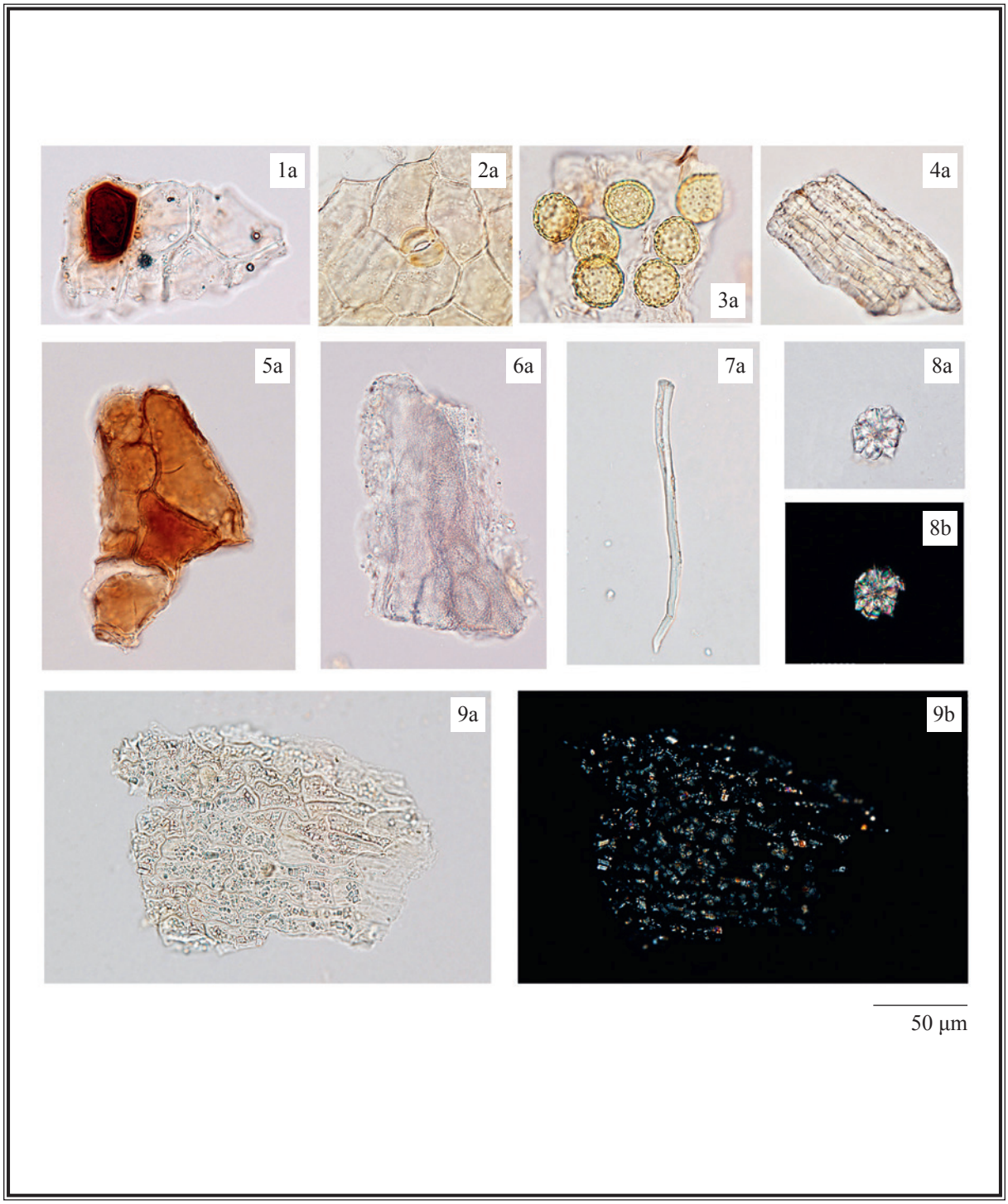

Figure 3 Microscopic features of powder of Kochiae Fructus

1. Epidermal cells of persistent perianth 2. Stoma from persistent perianth
3. Pollen grains from persistent perianth 4. Stone cells 5. Testa cells 6. Perisperm cells
7. Non-glandular hair 8. Cluster of calcium oxalate 9. Pericarp cells

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

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

Standard solution

Momordin Ic standard solution

Weigh 1.0 mg of momordin Ic CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of water, butan-1-ol and glacial acetic acid (12:7:1, v/v). Shake well and use the upper layer.

Spray reagent

Mix cautiously 25 mL of sulphuric acid (20%, v/v) into 25 mL of ice-cold glacial acetic acid. Add 2.5 mL of *p*-anisaldehyde. Add further 50 mL of sulphuric acid (20%, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately momordin Ic standard solution (5 µL) and the test solution (3 µL) to the plate. 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 until the spots or bands become visible (about 8 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 momordin Ic.

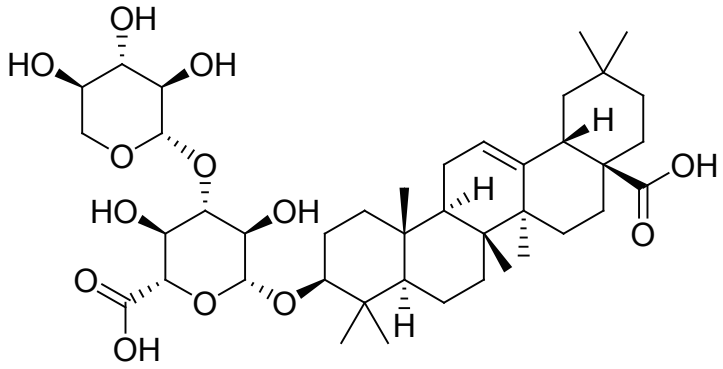

Figure 4 Chemical structure of momordin Ic

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

Standard solution

Momordin Ic standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 1.0 mg of momordin Ic CRS and dissolve in 2 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with ethanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 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 methanol, water and glacial acetic acid (85:15:0.2, v/v). The elution time is about 60 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of momordin Ic Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of momordin Ic should not be more than 5.0%; the RSD of the retention time of momordin Ic peak should not be more than 2.0%; the column efficiency determined from momordin Ic peak should not be less than 3000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject momordin Ic Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of momordin Ic peak in the chromatogram of momordin Ic Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify momordin Ic peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of momordin Ic Std-FP. The retention times of momordin Ic 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 four characteristic peaks of Kochiae Fructus extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Kochiae Fructus extract

Peak No.	RRT	Acceptable Range
1	0.85	± 0.03
2 (marker, momordin Ic)	1.00	-
3	2.42	± 0.03
4	3.44	± 0.03

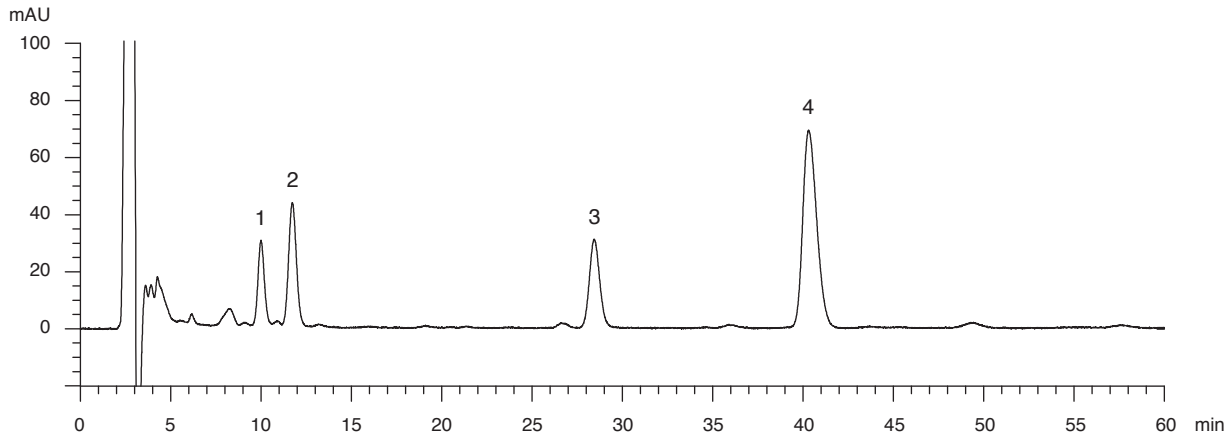

Figure 5 A reference fingerprint chromatogram of Kochiae Fructus extract

For positive identification, the sample must give the above four 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 *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 3.0%.

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 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 2.0 mg of momordin Ic CRS and dissolve in 2 mL of methanol.

Momordin Ic standard solution for assay, Std-AS

Measure accurately the volume of the momordin Ic Std-Stock, dilute with methanol to produce a series of solutions of 50, 100, 300, 500, 1000 mg/L for momordin Ic.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (220 W) the mixture for 30 min. Allow to stand for 15 min. Filter the supernatant and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with 5 mL of methanol. Combine the solution. Repeat the extraction for three more times. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-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 (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. The mobile phase is a mixture of methanol, water and glacial acetic acid (80:20:0.2, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 µL of momordin Ic Std-AS (300 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of momordin Ic should not be more than 5.0%; the RSD of the retention time of momordin Ic peak should not be more than 2.0%; the column efficiency determined from momordin Ic peak should not be less than 5000 theoretical plates.

The *R* value between momordin Ic 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 momordin Ic Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of momordin Ic against the corresponding concentrations of momordin Ic Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify momordin Ic peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of momordin Ic Std-AS. The retention times of momordin Ic 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 momordin Ic in the test solution, and calculate the percentage content of momordin Ic in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 2.3% of momordin Ic ($C_{41}H_{64}O_{13}$), calculated with reference to the dried substance.