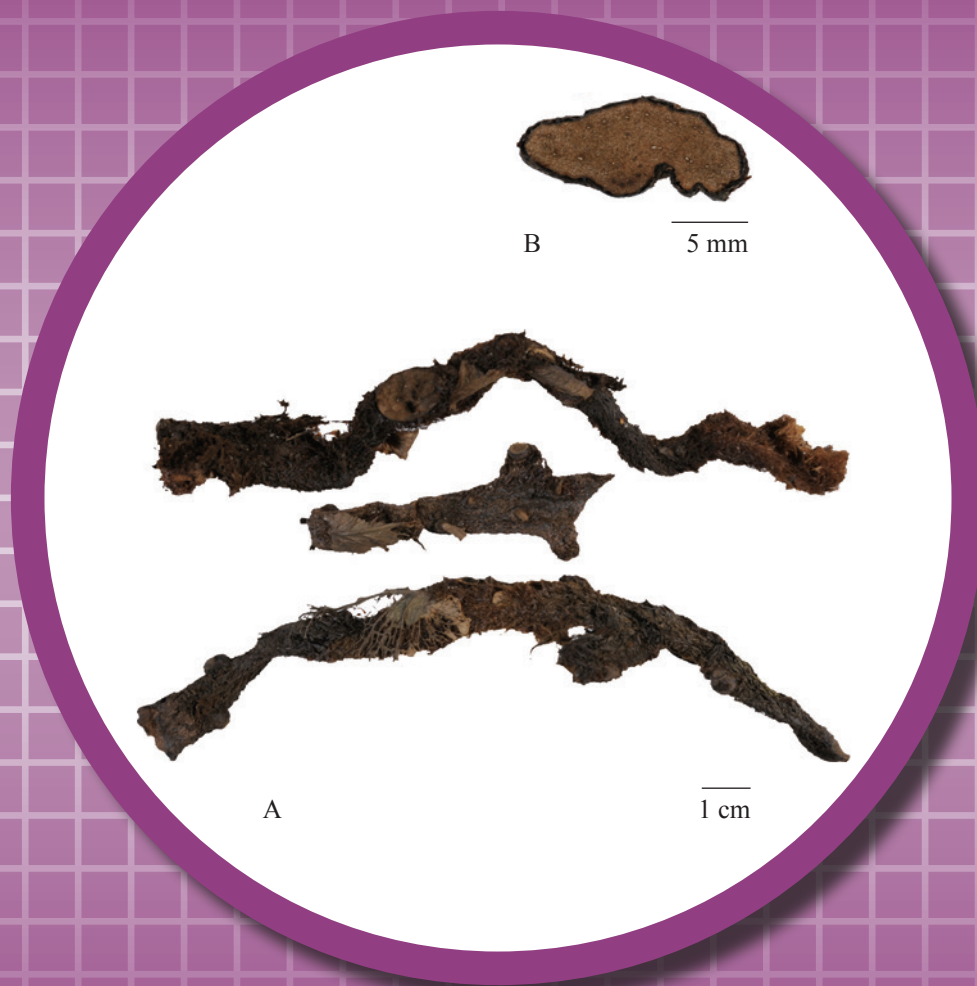


# Drynariae Rhizoma



**Figure 1** A photograph of Drynariae Rhizoma

A. Drynariae Rhizoma

B. Magnified image of transverse section of rhizome

Drynariae Rhizoma

1. NAMES

Official Name: Drynariae Rhizoma

Chinese Name: 骨碎補

Chinese Phonetic Name: Gusuibu

2. SOURCE

Drynariae Rhizoma is the dried rhizome of *Drynaria fortunei* (Kunze) J. Sm. (Polypodiaceae). The rhizome is collected all year round, soils removed, then dried under the sun or scorched off the hairs (ramenta) to obtain Drynariae Rhizoma.

3. DESCRIPTION

Flattened long slat-shaped or lacerated strip, mostly curved, branched, 2-19.5 cm long, 0.5-2.5 cm wide, 2-8 mm thick. Externally densely covered with deep brown to dark brown hairy ramenta, and changing to brown or dark brown when burnt; the upper surface and both sides marked by protuberant or dented circular frond scars, rarely by frond-bases and remnants of fibrous roots. Texture fragile and light in weight, easily broken. Fracture reddish-brown, vascular bundles yellow dotted and arranged in a ring. Odour slight; taste bland and slightly astringent (Fig. 1).

4. IDENTIFICATION

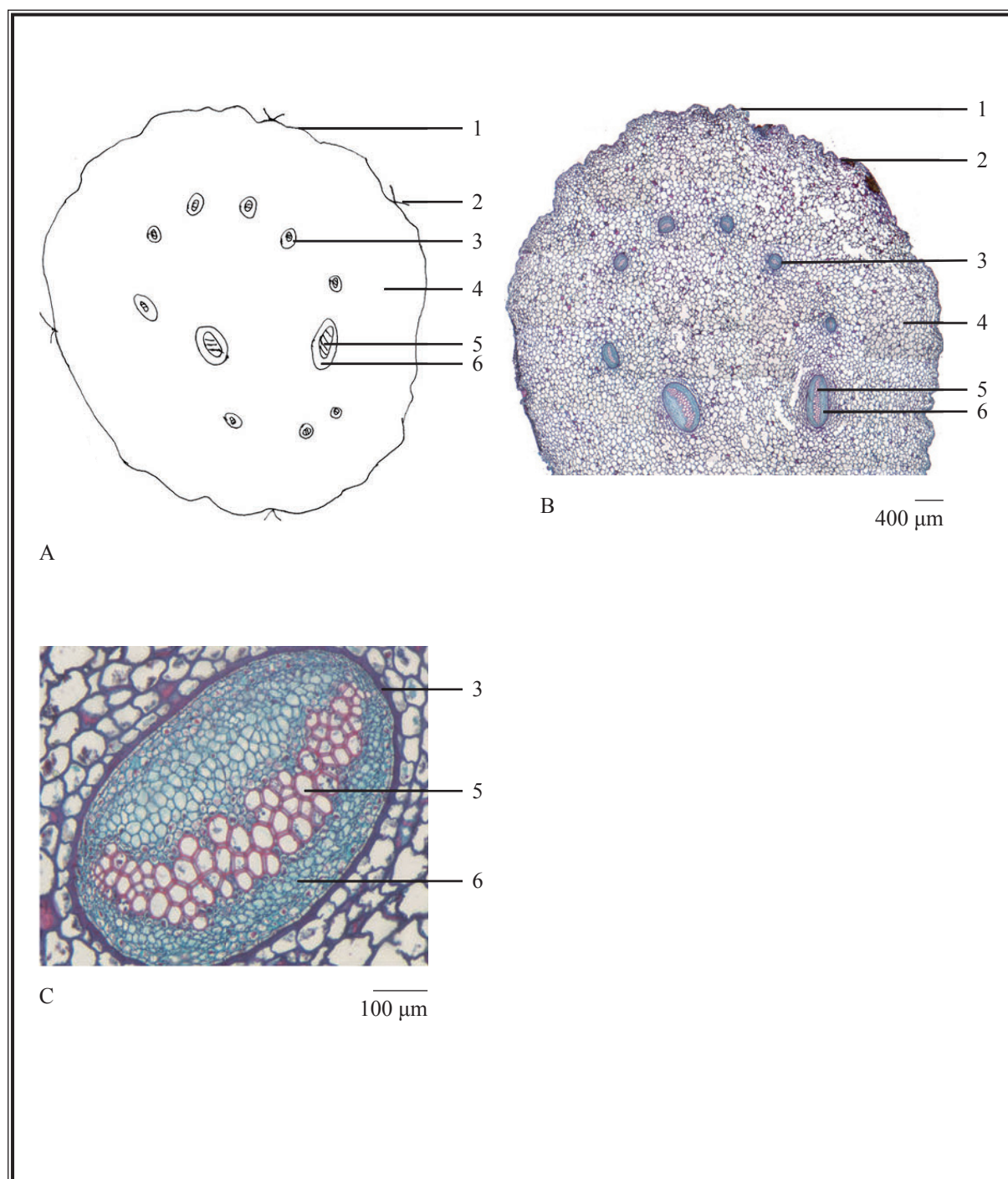
4.1 Microscopic Identification (Appendix III)

**Transverse section**

Epidermis consists of 1 layer of cells, subrounded or oblong, with relatively thickened outer wall. Basal part of scale leaves located at dented region of epidermis, consisting of 3-4 layers of cells, with thicken wall, containing reddish-brown pigments. Parenchymatous cells subrounded or irregularly undulately curved. Endodermis cells tangentially elongated; casparian dots indistinct. Vascular bundles amphicribal, 11-28 bundles arranged in a flattened annular ring, surrounded by endodermis. Tracheids of xylem mainly reticulate, 7-82 μm in diameter (Fig. 2).

**Powder**

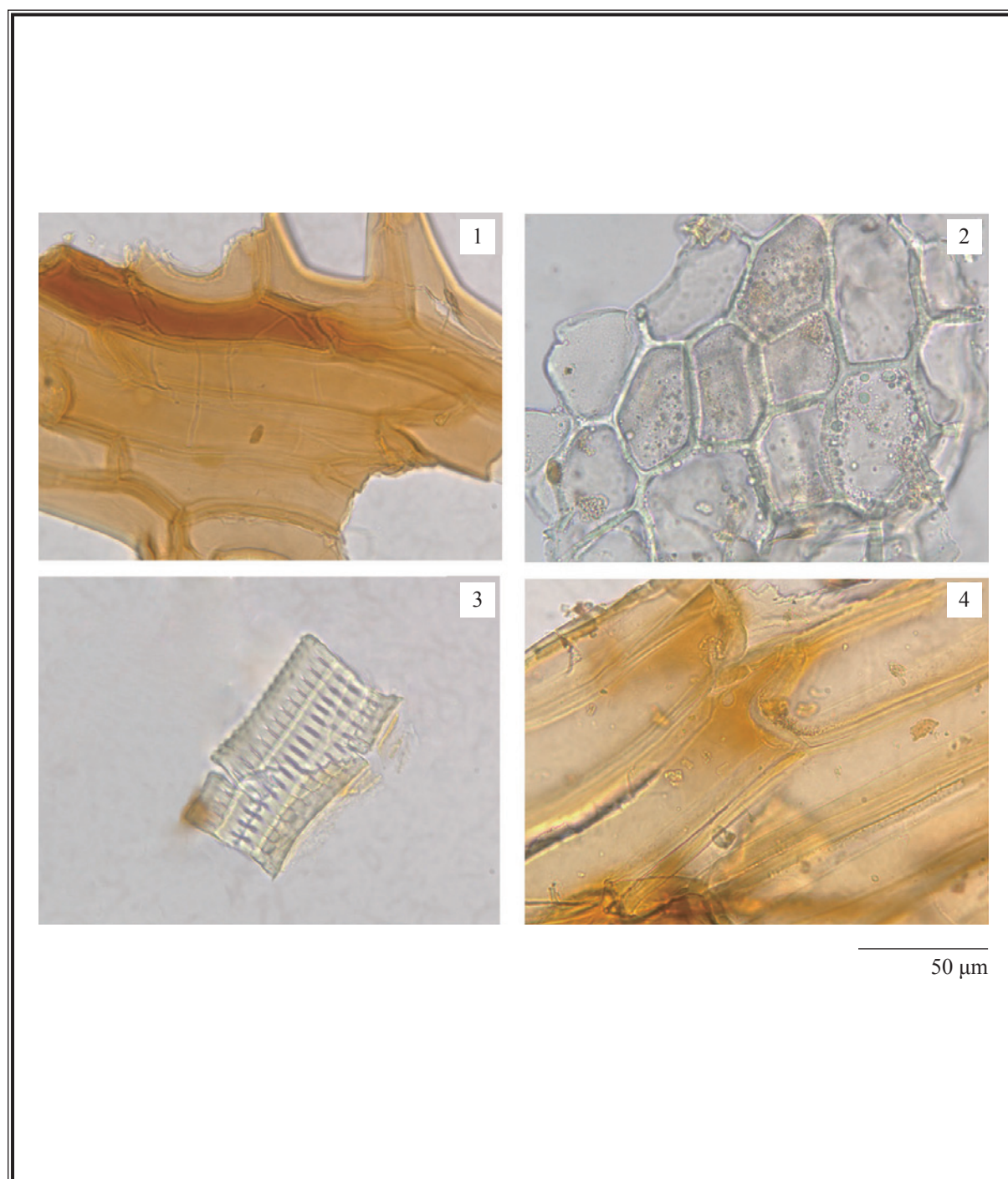
Colour brown. Fragments of scale leaf yellowish-brown or reddish-brown, cells of scales elongated or irregular in shape, some contain reddish-brown contents. Cortex cells subrectangular or subpolygonal, slightly lignified or unlignified, pit canals obvious; cells near to epidermis relatively small, walls slightly undulate, pit canals sparse; cells near to endodermis with thick walls, pit canals obvious. Tracheids mainly reticulate, colourless, yellow or yellowish-brown, 7-82 µm in diameter. Fibres usually in bundles, orange-yellow or reddish-brown, fusiform, tapering at both ends, walls thick, pit canals mostly unobvious (Fig. 3).



**Figure 2** Microscopic features of transverse section of *Drynariae Rhizoma*

A. Sketch B. Section illustration C. Endodermis and vascular bundles

1. Epidermis 2. Basal part of scale leaves 3. Endodermis 4. Parenchyma 5. Xylem 6. Phloem



**Figure 3** Microscopic features of powder of *Drynariae Rhizoma* (under the light microscope)

1. Fragments of scale leaf    2. Cortex cells    3. Tracheid    4. Fibres

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

### Standard solution

*Naringin standard solution*

Weigh 2.5 mg of naringin CRS (Fig. 4) and dissolve in 5 mL of ethanol (70%).

### Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (8:1:1, v/v).

### Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

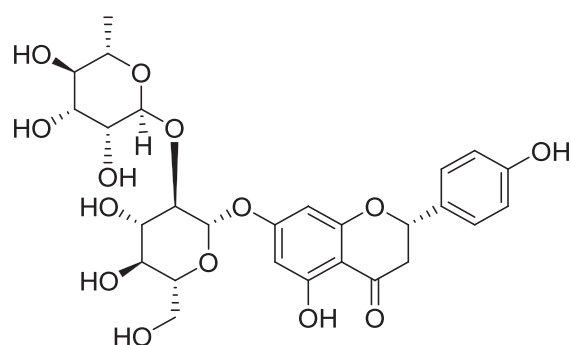
### Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about  $3000 \times g$  for 5 min. Filter the mixture.

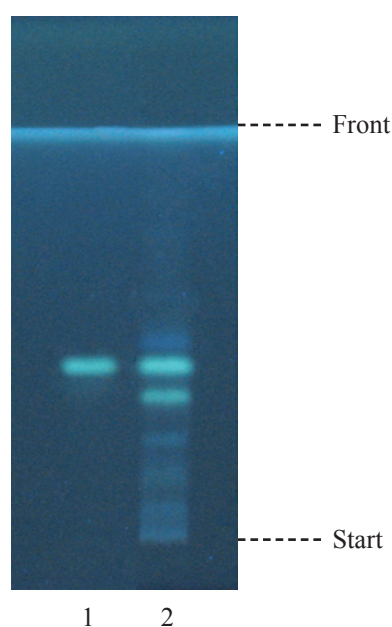
### Procedure

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 naringin standard solution (1  $\mu$ L) and the test solution (2  $\mu$ L) 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 2 min). 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 naringin



**Figure 5** A reference HPTLC chromatogram of Drynariae Rhizoma extract observed under UV light (366 nm) after staining

1. Naringin 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 naringin (Fig. 5).

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

#### Standard solution

*Naringin standard solution for fingerprinting, Std-FP (100 mg/L)*

Weigh 1.0 mg of naringin CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (200 W) the mixture for 1 h. Centrifuge at about 3000 × g for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (283 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) | Acetonitrile (%<br>v/v) | 0.5% Acetic acid (%<br>v/v) | Elution         |
|------------|-------------------------|-----------------------------|-----------------|
| 0 – 5      | 5                       | 95                          | isocratic       |
| 5 – 15     | 5 → 10                  | 95 → 90                     | linear gradient |
| 15 – 50    | 10 → 25                 | 90 → 75                     | linear gradient |
| 50 – 60    | 25                      | 75                          | isocratic       |

System suitability requirements

Perform at least five replicate injections, each using 5 μL of naringin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of naringin should not be more than 5.0%; the RSD of the retention time of naringin peak should not be more than 2.0%; the column efficiency determined from naringin peak should not be less than 200000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

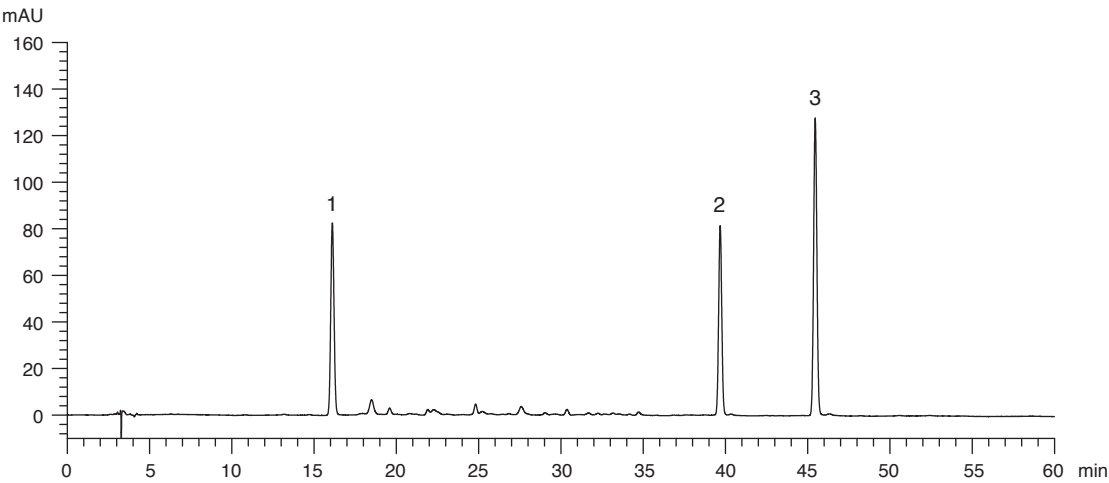
Separately inject naringin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of naringin peak in the chromatogram of naringin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify naringin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of naringin Std-FP. The retention times of naringin 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 Drynariae Rhizoma extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the three characteristic peaks of Drynariae Rhizoma extract

| Peak No.             | RRT  | Acceptable Range |
|----------------------|------|------------------|
| 1                    | 0.35 | ± 0.03           |
| 2                    | 0.87 | ± 0.03           |
| 3 (marker, naringin) | 1.00 | -                |



**Figure 6** A reference fingerprint chromatogram of Drynariae Rhizoma 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 (except cadmium should not be more than 2.5 mg/kg).
- 5.2 **Pesticide Residues** (*Appendix VI*): meet the requirements.
- 5.3 **Mycotoxins – Aflatoxins** (*Appendix VII*): meet the requirements.
- 5.4 **Sulphur Dioxide Residues** (*Appendix XVII*): meet the requirements.
- 5.5 **Foreign Matter** (*Appendix VIII*): not more than 2.0%.

## 5.6 Ash (*Appendix IX*)

Total ash: not more than 8.0%.

Acid-insoluble ash: not more than 2.5%.

## 5.7 Water Content (*Appendix X*)

Oven dried method: not more than 15.0%.

# 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 9.0%.

Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

# 7. ASSAY

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

## Standard solution

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

Weigh accurately 10.0 mg of naringin CRS and dissolve in 10 mL of ethanol (70%).

*Naringin standard solution for assay, Std-AS*

Measure accurately the volume of the naringin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 5, 10, 20, 50, 75 mg/L for naringin.

## Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (200 W) the mixture for 1 h. Centrifuge at about  $3000 \times g$  for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45- $\mu$ m PTFE filter.

## Chromatographic system

The liquid chromatograph is equipped with a DAD (283 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m 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<br>(min) | Acetonitrile<br>(%, v/v) | 0.5% Acetic acid<br>(%, v/v) | Elution         |
|---------------|--------------------------|------------------------------|-----------------|
| 0 – 20        | 5 → 50                   | 95 → 50                      | linear gradient |
| 20 – 30       | 50                       | 50                           | isocratic       |

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of naringin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of naringin should not be more than 5.0%; the RSD of the retention time of naringin peak should not be more than 2.0%; the column efficiency determined from naringin peak should not be less than 150000 theoretical plates.

The *R* value between naringin 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 naringin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of naringin against the corresponding concentrations of naringin Std-AS. Obtain the slope, y-intercept and the *r*<sup>2</sup> value from the 5-point calibration curve.

**Procedure**

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify naringin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of naringin Std-AS. The retention times of naringin 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 naringin in the test solution, and calculate the percentage content of naringin in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The sample contains not less than 0.50% of naringin (C<sub>27</sub>H<sub>32</sub>O<sub>14</sub>), calculated with reference to the dried substance.

**8. CAUTION**

This CMM should be used after proper processing (such as decoction).