Cinnamomi Cortex

Figure 1 A photograph of Cinnamomi Cortex

1. NAMES

Official Name: Cinnamomi Cortex

Chinese Name: 肉桂

Chinese Phonetic Name: Rougui

2. SOURCE

Cinnamomi Cortex is the dried stem bark of *Cinnamomum cassia* Presl (Lauraceae). The bark is frequently collected in autumn, and dried in a shaded area to obtain Cinnamomi Cortex.

3. DESCRIPTION

Channelled or quilled, 3.5-50 cm long, 0.5-6 cm wide when fully extended, 1-6 mm thick. Externally greyish-brown, slightly rough, with irregular fine wrinkles and transverse protuberant lenticels, sometimes showing greyish-white streaks; the inner surface reddish-brown, slightly even, with fine longitudinal striations and exhibiting oily trace on scratching. Texture hard and fragile, easily broken. Fracture uneven, the outer layer brown and relatively rough, the inner layer reddish-brown and oily, a yellowish-brown line visible between the two layers. Odour strongly aromatic; taste sweet and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells, the innermost layer of cells with thickened and lignified outer walls. Cortex scattered with stone cells and oil cells. Pericycle stone cells in groups, arranged in an interrupted ring; accompanied by fibre bundles outside, the outer walls of stone cells usually thinner. Phloem rays 1-2 rows of cells wide; containing fine raphides of calcium oxalate. Fibres usually 2-3 in bundles. Oil cells scattered throughout (Fig. 2).

Powder

Colour reddish-brown. Fibres mostly scattered singly, long fusiform, 69-618 μm long, 15-49 μm in diameter, walls thick and lignified, pits indistinct; polychromatic under the polarized microscope. Stone cells subrectangular or subrounded, 22-136 μm in diameter, walls thickened and some with one thin side. Oil cells occasionally found, broken. Cork cells polygonal, containing reddish-brown masses. Raphides of calcium oxalate scattered, relatively fine, varying in length; bright yellowish-white under the polarized microscope (Fig. 3).

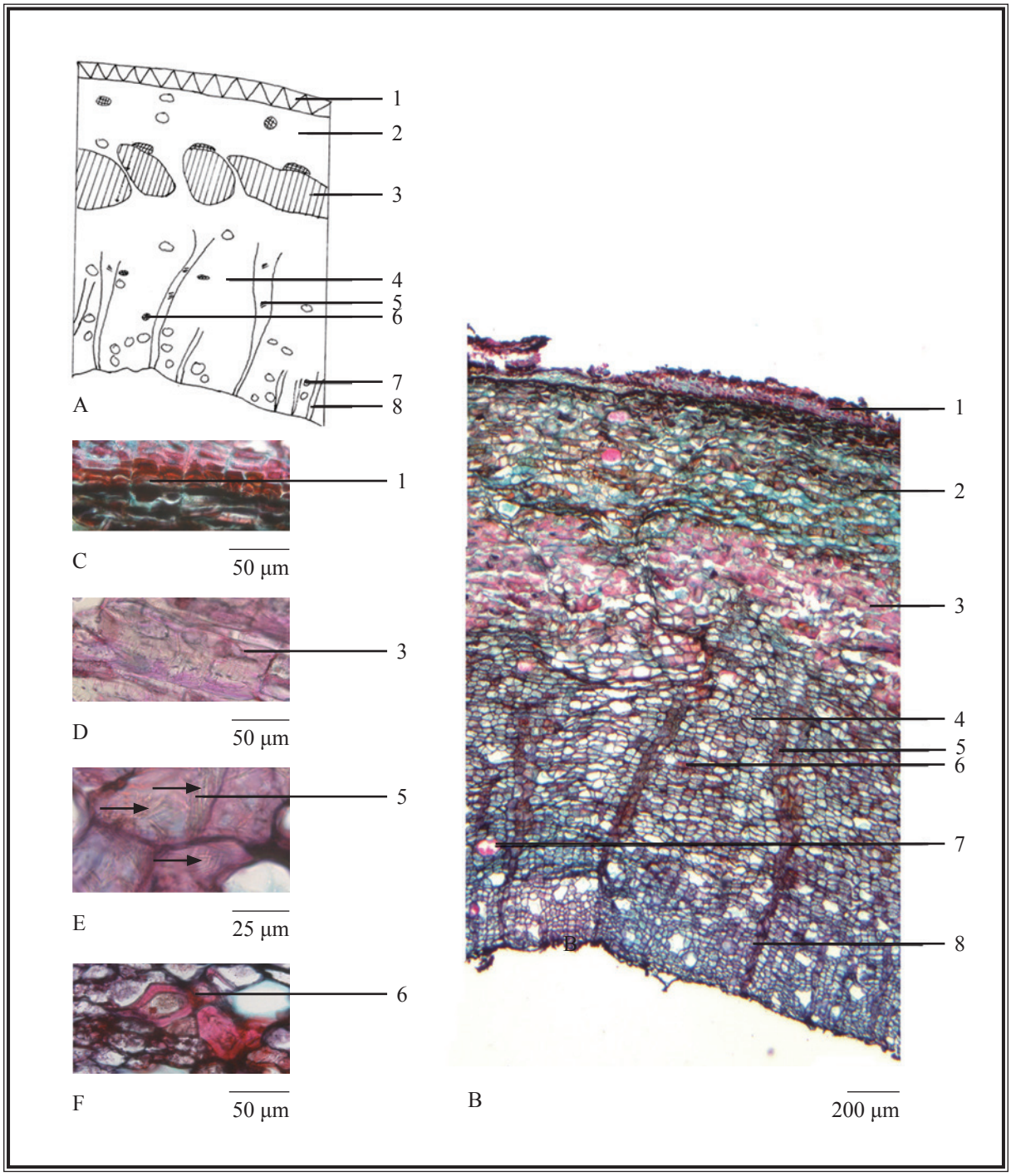


Figure 2 Microscopic features of transverse section of Cinnamomi Cortex

A. Sketch B. Section illustration C. The innermost layer of cork cells
D. Groups of stone cell at pericycle E. Ray cells containing raphides of calcium oxalate F. Fibres

1. Cork 2. Cortex 3. Pericycle 4. Phloem 5. Raphides of calcium oxalate 6. Fibre 7. Oil cell
8. Phloem ray

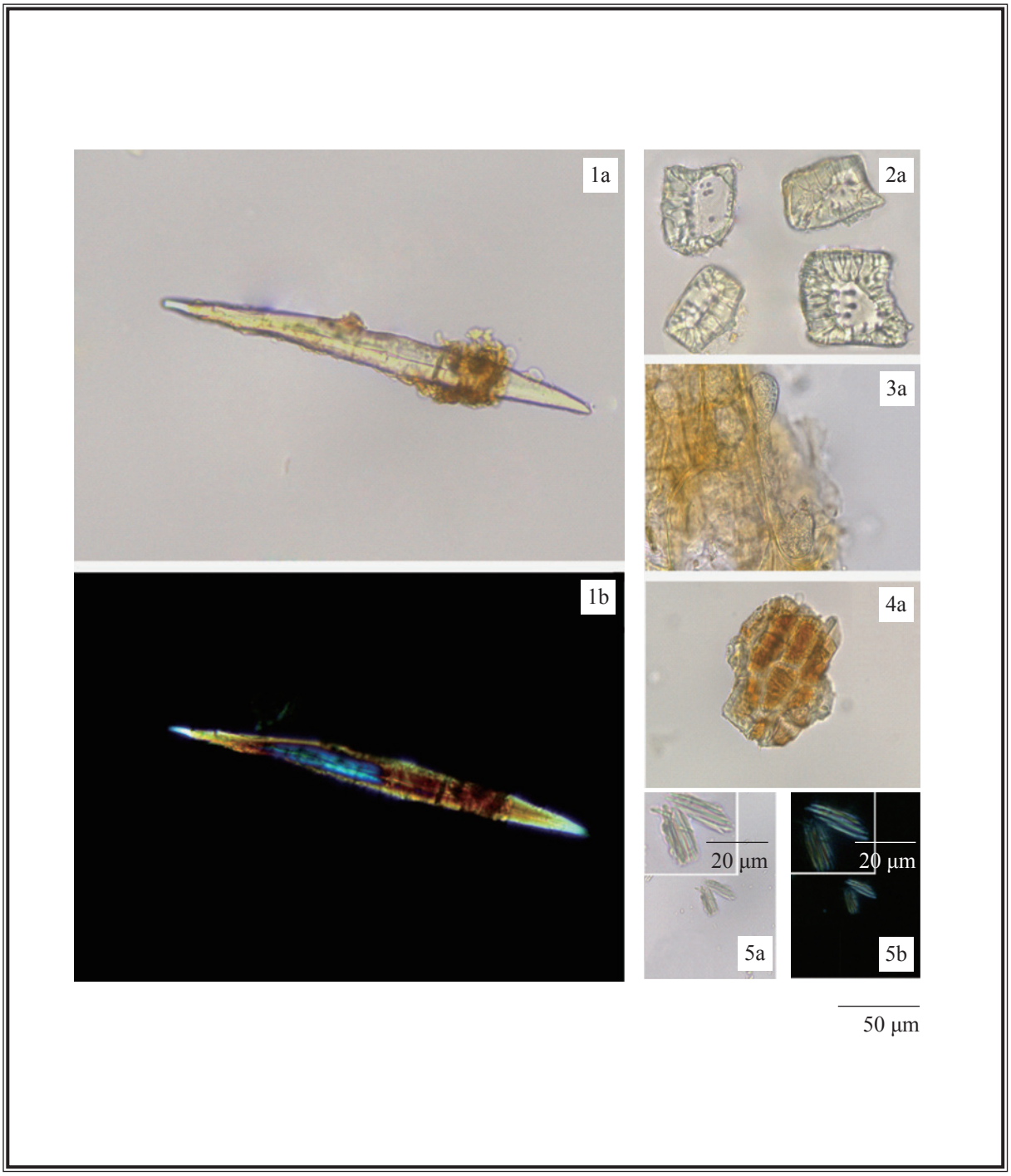


Figure 3 Microscopic features of powder of Cinnamomi Cortex

1. Fibre 2. Stone cells 3. Oil cells 4. Cork cells 5. Raphides 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 solutions

Cinnamaldehyde standard solution

Weigh 5.0 mg of cinnamaldehyde CRS (Fig. 4) and dissolve in 5 mL of ethanol (95%).

Cinnamic acid standard solution

Weigh 2.5 mg of cinnamic acid CRS (Fig. 4) and dissolve in 5 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and formic acid (4:1:0.1, v/v).

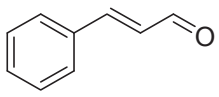
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (150 W) the mixture for 45 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 cinnamaldehyde standard solution (5 µL), cinnamic acid standard solution (1 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. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

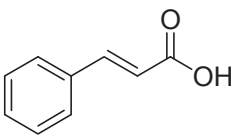


Figure 4 Chemical structures of (i) cinnamaldehyde and (ii) cinnamic acid

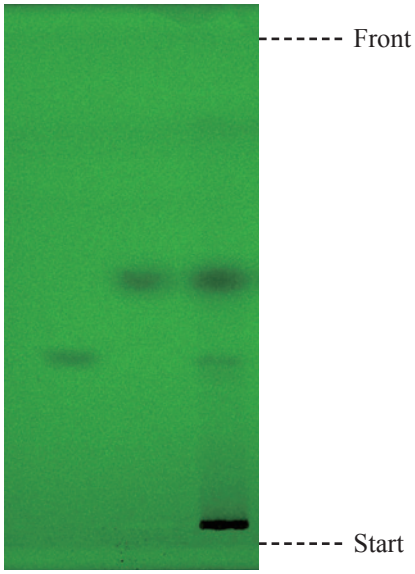


Figure 5 A reference HPTLC chromatogram of Cinnamomi Cortex extract observed under UV light (254 nm)

1. Cinnamic acid standard solution 2. Cinnamaldehyde 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 cinnamaldehyde and cinnamic acid (Fig. 5).

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

Standard solutions

Cinnamaldehyde standard solution for fingerprinting, Std-FP (80 mg/L)

Weigh 0.8 mg of cinnamaldehyde CRS and dissolve in 10 mL of ethanol (70%).

Cinnamic acid standard solution for fingerprinting, Std-FP (1 mg/L)

Weigh 0.1 mg of cinnamic acid CRS and dissolve in 100 mL of ethanol (70%).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 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 (290 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 (%, v/v)	0.5% Acetic acid (%, v/v)	Elution
0 – 10	28	72	isocratic
10 – 20	28 → 32	72 → 68	linear gradient
20 – 30	32 → 40	68 → 60	linear gradient
30 – 40	40 → 60	60 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of cinnamaldehyde Std-FP and cinnamic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamaldehyde and cinnamic acid should not be more than 5.0%; the RSD of the retention times of cinnamaldehyde and cinnamic acid peaks should not be more than 2.0%; the column efficiencies determined from cinnamaldehyde and cinnamic acid peaks should not be less than 25000 and 20000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Cinnamomi Cortex extract

Peak No.	RRT	Acceptable Range
1 (marker, cinnamic acid)	1.00	-
2 (cinnamaldehyde)	1.31	± 0.03
3	1.64	± 0.06

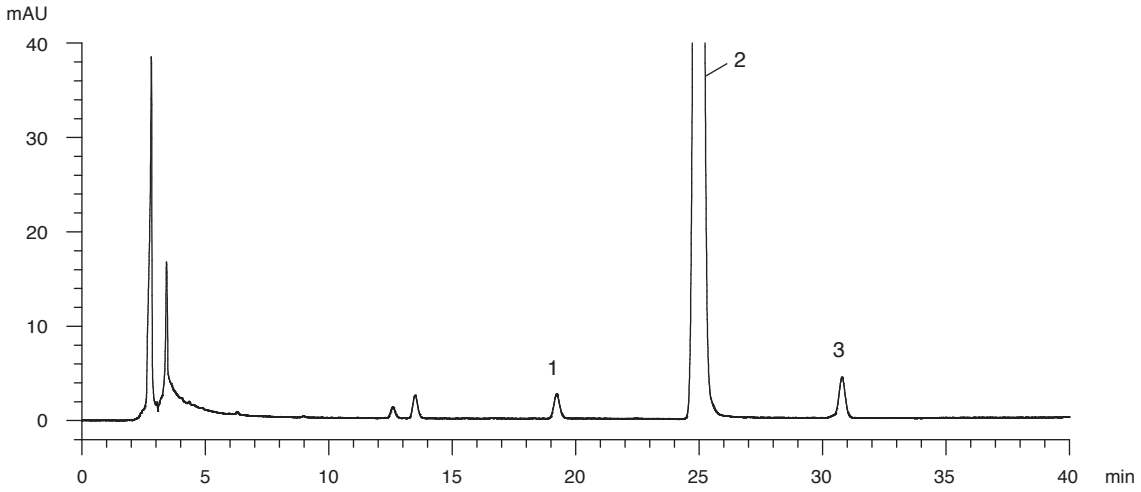


Figure 6 A reference fingerprint chromatogram of Cinnamomi Cortex extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 8.0%.
Ethanol-soluble extractives (hot extraction method): not less than 10.0%.

7. ASSAY

7.1 Assay of cinnamaldehyde and cinnamic acid

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

Standard solution

Mixed cinnamaldehyde and cinnamic acid standard stock solution, Std-Stock (200 mg/L for cinnamaldehyde and 10 mg/L for cinnamic acid)

Weigh accurately 2.0 mg of cinnamaldehyde CRS and 0.1 mg of cinnamic acid CRS, and dissolve in 10 mL of ethanol (70%).

Mixed cinnamaldehyde and cinnamic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed cinnamaldehyde and cinnamic acid Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 10, 20, 30, 40, 100 mg/L for cinnamaldehyde and 0.1, 0.2, 0.5, 1, 2 mg/L for cinnamic acid.

Test solution

Weigh accurately 0.1 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. 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-μm PTFE 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 flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.5% Acetic acid (%, v/v)	Elution
0 – 10	28	72	isocratic
10 – 20	28 → 32	72 → 68	linear gradient
20 – 30	32 → 40	68 → 60	linear gradient
30 – 40	40 → 60	60 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of the mixed cinnamaldehyde and cinnamic acid Std-AS (30 mg/L for cinnamaldehyde and 0.5 mg/L for cinnamic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamaldehyde and cinnamic acid should not be more than 5.0%; the RSD of the retention times of cinnamaldehyde and cinnamic acid peaks should not be more than 2.0%; the column efficiencies determined from cinnamaldehyde and cinnamic acid peaks should not be less than 25000 and 20000 theoretical plates respectively.

The *R* value between cinnamaldehyde peak and the closest peak; and the *R* value between cinnamic acid 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 cinnamaldehyde and cinnamic acid Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of cinnamaldehyde and cinnamic acid against the corresponding concentrations of the mixed cinnamaldehyde and cinnamic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify cinnamaldehyde and cinnamic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed cinnamaldehyde and cinnamic acid Std-AS. The retention times of cinnamaldehyde and cinnamic acid 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 cinnamaldehyde and cinnamic acid in the test solution, and calculate the percentage contents of cinnamaldehyde and cinnamic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.7% of the total content of cinnamaldehyde (C_9H_8O) and cinnamic acid ($C_9H_8O_2$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 50 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 300 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method B).

Limits

The sample contains not less than 1.2% (v/w) of volatile oil.