Periplocae Cortex

Figure 1 A photograph of Periplocae Cortex

A. Periplocae Cortex B. Magnified outer surface of root bark
C. Magnified inner surface of root bark

1. NAMES

Official Name: Periplocae Cortex

Chinese Name: 香加皮

Chinese Phonetic Name: Xiangjiapi

2. SOURCE

Periplocae Cortex is the dried root bark of *Periploca sepium* Bge. (Asclepiadaceae). The root is collected in spring and autumn, stripped off the root bark, then dried under the sun to obtain Periplocae Cortex.

3. DESCRIPTION

Quilled or channelled pieces of bark, a few broken up irregularly, 2.5-23 cm long, 3-15 mm wide, 1-5 mm thick. Externally greyish-brown or yellowish-brown, cork soft, lax and usually scaly, easily exfoliated; the inner surface pale yellow or pale yellowish-brown, relatively smooth, with fine longitudinal striations. Texture fragile and light in weight, easily broken. Fracture uneven, yellowish-white. Odour distinctively aromatic; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 3-30 layers of cells, brownish-yellow. Cortex broad, scattered with prisms of calcium oxalate, stone cells and laticiferous tubes. Stone cells square or subpolygonal, mainly in groups. Phloem rays 1-5 rows of cells wide. Phloem scattered with prisms of calcium oxalate. Laticiferous tubes located in cortex and phloem (Fig. 2).

Powder

Colour pale brown. Stone cells rectangular or subpolygonal, 20-123 μm in diameter. Laticiferous tubes contain oily granules. Prisms of calcium oxalate 4-37 μm in diameter; polychromatic under the polarized microscope. Cork cells brownish-yellow, polygonal. Starch granules numerous, simple starch granules subrounded or oblong, 3-18 μm in diameter; compound starch granules composed of 2-6 units (Fig. 3).

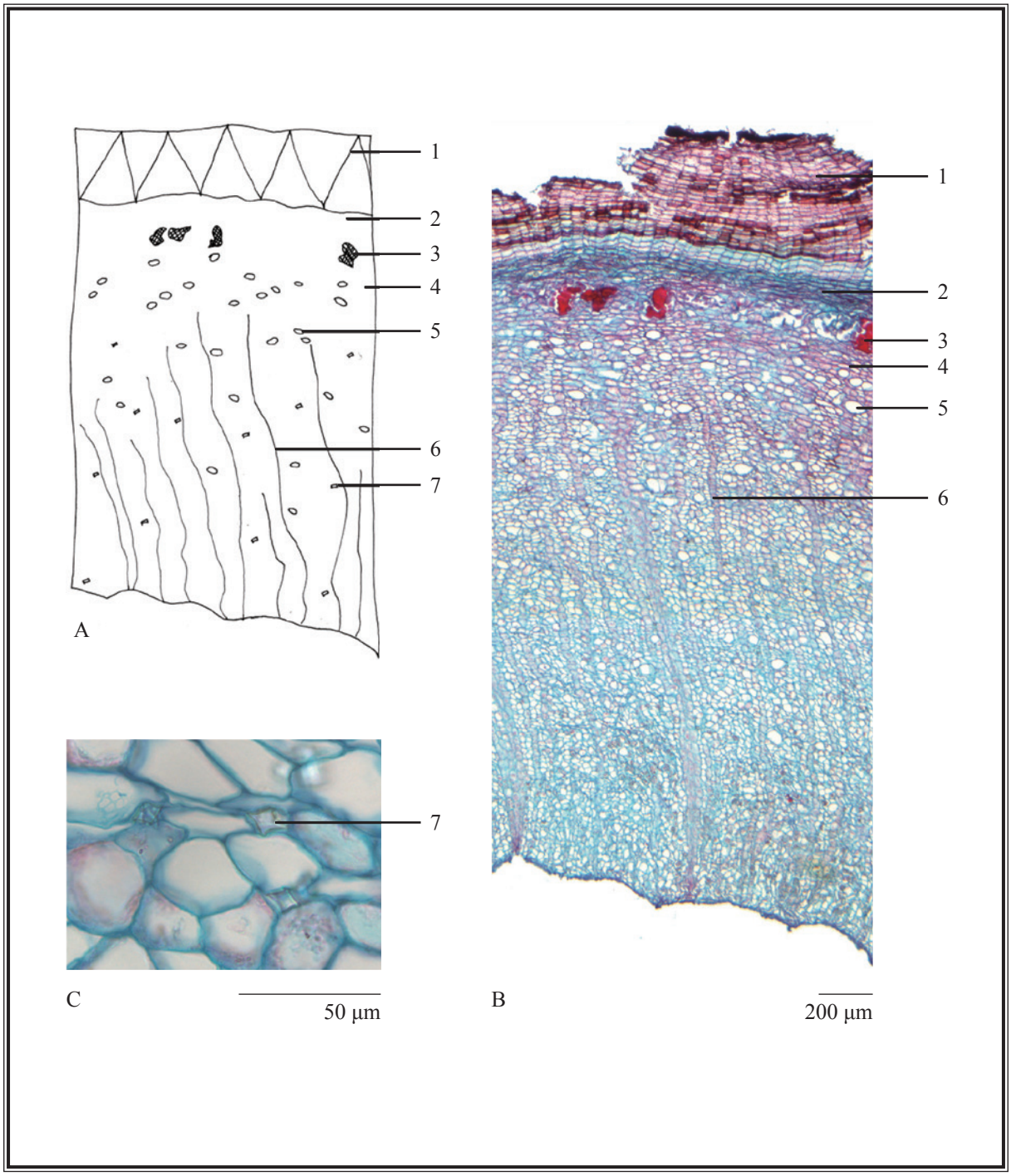


Figure 2 Microscopic features of transverse section of Periplocae Cortex

A. Sketch B. Section illustration C. Prisms of calcium oxalate

1. Cork 2. Cortex 3. Stone cells 4. Phloem 5. Laticiferous tube
6. Phloem ray 7. Prism of calcium oxalate

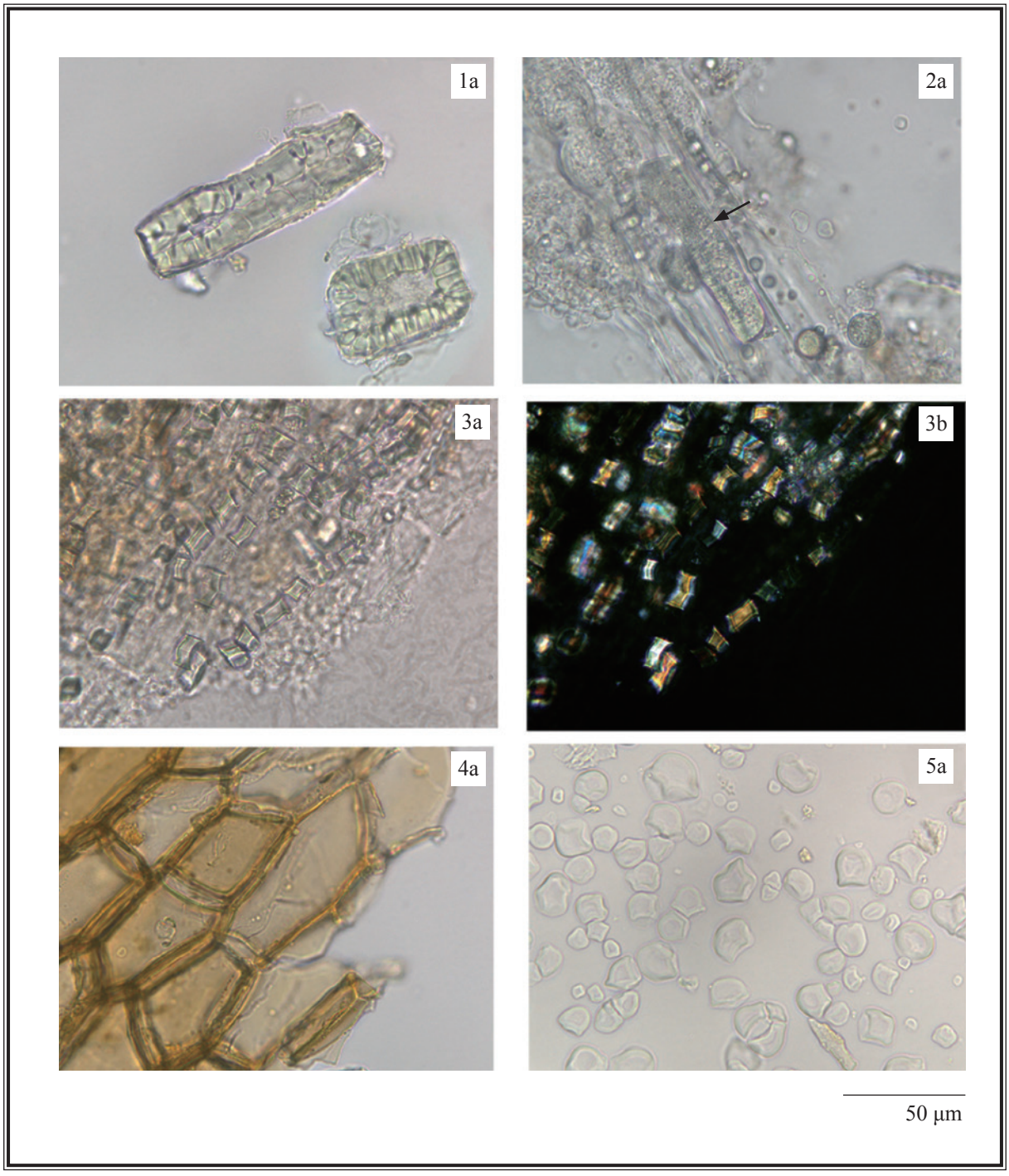


Figure 3 Microscopic features of powder of Periplocae Cortex

1. Stone cells 2. Laticiferous tubes 3. Prisms of calcium oxalate
4. Cork cells 5. Starch granules

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

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

(I) Identification of 4-methoxysalicylaldehyde

Standard solution

4-methoxysalicylaldehyde standard solution

Weigh 2.0 mg of 4-methoxysalicylaldehyde CRS (Fig. 4) and dissolve in 2 mL of ethanol.

Developing solvent system

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

Spray reagent

Weigh 0.1 g of 2,4-dinitrophenylhydrazine and dissolve in 100 mL of a mixture of ethanol and hydrochloric acid (99:1, v/v).

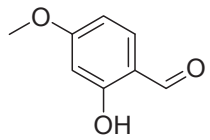
Test solution

Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of ethanol. Sonicate (150 W) the mixture for 30 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 4-methoxysalicylaldehyde standard solution (2 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. Spray the plate evenly with the spray reagent and dry in air. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

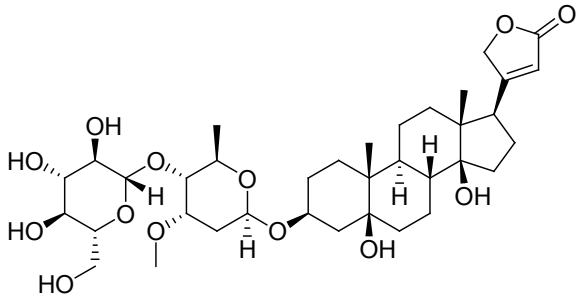


Figure 4 Chemical structures of (i) 4-methoxysalicylaldehyde and (ii) periplocin

Figure 5 A reference HPTLC chromatogram of Periplocae Cortex extract observed under visible light after staining

1. 4-methoxysalicylaldehyde 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 4-methoxysalicylaldehyde (Fig. 5).

(II) Identification of periplocin

Standard solution

Periplocin standard solution

Weigh 1.0 mg of periplocin CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, glacial acetic acid and water (3:1:1, 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 25-mL conical flask, then add 5 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Filter the mixture. Make appropriate dilution where necessary.

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 periplocin standard solution (0.5 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. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

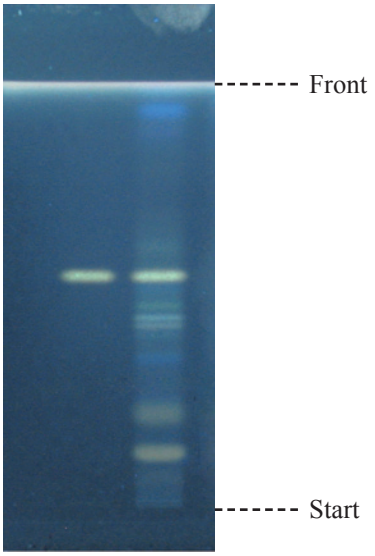


Figure 6 A reference HPTLC chromatogram of Periplocae Cortex extract observed under UV light (366 nm) after staining

1. Periplocin 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 periplocin (Fig. 6).

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

Standard solutions

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

Weigh 0.3 mg of 4-methoxysalicylaldehyde CRS and dissolve in 10 mL of ethanol (50%).

Periplocin standard solution for fingerprinting, Std-FP (120 mg/L)

Weigh 1.2 mg of periplocin CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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)	Trifluoroacetic acid:Acetonitrile (0.03:99.97, v/v)	0.03% Trifluoroacetic acid (%, v/v)	Elution
0 – 18	10 → 20	90 → 80	linear gradient
18 – 44	20 → 70	80 → 30	linear gradient
44 – 60	70	30	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 µL of 4-methoxysalicylaldehyde Std-FP and periplocin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 4-methoxysalicylaldehyde and periplocin should not be more than 5.0%; the RSD of the retention times of 4-methoxysalicylaldehyde and periplocin peaks should not be more than 2.0%; the column efficiencies determined from 4-methoxysalicylaldehyde and periplocin peaks should not be less than 200000 and 250000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and 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. 7).

Procedure

Separately inject 4-methoxysalicylaldehyde Std-FP, periplocin Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of 4-methoxysalicylaldehyde and periplocin peaks in the chromatograms of 4-methoxysalicylaldehyde Std-FP, periplocin Std-FP and the retention times of the three characteristic peaks (Fig. 7) in the chromatogram of the test solution. Identify 4-methoxysalicylaldehyde and periplocin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of 4-methoxysalicylaldehyde Std-FP and periplocin Std-FP. The retention times of 4-methoxysalicylaldehyde and periplocin 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 Periplocae Cortex extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2 (periplocin)	0.85	± 0.03
3 (marker, 4-methoxysalicylaldehyde)	1.00	-

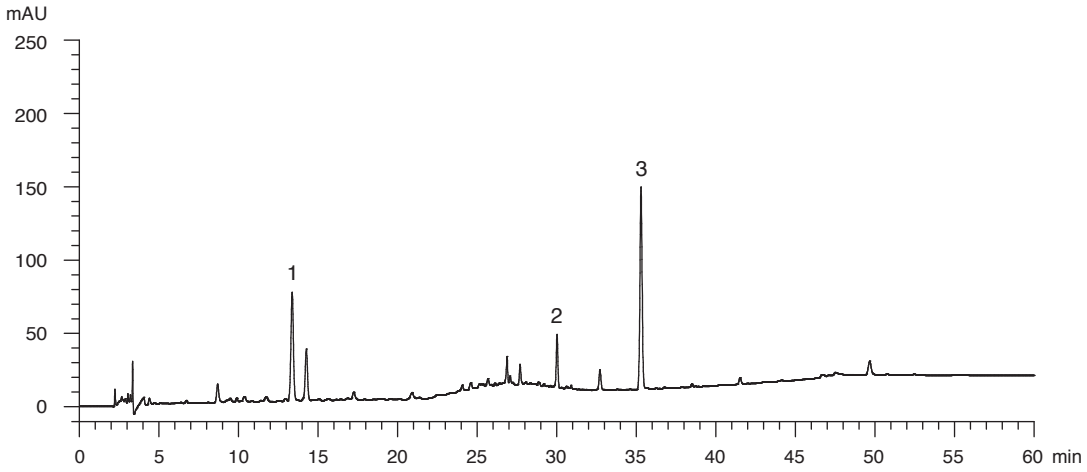


Figure 7 A reference fingerprint chromatogram of Periplocae Cortex extract

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. 7).

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.

Acid-insoluble ash: not more than 4.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 19.0%.
Ethanol-soluble extractives (cold extraction method): not less than 19.0%.

7. ASSAY

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

Standard solution

4-methoxysalicylaldehyde standard stock solution, Std-Stock (125 mg/L)
Weigh accurately 1.25 mg of 4-methoxysalicylaldehyde CRS and dissolve in 10 mL of ethanol (50%).
4-methoxysalicylaldehyde standard solution for assay, Std-AS
Measure accurately the volume of the 4-methoxysalicylaldehyde Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1, 10, 40, 100, 125 mg/L for 4-methoxysalicylaldehyde.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (278 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 0.05% trifluoroacetic acid and acetonitrile (71:29, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of 4-methoxysalicylaldehyde Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 4-methoxysalicylaldehyde should not be more than 5.0%; the RSD of the retention time of 4-methoxysalicylaldehyde peak should not be more than 2.0%; the column efficiency determined from 4-methoxysalicylaldehyde peak should not be less than 10000 theoretical plates.

The *R* value between 4-methoxysalicylaldehyde 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 4-methoxysalicylaldehyde Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of 4-methoxysalicylaldehyde against the corresponding concentrations of 4-methoxysalicylaldehyde Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify 4-methoxysalicylaldehyde peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 4-methoxysalicylaldehyde Std-AS. The retention times of 4-methoxysalicylaldehyde 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 4-methoxysalicylaldehyde in the test solution, and calculate the percentage content of 4-methoxysalicylaldehyde in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.37% of 4-methoxysalicylaldehyde ($C_8H_8O_3$), calculated with reference to the dried substance.