Piperis Retrofracti Fructus

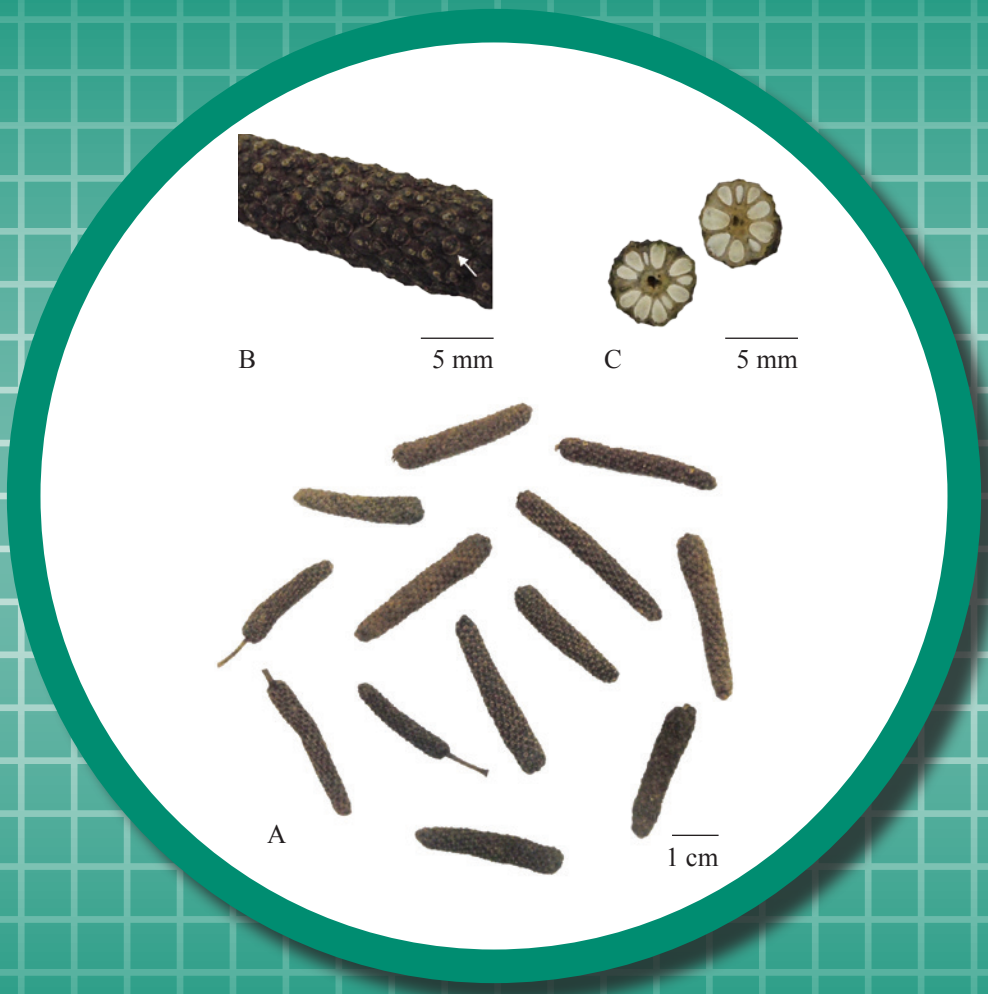

Figure 1 A photograph of Piperis Retrofracti Fructus

A. Piperis Retrofracti Fructus B. Magnified outer surface (bract→)
C. Magnified transverse section of fruit-spike

1. NAMES

Official Name: Piperis Retrofracti Fructus

Chinese Name: 長果蓽茇

Chinese Phonetic Name: Changguobibo

2. SOURCE

Piperis Retrofracti Fructus is the dried fruit-spike of *Piper retrofractum* Vahl (Piperaceae). The fruit-spike is collected when it is ripe, foreign matter removed, then dried under the sun to obtain Piperis Retrofracti Fructus.

3. DESCRIPTION

Cylindrical, sometimes slightly curved, 1.7-4.9 cm long, 3-9 mm in diameter, sometimes with remnants of peduncle. Externally blackish-brown or brown, aggregated by numerous small berries, showing obliquely and regularly arranged protuberances, sometimes with round bracts between berries. Texture hard and fragile, easily broken. Fracture irregular, with berries radially arranged on the cut surface. Odour characteristic; taste pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Bract located between berries, consisting of parenchymatous cells, with oil cells and small vascular bundles scattered. Exocarp consists of 1 layer of cells. Mesocarp consists of several layers of collenchymatous cells and few stone cells on the outer side, parenchymatous cells on the inner side, oil cells and small vascular bundles scattered. Oil cell layer located near the endocarp. Endocarp consists of 1 layer of parenchymatous cells. Testa consists of 2-3 layers of cells. Perisperm cells filled with tiny starch granules. Spicate axis consists of parenchymatous cells, hollow in centre; collateral vascular with fibres surrounded radically arranged (Fig. 2).

Powder

Colour brown. Stone cells scattered singly, in groups or present in parenchyma, pale yellow, subrounded, subpolygonal or long-ovoid, 25-168 μm long, 18-86 μm in diameter, with distinct pits, pit canals and striations; yellowish-white or orange-white under the polarized microscope. Cells of endocarp long-polygonal or subpolygonal in surface view, anticlinal walls irregular beaded-thickened, often adhere brown cells of testa on the lower layer. Cells of testa yellowish-brown or reddish-brown, polygonal or long-polygonal in surface view. Oil cells mainly present in parenchyma, subrounded, 27-71 μm in diameter, some contain yellowish-brown secretions. Perisperm cells rectangular or polygonal, filling with tiny starch granules; pale white under the polarized microscope. Vessels mainly scalariform, spiral and reticulate vessels also present, 4-25 μm in diameter (Fig. 3).

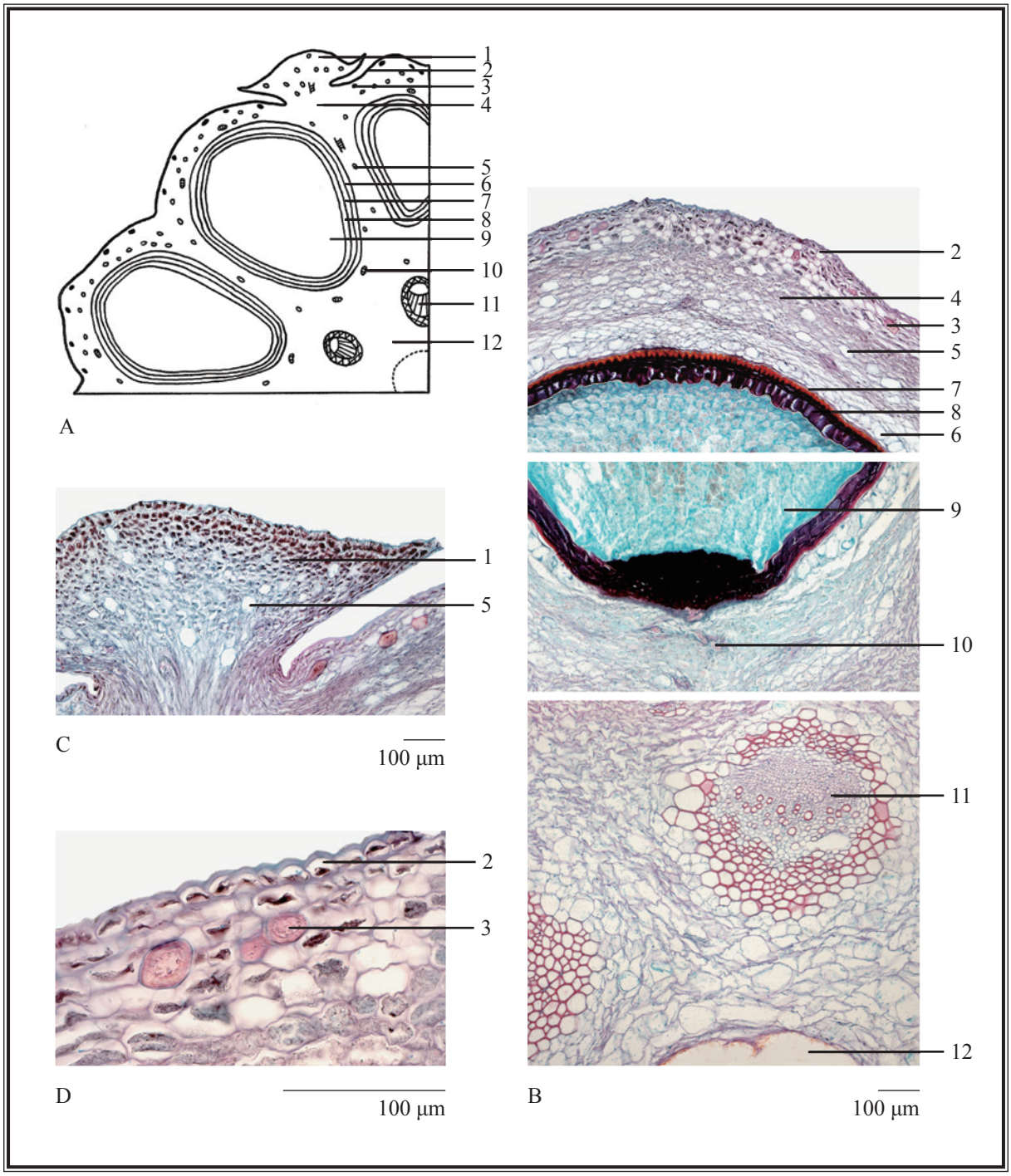

Figure 2 Microscopic features of transverse section of Piperis Retrofracti Fructus

A. Sketch B. Section illustration C. Bract D. Stone cells

1. Bract 2. Exocarp 3. Stone cells 4. Mesocarp 5. Oil cells 6. Oil cell layer
7. Endocarp 8. Testa 9. Perisperm 10. Small vascular bundles
11. Vascular bundle of spicate axis 12. Spicate axis

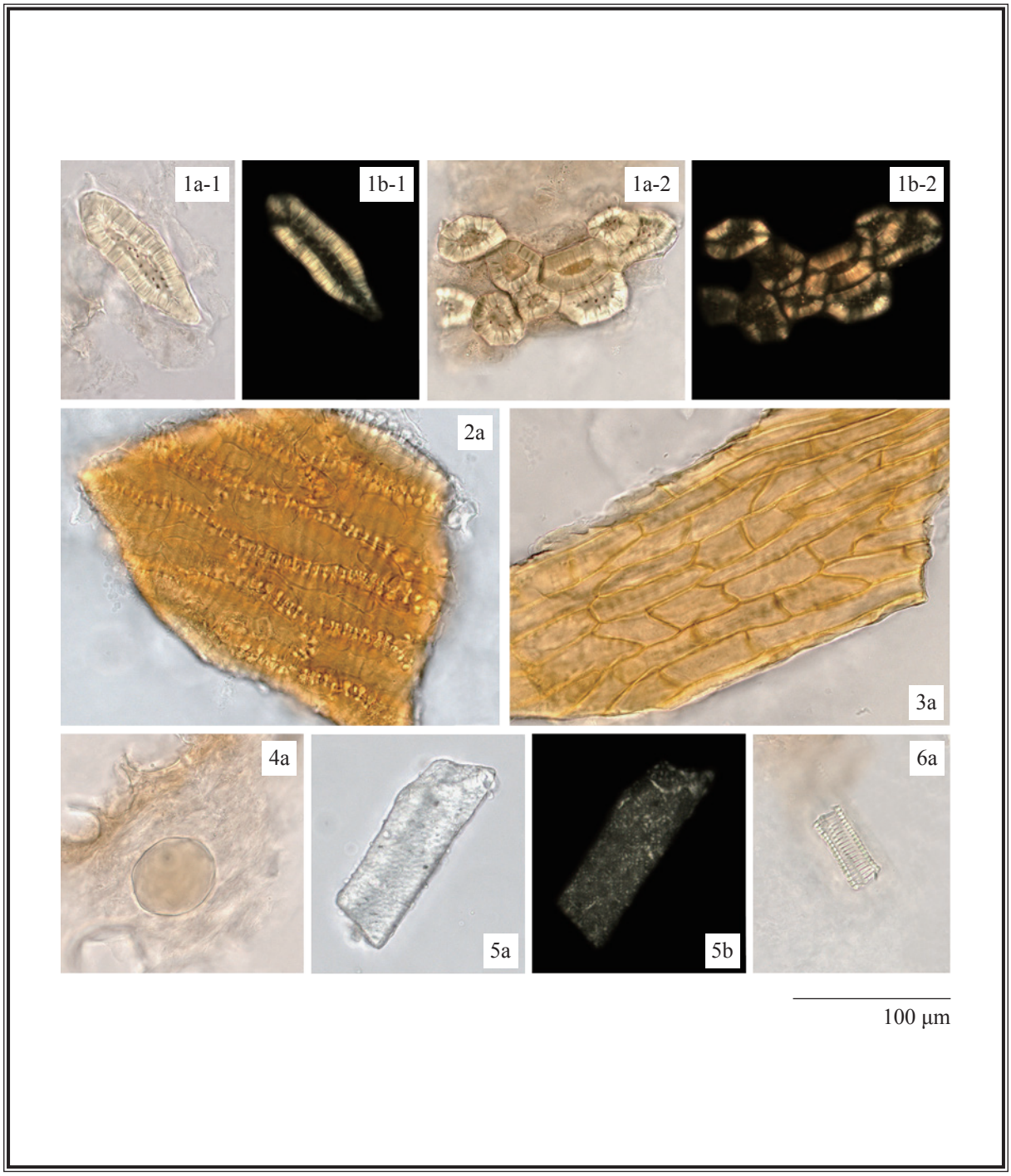

Figure 3 Microscopic features of powder of Piperis Retrofracti Fructus

1. Stone cells 2. Cells of endocarp 3. Cells of testa 4. Oil cell 5. Perisperm cell 6. Vessel

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

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

Standard solution

Piperine standard solution

Weigh 2.5 mg of piperine CRS (Fig. 4) and place it in a 5-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Developing solvent system

Prepare a mixture of methanol and water (9:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 0.5 g of the freshly powdered sample and place it in a 15-mL centrifuge tube wrapped in aluminium foil, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter through a 0.45-μm nylon filter. Freshly prepare the test solution.

Procedure

Carry out the method by using a HPTLC RP-18 F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately piperine standard solution (2 μL) and the test solution (1.5 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 6 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 3-5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

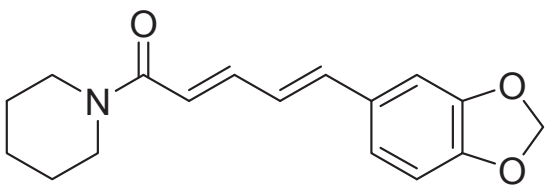

Figure 4 Chemical structure of piperine

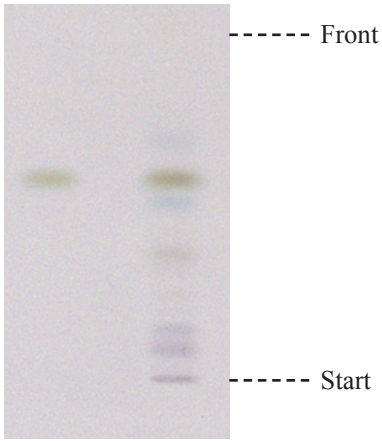

Figure 5 A reference HPTLC chromatogram of Piperis Retrofracti Fructus extract observed under visible light after staining

1. Piperine 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 piperine (Fig. 5).

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

Standard solution

Piperine standard solution for fingerprinting, Std-FP (12 mg/L)

Weigh 0.12 mg of piperine CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol (70%). Freshly prepare the standard solution.

Test solution

Weigh 0.1 g of the freshly powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 40 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Pipette 4 mL of supernatant to a 10-mL amber glass volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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)	Methanol (%, v/v)	0.1% Formic acid (%, v/v)	Elution
0 – 20	70 → 80	30 → 20	linear gradient
20 – 25	80 → 90	20 → 10	linear gradient
25 – 40	90	10	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of piperine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of piperine should not be more than 5.0%; the RSD of the retention time of piperine peak should not be more than 2.0%; the column efficiency determined from piperine peak should not be less than 15000 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. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Piperis Retrofracti Fructus extract

Peak No.	RRT	Acceptable Range
1	0.92	± 0.03
2 (marker, piperine)	1.00	-
3	1.50	± 0.03
4	2.21	± 0.07
5	2.75	± 0.12

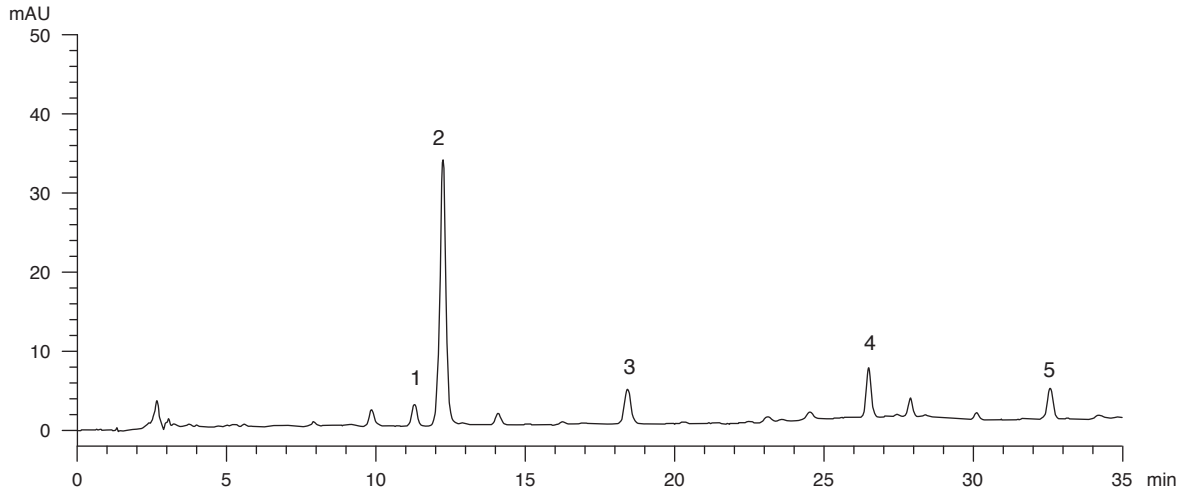

Figure 6 A reference fingerprint chromatogram of Piperis Retrofracti Fructus extract

For positive identification, the sample must give the above five 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 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 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 9.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

7.1 Assay of Piperine

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

Standard solution

Piperine standard stock solution, Std-Stock (600 mg/L)

Weigh accurately 6.0 mg of piperine CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol (70%). Freshly prepare the standard solution.

Piperine standard solution for assay, Std-AS

Measure accurately the volume of the piperine Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1.2, 3, 6, 30, 60 mg/L for piperine. Store in amber glass volumetric flask.

Test solution

Weigh accurately 0.1 g of the freshly powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 40 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Pipette 4 mL of supernatant to a 10-mL amber glass volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The liquid chromatograph is equipped with a DAD (343 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)	Methanol (%, v/v)	0.1% Formic acid (%, v/v)	Elution
0 – 20	70 → 80	30 → 20	linear gradient
20 – 25	80 → 90	20 → 10	linear gradient
25 – 40	90	10	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of piperine Std-AS (6 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of piperine should not be more than 5.0%; the RSD of the retention time of piperine peak should not be more than 2.0%; the column efficiency determined from piperine peak should not be less than 15000 theoretical plates.

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

Limits

The sample contains not less than 2.5% of piperine ($C_{17}H_{19}NO_3$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 80 g of the freshly powdered sample and place it in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 0.60% (v/w) of volatile oil.