Linderae Radix

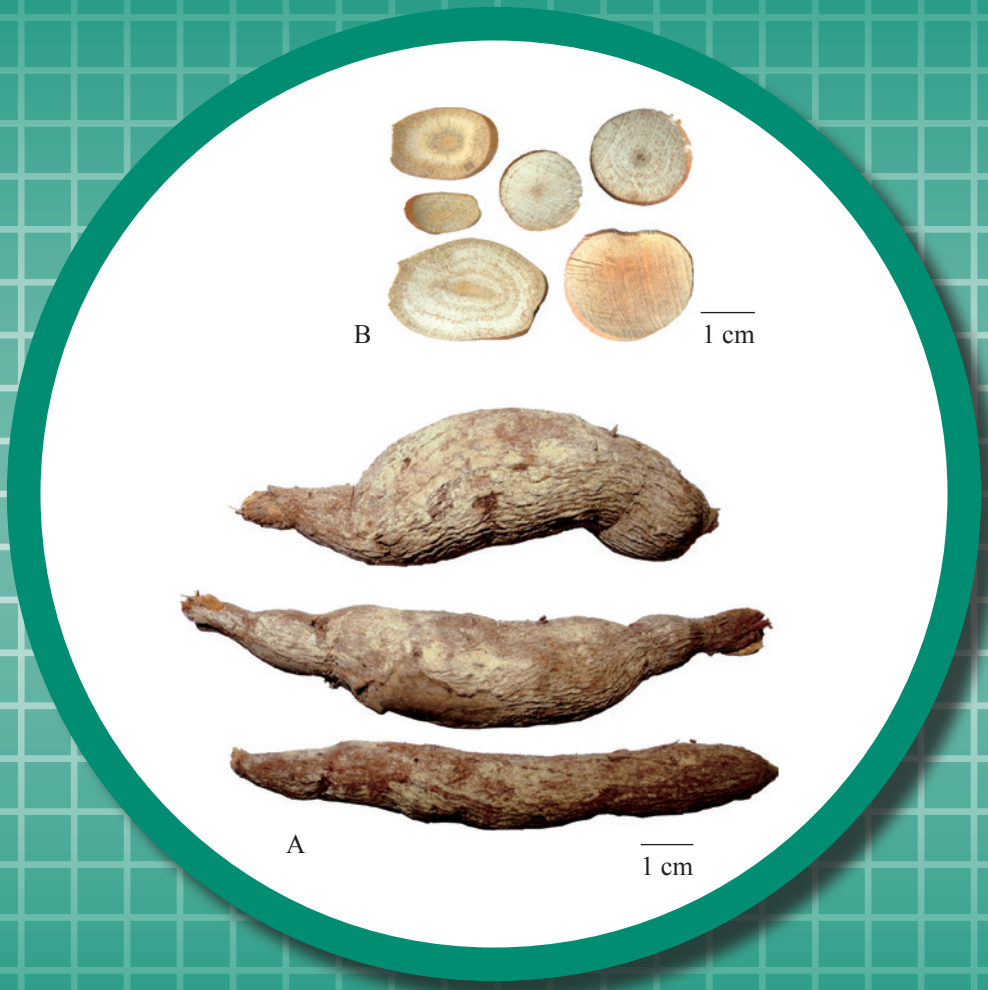


Figure 1 A photograph of Linderae Radix

A. Linderae Radix B. Magnified slices of root tuber

1. NAMES

Official Name: Linderae Radix

Chinese Name: 烏藥

Chinese Phonetic Name: Wuyao

2. SOURCE

Linderae Radix is the dried root tuber of *Lindera aggregata* (Sims) Kosterm. (Lauraceae). The root tuber is collected all year round, rootlets removed, washed clean, then dried under the sun to yield the intact form. Alternatively, the root tuber is sliced while fresh, and dried under the sun to obtain sliced form of Linderae Radix.

3. DESCRIPTION

Mostly fusiform, slightly curved, some constricted in the middle to be moniliform, 6-15 cm long, 10-30 mm in diameter, thicker distally. Externally yellowish-brown, with longitudinal wrinkles and sparse rootlet scars. Texture hard. Slice 0.5-3 mm thick, transversely cut surface yellowish-white to yellowish-brown, deeper colour in the centre, with rays radiate, annual rings visible. Odour aromatic; taste slightly bitter and pungent, with a cooling sensation (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of more than 10 layers of cork cells, cells mostly flattened, arranged regularly, sometimes broken. Cortex mainly consists of several layers of parenchymatous cells, scattered with numerous elliptical or subrounded oil cells, oil cells filled with oil contents. Phloem scattered with oil cells. Parenchymatous cells easily broken in phloem and cortex, and forming some furrows. Cambium in a ring, distinct. Xylem occupies the major portion of the root, annual ring distinct. Vessels mostly arranged in a single or several rows, or scattered singly, subpolygonal or subrounded. Xylem ray 1-4 rows of cells wide, cells subrectangular, mostly elongated tangentially, some relatively small. Parenchymatous cells contain numberous starch granules (Fig. 2).

Powder

Colour yellowish-white. Starch granules extremely numerous, simple starch granules subspherical, oblong or ovoid, 4-40 μm in diameter, hilum pointed, V-shaped or slit-shaped; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-4 units. Xylem fibres mostly in bundles, pale yellow, 20-30 μm in diameter, walls 3-5 μm thick, with simple pits, lumens filled with starch granules; bright white to yellowish-white under the polarized microscope. Phloem fibres mostly scattered singly, nearly colourless, long spindle-shaped, 15-30 μm in diameter, walls extremely thickened, pit canals indistinct; bright yellowish-white under the polarized microscope. Vessels bordered-pitted, 23-70 μm in diameter, bordered-pits arranged densely. Xylem rays cells subsquare, subrectangular or subpolygonal, with slightly thickened walls and dense pits. Oil cells scattered singly or several in groups, rounded or long-rounded, containing yellowish-brown to brown oily contents (Fig. 3).

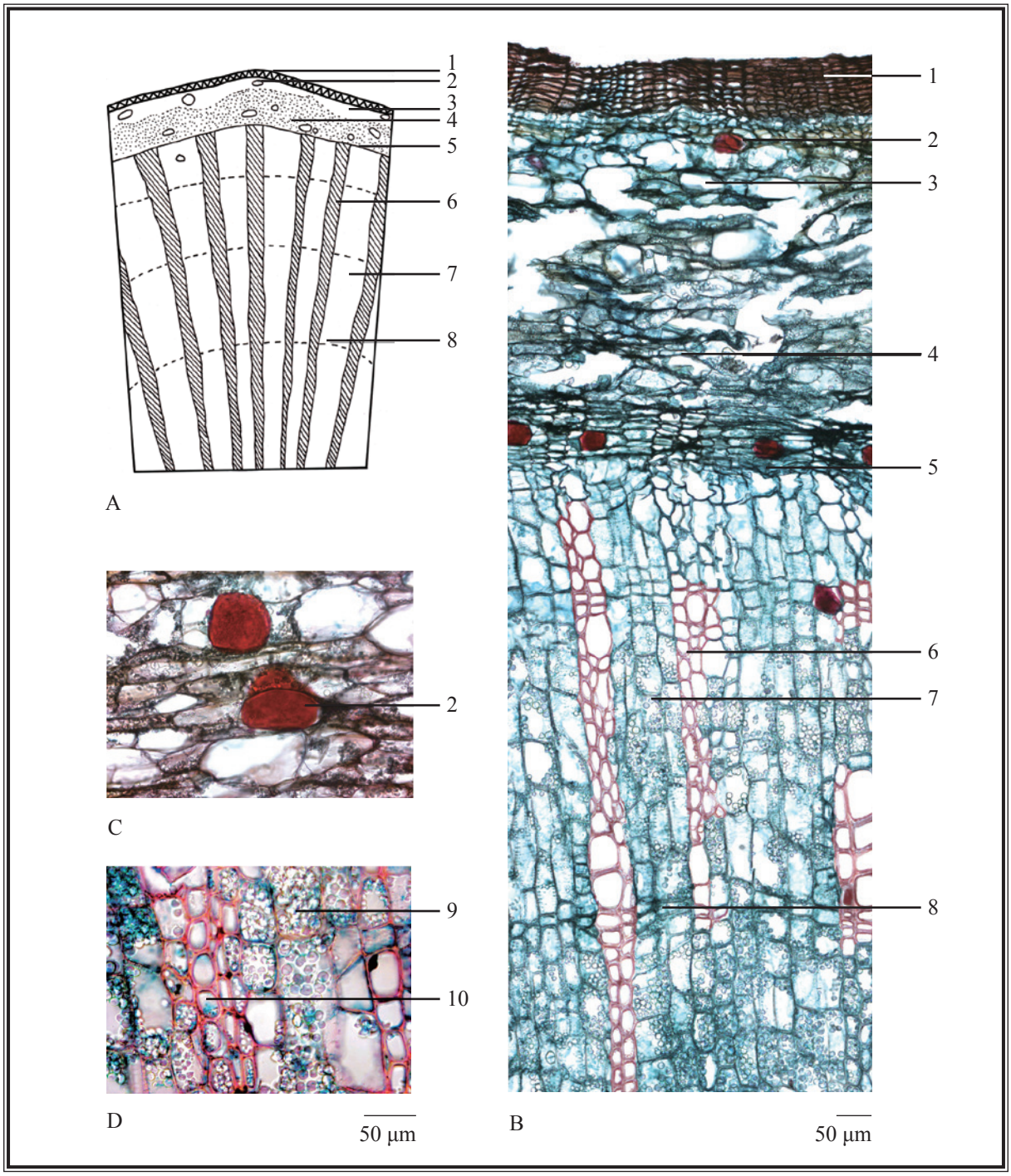


Figure 2 Microscopic features of transverse section of Linderae Radix

A. Sketch B. Section illustration C. Oil cells D. Starch granules

1. Cork 2. Oil cells 3. Cortex 4. Phloem 5. Cambium 6. Xylem
7. Xylem ray 8. Annual ring 9. Starch granules 10. Vessels

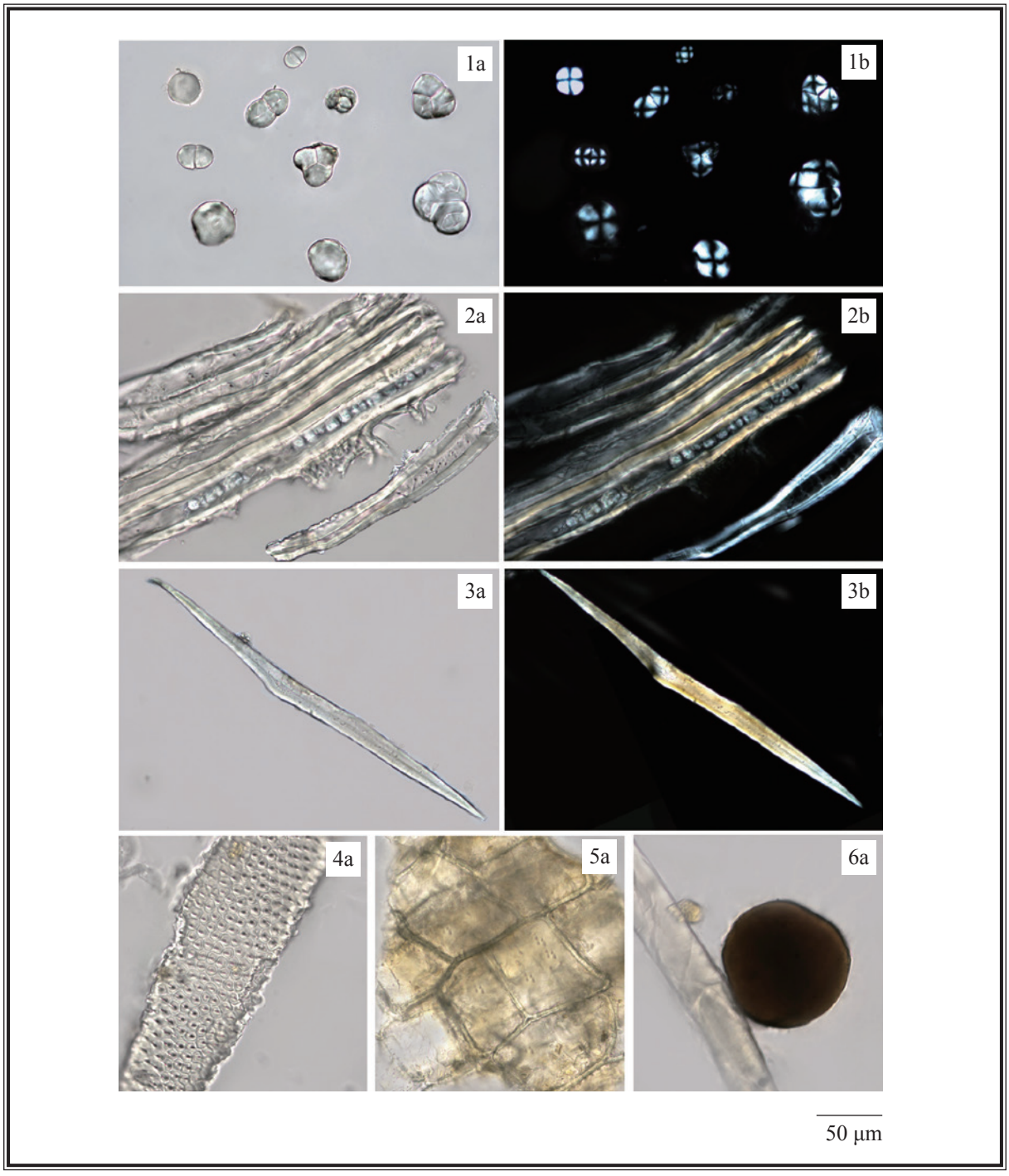


Figure 3 Microscopic features of powder of Linderae Radix

1. Starch granules 2. Xylem fibres 3. Phloem fibre 4. Bordered-pitted vessel
5. Xylem ray cells 6. Oil cell

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

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

Standard solution

Linderane standard solution

Weigh 0.75 mg of linderane CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of *n*-hexane and ethyl acetate (5:1, v/v).

Spray reagent

Weigh 1 g of vanillin and dissolve in 100 mL of sulphuric acid.

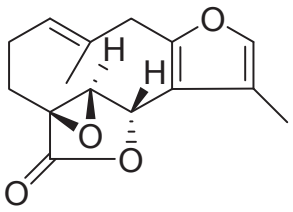
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol. Sonicate (100 W) the mixture for 40 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 linderane standard solution and the test solution (6 μL each) 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 7 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 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

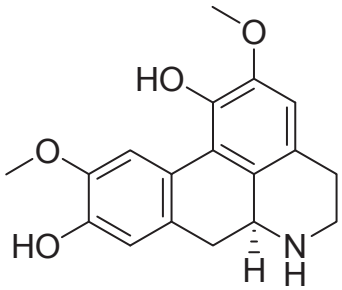


Figure 4 Chemical structures of (i) linderane and (ii) norisoboldine

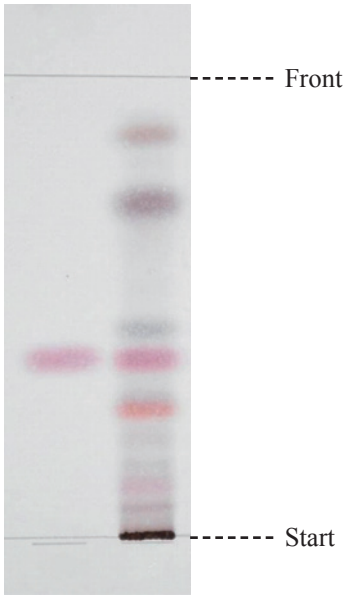


Figure 5 A reference HPTLC chromatogram of Linderae Radix extract observed under visible light after staining

1. Linderane 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 linderane (Fig. 5).

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

Standard solution

Linderane standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of linderane CRS and dissolve in 10 mL of acetone.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of acetone. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 25 mL of acetone. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (235 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)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	70 → 55	30 → 45	linear gradient
20 – 40	55 → 40	45 → 60	linear gradient
40 – 60	40 → 20	60 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of linderane Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of linderane should not be more than 5.0%; the RSD of the retention time of linderane peak should not be more than 2.0%; the column efficiency determined from linderane peak should not be less than 80000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Linderae Radix extract

Peak No.	RRT	Acceptable Range
1	0.78	± 0.03
2	0.88	± 0.03
3 (marker, linderane)	1.00	-

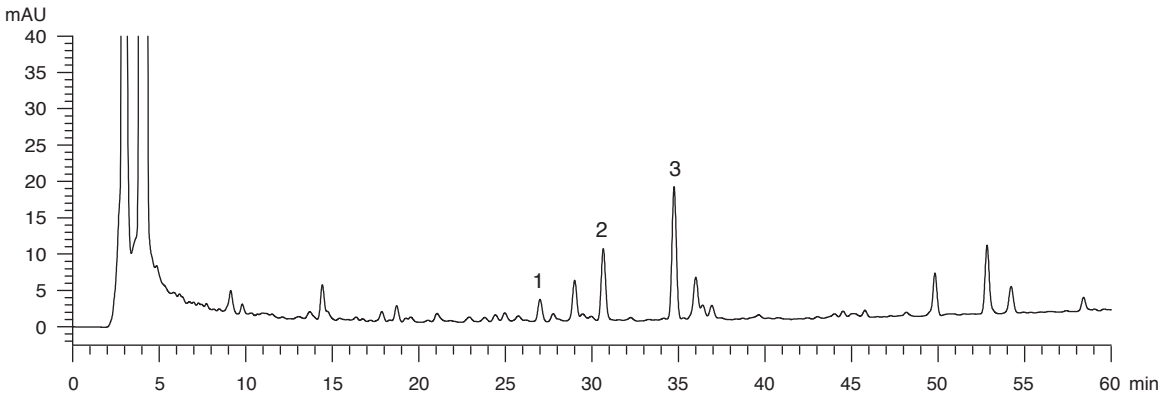


Figure 6 A reference fingerprint chromatogram of Linderae Radix extract

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 1.5%.
Acid-insoluble ash: not more than 1.0%.

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 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

7.1 Assay of Linderane

Standard solution

Linderane standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 1.0 mg of linderane CRS and dissolve in 2 mL of methanol.

Linderane standard solution for assay, Std-AS

Measure accurately the volume of the linderane Std-Stock, dilute with methanol to produce a series of solutions of 10, 20, 40, 60, 100 mg/L for linderane.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for

5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants 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 (235 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 water and acetonitrile (58:42, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of linderane Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of linderane should not be more than 5.0%; the RSD of the retention time of linderane peak should not be more than 2.0%; the column efficiency determined from linderane peak should not be less than 8000 theoretical plates.

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

Limits

The sample contains not less than 0.14% of linderane ($C_{15}H_{16}O_4$), calculated with reference to the dried substance.

7.2 Assay of Norisoboldine

Standard solution

Norisoboldine standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 2.0 mg of norisoboldine CRS (Fig. 4) and dissolve in 1 mL of a mixture of 0.5% hydrochloric acid and methanol (1:2, v/v).

Norisoboldine standard solution for assay, Std-AS

Measure accurately the volume of the norisoboldine Std-Stock, dilute with a mixture of 0.5% hydrochloric acid and methanol (1:2, v/v) to produce a series of solutions of 30, 60, 90, 120, 150 mg/L for norisoboldine.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of a mixture of 0.5% hydrochloric acid and methanol (1:2, v/v). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with a mixture of 0.5% hydrochloric acid and methanol (1:2, v/v). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 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 0.1% triethylamine with 0.5% formic acid and acetonitrile (90:10, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of norisoboldine Std-AS (90 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of norisoboldine should not be more than 5.0%; the RSD of the retention time of norisoboldine peak should not be more than 2.0%; the column efficiency determined from norisoboldine peak should not be less than 6000 theoretical plates.

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

Limits

The sample contains not less than 0.40% of norisoboldine ($C_{18}H_{19}NO_4$), calculated with reference to the dried substance.