Arnebiae Radix

Figure 1 A photograph of Arnebiae Radix

1. NAMES

Official Name: Arnebiae Radix

Chinese Name: 紫草

Chinese Phonetic Name: Zicao

2. SOURCE

Arnebiae Radix is the dried root of *Arnebia euchroma* (Royle) Johnst. (Boraginaceae). The root is collected in spring or autumn, soil removed, and dried under the sun to obtain Arnebiae Radix.

3. DESCRIPTION

Irregularly long-cylindrical, usually twisted, 7-20 cm long, 10-25 mm in diameter. Externally purplish-red to purplish-brown; bark lax, striped-lamellar, usually more than 10 overlapped layers and easily exfoliated. Apex of the root sometimes bearing branched remnants of stem. Texture lax, soft and light in weight, easily broken. Fracture uneven, wood relatively small, yellowish-white to yellow. Odour characteristic; taste slightly bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Rhytidome thick, showing striped-lamellar, many layers overlapped, easily exfoliated. Phloem relatively broad, separating by lines of cell that are arranged longitudinally and transversely, forming many large gaps of tissue. Xylem occupies the larger portion of the root, with clefts; xylem vessels mostly arranged radially, the xylem sometimes off centre to one side, in which case, the phloem in the outside nearly degenerated (Fig. 2).

Powder

Colour dark purplish-red. Suberized cells numerous, polygonal or rounded-polygonal in surface view, walls straight or slightly curved, filling with purplish-red pigment substances, pigment substances gradually dissolved and cells became brown in colour after treatment with a solution of chloral hydrate. Parenchymatous cells relatively numerous, pale brown, mostly filled with purplish-red substances. Vessels mainly reticulate, few bordered-pitted, pale brown or brown, 14-83 μm in diameter, often connected with brown parenchymatous cells. Non-glandular hairs unicellular, extremely long, straight or slightly curved, mostly broken, 13-56 μm in diameter, base enlarged into trumpet-like, walls with longitudinal striations, some lumens contain purplish-red pigments (Fig. 3).

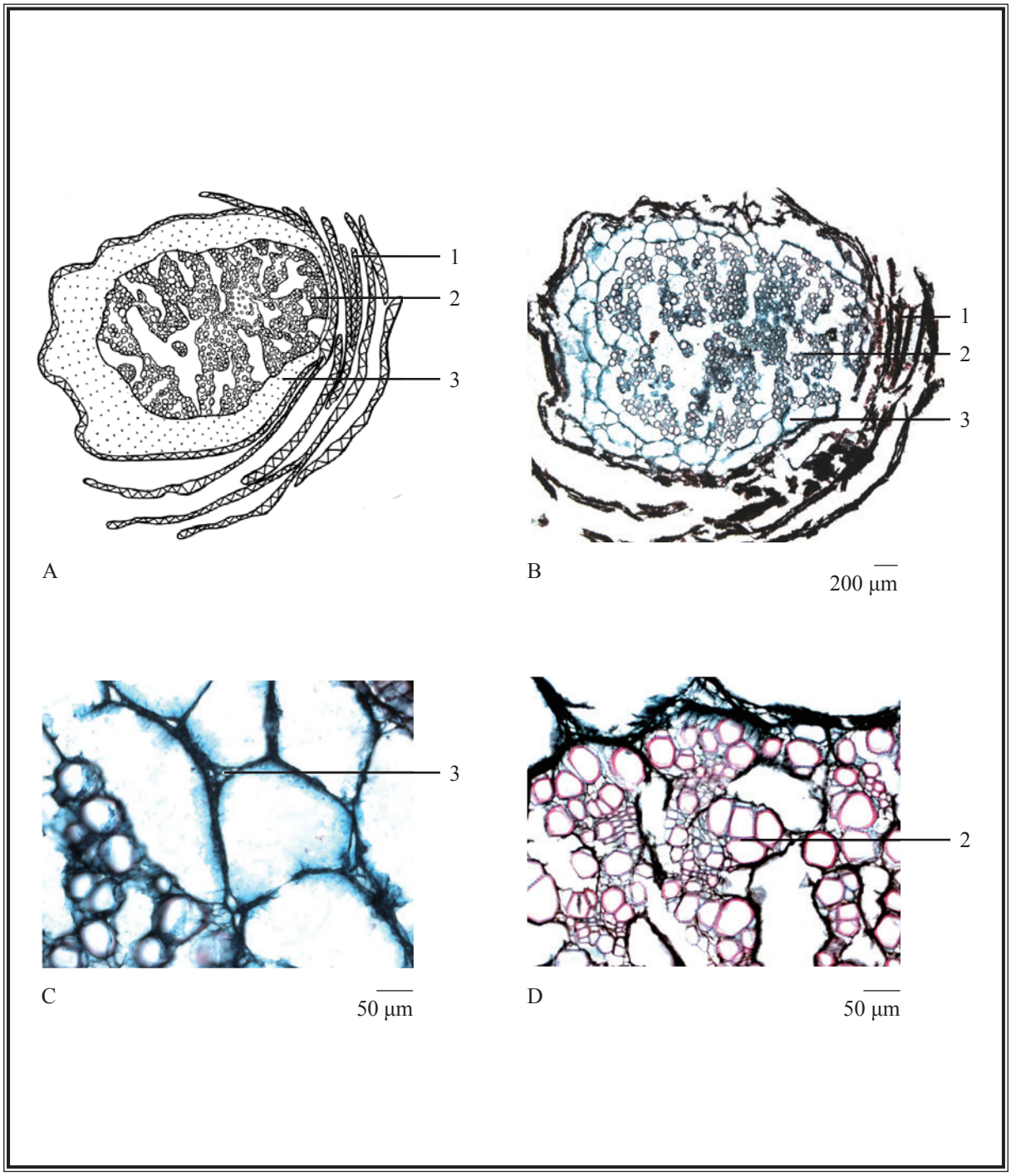

Figure 2 Microscopic features of transverse section of Arnebiae Radix

A. Sketch B. Section illustration C. Phloem D. Xylem

1. Rhytidome 2. Xylem 3. Phloem

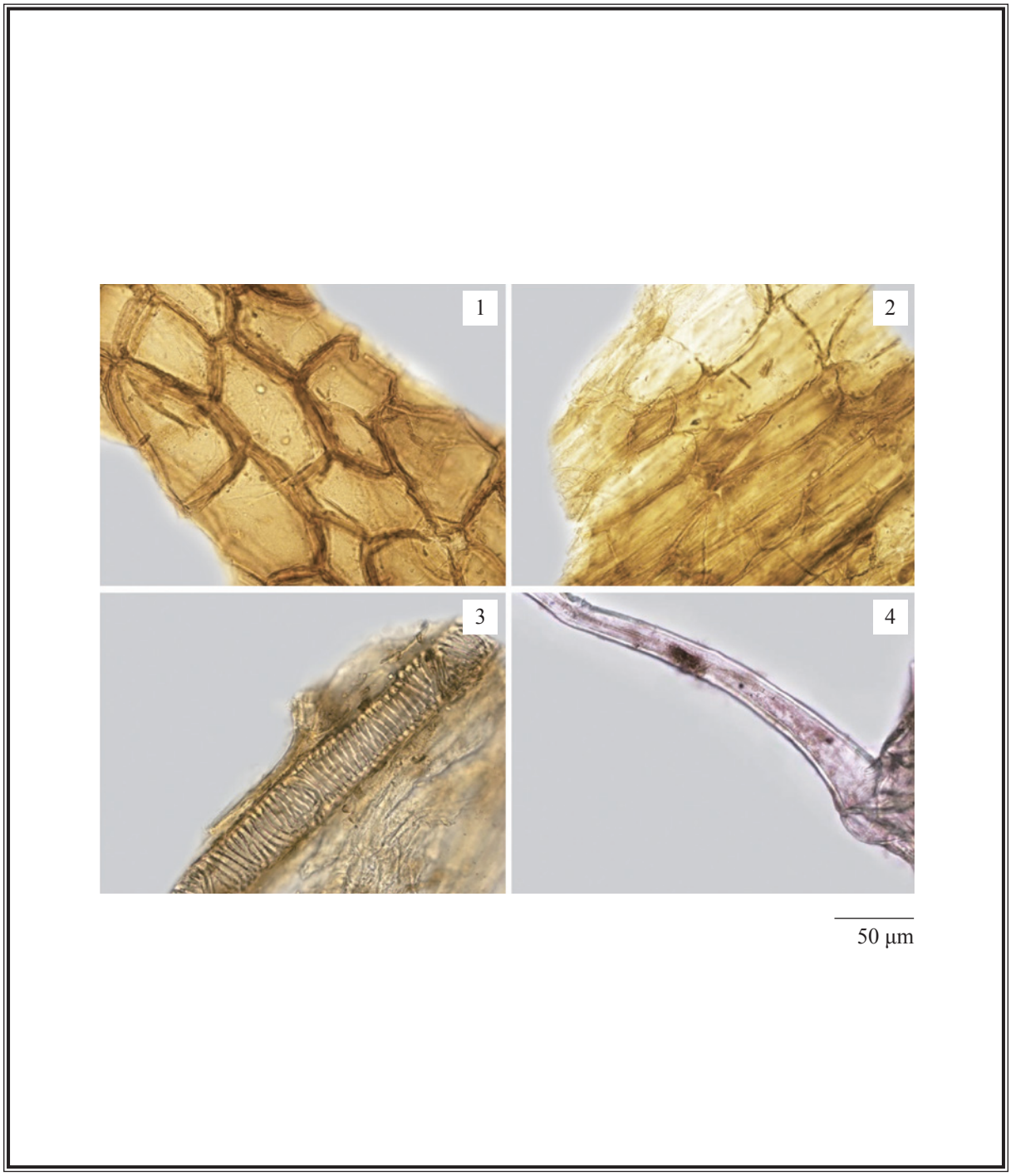

Figure 3 Microscopic features of powder of Arnebiae Radix (under the light microscope)

1. Suberized cells 2. Parenchymatous cells 3. Vessels 4. Non-glandular hair

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

Standard solution

β-Acetoxyisovalerylalkannin standard solution

Weigh 2.0 mg of β-acetoxyisovalerylalkannin CRS (Fig. 4) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with petroleum ether (60-80°C).

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and formic acid (9:2:0.2, 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 petroleum ether (60-80°C). Sonicate (100 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of petroleum ether.

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 β-acetoxyisovalerylalkannin standard solution and the test solution (2 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. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

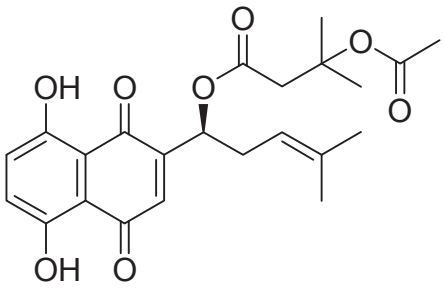

Figure 4 Chemical structure of β-acetoxyisovalerylalkannin

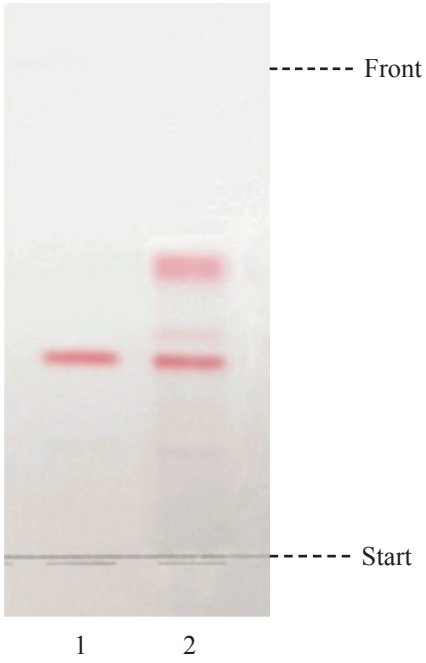

Figure 5 A reference HPTLC chromatogram of Arnebiae Radix extract observed under visible light

1. β-Acetoxyisovalerylalkannin 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 β-acetoxyisovalerylalkannin (Fig. 5).

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

Standard solution

β-Acetoxyisovalerylalkannin standard solution for fingerprinting, Std-FP (140 mg/L)

Weigh 1.4 mg of β-acetoxyisovalerylalkannin CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol.

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 methanol. Transfer the solution to a 10-mL volumetric flask 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 (516 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% formic acid and acetonitrile (30:70, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of β-acetoxyisovalerylalkannin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of β-acetoxyisovalerylalkannin should not be more than 5.0%; the RSD of the retention time of β-acetoxyisovalerylalkannin peak should not be more than 2.0%; the column efficiency determined from β-acetoxyisovalerylalkannin peak should not be less than 16000 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 β-acetoxyisovalerylalkannin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of β-acetoxyisovalerylalkannin peak in the chromatogram of β-acetoxyisovalerylalkannin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify β-acetoxyisovalerylalkannin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of β-acetoxyisovalerylalkannin Std-FP. The retention times of β-acetoxyisovalerylalkannin 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 four characteristic peaks of Arnebiae Radix extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Arnebiae Radix extract

Peak No.	RRT	Acceptable Range
1	0.66	± 0.03
2 (marker, β-acetoxyisovalerylalkannin)	1.00	-
3	1.25	± 0.03
4	1.67	± 0.03

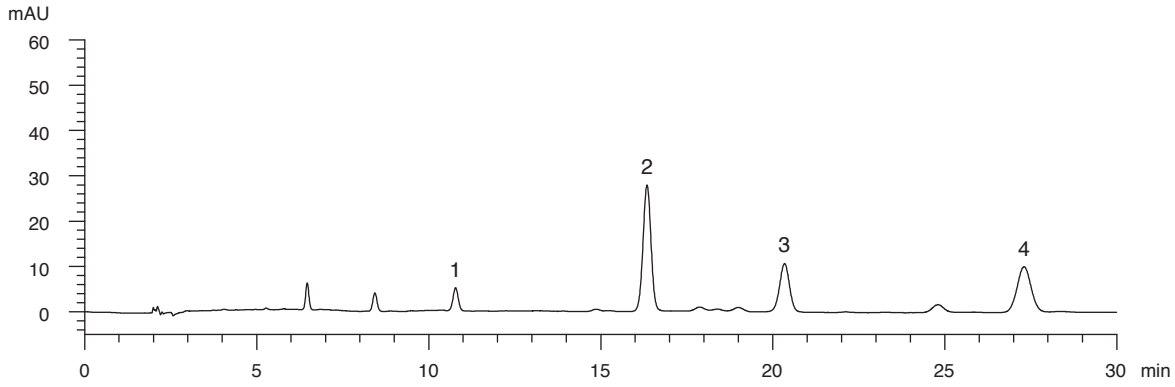

Figure 6 A reference fingerprint chromatogram of Arnebiae Radix extract

For positive identification, the sample must give the above four 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 15.0%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 2.0%.
Ethanol-soluble extractives (hot extraction method): not less than 1.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 4.0 mg of β-acetoxyisovalerylalkannin CRS and place it in a 2-mL amber glass volumetric flask. Make up to the mark with methanol.

β-Acetoxyisovalerylalkannin standard solution for assay, Std-AS

Measure accurately the volume of the β-acetoxyisovalerylalkannin Std-Stock, dilute with methanol to produce a series of solutions of 6, 30, 60, 140, 460 mg/L for β-acetoxyisovalerylalkannin.

Test solution

Weigh accurately 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 methanol. Transfer the solution to a 10-mL volumetric flask 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 (516 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% formic acid and acetonitrile (30:70, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of β-acetoxyisovalerylalkannin Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of β-acetoxyisovalerylalkannin should not be more than 5.0%; the RSD of the retention time of β-acetoxyisovalerylalkannin peak should not be more than 2.0%; the column efficiency determined from β-acetoxyisovalerylalkannin peak should not be less than 16000 theoretical plates.

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

Limits

The sample contains not less than 0.10% of β-acetoxyisovalerylalkannin ($C_{23}H_{26}O_8$), calculated with reference to the dried substance.