Inulae Radix

Figure 1 A photograph of Inulae Radix

A. Inulae Radix B. Transverse section of root
C. Magnified image of transverse section of root

1. NAMES

Official Name: Inulae Radix

Chinese Name: 土木香

Chinese Phonetic Name: Tumuxiang

2. SOURCE

Inulae Radix is the dried root of *Inula helenium* L. (Asteraceae). The root is collected in autumn, soil and foreign matter removed, then dried under the sun to obtain Inulae Radix.

3. DESCRIPTION

Conical, slightly curved, 5-29 cm long. Externally yellowish-brown or dark brown, with longitudinal wrinkles and fibrous root scars. Root vertex large with sunken stem scars and remnants of leaf sheath, surrounded by cylindrical rootlets. Texture hard, uneasily broken. Fracture slightly even, yellowish-white to pale greyish-yellow, with dented-dotted oil cavities. Odour slightly fragrant; taste bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex consists of around 10 layers of tangential elongated parenchymatous cells. Phloem broad. Cambium arranged in ring, indistinct. Xylem vessel rare, singly scattered or several in groups, arranged radially. Xylem fibres rare, in bundles, surrounding the vessels at the centre of xylem. Oil cavity 50-350 μm in diameter, scattered in the phloem and xylem (Fig. 2).

Powder

Colour pale yellowish-brown to brown. Inulin abundant, colourless, in irregular pieces; white under the polarized microscope. Vessels mainly reticulate, 10-83 μm in diameter. Cork cells yellowish-brown, polygonal. Oil cavities occasionally found, mostly fractured. Xylem fibres long-fusiform, with oblique ends and oblique pits (Fig. 3).

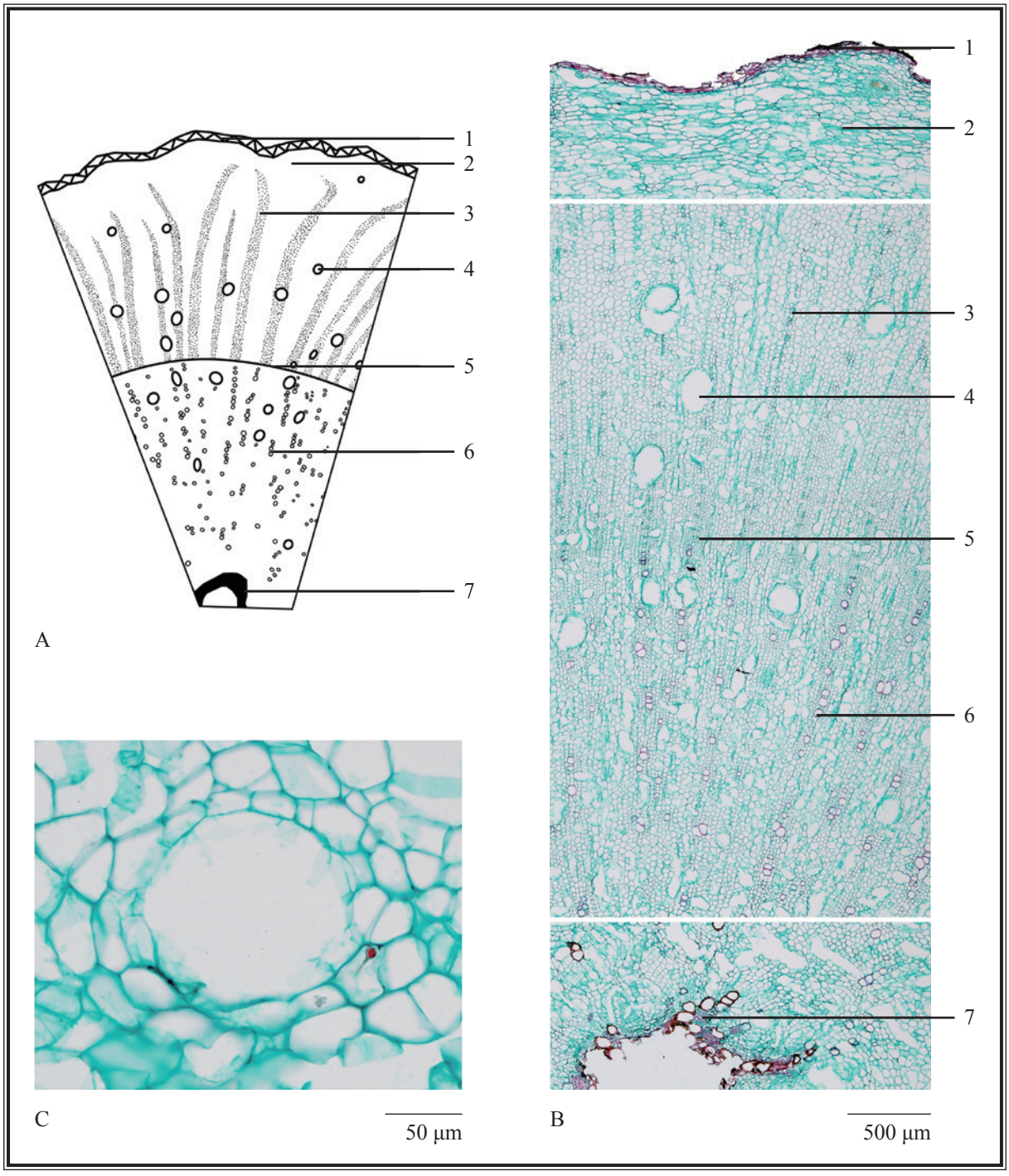

Figure 2 Microscopic features of transverse section of Inulae Radix

A. Sketch B. Section illustration C. Oil Cavity

1. Cork 2. Cortex 3. Phloem 4. Oil cavity 5. Cambium 6. Xylem 7. Xylem fibres

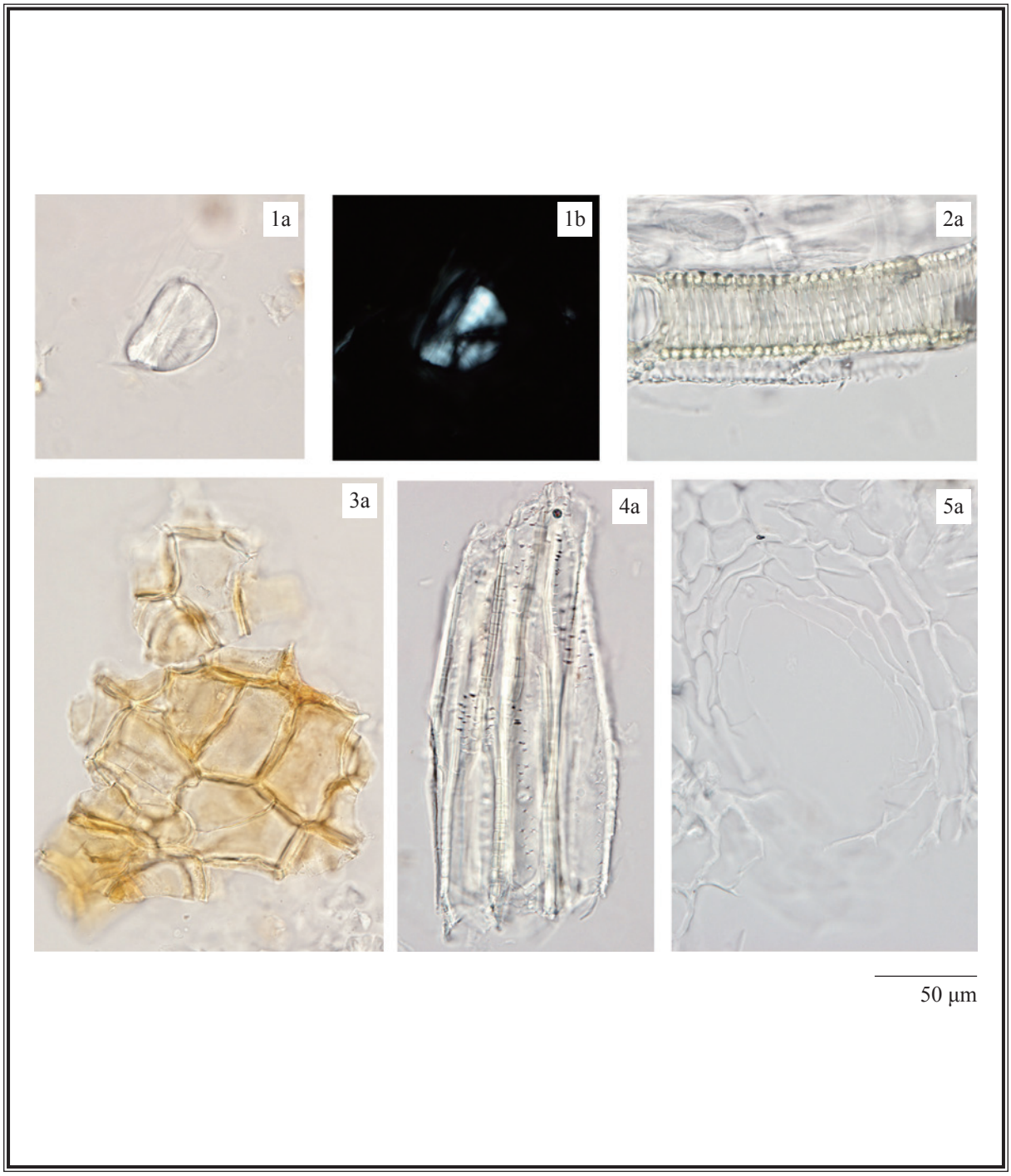

Figure 3 Microscopic features of powder of Inulae Radix

1. Inulin 2. Reticulate vessel 3. Cork cells 4. Xylem fibres 5. Fragment of oil cavity

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

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

Standard solutions

Alantolactone standard solution

Weigh 1.0 mg of alantolactone CRS (Fig. 4) and dissolve in 1 mL of methanol.

Isoalantolactone standard solution

Weigh 1.0 mg of isoalantolactone 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

Mix cautiously 25 mL of sulphuric acid (20%, v/v) into 25 mL of ice-cold glacial acetic acid. Add 2.5 mL of *p*-anisaldehyde. Add further 50 mL of sulphuric acid (20%, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (220 W) the mixture for 30 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 5 mL of methanol.

Procedure

Carry out the method by using a TLC plate of silica gel with 5% silver nitrate and a freshly prepared developing solvent system as described above. Apply separately alantolactone standard solution (2 μL), isoalantolactone standard solution (2 μL) and the test solution (1 μL) to the plate. Develop over a path of about 8 cm and analyze within 30 min. 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 15 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

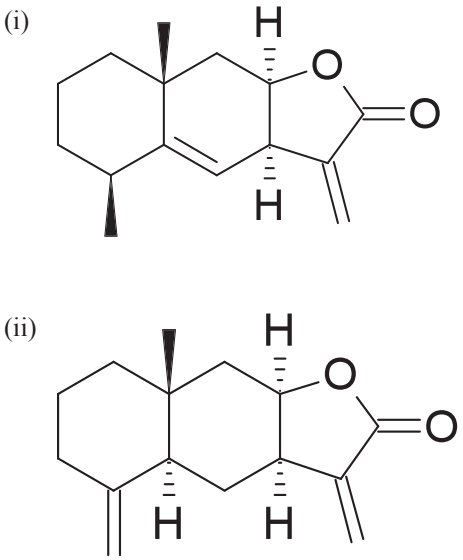

Figure 4 Chemical structures of (i) alantolactone and (ii) isoalantolactone

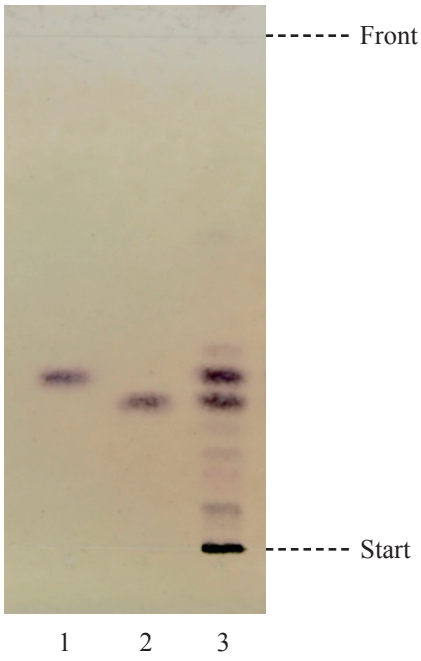

Figure 5 A reference TLC chromatogram of Inulae Radix extract observed under visible light after staining

1. Alantolactone standard solution 2. Isoalantolactone 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 alantolactone and isoalantolactone (Fig. 5).

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

Standard solutions

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

Weigh 0.5 mg of alantolactone CRS and dissolve in 10 mL of methanol.

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

Weigh 0.5 mg of isoalantolactone CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. 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 (194 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% phosphoric acid and acetonitrile (45:55, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of alantolactone Std-FP and isoalantolactone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of alantolactone and isoalantolactone should not be more than 5.0%; the RSD of the retention times of alantolactone and isoalantolactone peaks should not be more than 2.0%; the column efficiencies determined from alantolactone and isoalantolactone peaks should not be less than 10000 theoretical plates.

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 1.0 (Fig. 6).

Procedure

Separately inject alantolactone Std-FP, isoalantolactone Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of alantolactone and isoalantolactone peaks in the chromatograms of alantolactone Std-FP, isoalantolactone Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify alantolactone and isoalantolactone peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of alantolactone Std-FP and isoalantolactone Std-FP. The retention times of alantolactone and isoalantolactone 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 four characteristic peaks of Inulae Radix extract are listed in Table 1.

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

Peak No.	RRT	Acceptable Range
1	0.87	± 0.03
2 (isoalantolactone)	0.94	± 0.03
3 (marker, alantolactone)	1.00	-
4	1.16	± 0.03

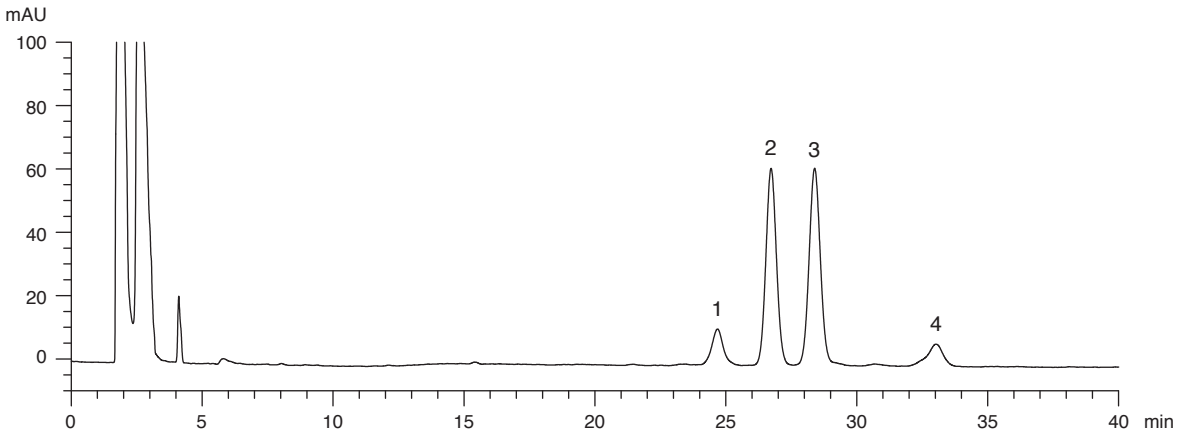

Figure 6 A reference fingerprint chromatogram of Inulae Radix extract

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 6.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 51.0%.
Ethanol-soluble extractives (cold extraction method): not less than 35.0%.

7. ASSAY

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

Standard solution

Mixed alantolactone and isoalantolactone standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of alantolactone CRS and 2.0 mg of isoalantolactone CRS, and dissolve in 10 mL of ethyl acetate.

Mixed alantolactone and isoalantolactone standard solution for assay, Std-AS

Measure accurately the volume of the mixed alantolactone and isoalantolactone Std-Stock, dilute with ethyl acetate to produce a series of solutions of 3, 5, 10, 20, 30 mg/L for both alantolactone and isoalantolactone.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of ethyl acetate. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Wash the residues for two times each with 5 mL of ethyl acetate. Combine the solutions and make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-5, 0.32 mm × 30 m) of which the internal wall is covered with (5%- phenyl)-methylpolysiloxane in a layer about 0.25 µm thick. The injection temperature is at 260°C. The detector temperature is at 280°C. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 2) –

Table 2 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 30	190	-
30 – 35	190 → 240	10
35 – 40	240	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of the mixed alantolactone and isoalantolactone Std-AS (10 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of alantolactone and isoalantolactone should not be more than 5.0%; the RSD of the retention times of alantolactone and isoalantolactone peaks should not be more than 2.0%; the column efficiencies determined from alantolactone and isoalantolactone peaks should not be less than 30000 theoretical plates.

The *R* value between alantolactone peak and the closest peak; and the *R* value between isoalantolactone 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 alantolactone and isoalantolactone Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of alantolactone and isoalantolactone against the corresponding concentrations of the mixed alantolactone and isoalantolactone Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify alantolactone and isoalantolactone peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed alantolactone and isoalantolactone Std-AS. The retention times of alantolactone and isoalantolactone 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 alantolactone and isoalantolactone in the test solution, and calculate the percentage contents of alantolactone and isoalantolactone in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 2.3% of the total content of alantolactone ($C_{15}H_{20}O_2$) and isoalantolactone ($C_{15}H_{20}O_2$), calculated with reference to the dried substance.