# Homalomenae Rhizoma


**Figure 1** A photograph of Homalomenae Rhizoma

A. Homalomenae Rhizoma B. Magnified image of fracture surface
C. Magnified image of cut surface

## 1. NAMES

Official Name: Homalomenae Rhizoma

Chinese Name: 千年健

Chinese Phonetic Name: Qiannianjian

## 2. SOURCE

Homalomenae Rhizoma is the dried rhizome of *Homalomena occulta* (Lour.) Schott (Araceae). The rhizome is collected in spring and autumn, washed clean, outer bark and foreign matter removed, then dried under the sun to obtain Homalomenae Rhizoma.

## 3. DESCRIPTION

Cylindrical, sometimes slightly flattened, straight or curved, rarely branched, 4-21 mm in diameter. Externally yellowish-brown to reddish-brown, coarse, with distinct longitudinal furrows, occasionally with rounded root scars and leaf bases remnants at the node. Texture hard, when broken, with yellow brush-like fibre bundles on broken side, holes on the other side; cut surface yellowish-white, reddish-brown or pinkish-purple, with sparse brown spots. Odour characteristic and aromatic; taste pungent, slightly bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse Section**

Cork consists of several layers of flattened cells, sometimes removed. Ground tissue scattered with clusters of calcium oxalate, fibre bundles, secretory cavities, vascular bundles and mucilage cells containing raphides of calcium oxalate, sometimes also with pigment masses. Fibres with thickened wall, in bundles, lignified. Secretory cavities large, varied in size, surrounded by suberized cells. Vascular bundles collateral and amphivasal, usually accompanied by fibre bundles (Fig. 2).

**Powder**

Colour yellowish-brown to reddish-brown. Clusters of calcium oxalate numerous, 17-68 μm in diameter; polychromatic under the polarized microscope. Raphides of calcium oxalate slender, needle-shaped, 20-131 μm long, usually in bundles, present in the mucilage cells or scattered; polychromatic under the polarized microscope. Fibres colourless or pale pinkish-purple, 8-83 μm in diameter, usually broken, sometimes in bundles, walls slightly thick or thick, lignified, with distinct pits and pit canals; bright white under the polarized microscope. Cork cells pale brown, subrectangular, subsquare, polygonal or elongated-polygonal in surface view. Stone cells occasionally found, subsquare or rectangular, single or in groups; yellowish-white under the polarized microscope. Vessels usually in groups, 6-68 μm in diameter, mainly spiral and scalariform (Fig. 3).

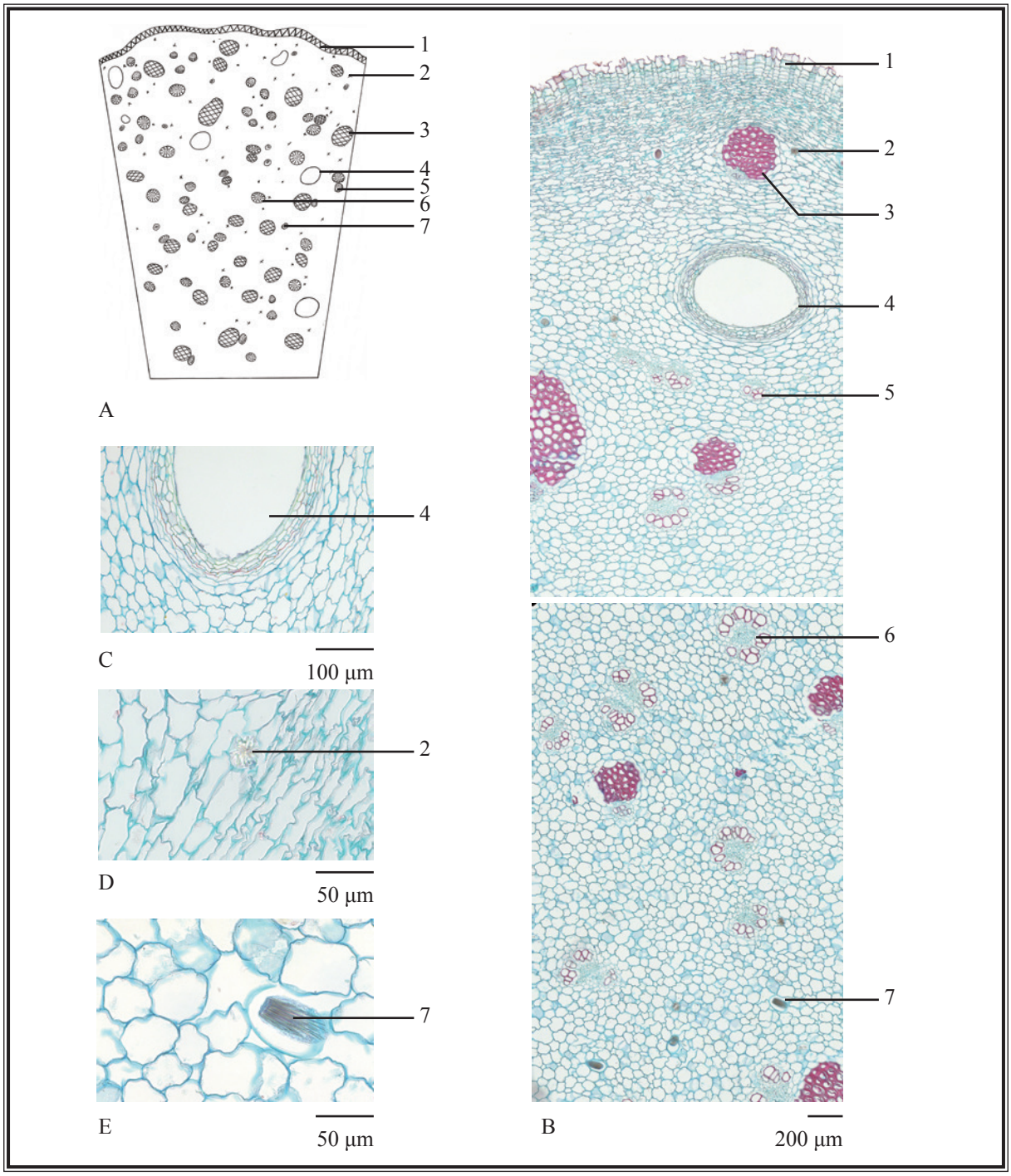


**Figure 2** Microscopic features of transverse section of Homalomenae Rhizoma

A. Sketch B. Section illustration C-E. Section magnified

1. Cork 2. Cluster of calcium oxalate 3. Fibre bundle 4. Secretory cavity
5. Collateral vascular bundle 6. Amphivasal vascular bundle
7. Mucilage cell containing raphides of calcium oxalate

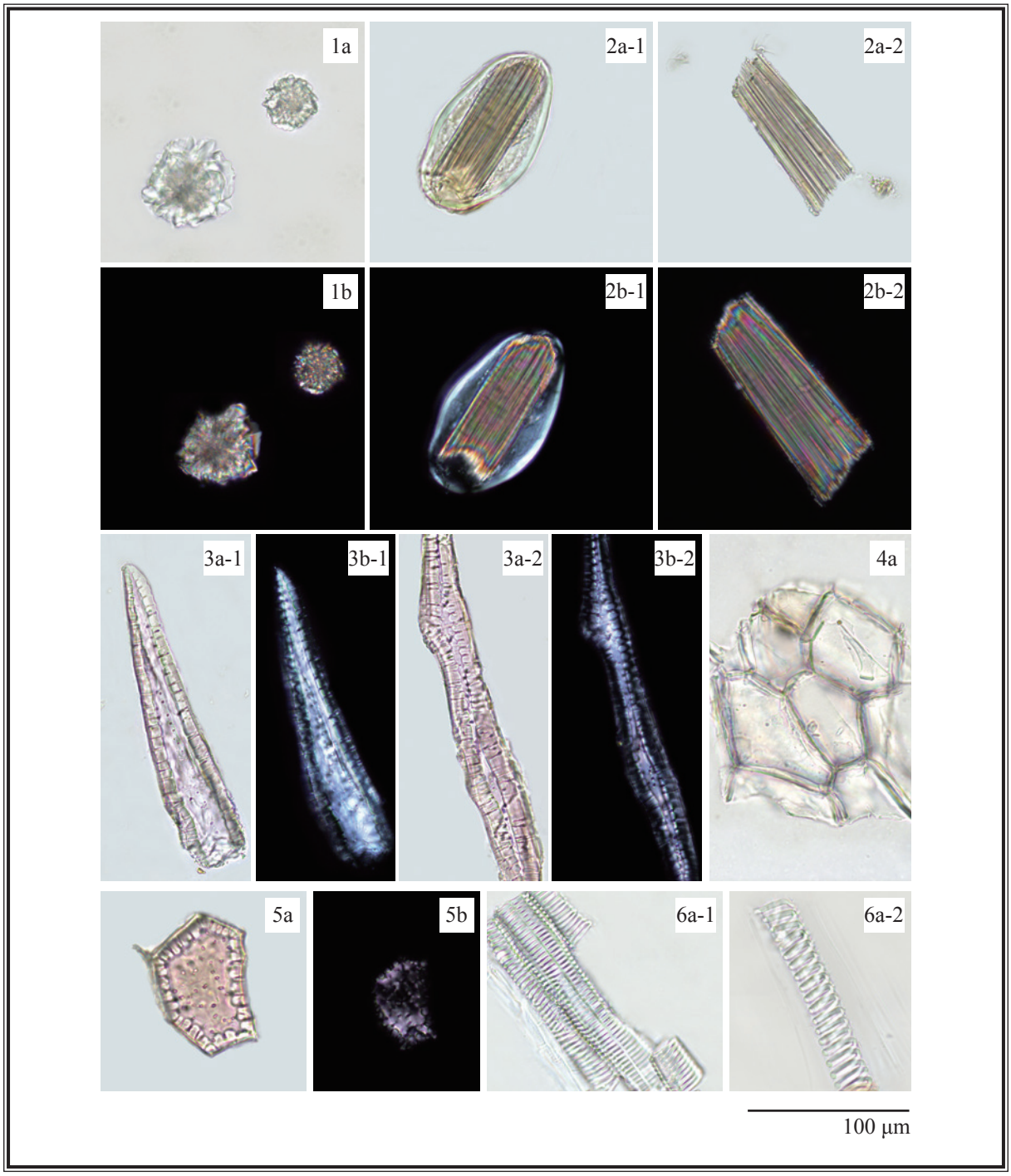


**Figure 3** Microscopic features of powder of Homalomenae Rhizoma

1. Clusters of calcium oxalate 2. Raphides of calcium oxalate (2-1 in mucilage cell, 2-2 scattered)
3. Fibres (3-1 wall slightly thick, 3-2 wall thick) 4. Cork cells 5. Stone cell
6. Vessels (6-1 scalariform vessels, 6-2 spiral vessel)

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

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

**Standard solution**

*Linalool standard solution*

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

**Developing solvent system**

Prepare a mixture of dichloromethane and ethyl acetate (9:1, v/v).

**Spray reagent**

Add slowly 10 mL of sulphuric acid to 100 mL of ethanol and dissolve 6 g of vanillin. Freshly prepare the reagent.

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of *n*-hexane. Cap the flask. Sonicate (300 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 methanol. Filter through a 0.45-μm nylon filter.

**Procedure**

Carry out the method by using a HPTLC silica gel G60 plate and a freshly prepared developing solvent system as described above. Apply separately linalool standard solution and the test solution (2 μL each) to the plate. 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 visible light. Calculate the $R_f$ value by using the equation as indicated in Appendix IV (A).

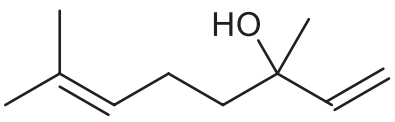


**Figure 4** Chemical structure of linalool

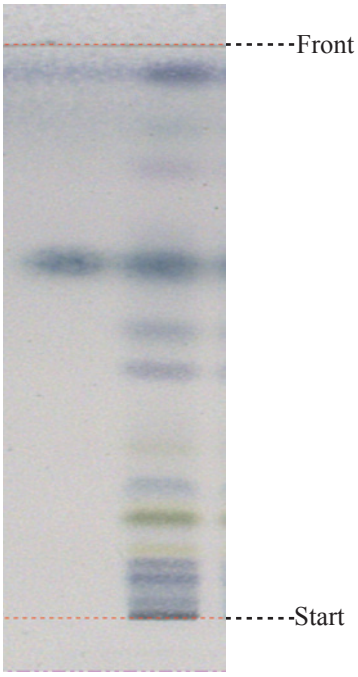


**Figure 5** A reference HPTLC chromatogram of Homalomenae Rhizoma extract observed under visible light after staining

1. Linalool 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 linalool (Fig. 5).

## 4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

**Standard solution**

*Linalool standard solution for fingerprinting, Std-FP (15 mg/L)*

Weigh 0.3 mg of linalool CRS and dissolve in 20 mL of *n*-hexane.

**Test solution**

Weigh 0.25 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of *n*-hexane. Cap the flask. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with *n*-hexane. Combine the solutions and make up to the mark with *n*-hexane. Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The gas chromatograph is equipped with a FID and a capillary column (DB-5 MS, 0.25 mm × 30 m) of which the internal wall is covered with phenyl arylene polymer in a layer about 0.25 µm thick. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 4:1 is used. Programme the chromatographic system as follows (Table 1) –

**Table 1** Chromatographic system conditions

| Time (min) | Temperature (ºC) | Rate (ºC/min) |
|---|---|---|
| 0 – 15 | 70 → 100 | 2 |
| 15 – 43 | 100 → 240 | 5 |
| 43 – 45 | 240 → 280 | 20 |

**System suitability requirements**

Perform at least five replicate injections, each using 2 µL of linalool Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of linalool should not be more than 5.0%; the RSD of the retention time of linalool peak should not be more than 2.0%; the column efficiency determined from linalool peak should not be less than 150000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

**Procedure**

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

**Table 2** The RRTs and acceptable ranges of the five characteristic peaks of Homalomenae Rhizoma extract

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 (marker, linalool) | 1.00 | - |
| 2 | 1.58 | ± 0.03 |
| 3 | 2.63 | ± 0.03 |
| 4 | 2.70 | ± 0.03 |
| 5 | 2.79 | ± 0.03 |

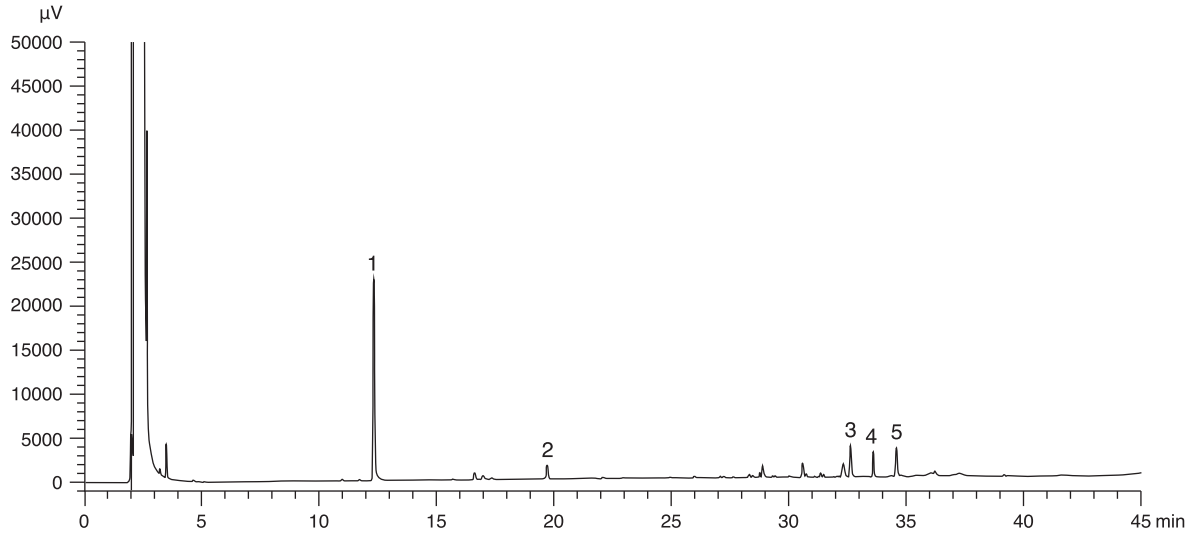


**Figure 6** A reference GC fingerprint chromatogram of Homalomenae Rhizoma extract

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 GC fingerprint chromatogram (Fig. 6).

## 5. TESTS

**5.1 Heavy Metals** *(Appendix V)*: The CMM shall meet the requirements for arsenic, lead and mercury as specified in Appendix V. For cadmium, Homalomenae Rhizoma should meet the specified limit of not more than 2.0 mg/kg, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limit for cadmium specified in Appendix V shall be applied.

**5.2 Pesticide Residues** *(Appendix VI)*: meet the requirements.

**5.3 Mycotoxins – Aflatoxins** *(Appendix VII)*: meet the requirements.

**5.4 Sulphur Dioxide Residues** *(Appendix XVI)*: meet the requirements.

**5.5 Foreign Matter** *(Appendix VIII)*: not more than 2.0%.

**5.6 Ash** *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 0.5%.

**5.7 Water Content** *(Appendix X)*

Toluene distillation method: not more than 8.0%.

## 6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 16.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

## 7. ASSAY

### 7.1 Assay of Linalool

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

**Standard solution**

*Linalool standard stock solution, Std-Stock (800 mg/L)*

Weigh accurately 4.0 mg of linalool CRS and dissolve in 5 mL of *n*-hexane.

*Linalool standard solution for assay, Std-AS*

Measure accurately the volume of the linalool Std-Stock, dilute with *n*-hexane to produce a series of solutions of 0.8, 4, 16, 64, 120 mg/L for linalool.

**Test solution**

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of *n*-hexane. Cap the flask. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with *n*-hexane. Combine the solutions and make up to the mark with *n*-hexane. Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The gas chromatograph is equipped with a FID and a capillary column (DB-5 MS, 0.25 mm × 30 m) of which the internal wall is covered with phenyl arylene polymer in a layer about 0.25 µm thick. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 4:1 is used. Programme the chromatographic system as follows (Table 3) –

**Table 3** Chromatographic system conditions

| Time (min) | Temperature (ºC) | Rate (ºC/min) |
|---|---|---|
| 0 – 15 | 70 → 100 | 2 |
| 15 – 43 | 100 → 240 | 5 |
| 43 – 45 | 240 → 280 | 20 |

**System suitability requirements**

Perform at least five replicate injections, each using 2 µL of linalool Std-AS (16 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of linalool should not be more than 5.0%; the RSD of the retention time of linalool peak should not be more than 2.0%; the column efficiency determined from linalool peak should not be less than 150000 theoretical plates.

The *R* value between linalool peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

**Calibration curve**

Inject a series of linalool Std-AS (2 µL each) into the GC system and record the chromatograms. Plot the peak areas of linalool against the corresponding concentrations of linalool Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

**Procedure**

Inject 2 µL of the test solution into the GC system and record the chromatogram. Identify linalool peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of linalool Std-AS. The retention times of linalool 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 linalool in the test solution, and calculate the percentage content of linalool in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The sample contains not less than 0.20% of linalool ($C_{10}H_{18}O$), calculated with reference to the dried substance.

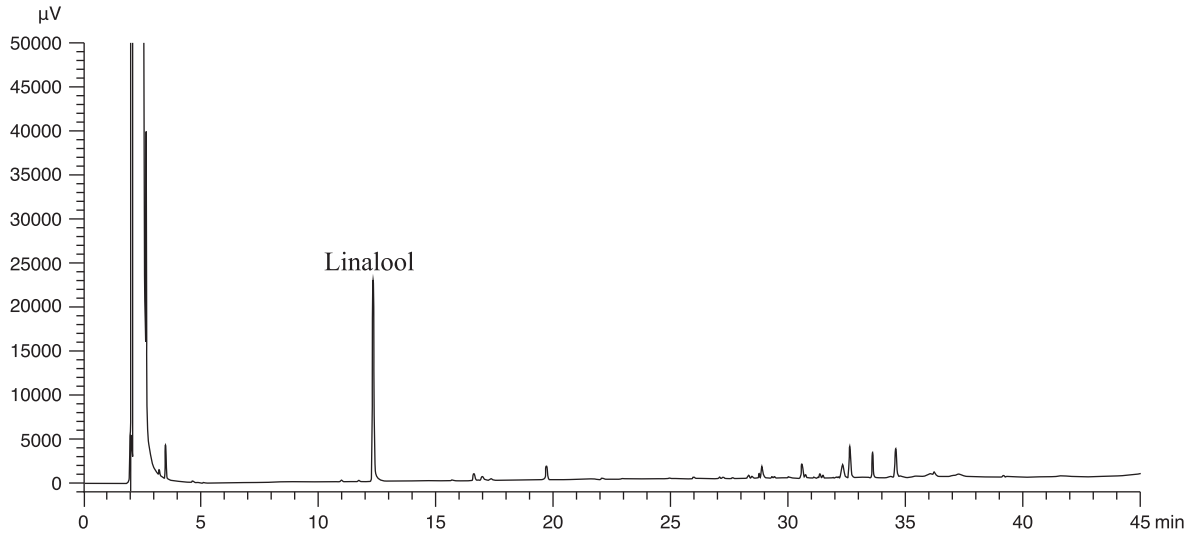


**Figure 7** A reference GC assay chromatogram of Homalomenae Rhizoma extract

### 7.2 Assay of Volatile Oil

Weigh accurately 70 g of the 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 0.53% (v/w) of volatile oil.

## 8. CAUTION

This CMM should be used after proper processing (such as decoction).