# Cyperi Rhizoma


**Figure 1** A photograph of Cyperi Rhizoma

A. Cyperi Rhizoma B. Magnified rhizome C. Fracture of unsteamed rhizome
D. Fracture of steamed rhizome

## 1. NAMES

Official Name: Cyperi Rhizoma

Chinese Name: 香附

Chinese Phonetic Name: Xiangfu

## 2. SOURCE

Cyperi Rhizoma is the dried rhizome of *Cyperus rotundus* L. (Cyperaceae). The rhizome is collected in autumn and fibrous roots are burnt off, dried under the sun; or boiled briefly or steamed thoroughly, then dried under the sun to obtain Cyperi Rhizoma.

## 3. DESCRIPTION

Fusiform, some slightly curved, 1.1-3.7 cm long, 4-11 mm in diameter. Externally dark brown to blackish-brown, with longitudinal wrinkles and 4-13 slightly prominent annular nodes containing brown fibrous roots and remnants of root-scars. For those rhizomes with completely removed fibrous roots, annular nodes indistinct, outer surface relatively smoother. Texture hard. Fracture of steamed rhizomes yellowish-brown to reddish-brown, horny; fracture of unsteamed rhizomes white and starchy, with obvious endodermis ring, stele dark, with scattered dotted vascular bundles. Odour aromatic; taste slightly bitter (Fig. 1).

## 4. IDENTIFICATION

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

**Transverse section**

Epidermal cells pale yellow to brownish-yellow, always accompanied by hypodermal fibre bundles. Hypodermis underneath epidermis, consisting of 2-3 layers of sclerenchymatous cells. Cortex consists of parenchymatous cells, numerous secretory cells and a few collateral vascular bundles. Collateral vascular bundles surrounded by endodermis. Endodermal cells subsquare to rectangular, arranged in a ring. Stele consists of numerous amphivasal vascular bundles, secretory cells and parenchymatous cells. Parenchymatous cells filled with starch granules in unsteamed rhizome or starch gelatinous masses in steamed rhizome (Fig. 2).

**Powder**

Colour pale brown to brown. Hypodermal fibres in bundles, reddish-brown to dark brown, 5-25 μm in diameter, wall thickened. Epidermal cells pale yellow to brownish-yellow, polygonal, 12-34 μm in diameter. Secretory cells subrounded, 20-72 μm in diameter, containing pale yellowish-brown to reddish-brown secretion, with 4-10 adjacent cells radially arranged in a circle, mostly broken. Parenchymatous cells numerous, subrounded, containing starch granules in unsteamed rhizome or starch gelatinous masses in steamed rhizome. Hypodermal cells (sclerenchymatous cells) pale yellow, subsquare, subrounded or irregular, walls slightly thickened. Starch granules numerous, subrounded or ovoid to ellipsoid, 4-29 μm in diameter, hilum indistinct; black and cruciate-shaped under the polarized microscope. Stone cells rare, reddish-brown, subsquare, subrounded or subpolygonal, wall thickened (Fig. 3).

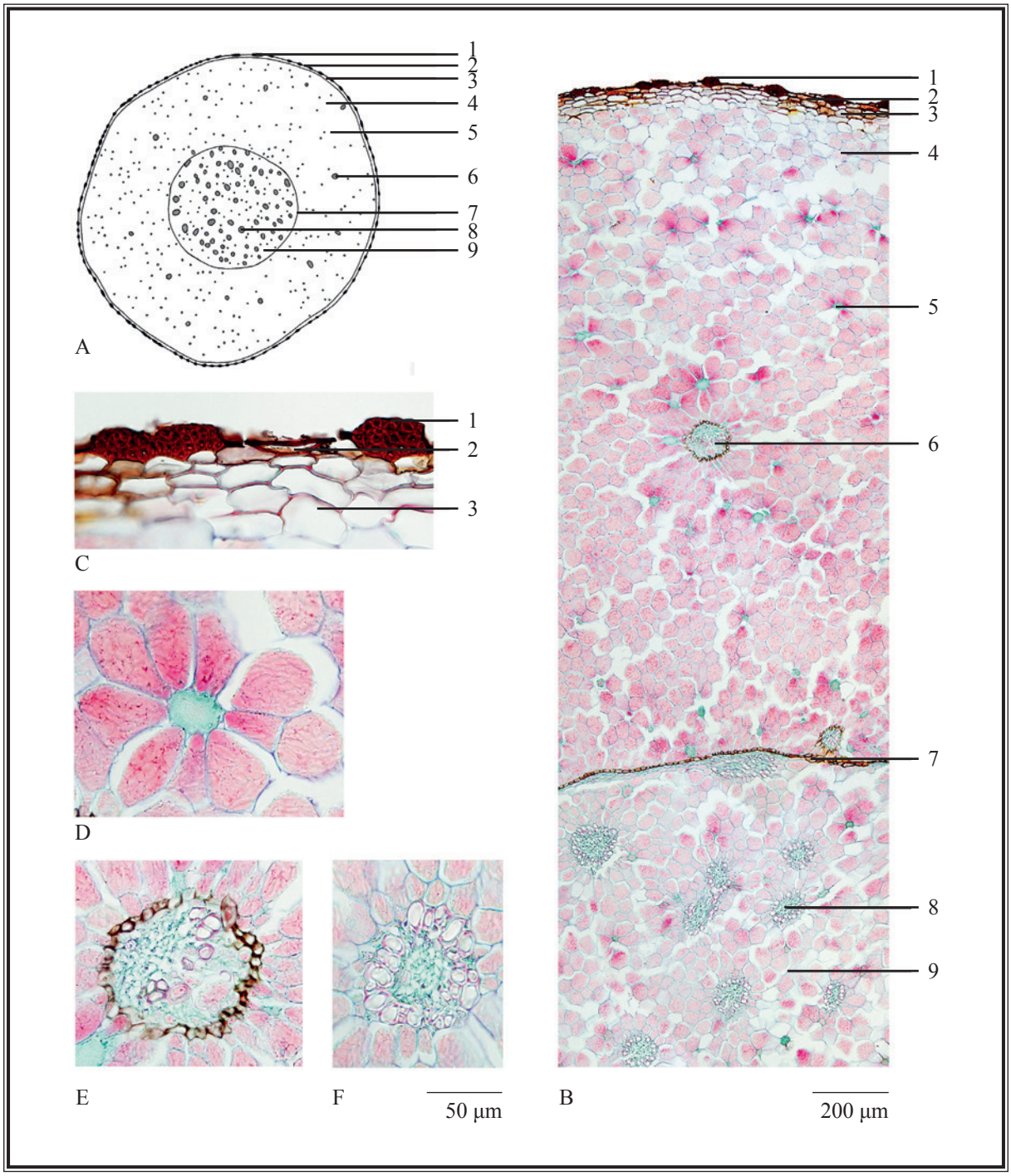


**Figure 2** Microscopic features of transverse section of Cyperi Rhizoma

A. Sketch B. Section illustration C. Epidermal layer D. Secretory cell
E. Vascular bundle (collateral type) F. Vascular bundle (amphivasal type)

1. Hypodermal fibre bundles 2. Epidermis 3. Hypodermis 4. Cortex
5. Secretory cell 6. Vascular bundle (collateral type) 7. Endodermis
8. Vascular bundle (amphivasal type) 9. Stele

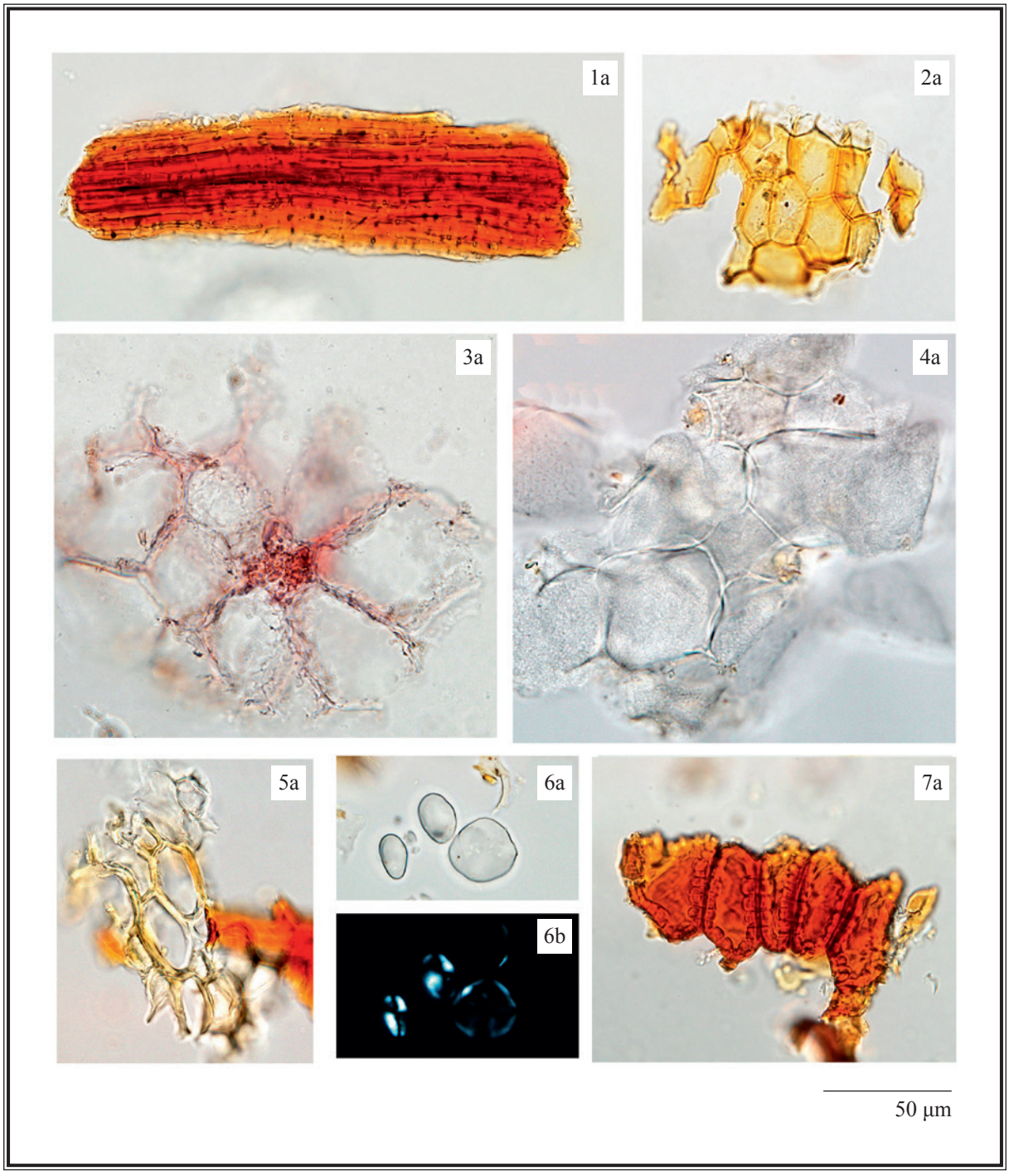


**Figure 3** Microscopic features of powder of Cyperi Rhizoma

1. Hypodermal fibre bundle 2. Epidermal cells 3. Secretory cell
4. Parenchymatous cells containing starch gelatinous masses
5. Hypodermal cells (sclerenchymatous cells) 6. Starch granules 7. Stone cells

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

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

**Standard solution**

*α-Cyperone standard solution*

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

**Developing solvent system**

Prepare a mixture of n-hexane, ethyl acetate and glacial acetic acid (9:1:0.1, v/v).

**Spray reagent**

Weigh 1.5 g of 2,4-dinitrophenylhydrazine and dissolve in 20 mL of sulphuric acid (50%, v/v). Transfer the solution to a 100-mL volumetric flask and make up to the mark with water. Filter the solution.

**Test solution**

Weigh 3.0 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 the mixture.

**Procedure**

Carry out the method by using a HPTLC silica gel $F_{254}$ plate and a freshly prepared developing solvent system as described above. Apply separately α-cyperone standard solution (3 µL) and the test solution (6 µL) 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 dry it in air until the spots or bands become visible (about 30 min). Examine the plate under visible light. Calculate the $R_f$ value by using the equation as indicated in Appendix IV(A).

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 α-cyperone.

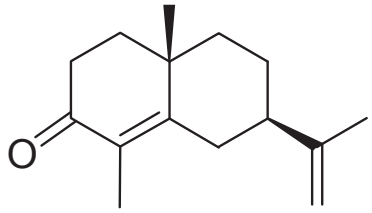


**Figure 4** Chemical structure of α-cyperone

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

### Standard solution

*α-Cyperone standard solution for fingerprinting, Std-FP (100 mg/L)*

Weigh 1.0 mg of α-cyperone CRS and dissolve in 10 mL of n-hexane.

### Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of n-hexane. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. 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 (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 250°C. The detector temperature is at 250°C. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 1) –

**Table 1** Chromatographic system conditions

| Time (min) | Temperature (°C) | Rate (°C/min) |
|---|---|---|
| 0 – 40 | 120 → 160 | 1 |

**System suitability requirements**

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

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

**Procedure**

Separately inject α-cyperone Std-FP and the test solution (2 μL each) into the GC system and record the chromatograms. Measure the retention time of α-cyperone peak in the chromatogram of α-cyperone Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify α-cyperone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of α-cyperone Std-FP. The retention times of α-cyperone 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 Cyperi Rhizoma extract are listed in Table 2.

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

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 (cyperene) | 0.36 | ± 0.03 |
| 2 (selinene) | 0.48 | ± 0.03 |
| 3 | 0.88 | ± 0.03 |
| 4 | 0.95 | ± 0.03 |
| 5 (marker, α-cyperone) | 1.00 | - |

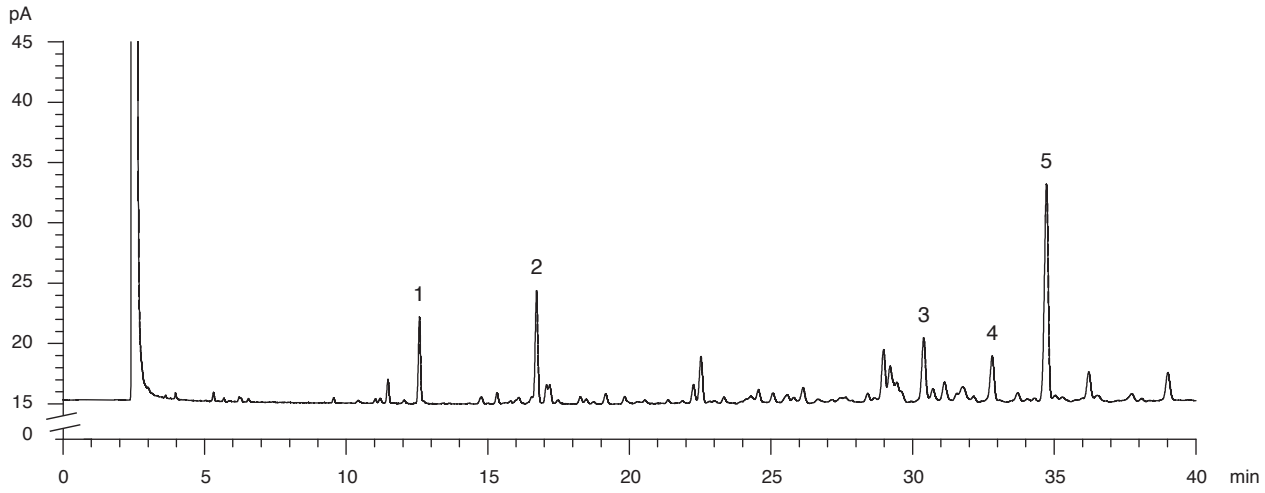


**Figure 5** A reference GC fingerprint chromatogram of Cyperi Rhizoma extract

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. 5).

## 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 2.0%.

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

Total ash: not more than 3.5%.
Acid-insoluble ash: not more than 1.0%.

**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 11.0%.
Ethanol-soluble extractives (hot extraction method): not less than 13.0%.

## 7. ASSAY

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

**Standard solution**

*α-Cyperone standard stock solution, Std-Stock (20 mg/L)*

Weigh accurately 1.0 mg of α-cyperone CRS and dissolve in 50 mL of methanol.

*α-Cyperone standard solution for assay, Std-AS*

Measure accurately the volume of the α-cyperone Std-Stock, dilute with methanol to produce a series of solutions of 1, 2, 6, 10, 15 mg/L for α-cyperone.

**Test solution**

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 15 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for three more times. Combine the filtrates 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 (253 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 acetonitrile and water (60:40, v/v). The elution time is about 40 min.

**System suitability requirements**

Perform at least five replicate injections, each using 20 μL of α-cyperone Std-AS (6 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of α-cyperone should not be more than 5.0%; the RSD of the retention time of α-cyperone peak should not be more than 2.0%; the column efficiency determined from α-cyperone peak should not be less than 9000 theoretical plates.

The *R* value between α-cyperone 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 α-cyperone Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of α-cyperone against the corresponding concentrations of α-cyperone Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

**Procedure**

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify α-cyperone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of α-cyperone Std-AS. The retention times of α-cyperone 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 α-cyperone in the test solution, and calculate the percentage content of α-cyperone in the sample by using the equations as indicated in Appendix IV(B).

**Limits**

The sample contains not less than 0.088% of α-cyperone ($C_{15}H_{22}O$), calculated with reference to the dried substance.