

# Rhizoma Cyperi – *Xiangfu*

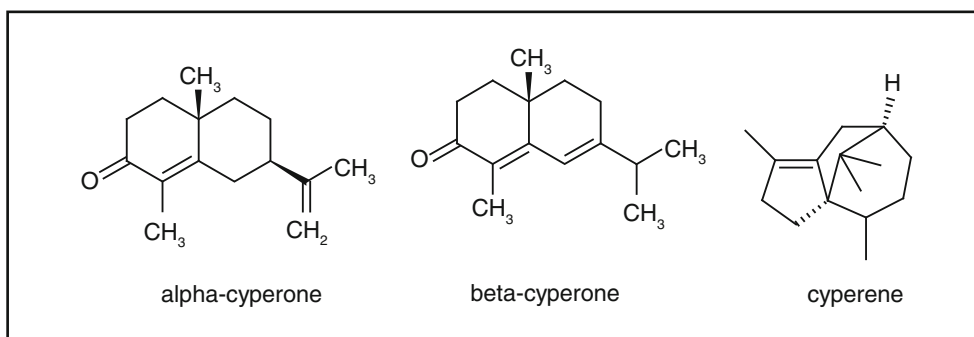
|   |   |
|---|---|
| <b>Pharmacopoeia:</b> <sup>[1]</sup>                | Pharmacopoeia of the People's Republic of China, English Edition<br>Vol. I, 2005/2010   |
| <b>Official drug:</b> <sup>[1]</sup>                | Nut grass Galingale Rhizome is the dried rhizome of <i>Cyperus rotundus</i> L. (Fam. Cyperaceae).<br><br>The drug is collected in autumn, burnt off the fibrous roots, boiled briefly or steamed thoroughly and dried in the sun, or dried in the sun directly after burning off the fibrous roots.   |
| <b>Origin:</b> <sup>[2, 3, 19]</sup>                | Chinese Provinces Guangdong, Sichuan, Henan, Zhejiang, Anhui, Shandong and Hunan.   |
| <b>Descriptions of the drug:</b> <sup>[1]</sup>     | Frequently fusiform, some slightly curved, 2–3.5 cm long, 0.5–1 cm in diameter. Externally dark brown or blackish-brown, longitudinally wrinkled and with 6–10 slightly prominent annular nodes with brown fibrous roots and broken roots; or slightly smooth and exhibiting indistinct annular nodes on the ones of fibrous roots completely removed. Texture hard, fracture of steamed rhizomes appearing yellowish-brown or reddish-brown, horny; fracture of the unsteamed ones white and starchy, an endodermis ring obvious, stele darkened in colour, with scattered dotted vascular bundles. Odour, aromatic; taste, slightly bitter. |
| <b>Pretreatment of the raw drug:</b> <sup>[1]</sup> | Remove fibrous roots and foreign matter, pound to pieces or cut into thin slices.   |
| <b>Processing:</b> <sup>[1]</sup>                   | <u>Cyperi Rhizoma (processed with vinegar)</u><br><br>Stir-bake the pieces or slices of Cyperi Rhizoma as described under the method for stir-baking with vinegar (Appendix II D) to dryness.   |
| <b>Medicinal use:</b> <sup>[2]</sup>                | For the treatment of digestive disorders, vomitus, menstrual disorders, internal bleeding, acute hearing loss, otitis media, migraine, and depression.  |

**Effects and indications of Rhizoma Cyperi according to Traditional Chinese Medicine<sup>[1, 4, 5]</sup>**

|                           |   |
|---------------------------|---|
| Taste:                    | Acrid, sweet, bitter  |
| Temperature:              | Neutral, with tendency to cold  |
| Channels entered:         | <i>Orbis hepaticus, orbis lienalis, orbis tricolorii</i>  |
| Effects (functions):      | To remove stagnation of <i>qi</i> , regulate menstruation and relieve pain (2005).<br><br>To soothe the liver to resolve depression, regulate <i>qi</i> and soothe the middle, regulate menstruation and relieve pain (2010).   |
| Symptoms and indications: | Stagnation of the <i>liver-qi</i> characterized by distending pain in the chest, hypochondria and epigastrium, indigestion, feeling of stuffiness in the chest and epigastrium, abdominal colic, distending pain in the breast, menstrual disorders, amenorrhea or dysmenorrhoea (2005).<br><br>Liver depression and <i>qi</i> stagnation, distending pain in the chest and the hypochondrium, pain caused by genital disease, distending pain in the breasts. Spleen-stomach <i>qi</i> stagnation, stuffiness and oppression in the epigastrium and abdomen, pain, distention and fullness, menstrual irregularities, amenorrhea and dysmenorrhoea (2010). |

**Main constituents:**

- Sesquiterpeneoids<sup>[6, 7, 10, 12, 17, 20]</sup>  
Epi-guaidiol A, sugebiol, guaidiol A, sugetriol triacetate, cyperenoic acid, cyperotundone, rotundines A-C
- Norsesquiterpenes<sup>[7]</sup>  
norcyperone
- Essential oil<sup>[9–13, 17, 20]</sup>  
 $\alpha$ -cyperone,  $\beta$ -cyperone, cyperol, isocyperol, **cyperene**, cyprotene, cyperotundone, cypera 2,4-diene, caryophyllene, rotundine,  $\alpha$ -copaene,  $\alpha$ -selinene, epi- $\alpha$ -selinene,  $\beta$ -selinene, rotundene, valercene, ylanga-2,4-diene,  $\gamma$ -gurjune, trans calamenene,  $\delta$ -cadinene,  $\gamma$ -calacorene,  $\alpha$ -muurolene,  $\gamma$  muurolene, cadalene, nootkatene, mustakone,  $\alpha$ -copaene, isolongifolen-5-one +  $\gamma$ -gurjunenepoxide, (*E*)-pinocarveol, myrtenal, dihydrocarvone, verbenone, (*E*)-carveol, valencene
- Flavonoids<sup>[8, 12–14, 17]</sup>  
Vitexin, isovitexin, orientin, epiorientin
- Cardiac glycosides<sup>[12, 13, 17]</sup>
- Alkaloids<sup>[15]</sup>
- Saponins<sup>[15]</sup>



**Fig. 1:** Formulae of the main compounds of Rhizoma Cyperi <sup>[10]</sup>

### Reported Pharmacological Activities

Anti-inflammatory<sup>[6, 7, 12, 13, 15, 17, 20]</sup>

Anti-estrogenic activity<sup>[3, 7, 14]</sup>

Antimicrobial<sup>[14, 16]</sup>

Anthelmintic<sup>[7, 14]</sup>

Anti-histaminic<sup>[14]</sup>

Anti-emetic<sup>[7, 14]</sup>

Antipyretic<sup>[7, 12–15, 17, 20]</sup>

Antidiabetic<sup>[6, 7, 14, 20]</sup>

Anti-diarrhoeal activity<sup>[3, 7, 20]</sup>

Antimalarial<sup>[7, 15, 16, 20]</sup>

Antispasmodic <sup>[17]</sup>

Hepatoprotective <sup>[7]</sup>

Acetylcholinesterase inhibitory activity <sup>[6]</sup>

Protein glycation inhibitory activity <sup>[6]</sup>

Antidepressant <sup>[20]</sup>

Inhibition of nitric oxide and superoxide production<sup>[6, 20]</sup>

Hypotensive<sup>[7, 12, 13, 17]</sup>

Aphrodisiac<sup>[7]</sup>

Diuretic<sup>[7]</sup>

Sedative<sup>[7, 17]</sup>

Carminative<sup>[7]</sup>

Anticolic<sup>[7]</sup>

Stimulant<sup>[7]</sup>

Stomachic<sup>[7]</sup>

Removes renal calculi<sup>[7]</sup>

Emmenagogue activity <sup>[16]</sup>

**TLC-Fingerprint Analysis**

| Drug samples                              | Origin   |
|---|--|
| 1 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Sample of commercial drug obtained from HerbaSinica (origin: Zhejiang)           |
| 2 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Sample of commercial drug obtained from Herbasin (origin: unknown)               |
| 3 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Sample of commercial drug obtained from TCM-Clinic Bad Kötzing (origin: unknown) |
| 4 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Province Shandong (China)  |
| 5 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Province Hebei (China)   |
| 6 Rhizoma Cyperi/ <i>Cyperus rotundus</i> | Province Anhui (China)   |

| Reference compound Fig. 2a and 2b | R <sub>f</sub> |
|-----------------------------------|----------------|
| T $\alpha$ -Cyperone              | 0.41           |
| Reference compound Fig. 2c and 2d | R <sub>f</sub> |
| T $\alpha$ -Cyperone              | 0.34           |

- Extraction: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.
- Reference compound: 1 mg is dissolved in 1 ml ethyl acetate
- Separation parameters:
  - Plate: HPTLC Silica gel 60 F<sub>254</sub>, Merck
  - Applied amounts: Rhizoma Cyperi extracts: each 10  $\mu$ l  
Reference compound: 10  $\mu$ l
  - Solvent system: Toluene + ethyl acetate + glacial acetic acid (92+5+5)

## Detection:

1. Without chemical treatment (Fig. 2a)

254 nm

2. Dinitrophenylhydrazine reagent (Fig. 2b)

1.5 g 2,4-dinitrophenylhydrazine are dissolved in 20 ml sulphuric acid (25 %), filled up with water to 100 ml and filtered.

After spraying with 10 ml, the plate is evaluated after 10 min in VIS.

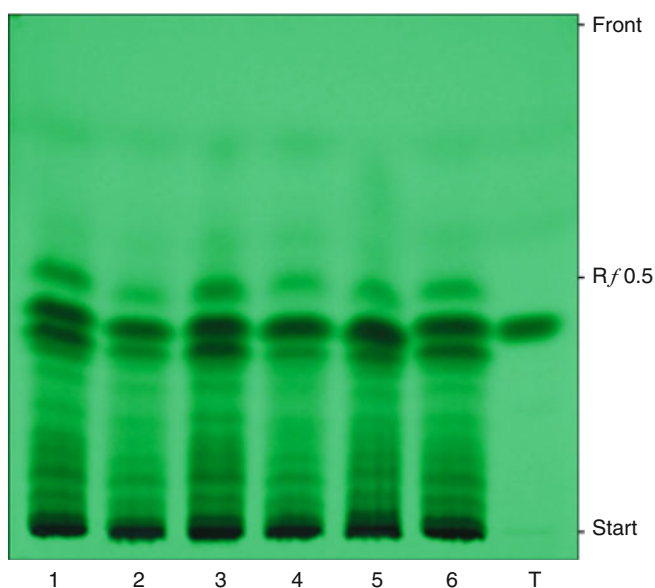
3. Anisaldehyde – Sulphuric acid reagent (Fig. 2c and 2d)

0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

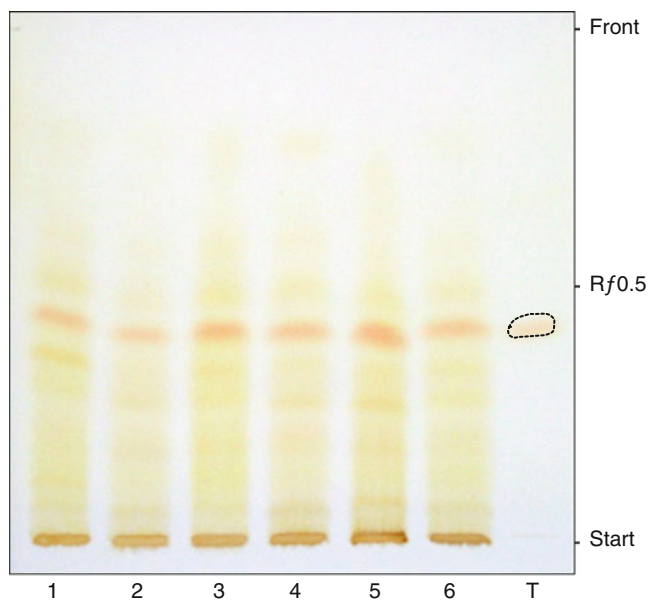
The plate is sprayed with 10 ml, heated at 100 °C for 5 min, then evaluated in VIS and under 366 nm.

Note: The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

## 4. Description:



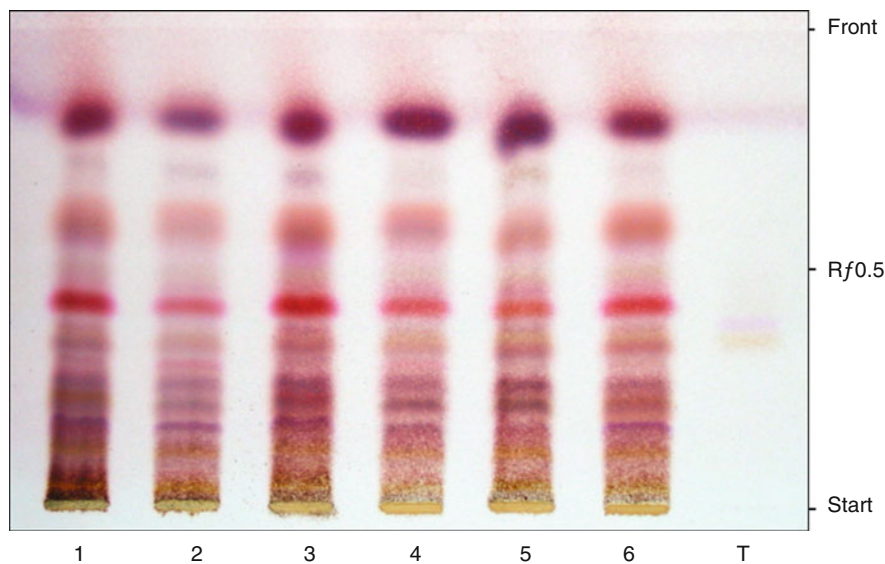
**Fig. 2a:** Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi without chemical treatment (UV 254 nm)



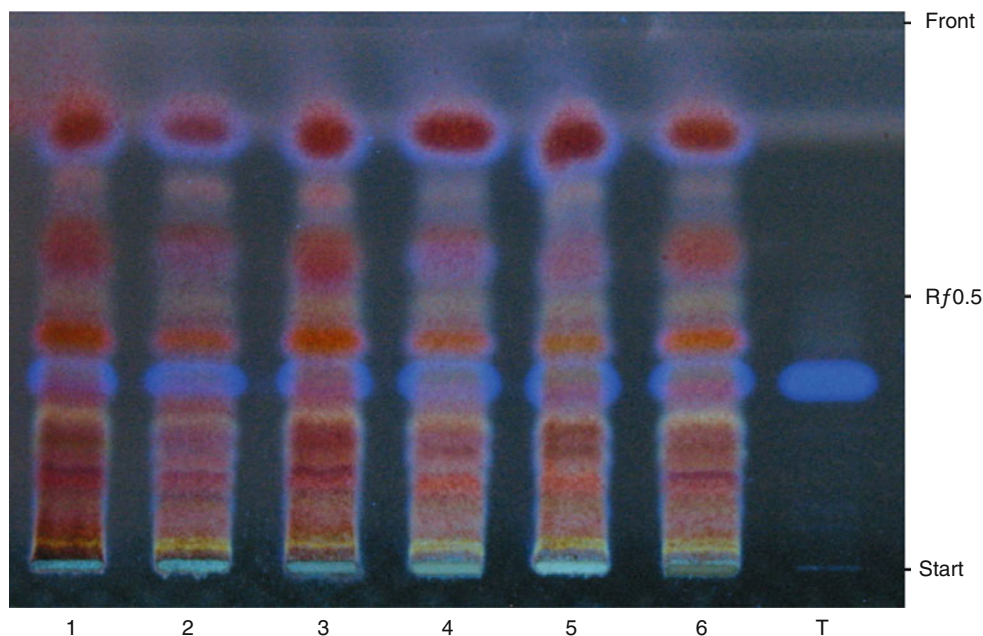
**Fig. 2b:** Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with 2,4-dinitrophenylhydrazine (VIS)

**Figure 2a** shows the six samples of Rhizoma Cyperi under UV 254 nm without chemical treatment. In all samples several black zones are detectable in the  $R_f$ – range from the start up to 0.5. The main zone at  $R_f=0.41$  (T) could be identified as  $\alpha$ -cyperone. The second zone at  $R_f=0.39$  might be  $\beta$ -cyperone.

After spraying with 2,4-dinitrophenylhydrazin (**Fig. 2b**) the zones appeared in yellow/orange colours. In all samples the orange spot of  $\alpha$ -cyperone at  $R_f=0.41$  is clearly detectable.



**Fig. 2c:** Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (VIS)



**Fig. 2d:** Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (UV 366 nm)

**Fig. 2c and d:** With the solvent system generally used for essential oils several pink and violet zones from the start up to  $R_f=0.85$  are detectable. In VIS (**Fig. 2c**)  $\alpha$ -cyperone is not exactly distinguishable, but under UV 366 nm (**Fig. 2d**) the compound can be detected by a light blue coloured spot at  $R_f=0.34$ .

### HPLC-Fingerprint Analysis <sup>[18]</sup>

1. Sample preparation: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, Type 0.45  $\mu\text{m}$ .
2. Injection volume: Rhizoma Cyperi extract: each 10.0  $\mu\text{l}$
3. HPLC parameter:
 

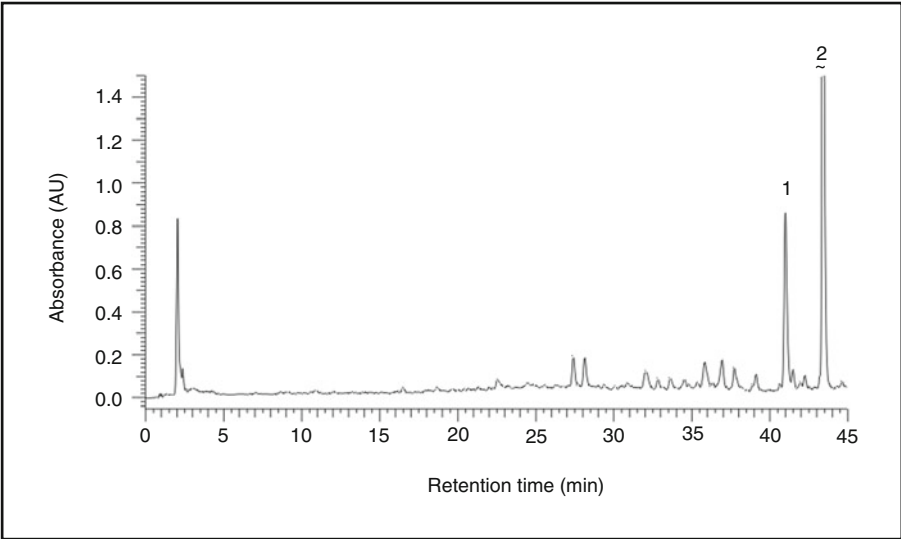
|                    |   |
|--------------------|---|
| Apparatus:         | MERCK HITACHI D-6000 A Interface<br>MERCK HITACHI L-4500 A Diode Array Detector<br>MERCK HITACHI AS-2000 Autosampler<br>MERCK HITACHI L-6200 A Intelligent Pump |
| Separation column: | LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 $\mu\text{m}$ ), Merck  |
| Precolumn:         | LiChroCART® 4-4 LiChrospher® 100 RP-18, Merck   |
| Solvent:           | A: water (Millipore Ultra Clear UV plus® filtered)<br>B: methanol (VWR)   |
| Gradient:          | 10–100 % B in 45 min, total runtime: 45 min   |
| Flow:              | 1 ml/min  |
| Detection:         | 254 nm  |

Retention times of the main peaks recorded at 254 nm

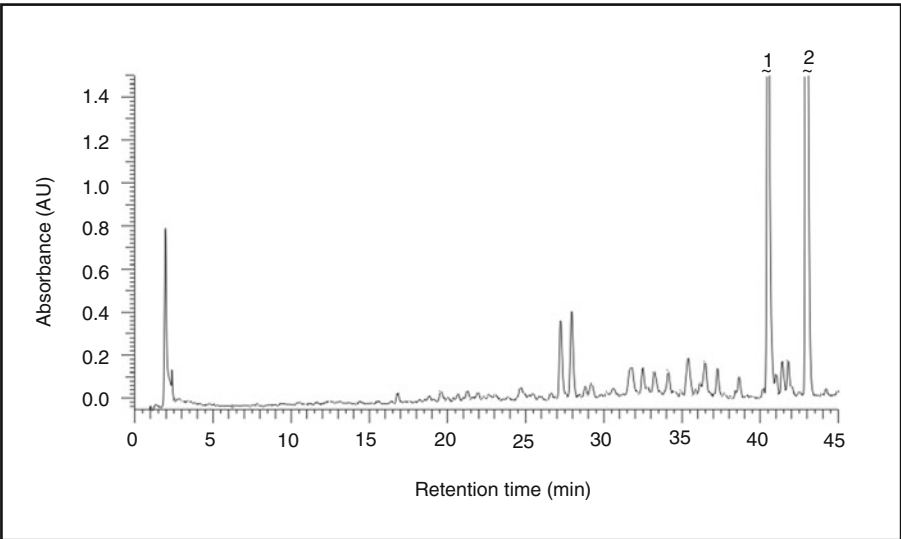
| Peak | Rt (min) | Compound            |
|------|----------|---------------------|
| 1    | 40.8     | $\beta$ -Cyperone ? |
| 2    | 43.2     | $\alpha$ -Cyperone  |

4. Description of the HPLC-Figures

In the Rt – range 27.0–39.0 there a several minor peaks in both samples. The two main peaks at Rt 40.8 and 43.2 can be assigned to  $\beta$ - and  $\alpha$ -cyperone, respectively.

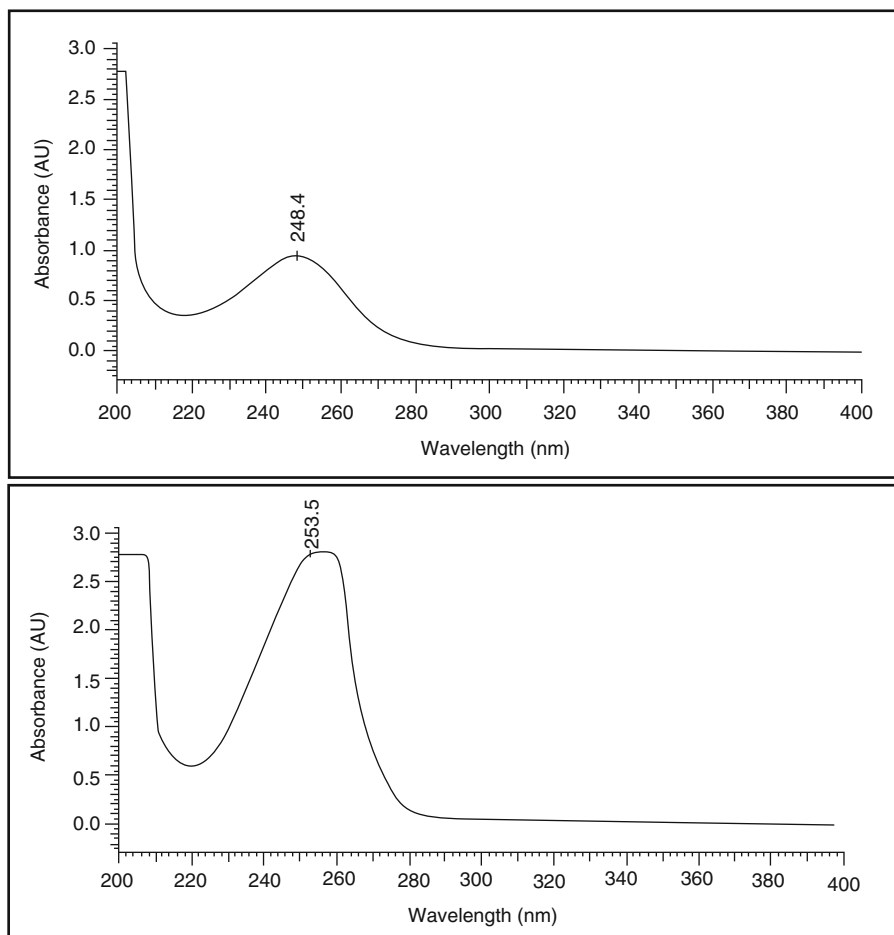


**Fig. 3a:** HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 2



**Fig. 3b:** HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 6





**Fig. 4:** On line UV-spectra of main peaks of Rhizoma Cyperi

**Note:** Rhizoma Cyperi should contain not less than 1.0 % of volatile oil, according to the Chinese Pharmacopoeia <sup>[1]</sup>.

## Conclusion

The identity of Rhizoma Cyperi can be easily determined by TLC- and HPLC-analysis using MeOH-extract or essential oil by means of the characteristic  $\alpha$ - $\beta$ -cyperone dublett in HPLC.

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Chromatographic Fingerprint Analysis of Herbal  
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