Rhizoma Gastrodiae

Figure 1 A photograph of Rhizoma Gastrodiae
1. NAMES

Official Name: Rhizoma Gastrodiae

Chinese Name: 天麻

Chinese Phonetic Name: Tianma

2. SOURCE

Rhizoma Gastrodiae is the dried tuber of Gastrodia elata Bl. (Orchidaceae). The tuber is collected from winter to spring, washed clean immediately, steamed thoroughly, spread out and dried at a temperature not more than 60˚C to obtain Rhizoma Gastrodiae.

3. DESCRIPTION

Ellipsoid or slat-shaped, slightly compressed, shrunken and somewhat curved, 5-17 cm long, 14-43 mm wide, 5-26 mm thick. Externally yellowish-white to pale yellowish-brown, with longitudinal wrinkles and many transverse annulations arranged along latent buds, brown thread sometimes visible. Apex with reddish-brown to deep brown parrot-beak-shaped buds or remains of stem; the lower end with a rounded scar. Texture hard and uneasily broken, fracture fairly even, yellowish-white to brownish, horny. Odour slight; taste sweetish (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

The transverse section sometimes shows remains of epidermis; the hypodermis consists of 2-3 rows of suberized and tangentially elongated cells. Cortex cells are elongated tangentially, some contain raphides of calcium oxalate. In older tubers, one to several rows of sclerenchyma cells present in cortex adjacent to the hypodermis. Stele large, with numerous small amphicribral or collateral vascular bundles, plenty of parenchyma cells contained gelatinized polysaccharides were visible, raphides of calcium oxalate scattered in parenchyma cells (Fig. 2).

Powder

Colour yellowish-white to yellowish-brown. Needle crystals of calcium oxalate are found in bundles or scattered, their length varied, the longer one up to 95 µm, showing a polychrome when examined under a polarized microscope. When mounted in glycerol-acetic acid test solution,
parenchyma cells containing gelatinized polysaccharides appear colourless, while some cells containing long-ellipsoid or subglobose granules appear as a brown or brownish-purple colour when iodine solution is added. Sclerenchyma cells are sometimes visible, ellipsoid to subpolygonal, the wall 3-9 µm thick, lignified, distinctly pitted; pitted walls showing a black, cross shape when examined under a polarized microscope (Fig. 3).

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

**Standard solution**

*Gastrodin standard solution*

Weigh 1.0 mg of gastrodin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

**Developing solvent system**

Prepare a mixture of ethyl acetate, ethanol and water (45:10:1, v/v).

**Spray reagent**

Mix 1 mL of dilute sulphuric acid (50%, v/v) and 10 mL of \( p \)-hydroxybenzaldehyde in ethanol (2%, w/v). Freshly prepare the reagent.

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (560 W) the mixture for 60 min. Centrifuge at about 3000 x \( g \) for 5 min. Transfer the supernatant 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 ethanol.

**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 gastrodin standard solution and the test solution (4 µL each) to the plate. Develop over a path of about 5 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 120ºC 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).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the \( R_f \) value, corresponding to those of gastrodin.
Figure 2  Microscopic features of transverse section of Rhizoma Gastrodiae

A. Sketch  B. Section illustration  C. Raphides of calcium oxalate  D. Vascular bundle

Figure 3  Microscopic features of powder of Rhizoma Gastrodiae

1. Raphides of calcium oxalate  2. Parenchyma cells containing gelatinized polysaccharides
3. Sclerenchyma cells

a. Features under the light microscope  b. Features under the polarized microscope
4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

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

Weigh 1.0 mg of gastrodin CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL test tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (230 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.05% Trifluoroacetic acid (%)</th>
<th>Trifluoroacetic acid : Acetonitrile (0.05:99.95, v/v) (%)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>100 → 70</td>
<td>0 → 30</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

System suitability requirements

Perform at least five replicate injections, each using 10 µL of gastrodin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of gastrodin should not be more than 5.0%; the RSD of the retention time of gastrodin peak should not be more than 2.0%; the column efficiency determined from gastrodin peak should not be less than 20000 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. 5).
Procedure

Separately inject gastrodin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of gastrodin peak in the chromatogram of gastrodin Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify gastrodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of gastrodin Std-FP. The retention times of gastrodin 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 six characteristic peaks of Rhizoma Gastrodiae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Rhizoma Gastrodiae extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (marker, gastrodin)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.31 (vs peak 1)</td>
<td>± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>1.71 (vs peak 1)</td>
<td>± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>1.28 (vs peak 3)</td>
<td>± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>1.36 (vs peak 3)</td>
<td>± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>1.50 (vs peak 3)</td>
<td>± 0.04</td>
</tr>
</tbody>
</table>

Figure 5 A reference fingerprint chromatogram of Rhizoma Gastrodiae extract
For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference 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 Foreign Matter (Appendix VIII): not more than 1.0%.

5.5 Ash (Appendix IX)

Total ash: not more than 3.0%.
Acid-insoluble ash: not more than 0.5%.

5.6 Water Content (Appendix X): not more than 14.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 20.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution
Gastrodin standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 10.0 mg of gastrodin CRS and dissolve in 10 mL of methanol.
Gastrodin standard solution for assay, Std-AS
Measure accurately the volume of the gastrodin Std-Stock, dilute with methanol to produce a series of solutions of 5, 50, 100, 150, 200 mg/L for gastrodin.

Test solution
Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × g for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times
Rhizoma Gastrodiae

Each with 5 ml of ethanol (50%). Combine the extracts and make up to the mark with ethanol (50%). Filter through a 0.45-µm RC filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Water (% v/v)</th>
<th>Acetonitrile (% v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>100 → 80</td>
<td>0 → 20</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20–30</td>
<td>80 → 0</td>
<td>20 → 100</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of gastrodin Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of gastrodin should not be more than 5.0%; the RSD of the retention time of gastrodin peak should not be more than 2.0%; the column efficiency determined from gastrodin peak should not be less than 25000 theoretical plates.

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

**Limits**

The sample contains not less than 0.41% of gastrodin (C₁₃H₁₈O₇), calculated with reference to the dried substance.