**GINKGO LEAF**

*Ginkgonis folium*

**DEFINITION**

Whole or fragmented, dried leaf of *Ginkgo biloba* L.

**Content**: not less than 0.5 per cent of flavonoids, calculated as flavone glycosides (M, 757) (dried drug).

**CHARACTERS**

Greyish or yellowish-green or yellowish-brown.

**IDENTIFICATION**

A. The upper surface of ginkgo leaf is slightly darker than the lower surface. The petioles of the leaf are about 4 cm to 9 cm long. The lamina is about 4 cm to 10 cm wide, fan-shaped, usually bilobate or sometimes undivided. Both surfaces are smooth, and the venation dichotomous, the veins appearing to radiate from the base; they are equally prominent on both surfaces. The distal margin is incised, irregularly and to different degrees, and irregularly lobate or emarginate. The lateral margins are entire and taper towards the base.

B. Reduce to a powder (355) (2.9.12). The powder is greyish or yellowish-green or yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows irregularly-shaped fragments of the lamina in surface view, the upper epidermis consisting of elongated cells with irregularly sinuous walls, the lower epidermal cells smaller, with a finely striated cuticle and each cell shortly papillose; stomata about 60 µm, large, deeply sunken with 6 to 8 subsidiary cells, are more numerous in the lower epidermis; abundant large cluster crystals of calcium oxalate of various sizes in the mesophyll; fragments of fibro-vascular tissue from the petiole and veins.

C. Thin-layer chromatography (2.2.27).
Test solution. To 2.0 g of the powdered drug (710) (2.9.12) add 10 ml of methanol R. Heat in a water-bath at 65 °C for 10 min. Shake frequently. Allow to cool to room temperature and filter.

Reference solution. Dissolve 1.0 mg of chlorogenic acid R and 3.0 mg of rutin R in 20 ml of methanol R.

Plate: TLC silica gel plate R.


Application: 20 µl, as bands.

Development: over a path of 17 cm.

Drying: at 100-105 °C.

Detection: spray the warm plate with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R. Subsequently spray with the same volume of a 50 g/l solution of diphenylboric acid aminoethyl ester R in methanol R. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other weaker fluorescent zones may be present in the chromatogram obtained with the test solution.

<table>
<thead>
<tr>
<th>Top of the plate</th>
<th>Test solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid: a light blue fluorescent zone</td>
<td>A yellowish-brown fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>A green fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>2 yellowish-brown fluorescent zones</td>
</tr>
<tr>
<td></td>
<td>An intense light blue fluorescent zone sometimes overlapped by a greenish-brown fluorescent zone</td>
</tr>
<tr>
<td>Rutin: a yellowish-brown fluorescent zone</td>
<td>A green fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>2 yellowish-brown fluorescent zones</td>
</tr>
<tr>
<td></td>
<td>A green fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>A yellowish-brown fluorescent zone</td>
</tr>
</tbody>
</table>

Reference solution: Test solution

Flow rate: 1.0 ml/min.

Detector: spectrophotometer at 370 nm.

Injection: 10 µl.

Relative retention with reference to quercetin (retention time = about 12.5 min): kaempferol = about 1.4; isorhamnetin= about 1.5.

System suitability:

- resolution: minimum 1.5 between the peaks due to kaempferol and to isorhamnetin.

Do not take into account peaks eluting before the quercetin peak or after the isorhamnetin peak in the chromatogram obtained with the test solution.

Calculate the percentage content of flavonoids, expressed as flavone glycosides, from the expression:

\[
2 \times \frac{F_1 \times m_1 \times 2.514 \times p}{F_2 \times m_2} 
\]

where:

- \(F_1\) = sum of the areas of all the considered peaks in the chromatogram obtained with the test solution,
- \(F_2\) = area of the peak corresponding to quercetin in the chromatogram obtained with the reference solution,
- \(m_1\) = mass of quercetin used to prepare the reference solution, in grams,
- \(m_2\) = mass of the drug to be examined used to prepare the test solution, in grams,
- \(p\) = percentage content of anhydrous quercetin in quercetin dihydrate R.

Tests

Foreign matter (2.8.2): maximum 5 per cent of stems and 2 per cent of other foreign matter.

Loss on drying (2.2.32): maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) (2.9.12) by drying in an oven at 105 °C for 2 h.

Total ash (2.4.16): maximum 11.0 per cent.

Assay

Flavonoids. Liquid chromatography (2.2.29).

Test solution. Heat 2.500 g of the powdered drug (710) (2.9.12) in 50 ml of a 60 per cent V/V solution of acetone R under a reflux condenser for 30 min. Filter and collect the filtrate. Extract the drug residue a second time in the same manner, using 40 ml of a 60 per cent V/V solution of acetone R and filter. Collect the filtrates and dilute to 100.0 ml with a 60 per cent V/V solution of acetone R. Evaporate 50.0 ml of the solution to eliminate the acetone and transfer to a 50.0 ml vial, rinsing with 30 ml of methanol R. Add 4.4 ml of hydrochloric acid R1, dilute to 50.0 ml with water R and centrifuge. Place 10 ml of the supernatant liquid in a 10 ml brown-glass vial. Close with a rubber seal and an aluminium cap and heat on a water-bath for 25 min. Allow to cool to room temperature.

Reference solution. Dissolve 10.0 mg of quercetin dihydrate R in 20 ml of methanol R. Add 15.0 ml of dilute hydrochloric acid R and 5 ml of water R and dilute to 50.0 ml with methanol R.

Column:

- stationary phase: octadecylsilil silica gel for chromatography R (5 µm),
- size: l = 0.125 m, Ø = 4 mm,
- temperature: 25 °C.

Mobile phase:

- mobile phase A: 0.3 g/l solution of phosphoric acid R adjusted to pH 2.0,
- mobile phase B: methanol R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>1 - 20</td>
<td>60 → 45</td>
<td>40 → 55</td>
</tr>
<tr>
<td>20 - 21</td>
<td>45 → 0</td>
<td>55 → 100</td>
</tr>
<tr>
<td>21 - 25</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

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See the information section on general monographs (cover pages)