Reference solution. Dissolve 1.0 mg of chlorogenic acid R and 2.5 mg of hyperoside R in 10 ml of methanol R.
Plate: TLC silica gel plate R.
Application: 20 µl as bands.
Development: over a path of 15 cm.
Drying: at 100-105 °C.
Detection: spray with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R. Subsequently spray with a 50 g/l solution of macrogol 400 R in methanol R. Allow the 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 solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.

<table>
<thead>
<tr>
<th>Top of the plate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoside: a yellowish-orange fluorescent zone</td>
<td>A yellowish-green fluorescent zone (vitexin)</td>
</tr>
<tr>
<td>Chlorogenic acid: a light blue fluorescent zone</td>
<td>A light blue fluorescent zone (hyperoside)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Test solution</th>
</tr>
</thead>
</table>

Tests

Foreign matter (2.8.2): maximum 8 per cent of lignified branches with a diameter greater than 2.5 mm and maximum 2 per cent of other foreign matter.

Loss on drying (2.2.32): maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) by drying in an oven at 100-105 °C for 2 h.

Total ash (2.4.16): maximum 10.0 per cent.

Assay

Stock solution. In a 200 ml flask introduce 0.400 g of the powdered drug (250) and 40 ml of alcohol (60 per cent V/V) R. Heat in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter through a plug of absorbent cotton into a 100 ml volumetric flask. Transfer the absorbent cotton with the drug residue back into the 200 ml flask, add 40 ml of alcohol (60 per cent V/V) R and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 ml volumetric flask as previously. Rinse the 200 ml flask and filter with a further quantity of alcohol (60 per cent V/V) R and transfer to the same 100 ml volumetric flask. Dilute to volume with alcohol (60 per cent V/V) R and filter.

Test solution. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the same 25 ml volumetric flask as previously. Add 10.0 ml of a solution containing 25.0 g/l of boric acid R and 20.0 g/l of oxalic acid R in anhydrous formic acid R and dilute to 25.0 ml with anhydrous acetic acid R.

Compensation liquid. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer it into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the same 25 ml volumetric flask as previously. Add 10.0 ml of anhydrous formic acid R and dilute to 25.0 ml with anhydrous acetic acid R.

Measure the absorbance (2.2.25) of the test solution at 410 nm after 30 min. Calculate the percentage content of total flavonoids expressed as hyperoside from the expression:

\[
A \times \frac{1.235}{m}
\]

i.e. taking the value of the specific absorbance of hyperoside at 410 nm to be 405.

\[
A = \text{absorbance of the test solution at 410 nm,}
\]

\[
m = \text{mass of the drug to be examined, in grams.}
\]

01/2005:1865

Hawthorn leaf and flower dry extract

Crataegi folii cum flore extractum siccum

Definition

Extract produced from Hawthorn leaf and flower (1432).

Content:

– for aqueous extracts: minimum 2.5 per cent of flavonoids, expressed as hyperoside \( (C_{21}H_{20}O_{12}; M_r 464.4) \) (dried extract);

– for hydroalcoholic extracts: minimum 6.0 per cent of flavonoids, expressed as hyperoside \( (C_{21}H_{20}O_{12}; M_r 464.4) \) (dried extract).

Production

The extract is produced from the drug by a suitable procedure using either water or a hydroalcoholic solvent equivalent in strength to a minimum of 45 per cent V/V ethanol.

Characters

Appearance: light brown or greenish-brown powder.

Identification

Thin-layer chromatography (2.2.27).

Test solution. Suspend 0.2 g of the extract to be examined in 20 ml of alcohol (70 per cent V/V) R and filter.

Reference solution. Dissolve 1 mg of chlorogenic acid R, 2.5 mg of hyperoside R and 2.5 mg of rutin R in 10 ml of methanol R.

Plate: TLC silica gel plate R.


Application: 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development: over a path of 15 cm.

Drying: at 100-105 °C.
Detection: spray the plate whilst hot with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R; subsequently spray the plate with a 50 g/l solution of macrogol 400 R in methanol R; allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm.

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

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<th>Top of the plate</th>
<th>Test solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoside: a yellowish-orange fluorescent zone</td>
<td>A light yellow fluorescent zone</td>
</tr>
<tr>
<td>Chlorogenic acid: a light blue fluorescent zone</td>
<td>A yellowish-green fluorescent zone (vitexin 2-rhamnoside)</td>
</tr>
<tr>
<td>Rutin: a yellowish-orange fluorescent zone</td>
<td>A yellowish-orange fluorescent zone (rutin)</td>
</tr>
</tbody>
</table>

Reference solution

<table>
<thead>
<tr>
<th>Test solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = absorbance at 410 nm.</td>
</tr>
<tr>
<td>m = mass of the extract to be examined, in grams.</td>
</tr>
</tbody>
</table>

HEPARIN CALCIUM

Heparinum calcicum

DEFINITION

Heparin calcium is a preparation containing the calcium salt of a sulphated glucosaminoglycan present in mammalian tissues. On complete hydrolysis, it liberates D-glucosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulphuric acid. It has the characteristic property of delaying the clotting of freshly shed blood. The potency of heparin calcium intended for parenteral administration is not less than 150 IU/mg, calculated with reference to the dried substance. The potency of heparin calcium not intended for parenteral administration is not less than 120 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is prepared from the lungs of oxen or from the intestinal mucosa of oxen, pigs or sheep.

It is produced by methods of manufacturing designed to minimise or eliminate microbial contamination and substances lowering blood pressure.

CHARACTERS

A white or almost white powder, moderately hygroscopic, freely soluble in water.

IDENTIFICATION

A. It delays the clotting of recalcified citrated sheep plasma (see Assay).
B. 0.40 g in water R and dilute to 10.0 ml with the same solvent. The specific optical rotation (2.2.7) is not less than + 35.
C. Examine by zone electrophoresis (2.2.31) using agarose for electrophoresis R as the supporting medium. To equilibrate the agarose and as electrolyte solution use a mixture of 50 ml of glacial acetic acid R and 800 ml of water R adjusted to pH 3 by addition of lithium hydroxide R and diluted to 1000.0 ml with water R. Test solution. Dissolve 25 mg of the substance to be examined in water R and dilute to 10 ml with the same solvent.

Reference solution. Dilute heparin sodium BRP with an equal volume of water R.

Apply separately to the strip 2 µl to 3 µl of each solution. Pass a current of 1 mA to 2 mA per centimetre of strip width at a potential difference of 300 V for about 10 min. Stain the strips using a 1 g/l solution of toluidine blue R and remove the excess by washing. The ratio of the mobility of the band in the electropherogram obtained with the test solution to the mobility of the band in the electropherogram obtained with the reference solution is 0.9 to 1.1.

D. It gives the reactions of calcium (2.3.1).

TESTS

Appearance of solution. Dissolve a quantity equivalent to 50 000 IU in water R and dilute to 10 ml with the same solvent. The solution is clear (2.2.1) and not more intensely coloured than degree 5 of the range of reference solutions of the most appropriate colour (2.2.2. Method II).