through a paper filter. Add the paper filter, cut into pieces, to the residue in the round-bottomed flask, add 50 ml of a mixture of equal volumes of methanol R and water R and heat under a reflux condenser in a water-bath at 50-60 °C for 30 min, shaking frequently. Repeat this procedure twice. To the combined filtrate add 3.00 ml of the internal standard solution and evaporate to 18 ml under reduced pressure. Rinse the round-bottomed flask with water R and dilute, with the washings, to 20.0 ml. Elute the solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of kieselguhr for chromatography R. Allow to stand for 20 min. Elute with 200 ml of a mixture of equal volumes of ethyl acetate R and methylene chloride R. Evaporate the eluate to dryness in a 250 ml round-bottomed flask. Dissolve the residue in 10.0 ml of methanol R and add 10.0 ml of water R. Add 7.0 g of neutral aluminium oxide R, shake for 120 s, centrifuge at 5000 g for 10 min and filter through a paper filter. Evaporate 10.0 ml of the filtrate to dryness. Dissolve the residue in 3.0 ml of a mixture of equal volumes of methanol R and water R and filter.

Column:
- size: l = 0.12 m, Ø = 4 mm;
- stationary phase: octadecyldsidyl silica gel for chromatography R (4 µm).

Mobile phase:
- mobile phase A: water R;
- 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 - 3</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>3 - 20</td>
<td>62 → 55</td>
<td>38 → 45</td>
</tr>
<tr>
<td>20 - 30</td>
<td>55</td>
<td>45</td>
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<tr>
<td>30 - 55</td>
<td>55 → 45</td>
<td>45 → 55</td>
</tr>
<tr>
<td>55 - 57</td>
<td>45</td>
<td>55 → 100</td>
</tr>
<tr>
<td>57 - 70</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>70 - 90</td>
<td>3</td>
<td>38</td>
</tr>
</tbody>
</table>

Flow rate: 1.2 ml/min.

Detection: spectrophotometer at 225 nm.

Injection: a 20 µl loop injector.

Calculate the percentage content of total sesquiterpene lactones, expressed as dihydrohelenalin tiglate, using the following expression:

\[
\frac{S_{LS} \times C \times V \times 1.187 \times 100}{S_{S} \times m \times 1000}
\]

- \(S_{LS}\) = area of all peaks due to sesquiterpene lactones appearing after the santinon peak in the chromatogram obtained with the test solution;
- \(S_{S}\) = area of the peak due to santinon in the chromatogram obtained with the test solution;
- \(m\) = mass of the drug to be examined, in grams;
- \(C\) = concentration of santinon in the internal standard solution used for the test solution, in milligrams per millilitre;
- \(V\) = volume of the internal standard solution used for the test solution, in millilitres;
- 1.187 = peak correlation factor between dihydrohelenalin tiglate and santinon.

**TESTS**

**Calendula officinalis - Heterotheca inuloides.** Thin-layer chromatography (2.2.27). Test solution. The tincture to be examined.

Reference solution. Dissolve 2.0 mg of caffeic acid R, 2.0 mg of chlorogenic acid R and 5.0 mg of rutin R in methanol R and dilute to 30.0 ml with the same solvent.

Plate: TLC silica gel plate R (5-40 µm) or TLC silica gel plate R (2-10 µm).


Application: 30 µl [or 8 µl] as bands.

Development: over a path of 15 cm [or 8 cm].

Drying: at 80-105 °C.

Detection: spray the plate whilst still hot with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R and then with a 50 g/l solution of macrogol 400 R in methanol R. Heat 5 min at 100 - 105 °C. Allow the plate to dry in air and examine in ultraviolet light at 365 nm.

Results: the chromatogram obtained with the reference solution shows in the lower part an orange-yellow fluorescent zone (rutin), in the middle part a fluorescent zone due to chlorogenic acid and in the upper part a light bluish fluorescent zone (caffeic acid). The chromatogram obtained with the test solution does not show any fluorescent orange-yellow zone corresponding to rutin in the chromatogram obtained with the reference solution and no zone below the zone corresponding to rutin.

**Ethanol (2.9.10):** the final alcohol concentration is not less than 90 per cent of that of the initial extraction solvent.
Methanol and 2-propanol (2.9.11): maximum 0.05 per cent V/V of methanol and maximum 0.05 per cent V/V of 2-propanol.

Dry residue (2.8.16): minimum of 1.7 per cent.

ASSAY

Liquid chromatography (2.2.29).

Internal standard solution. Dissolve immediately before use 0.010 g accurately weighed of santonin R and 0.02 g of butyl 4-hydroxybenzoate R in 10.0 ml of methanol R.

Test solution. In a round-bottomed flask introduce 5.00 g of the tincture to be examined, add 2.00 ml of internal standard solution and 3 g of anhydrous aluminium oxide R, shake for 120 s and filter through a paper filter. Rinse the round-bottomed flask and filter with 5 ml of a mixture of equal volumes of methanol R and water R and filter. Evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of a mixture of 20 volumes of water R and 80 volumes of methanol R and filter by a membrane filter (porosity: 0.45 µm).

Reference solution. Dissolve 0.02 g of methyl 4-hydroxybenzoate R and 0.02 g of ethyl 4-hydroxybenzoate R in methanol R and dilute to 10.0 ml with the same solvent.

Column:
- size: l = 0.12 m, Ø = 4 mm,
- stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 µm),
- temperature: 20 °C.

Mobile phase:
- mobile phase A: water R;
- mobile phase B: methanol R;

<table>
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</tr>
<tr>
<td>30 - 55</td>
<td>55 → 45</td>
<td>45 → 55</td>
</tr>
</tbody>
</table>

Flow rate: 1.2 ml/min.

Detector: spectrophotometer at 225 nm.

Injection: 20 µl.

Relative retention with reference to santonin (retention time = about 9.5 min): butyl 4-hydroxybenzoate = about 4.6.

System suitability: reference solution:
- resolution: minimum 5 between the peaks due to methyl 4-hydroxybenzoate and ethyl 4-hydroxybenzoate.

Calculate the percentage of lactone sesquiterpenes, expressed as dihydrohelenalin tiglate from the expression:

\[
\frac{F_1 \times C \times V \times 1.187}{F_2 \times m \times 10}
\]

\(F_1\) = area of all peaks appearing between the peaks due to santonin and butyl 4-hydroxybenzoate in the chromatogram obtained with the test solution,

\(F_2\) = area of the peak due to santonin in the chromatogram obtained with the test solution,

\(m\) = mass of the tincture to be examined, in grams,

\(C\) = concentration of santonin in the internal standard solution used for the test solution in milligrams per millilitre,

\(V\) = volume of internal standard solution used for the test solution, in millilitres.

1.187 = peak correlation factor between dihydrohelenalin tiglate and santonin.

ARTICAINE HYDROCHLORIDE

Articaini hydrochloridum

\[
\text{C}_{13}\text{H}_{21}\text{ClN}_2\text{O}_3\text{S}
\]

M, 320.8

[23964-57-0]

DEFINITION

Methyl 4-methyl-3-[(2RS)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylate hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water and in alcohol.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 50.0 mg in a 1 g/l solution of hydrochloric acid R and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 100.0 ml with a 1 g/l solution of hydrochloric acid R. Examined between 200 nm and 350 nm (2.2.23), the solution shows an absorption maximum at 272 nm. The specific absorbance at the maximum is 290 to 320.

B. Infrared absorption spectrophotometry (2.2.24).

Preparation: place dropwise 20 µl of the test solution on 300 mg discs.

Test solution. Dissolve 0.1 g in 5 ml of water R, add 3 ml of a saturated solution of sodium hydrogen carbonate R and shake twice with 2 ml of methylene chloride R. Combine the methylene chloride layers, dilute to 5.0 ml with methylene chloride R and dry over anhydrous sodium sulphate R.

Comparison: articaine hydrochloride CRS.

C. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 20 mg of the substance to be examined in 5 ml of alcohol R.

Reference solution. Dissolve 20 mg of articaine hydrochloride CRS in 5 ml of alcohol R.

Plate: TLC silica gel F_{s4} plate R.


Application: 5 µl.

Development: over a path of 15 cm.