Chelidonium majus e floribus, ethanol. Digestio

Chelidonium, Flos, ethanol. Digestio

Fresh flowers of *Chelidonium majus* L.

Description

The yellow actinomorphic flowers grow in long-stemmed clusters of just a few flowers. The two sepals are light yellow, with occasional hairs, and drop off soon; the four petals are broadly ovate, and there are numerous yellow stamens. The short thick style bears a two-lobed stigma. The elongated bicarpellary ovary is unilocular and contains numerous ovules arranged in two rows.

Dosage forms

The mother tincture contains minimum 0.02 and maximum 0.10 per cent of alkaloids, calculated as chelidonine ($C_{20}H_{19}NO_5$; M_r 353,4).

Production

Prepare the mother tincture and liquid dilutions according to Method 18c.

Characteristics

The mother tincture is a brown-yellow liquid with an aromatic odour.

Identification

Thin-layer chromatography (2.2.27)

Test solution: Concentrate 10 ml of the mother tincture to about 5 ml on a water bath. Add to the residue 3 ml of dilute ammonia *R1* and shake the mixture with 10 ml of ether *R*. Collect the separate organic phase and dry over anhydrous sodium sulfate *R*, decant, evaporate to dryness and dissolve the residue in 1 ml of methanol *R*.

Reference solution: Dissolve 10 mg of berberine chloride *R* and 20 mg of methyl red *R* in 10 ml of methanol *R*.

Plate: TLC silica gel plate *R*

Mobile phase: formic acid *R*, water *R*, propanol *R* (1:9:90 *V*/*V*/*V*)

Application: 40 µl of the test solution and 10 µl of the reference solution; as bands of 20 mm

Development: over a path of 10 cm

Detection A: Allow the mobile phase to evaporate, then examine the chromatograms in ultraviolet light at 365 nm.

Results A: See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Additional fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A red fluorescent zone may be present
	A green-blue fluorescent zone
Methyl red: examined in daylight	
	A blue fluorescent zone
	A green fluorescent zone
Berberine chloride: a yellow-green fluorescent zone	A yellow-green fluorescent zone
Reference solution	**Test solution**

Detection B: Spray the plate with dilute potassium iodobismuthate solution *R* and then with sodium nitrite solution *R* and examine the chromatograms in daylight.

Results B: See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Additional brown or brown-yellow zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Methyl red: a strong brown zone	A strong brown zone
Berberine chloride: a brown zone	A strong brown zone
Reference solution	**Test solution**

Tests

Relative density (2.2.5): 0.900 to 0.915

Dry residue (H 2.2.6): minimum 1.4 per cent

Assay

Liquid chromatography (2.2.29)

Test solution: Dilute 1.00 g of the mother tincture with a mixture of 75 volumes of a 1.5 ml/l solution of trifluoroacetic acid *R* and 25 volumes of methanol *R2* to 5.0 ml. Filter the solution through a filter with a pore width of 0.45 µm.

Stock solution: Dissolve 10.0 mg of chelidonine *RH* in methanol *R2* to 10.0 ml.

Reference solution: Dilute 2.0 ml of the stock solution with a mixture of 75 volumes of a 1.5 ml/l solution of trifluoroacetic acid *R* and 25 volumes of methanol *R2* to 20.0 ml.

Column
- size: $l = 0{,}15$ m, $\varnothing = 4.6$ mm
- stationary phase: end-capped silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phases *R* (4 µm)
- temperature: 20 °C

Mobile phase
- mobile phase A: 1.5 ml/l solution of trifluoroacetic acid *R*
- mobile phase B: acetonitrile for chromatography *R*

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 – 5	73	27
5 – 18	73 → 40	27 → 60
18 – 19	40 → 0	60 → 100
19 – 20	0 → 73	100 → 27
20 – 27	73	27

Flow rate: 1.0 ml/min

Detection: spectrophotometer set at 291 nm

Injection: 25 µl

Relative retentions (with reference to chelidonine, t_R about 11 min)

- protopin: about 0.81
- alkaloid 1: about 1.03
- coptisin: about 1.06
- alkaloid 2: about 1.13
- alkaloid 3: about 1.18
- berberine: about 1.31

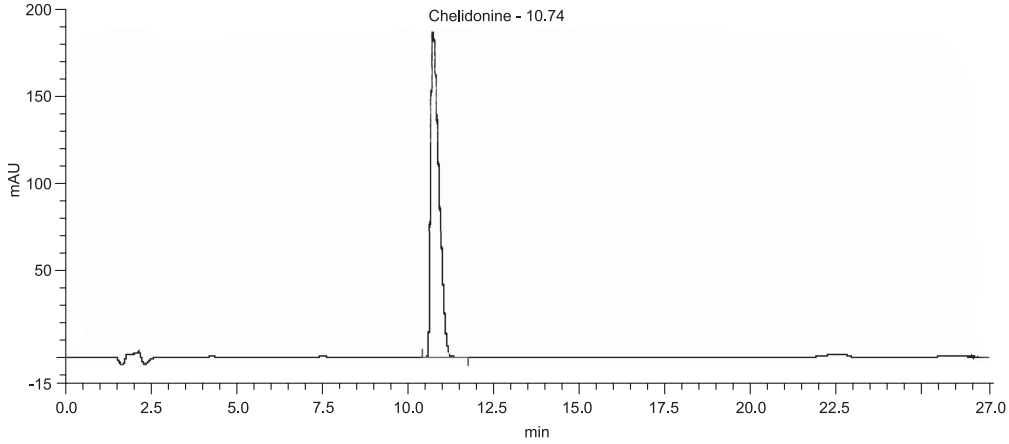
***Note**: Not all listed peaks need to be present and additional peaks may be present.*

System suitability: reference solution
- repeatability: maximum 2.0 per cent relative standard deviation for the area of the chelidonine peak after 6 injections
- Symmetry factor: maximum 2.0 for the peak due to chelidonine

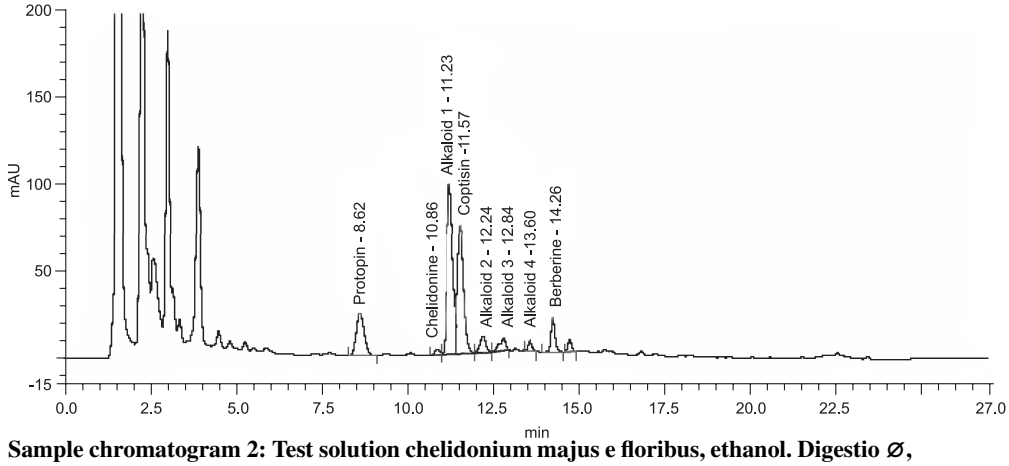
Calculate the content of total alkaloids in per cent (*m/m*), calculated as chelidonine, according to the following formula:

$$\frac{A_1 \cdot m_2 \cdot p}{A_2 \cdot m_1 \cdot 20}$$

A_1 = sum of the areas of all alkaloid peaks with a relative retention of 0.8 to 1.5 in the chromatogram obtained with the test solution
A_2 = area of the chelidonine peak in the chromatogram obtained with the reference solution
m_1 = mass of the mother tincture in grams
m_2 = mass of the reference substance chelidonine *RH* in grams
p = per cent content of chelidonine in the reference substance chelidonine *RH*



Sample chromatogram 1: Reference solution chelidonine (0.1 mg/ml)



Sample chromatogram 2: Test solution chelidonium majus e floribus, ethanol. Digestio ⌀, Method 18c

Storage

Store protected from light.