BLOODROOT CANADENSIS FOR HOMOEOPATHIC PREPARATIONS

SANGUINARIA CANADENSIS FOR HOMOEOPATHIC PREPARATIONS

Sanguinaria canadensis ad praeparationes homoeopathicas

DEFINITION

Dried, underground part of *Sanguinaria canadensis* L.

Content: minimum 2.5 per cent of the amount of sanguinarine and chelerythrine, expressed as sanguinarine chloride ($C_{20}H_{14}ClNO_4$; M_r 367.8) (dried drug).

IDENTIFICATION

A. Big, irregular, knotty, cylindrical fragments, 3-6 cm long and 6-12 mm large; slightly thinned ends; some flattened and tortuous fragments, others are tubercle-shaped. Reddish-brown surface sometimes dark, battered, wrinkled and annular. Inside surface with rounded scars left by the roots. Spongy, orange fracture, punctuated with red and brown in the middle. Adventitious, spindly roots about 1 mm in diameter.

B. Reduce bloodroot canadensis to a powder (355). The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R.* The powder shows the following elements: numerous fragments of cellulose parenchyma with ovoid cells with intercellular spaces; lactiferous fragments with slightly and regularly thickened cell-walls and reddish content; wood vessels fragments with punctuate or reticulate markings; scarce suber fragments consisting of polyhedral cells with dark brown cell-walls. Examine under a microscope using a solution of *glycerol* (50 per cent *V/V*) *R*: numerous starch granules either single or in groups of 2 or 3, ovoid to flattened, free or within parenchyma cells.

C. Thin layer chromatography (*2.2.27*).

Test solution. Add 30 mL of *ethanol (65 per cent V/V) R* to 3 g of powdered drug. Heat under a reflux condenser on a water-bath at 60 °C for 15 min. Allow the plate to cool. Filter.

Reference solution. Dissolve 5 mg of *sanguinarine chloride R* and 5 mg of *chelerythrine chloride R* in 10 mL of *ethanol* (*96 per cent*) *R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V).*

Application: 5 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Detection: Examine in ultraviolet light at 365 nm.

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

Top of the plate	
	A purplish-blue zone A purplish-blue zone A pinkish-brown zone
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Chelerythrine (chloride): a yellow zone Sanguinarine (chloride): an orange zone	A yellow zone (chelerythrine) An orange zone (sanguinarine)
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Reference solution	**Test solution**

TESTS

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

Total ash (*2.4.16*): maximum 6.0 per cent, determined on 1.0 g of powdered drug (355).

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 250 mL round-bottomed flask, place 2.500 g of powdered drug (355). Add 40 mL of *methanol R* and heat under a reflux condenser on a water-bath at 75 °C for 1 h. Allow to cool. Filter on a plug of absorbent cotton into a 100.0 mL volumetric flask. Dissolve the residue in 40 mL of *methanol* R and heat again under a reflux condenser on a water-bath at 75 °C for 1 h. Allow to cool. Filter on the same plug of absorbent cotton. Rinse the round-bottomed flask and the filter with *methanol R* and dilute to 100.0 mL with the same solvent. Take 2.0 mL of this solution and dilute to 20.0 mL with *methanol R.*

Reference solution. In a 100.0 mL volumetric flask, dissolve 10.0 mg of *sanguinarine chloride R* in *methanol R* and dilute to 100.0 mL with the same solvent. In a 20.0 mL volumetric flask, place 10.0 mL of this solution and dilute to 20.0 mL with *methanol R*.

Column:
– *size*: *l* = 0.25 m, *Ø* = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 30 °C.

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Mobile phase:
– *mobile phase A*: *phosphoric acid* (0.2 per cent *V/V*) *R*.
– *mobile phase B*: *methanol R.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 20	70 → 0	30 → 100
20 – 25	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 275 nm.

Injection: 5 µL.

Elution order: sanguinarine, chelerythrine.

System suitability:
- *Resolution*: minimum 2.0 between the peaks due to sanguinarine and chelerythrine.

Calculate the percentage content of sanguinarine and chelerythrine, expressed in sanguinarine chloride, from the expression:

$$\frac{(A_1 + A_2) \times m_2 \times 5 \times p}{A_3 \times m_1}$$

A_1 = area of the peak due to sanguinarine the chromatogram obtained with the test solution,
A_2 = area of the peak due to chelerythrine in the chromatogram obtained with the test solution,
A_3 = area of the peak due to sanguinarine chloride in the chromatogram obtained with the reference solution,
m_1 = mass of the drug sample, in grams,
m_2 = mass of the sample of sanguinarine chloride, in grams,
p = percentage content of sanguinarine chloride in *sanguinarine chloride R.*

STOCK

DEFINITION

Bloodroot canadensis mother tincture is prepared with ethanol (65 per cent *V/V*) using the dried, underground part of *Sanguinaria canadensis* L.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Adjusted content: 0.10 per cent to 0.30 per cent *m/m* of the amount of sanguinarine and chelerythrine, expressed as sanguinarine chloride ($C_{20}H_{14}ClNO_4$; M_r 367.8).

PRODUCTION

Method 1.1.10 (2371). Drug fragmented into segments about 1 cm long. Maceration time: 3 to 5 weeks.

CHARACTERS

Appearance: red liquid.

IDENTIFICATION

Thin layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *sanguinarine chloride R* and 5 mg of *chelerythrine chloride R* in 10 mL of *ethanol* (*96 per cent*) *R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V).*

Application: 5 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: Examine in ultraviolet light at 365 nm.

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

Top of the plate	
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Chelerythrine (chloride): a yellow zone Sanguinarine (chloride): an orange zone	A yellow zone (chelerythrine) An orange zone (sanguinarine)
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Reference solution	**Test solution**

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TESTS

Ethanol (*2.9.10*): 60 per cent *V/V* to 70 per cent *V/V.*

Dry residue (*2.8.16*): minimum 2.0 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 20.0 mL volumetric flask, place 1.000 g of mother tincture and dilute to 20.0 mL with *ethanol* (*60 per cent V/V*) *R.*

Reference solution. In a 100.0 mL volumetric flask, dissolve 10.0 mg of *sanguinarine chloride R* in *methanol R* and dilute to 100.0 mL with the same solvent. In a 20.0 mL volumetric flask, place 10.0 mL of this solution and dilute to 20.0 mL with *methanol R*.

Column:
– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).
– *temperature*: 30 °C.

Mobile phase:
– *mobile phase A*: *phosphoric acid* (0.2 per cent *V/V*) *R.*
– *mobile phase B*: *methanol R.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 20	70 → 0	30 → 100
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Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 275 nm.

Injection: 5 µL.

Elution order: sanguinarine, chelerythrine.

System suitability:
- *Resolution*: minimum 2.0 between the peaks due to sanguinarine and chelerythrine.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Calculate the percentage content *m/m* of sanguinarine and chelerythrine, expressed as sanguinarine chloride, from the expression:

$$\frac{(A_1 + A_2) \times m_2 \times 0.1 \times p}{A_3 \times m_1}$$

A_1 = area of the peak due to sanguinarine in the chromatogram obtained with the test solution,
A_2 = area of the peak due to chelerythrine in the chromatogram obtained with the test solution,
A_3 = area of the peak due to sanguinarine chloride in the chromatogram obtained with the reference solution,
m_1 = mass of the drug sample, in grams,
m_2 = mass of the sample of sanguinarine chloride, in grams,
p = percentage content of sanguinarine chloride in *sanguinarine chloride R.*

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.