

**OREGON GRAPE
FOR HOMOEOPATHIC PREPARATIONS
BERBERIS AQUIFOLIUM
FOR HOMOEOPATHIC PREPARATIONS**

***Berberis aquifolium* ad praeparationes homoeopathicas**
Other Latin name used in homoeopathy : ***Mahonia aquifolium***

DEFINITION

Dried root bark, entire or fragmented of *Berberis aquifolium* Pursh (= *Mahonia aquifolium* (Pursh) Nutt).

Content : minimum 1.0 per cent of total alkaloids, expressed as berberine (C₂₀H₁₉NO₅; M_r 353.4) (dried drug).

CHARACTERS

Yellow fluorescence under ultraviolet light at 365 nm.

Odour : bitter.

IDENTIFICATION

- A. Fragments usually flat of quite various size measuring up to 3-10 cm long and 50 mm to 3 cm thick. Brownish outside surface, wrinkled or cracked, seldom smooth. Dark yellow inside surface longitudinally striated. Clear fracture, marked with concentric striations. Remains of wood, bright yellow, sometimes sticking to the bark.
- B. Reduce the bark of the root to a powder (355). The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder presents the following characteristic elements : numerous fragments of brown suber composed of a great number of layers ; sclerous cells either free or in clusters ; liberous fibres, narrow and elongated with thickened cell-walls ; numerous prisms of calcium oxalate ; scarce reticulate or pitted vessels. Examine under a microscope using a 500 g/l solution of *glycerol R* : rounded starch granules, about 2-7 µm in diameter.

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

C. Thin-layer chromatography (2.2.27).

Test solution. Add 30 ml of ethanol (60 per cent V/V) R to 3.0 g of powdered drug (355). Heat on a water-bath at 60 °C, under a reflux condenser for 15 min. Allow to cool. Filter.

Reference solution. Dissolve 20 mg of berberine chloride R and 10 mg of sanguinarine nitrate R in 20 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: 20 µl as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection A: examine in daylight.

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

Top of the plate	
Berberine (chloride): a yellow zone Sanguinarine (nitrate): an orange zone	A yellow zone (berberine) A yellow zone
Reference solution	Test solution

Detection B: examine in ultraviolet light at 365 nm.

Results B: 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	
Berberine (chloride): an intense yellow zone Sanguinarine (nitrate): an orange zone	A purplish-blue zone An intense yellow zone (berberine) A greenish-yellow zone A greenish-yellow zone A blue zone
Reference solution	Test solution

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

Detection C: spray with *potassium iodobismuthate solution R*. Examine in daylight.

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

Top of the plate	
Berberine (chloride): an orange zone Sanguinarine (nitrate): an orange zone	An orange zone (berberine) A thin orange zone One to two faint orange zones
Reference solution	Test solution

TESTS

Berberis vulgaris. Examined under a microscope, the cross-section of the drug shows multiseriate medullary rays. Their absence shows adulteration by *Berberis vulgaris* L.

Loss on drying (2.2.32): maximum 8.0 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 6.0 per cent.

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

ASSAY

Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. Place 1.000 g of powdered drug (355) in a flask, add 20 ml of *ethanol* (60 per cent *V/V*) *R*. Shake for 30 min and filter into a 50.0 ml volumetric flask. Repeat the operation onto the residue. Dilute to 50.0 ml with *ethanol* (60 per cent *V/V*) *R*. Place 4.0 ml of this solution into a 50.0 ml volumetric flask and dilute with 0.05 *M sulphuric acid R* in *methanol R*.

Compensation liquid. 0.05 *M sulphuric acid R* in *methanol R*.

Immediately after the addition of the last reagent, measure the absorbance of the solution at 425 nm, in comparison with the compensation liquid.

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

2009.

Calculate the percentage content of total alkaloids, expressed as berberine, from the expression :

$$\frac{A \times 625}{163 \times m}$$

i.e. taking the specific absorbance of berberine, to be 163.

A = absorbance of the test solution at 425 nm,

m = mass of the sample, in grams.

STOCK

DEFINITION

Oregon grape mother tincture is prepared with ethanol (55 per cent *V/V*), using the dried root bark, entire or fragmented of *Berberis aquifolium* Pursh.

Adjusted content : minimum 0.10 per cent and maximum 0.30 per cent *m/m* of total alkaloids, expressed as berberine ($C_{20}H_{19}NO_5$; M_r 353.4).

CHARACTERS

Yellowish-brown to reddish-brown liquid.

PRODUCTION

Method 4c (2371). Powdered drug (710). Maceration time : 3-5 weeks.

IDENTIFICATION

Thin-layer chromatography (2.2.27).

Test solution. Mother tincture.

Reference solution. Dissolve 20 mg of *berberine chloride R* and 10 mg of *sanguinarine nitrate R* in 20 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 : 20 μ l as bands.

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

Development : over a path of 10 cm.

Drying : in air.

Detection A : examine in daylight.

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

Top of the plate	
----- Berberine (chloride) : a yellow zone Sanguinarine (nitrate) : an orange zone -----	A yellow zone (berberine) A yellow zone A yellow zone -----
Reference solution	Test solution

Detection B : examine in ultraviolet light at 365 nm.

Results B : 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	
----- Berberine (chloride) : an intense yellow zone Sanguinarine (nitrate) : an orange zone -----	A purplish-blue zone An intense yellow zone (berberine) A yellow zone One to two blue zones -----
Reference solution	Test solution

Detection C : spray with *potassium iodobismuthate solution R*. Examine in daylight.

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

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

2009.

Top of the plate	
----- Berberine (chloride): an orange zone Sanguinarine (nitrate): an orange zone -----	----- An orange zone (berberine) A thin orange zone An orange zone ----- One to two orange zones
Reference solution	Test solution

TESTS

Ethanol content (2.9.10): 50 per cent *V/V* to 60 per cent *V/V*.

Dry residue (2.8.16): minimum 0.9 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. In a 100.0 ml volumetric flask, place 2.000 g of mother tincture and dilute to 100.0 ml with 0.05 *M sulphuric acid R* in *methanol R*.

Compensation liquid. 0.05 *M sulphuric acid R* in *methanol R*.

Immediately after the addition of the last reagent, measure the absorbance of the solution at 425 nm, in comparison with the compensation liquid.

Calculate the percentage content (*m/m*) of total alkaloids, expressed as berberine, from the expression :

$$\frac{A \times 100}{163 \times m}$$

i.e. taking the specific absorbance of berberine, to be 163.

A = absorbance of the test solution at 425 nm,

m = mass of the mother tincture sample, in grams.