

**OLEANDER  
FOR HOMOEOPATHIC PREPARATIONS**

**NERIUM OLEANDER  
FOR HOMOEOPATHIC PREPARATIONS**

**Nerium oleander ad praeparationes homoeopathicas**  
Other Latin name used in homeopathy: **Oleander**

DEFINITION

Fresh leaf of *Nerium oleander* L.

IDENTIFICATION

- A. The fresh leaf of oleander is entire, shortly petioled, lanceolate, measuring about 12 cm long and 2 cm large. It is green on the upper side; the underside is paler and shows a very conspicuous midrib of a whitish colour and numerous, secondary, parallel veins. It has a coriaceous consistency.
- B. Take a fragment of abaxial epidermis of the leaf. Examine under a microscope, using *chloral hydrate solution R*: epidermis composed of polyhedral cells and unicellular covering trichomes with strongly thickened and finely echinulate cell-wall; numerous openings corresponding to stomatiferous cavities, more or less ovoid, bordered by small cells markedly cuticle-covered and unicellular covering trichomes bent or hooked-shaped.

TESTS

**Foreign matter** (2.8.2): maximum 5 per cent.

**Loss on drying** (2.2.32): minimum 50.0 per cent, determined on 5.0 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

**STOCK**

DEFINITION

Oleander mother tincture is prepared with ethanol (65 per cent V/V), using the fresh leaf of *Nerium oleander* L.

*Content*: minimum 0.050 per cent *m/m* of total flavonoids, expressed as rutin (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>, 3 H<sub>2</sub>O; *M<sub>r</sub>* 665).

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

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**PRODUCTION**

*Method 4c (2371).* Drug fragmented into 3-5 cm long segments. Maceration time: 3-5 weeks.

**CHARACTERS**

Brownish-green liquid.

**IDENTIFICATION**

Thin-layer chromatography (2.2.27).

*Test solution.* Mother tincture.

*Reference solution.* Dissolve 5 mg of *rutin R* and 5 mg of *hyperoside R* in 20 mL of *ethanol (96 per cent) R*.

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

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

*Application:* 20 µL [or 10 µL] as bands.

*Development:* over a path of 10 cm [or 6 cm].

*Drying:* in air.

*Detection:* first spray with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* then with a 50 g/L solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in the air for about 30 min. 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	
----- Hyperoside: an orange-yellow zone  -----	A red zone An orange zone  A blue zone A blue zone  -----
Rutin: an orange zone	An orange zone (rutin) An orange zone
<b>Reference solution</b>	<b>Test solution</b>

## TESTS

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

**Dry residue** (2.8.16): minimum 1.5 per cent *m/m*.

**Oleandrine**: maximum 0.030 per cent *m/m*.

Liquid chromatography (2.2.29).

*Test solution.* In a round-bottomed flask evaporate 1.250 g of mother tincture to dryness, under reduced pressure. Dilute the residue in 2.0 mL of *methanol R*.

*Reference solution.* In a 50.0 mL volumetric flask, dissolve 15.0 mg of *oleandrine R* in *methanol R* and dilute to 50.0 mL with the same solvent. Place 7.0 mL of this solution into a 20.0 mL volumetric flask and dilute to 20.0 mL with *methanol R*.

*Column:*

- size:  $l = 0.25$  m,  $\varnothing = 4.6$  mm,
- stationary phase: *octylsilyl silica gel for chromatography R* (5  $\mu$ m),
- temperature: 30 °C.

*Mobile phase:*

- mobile phase A: water *R*,
- mobile phase B: *acetonitrile R*.

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 – 10	60	40
10 – 20	60 □ 50	40 □ 50
20 – 23	50 □ 5	50 □ 95
23 – 35	5	95

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*Flow rate:* 1.0 mL/min.

*Detection:* spectrophotometer at 220 nm.

*Injection:* 10 µL.

Calculate the percentage content *m/m* of oleandrine, from the expression:

$$\frac{m_2 \times A_1 \times 0,014 \times p}{m_1 \times A_2}$$

$A_1$  : area of the peak due to oleandrine in the chromatogram obtained with the test solution,  
 $A_2$  : area of the peak due to oleandrine in the chromatogram obtained with the reference solution,  
 $m_1$  : mass of the mother tincture sample in the test solution, in grams,  
 $m_2$  : mass of oleandrine sample in the reference solution, in grams,  
 $p$  : percentage content of oleandrine in *oleandrine R*.  
ASSAY

Ultraviolet and visible absorption spectrophotometry (2.2.25).

*Stock solution.* Evaporate 1.500 g of mother tincture to dryness, under reduced pressure. Dissolve the residue in 25.0 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*.

*Test solution.* Place 5 mL of stock solution into a 25.0 mL volumetric flask, add 5 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Then add 10 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R*. Dilute to 25.0 mL with *glacial acetic acid R*.

*Compensation liquid of the test solution.* Place 5 mL of stock solution into a 25.0 mL volumetric flask, add 5 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Then add 10 mL of *anhydrous formic acid R*. Dilute to 25.0 mL with *glacial acetic acid R*.

*Reference stock solution.* In a 100.0 mL volumetric flask, dissolve 16.0 mg of *rutin CRS* in a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Dilute to 100.0 mL with the same mixture. In a 25.0 mL volumetric flask, place 5 mL of the solution and dilute to 25.0 mL with the same mixture.

*Reference solution.* Place 10 mL of reference stock solution into a 25.0 mL volumetric flask, add 10 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R*. Dilute to 25.0 mL with *glacial acetic acid R*.

*Compensation liquid of the reference solution.* Place 10 mL of reference stock solution into a 25.0 mL volumetric flask, add 10 mL of *anhydrous formic acid R*. Dilute to 25.0 mL with *glacial acetic acid R*.

Thirty min after the addition of the last reagent, measure the absorbance of the test solution at 420 nm, in comparison with the compensation liquid of the test solution, and the absorbance of the reference solution in comparison with the compensation liquid of the reference solution.

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Calculate the percentage content  $m/m$  of total flavonoids, expressed as rutin, from the expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 10}$$

$A_1$  = absorbance of the test solution,

$A_2$  = absorbance of the reference solution,

$m_1$  = mass of the mother tincture sample in the test solution, in grams,

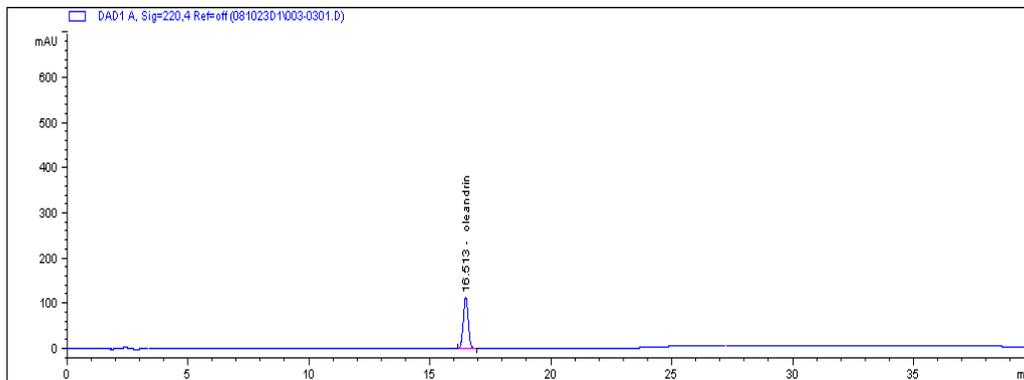
$m_2$  = mass of *rutin* sample in the reference solution, in grams,

$p$  = percentage content of rutin in *rutin CRS*.

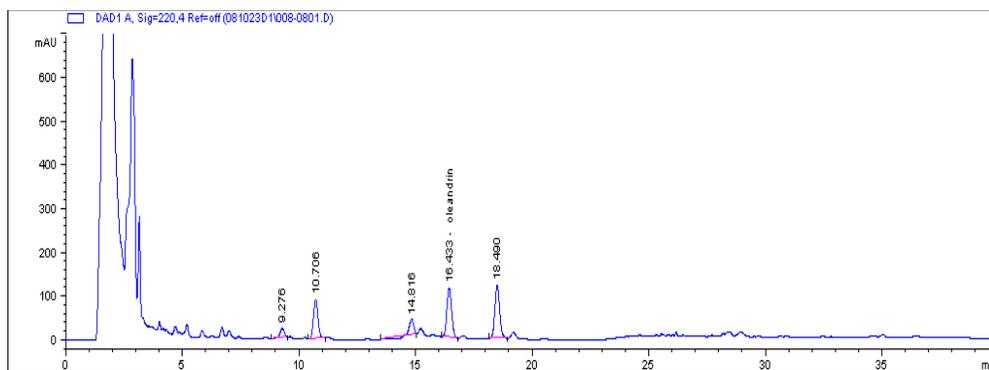
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## Standard LC profiles of the test « Oleandrine » for information



Standard LC profile of the reference solution



Standard LC profile of the test solution

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