

**PURGING CROTON
FOR HOMOEOPATHIC PREPARATIONS
CROTON TIGLIUM
FOR HOMOEOPATHIC PREPARATIONS**

Croton tiglium ad praeparationes homoeopathicas

DEFINITION

Dried seed of *Croton tiglium* L. (*Tiglium officinale* Klotsch).

IDENTIFICATION

Take all the precautions required: irritant product.

- A. Ovoid seed, 10-15 mm long and 7-10 mm large; convex back side, marked by a small, longitudinal arista; the face beneath with 2 more flattened sides separated by the prominent raphe; two side ribs surrounding the sides of the seed from the top occupied by the caruncle and the hilum down to the base, toward the chalaza. Seeds often deprived of this caruncle, small deciduous outgrowth wrapping the micropyle. Outside tegument, matt yellowish-brown, deprived of mottling and easily exfoliating, showing a second blackish, thorny envelope underneath. Cross-section of the triangular or rectangular seed with more or less swollen sides; presence of a third thin, silver envelope wrapping the oil-producing albumen containing 2 foliaceous cotyledons, inside the black, hard, brittle shell.
- B. Crush the seed. Examine under a microscope, using *chloral hydrate solution R*: fragments of the outside tegument comprising polyhedral cells with thin cell-walls, some layers of parenchyma with intercellular airspaces and a layer of very small, rounded cells; fragments of inside tegument composed of cells, markedly thickened in palisade; fragments of albumen cells, composed of polyhedral cells containing numerous droplets of oil.
- C. Thin-layer chromatography (2.2.27).
Test solution. Add 30 ml of *ethanol* (65 per cent V/V) *R* to 3 g of crushed drug. Heat under a reflux condenser at 60 °C for 15 min. Allow to cool. Filter.

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

Reference solution. Dissolve 10 mg of *linoleic acid R* and 2 mg of *linalol R* in 10 ml of *methanol R*.

Plate : TLC silica gel plate *R*.

Mobile phase : ethyl acetate *R*, toluene *R* (15:85 *V/V*).

Application : 30 µl as bands.

Development : over a path of 10 cm.

Drying : in air.

Detection : spray with a 50 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*. Heat at 100-105 °C for 10 min. Examine in daylight.

Results : 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	
----- Linalol : a greyish-blue zone -----	A greyish-blue zone -----
Linoleic acid : a greyish-blue zone	A large greyish-blue zone -----
Reference solution	Test solution

TESTS

Loss on drying (2.2.32): maximum 8.0 per cent, determined on 1.0 g of crushed drug by drying in an oven at 105 °C for 2 h.

Total ash (2.4.16): maximum 3.5 per cent, determined on 1.0 g of crushed drug.

STOCK

DEFINITION

Croton tiglium mother tincture is prepared with ethanol (65 per cent *V/V*), using the dried seed of *Croton tiglium* L. (*Tiglium officinale* Klotsch).

Adjusted content : minimum 0.020 per cent *m/m* and maximum 0.060 per cent *m/m* of total phorbol derivatives, expressed as phorbol ($C_{20}H_{28}O_6$; M_r 364.4).

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

PRODUCTION

Method 4c (2371). Crushed drug, fragmented into segments about 0.5 cm long. Maceration time : 3-6 weeks.

CHARACTERS

Yellow liquid.

IDENTIFICATION

Vigorously shake the mother tincture in order to homogenize the sample.

Thin-layer chromatography (2.2.27).

Test solution. Mother tincture.

Reference solution. Dissolve 10 mg of *linoleic acid R* and 2 mg of *linalol R* in 10 ml of *methanol R*.

Plate : TLC silica gel plate *R*.

Mobile phase : ethyl acetate *R*, toluene *R* (15:85 V/V).

Application : 30 µl as bands.

Development : over a path of 10 cm.

Drying : in air.

Detection : spray with a 50 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*. Heat at 100-105 °C for 10 min. Examine in daylight.

Results : 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	
----- Linalol : a greyish-blue zone -----	A greyish-blue zone -----
Linoelic acid : a greyish-blue zone	A large greyish-blue zone -----
Reference solution	Test solution

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

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TESTS

Vigorously shake the mother tincture in order to homogenize the sample.

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

Dry residue (2.8.16): minimum 0.7 per cent *m/m*.

ASSAY

Vigorously shake the mother tincture in order to homogenize the sample.

Liquid chromatography (2.2.29).

Test solution. In a 25.0 ml volumetric flask, dissolve 4.5 g of mother tincture accurately weighed in *methanol R* with sonicate for 10 s and dilute to 25.0 ml with the same solvent. Evaporate to dryness 10.0 ml of this solution, under reduced pressure. Dilute the residue with 5.0 ml of *methanol R*, 2.0 ml of 0.5 M *potassium hydroxide methanolic solution R*. Shake for 60 min, away from light. Add 2.0 ml of 0.5 M *hydrochloric acid methanolic solution R* and 5.0 ml of *phosphate buffer solution pH 7.2 R*. Transfer the whole quantity of the solution obtained into a 25.0 ml volumetric flask and dilute to 25.0 ml with *phosphate buffer solution pH 7.2 R*. Filter through 0.45 µm filter.

Reference solution. In a 25.0 ml volumetric flask, dissolve 5.0 g of *phorbol R* in *methanol R* and dilute to 25.0 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 the same solvent. Take 2.0 ml of this solution and dilute to 5.0 ml with *phosphate buffer solution pH 7.2 R*.

Column :

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

Mobile phase :

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

Time (min)	Mobile phase A (per cent <i>V/V</i>)	Mobile phase B (per cent <i>V/V</i>)
0-5	98	2
5-30	98 → 90	2 → 10
30-35	90 → 0	10 → 100

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

Flow rate : 1.0 ml/min.

Detection : spectrophotometer at 239 nm.

Injection : 20 μ l.

Retention time : phorbol = about 25 min.

Calculate the percentage content m/m of phorbol derivatives, expressed as phorbol, from the expression :

$$\frac{A_1 \times m_2 \times 0.5 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to phorbol in the chromatogram obtained with the test solution,

A_2 = area of the peak due to phorbol in the chromatogram obtained with the reference solution,

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

m_2 = mass of phorbol in the reference solution, in grams,

p = percentage content of phorbol in *phorbol R*.