# LILY OF THE VALLEY FOR HOMOEOPATHIC PREPARATIONS

# CONVALLARIA MAJALIS FOR HOMOEOPATHIC PREPARATIONS

Convallaria majalis ad praeparationes homoeopathicas

## DEFINITION

Whole, fresh, blooming plant, *Convallaria majalis* L.

## CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

Characteristic odour.

## IDENTIFICATION

A. Perennial herb with creeping rhizome bearing numerous rootlets. Stem 10 cm to 20 cm high with at the base 2 amplecant leaves, curvinervate, lanceolate-oval, about 4 cm large on 10 cm to 15 cm long. Inflorescence in unilateral raceme at the tip of the bare stalk. White, globular bell-shaped flowers with 6 curved teeth, 6 stamens, 3-carpel ovary topped by a short style.

B. Take a sample of epidermis from the underside of a leaf of lily of the valley. Examine under a microscope, using *chloral hydrate solution R.* Stomatiferous abaxial epidermis; elongated epidermis cells, more or less rectangular to parallelipipedic measuring about 100 µm long and 25 µm large; numerous stomata surrounded by four subsidiary cells (similar to the other epidermis cells), two are placed on either side of the stomata parallel to the ostiole, the other two are located at each end of the stoma.

## TESTS

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

**Loss on drying** (*2.2.32*): minimum 70.0 per cent, determined on 5.0 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

## STOCK

## DEFINITION

Lily of the valley mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement). The mother tincture is prepared with ethanol (65 per cent *V/V)*, using the whole, fresh, blooming plant, *Convallaria majalis* L.

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

*Content*: minimum 0.040 per cent *m/m* of cardenolic heterosides, expressed as digitoxin ($C_{41}H_{64}O_{12}$; $M_r$ 765).

CHARACTERS

*Appearance*: greenish-brown liquid.

IDENTIFICATION

A. Thin layer chromatography (*2.2.27*).

*Test solution.* Mother tincture.

*Reference solution*. Dissolve 10 mg of *aescin R* and 10 mg of *asiaticoside R* in 10 mL of *methanol R*.

*Plate*: *TLC silica gel plate R.*

*Mobile phase*: *glacial acetic acid R*, *water R*, *butanol R* (10:10:40 *V/V/V*).

*Application*: 50 µL, as bands.

*Development*: over a path of 10 cm.

*Drying*: in air.

*Detection*: spray with a 100 g/L solution of *sulfuric 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** | |
|---|---|
| | A purplish-pink zone |
| ----- | ----- |
| | A greyish-brown zone |
| Asiaticoside: a grey zone | |
| | A grey zone |
| ----- | ----- |
| Aescin: a purplish-grey zone | One to two more or less isolated brown zones |
| **Reference solution** | **Test solution** |

B. Thin layer chromatography (*2.2.27*).

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*Test solution.* Heat 10 mL of mother tincture to the boiling point for 2 min with 5 mL of *lead acetate solution R.* After cooling, centrifuge the mixture. Shake the supernatant with 15 mL of *methylene chloride R.* Collect the organic solution and dry it on *anhydrous sodium sulfate R*. Filter on cotton plug. Evaporate on a water-bath. Dissolve the residue in 1 mL of *ethanol* (70 per cent *V/V*) *R.*

*Reference solution*. Dissolve 2 mg of *convallatoxine R* and 5 mg of *digitoxin R* in 10 mL of *methanol R.*

*Plate*: *TLC silica gel plate R.*

*Mobile phase*: *water R, methanol R*, *ethyl acetate R* (8:11:81 *V/V/V*).

*Application*: 40 µL, as bands.

*Development*: over a path of 10 cm.

*Drying*: in air.

*Detection*: spray with a 10 g/L solution of *dinitrobenzoic acid R* in a mixture composed of equal quantities of *methanol R* and *potassium hydroxide 2 M*. 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** | |
|---|---|
| Digitoxin: a purple zone<br>----- | <br>----- |
| | A purple zone |
| Convallatoxine: a purple zone<br>----- | A purple zone (convallatoxine)<br>----- |
| | A purple zone |
| **Reference solution** | **Test solution** |

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

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).
*Prepare simultaneously the reference solution and the test solution.*

*Test solution.* In a round-bottomed flask, place 2.5 g of mother tincture accurately weighed, add 50.0 mL of *water R* then 5.0 mL of a 150 g/L solution of *lead acetate R.* Shake for some min, add 7.5 mL of a 40 g/L solution of *disodium hydrogen phosphate R*. Filter. Add 5 mL of 150 g/L *hydrochloric acid R* to 50.0 mL of the filtrate and heat under a reflux condenser on a water-bath for 1 h. Transfer into a separating funnel, rinse the round-bottomed flask twice with 5 mL of *water R*

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and shake 3 times with 25 mL of *methylene chloride R.* Combine the organic layers, dry them on *anhydrous sodium sulfate R* and dilute to 100.0 mL with *methylene chloride R*. Evaporate to dryness 40.0 mL of the organic solution. Dissolve the residue in 7.0 mL of *ethanol* (50 per cent *V/V*) *R* then add 2.0 mL of *dinitrobenzoic acid solution R* and 1.0 mL of *sodium hydroxide 1 M.*

*Reference solution.* Dissolve accurately 50.0 mg of *digitoxin CRS* in 50.0 mL of *ethanol* (*96 per cent*) *R.* Take 5.0 mL of this solution and dilute to 50.0 mL with *ethanol* (*96 per cent*) *R*. Take 5.0 mL of this solution. Add 25 mL of *water R* and 3 mL of 150 g/L *hydrochloric acid R*. Heat under a reflux condenser on a water-bath for 1 h. Transfer into a separating funnel, rinse the round-bottomed flask twice with 5 mL of *water R* and shake 3 times with 25 mL of *methylene chloride R*. Combine the organic layers, dry them on *anhydrous sodium sulfate R* and dilute to 100.0 mL with *methylene chloride R*. Evaporate to dryness 40.0 mL of the organic layer solution. Dissolve the residue in 7.0 mL of *ethanol* (50 per cent *V/V*) *R* then add 2.0 mL of *dinitrobenzoic acid solution R*, and 1.0 mL of *sodium hydroxide 1 M.*

*Compensation liquid.* Mix 1.0 mL of *sodium hydroxide 1 M*, 2.0 mL of *dinitrobenzoic acid solution R* and 7.0 mL of *ethanol* (50 per cent *V/V*) *R*.

Repeat the measure of the absorbance of the solutions over the first 12 min at 540 nm, until the maximum is reached in comparison with the compensation liquid.

Calculate the percentage content *m/m* of cardenolic heterosides, expressed as digitoxin, from the expression:

$$\frac{A_1 \times m_2 \times 1.25}{A_2 \times m_1}$$

$A_1$ = absorbance of the test solution,
$A_2$ = absorbance of the reference solution,
$m_1$ = mass of the mother tincture sample, in grams,
$m_2$ = mass of digitoxin sample in the reference solution, in grams.

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