YELLOW SWEET CLOVER FOR HOMOEOPATHIC PREPARATIONS

MELILOTUS OFFICINALIS FOR HOMOEOPATHIC PREPARATIONS

Melilotus officinalis ad praeparationes homoeopathicas

Other Latin name used in homoeopathy: **Melilotus**

DEFINITION

Fresh, blooming, aerial part of *Melilotus officinalis* (L.) Lam.

CHARACTERS

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

IDENTIFICATION

A. Herbaceous, biannual plant, erect, up to 90 cm high. Green, ridged stem. Glabrescent aerial parts. Alternate, petiolate, trifoliate leaves, each one accompanied by 2 lanceolate stipules. Numerous hairs conspicuous at the base of the petiole. Leaflets, greener on the upper side than on the underside, elongated, dentate, measuring up to 30 mm long and 20 mm wide. Inflorescence in a long raceme. Flowers up to 7 mm long; calyx with 5 unequal teeth, yellow petals whose banner is longer than the wings, themselves longer than the keel. Ovoid achene, yellowish-brown or turning darker when ripe, ending in a short point at the apex, transversally wrinkled, frequently located inside the calyx, even after maturity.

B. Take a fragment of abaxial epidermis of the leaf. Examine under a microscope, using *chloral hydrate solution R*: lamina epidermis composed of polygonal cells; bicellular covering trichomes, echinulate, bent at right angle; scarce secretory trichomes with a short bi or tri cellular foot and ovoid head, biseriate with 4 cells; anomocytic stomata (*2.8.3*).

TESTS

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

Loss on drying (*2.2.32*): minimum 45.0 per cent, determined on 5.0 g of finely-cut drug by drying in an oven at 105 °C for 2 h.

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

STOCK

DEFINITION

Yellow sweet clover mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Supplement). The mother tincture is prepared with ethanol (65 per cent *V/V*), using the fresh, flowering aerial part of *Melilotus officinalis* (L.) Lam.

Content: minimum 0.07 per cent *m/m* of coumarin ($C_9H_6O_2$; M_r 146.1).

CHARACTERS

Appearance: greenish-brown liquid.

Odour of coumarin.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 10 mg of *coumarin R* and 10 mg of *o-coumaric acid R* in 20 mL of *methanol R.*

Plate: *TLC silica gel plate R*.

Mobile phase: upper phase of the mixture: *dilute acetic acid R*, *ether R*, *toluene R* (10:50:50 *V/V/V*).

Application: 25 µL as bands.

Development: over a path of 15 cm.

Drying: in air.

Detection: spray with *2M alcoholic potassium hydroxide solution R.* 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.

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

Top of the plate	
Coumarin: a greenish-yellow zone -----	A greenish-yellow zone (coumarin) -----
o-coumaric acid: a greenish-yellow zone -----	A greenish-yellow zone (*o*-coumaric acid) may occur -----
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 1.5 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 100.0 mL volumetric flask, place 5.000 g of mother tincture. Dilute to 100.0 mL with *methanol R*.

Reference solution. In a 250.0 mL volumetric flask, dissolve 25.0 mg of *coumarin CRS* in *methanol R* and dilute to 250.0 mL with the same solvent.

Column:
– *size*: *l* = 0.25 m, Ø = 4 mm,
– *stationary phase*: *end-capped, octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R*, 5 g/L *phosphoric acid solution R* (22:78 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 275 nm.

Injection: 20 µL. Retention time of coumarin: about 19 min.

Calculate the percentage content of coumarin, from the expression:

$$\frac{A_1 \times m_2 \times 40}{m_1 \times A_2}$$

A_1 = area of the peak due to coumarin in the chromatogram obtained with the test solution,
A_2 = area of the peak due to coumarin in the chromatogram obtained with the reference solution,
m_1 = mass of the mother tincture in the test solution, in grams,
m_2 = mass of *coumarin CRS* in the reference solution, in grams.

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