MILK THISTLE FOR HOMOEOPATHIC PREPARATIONS CARDUUS MARIANUS FOR HOMOEOPATHIC PREPARATIONS

Carduus marianus ad praeparationes homoeopathicas
Other latin name used in homoeopathy : **Silybum marianum**

The herbal drug complies with the monograph *Milk thistle (1860)*.

STOCK

DEFINITION

Milk thistle mother tincture is prepared with ethanol (65 per cent *V*/*V*) using the dried, ripe, pappus-free fruit of *Silybum marianum* (L.) Gaertn.

Content : minimum 0.080 per cent *m*/*m* of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_r 482.4).

PRODUCTION

Method 4c (2371). Drug either whole or crushed. Maceration time : 3-5 weeks.

CHARACTERS

Yellow liquid.

IDENTIFICATION

Thin layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 2 mg of *silibinin R* and 5 mg of *taxifolin R* in 30 ml of *methanol R*.

Plate : TLC silica gel plate R.

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

Mobile phase : anhydrous formic acid R, acetone R, methylene chloride R (8.5:16.5:75 *V/V/V*).

Application : 30 µl, as bands.

Development : over a path of 10 cm.

Drying : at 100-105 °C.

Detection : first spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R,* while the plate is still warm, then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry 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	
Silibinin : a yellowish-green zone ------ Taxifolin : an orange zone ------	A yellowish-green zone (silibinin) ------ An orange zone (taxifolin) A yellowish-green zone(silicristin) ------
Reference solution	**Test solution**

TESTS

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

Dry residue (*2.8.16*) : minimum 0.4 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 50.0 ml volumetric flask, place 5.000 g of mother tincture and dilute to 50.0 ml with *methanol R*.

Reference solution (a). Dissolve 5.0 mg of *silibinin R* previously dried under vaccum in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dissolve 1.0 mg of *silicristin R* in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (c). Dissolve 1.0 mg of *silidianin R* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column :

— *size : l* = 0.125 m, Ø = 4 mm,

— *stationary phase : octadecylsilyl silica gel for chromatography R* (5 μm).

Mobile phase :

— *mobile phase A : phosphoric acid R, methanol R, water R* (0.5:35:65 *V/V/V*).

— *mobile phase B : phosphoric acid R, methanol R, water R* (0.5:50:50 *V/V/V*).

Intervalle (min)	**Phase mobile A (pour cent *V/V*)**	**Phase mobile B (pour cent *V/V*)**
0-28	100 → 0	0 → 100
28-35	0	100
35-36	0 → 100	100 → 0
36-51	100	0

Flow rate : 0.8 ml/min.

Detection : spectrophotometer at 288 nm.

Injection : 10 μl.

Retention time : silibinin B about 30 min. Where needed adjust the time periods of the gradient.

System suitability : reference solution (a).

— *Resolution :* minimum 1.8 between the peaks due to silibinin A and silibinin B.

With the help of the retention times determined from the chromatograms obtained with the reference solutions, locate the peaks due to silicristin, silidianin, silibinin A and silibinin B in the chromatogram obtained with the test solution. Locate the peaks due to isosilibinin A and isosilibinin B using the chromatogram below. The peak due to silidianin may vary in size, be missing or be the highest one. Calculate the area of the peaks due to silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B.

Calculate the percentage content *m/m* of silymarin, expressed as silibinin, from the expression :

$$\frac{(A_1 + A_2 + A_3 + A_4 + A_5 + A_6) \times m_2 \times p}{(A_7 + A_8) \times m_1}$$

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

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

A_2 = area of the peak due to silidianin in the chromatogram obtained with the test solution,

A_3 = area of the peak due to silibinin A in the chromatogram obtained with the test solution,

A_4 = area of the peak due to silibinin B in the chromatogram obtained with the test solution,

A_5 = area of the peak due to isosilibinin A in the chromatogram obtained with the test solution,

A_6 = area of the peak due to isosilibinin B in the chromatogram obtained with the test solution,

A_7 = area of the peak due to silibinin A in the chromatogram obtained with reference solution (a),

A_8 = area of the peak due to silibinin B in the chromatogram obtained with reference solution (a),

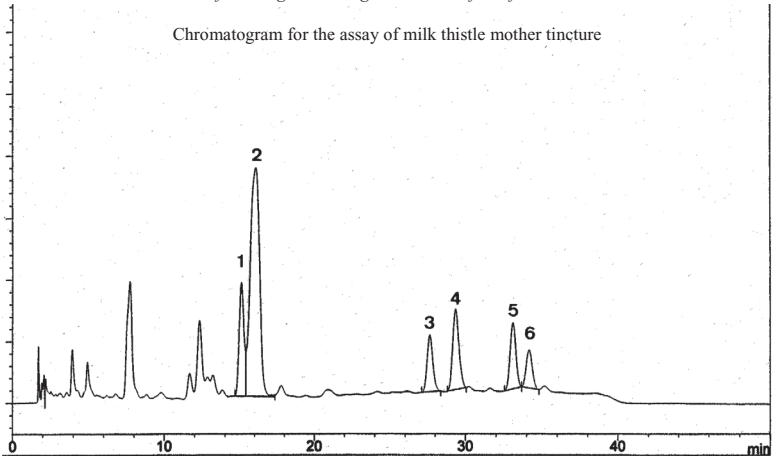
m_1 = mass of the mother tincture sample, in the test solution, in grams,

m_2 = mass of silibinin in reference solution (a), in grams,

p = percentage content of the amount of silibinin A and silibinin B in *silibinin R*.

The following chromatogram is shown for information

Chromatogram for the assay of milk thistle mother tincture



1. silicristin
2. silidianin
3. silibinin A
4. silibinin B
5. isosilibinin A
6. isosilibinin B

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