– the chromatogram obtained is similar to the chromatogram supplied with *milk thistle standardised dry extract CRS*.

Locate silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B by comparison with the chromatogram supplied with *milk thistle standardised dry extract CRS*. In the chromatogram obtained with the test solution the peak due to silidianin may vary in size, be absent or be present as the principal peak. Determine the area of the peaks due to silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B.

Calculate the percentage content of silymarin, calculated as silibinin, using the following expression:

$$\frac{(A1 + A2 + A3 + A4 + A5 + A6) \times m_1 \times p \times 1000}{(A7 + A8) \times m_2 \times (100 - d)}$$

$A1$ = area of the peak due to silicristin in the chromatogram obtained with the test solution;

$A2$ = area of the peak due to silidianin in the chromatogram obtained with the test solution;

$A3$ = area of the peak due to silibinin A in the chromatogram obtained with the test solution;

$A4$ = area of the peak due to silibinin B in the chromatogram obtained with the test solution;

$A5$ = area of the peak due to isosilibinin A in the chromatogram obtained with the test solution;

$A6$ = area of the peak due to isosilibinin B in the chromatogram obtained with the test solution;

$A7$ = area of the peak due to silibinin A in the chromatogram obtained with the reference solution;

$A8$ = area of the peak due to silibinin B in the chromatogram obtained with the reference solution;

m_1 = mass of *milk thistle standardised dry extract CRS* used to prepare the reference solution, in grams;

m_2 = mass of the drug to be examined, in grams;

p = combined percentage content of silibinin A and silibinin B in *milk thistle standardised dry extract CRS*;

d = percentage loss on drying of the drug.

Monographs M

01/2008:2071

MILK THISTLE DRY EXTRACT, REFINED AND STANDARDISED

Silybi mariani extractum siccum raffinatum et normatum

DEFINITION

Dry extract, refined and standardised, produced from *Milk-thistle fruit (1860)*.

Content: 90 per cent to 110 per cent of the nominal content of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_r 482.4), stated on the label. The nominal content of silymarin is within the range 30 per cent *m/m* to 65 per cent *m/m* (dried extract).

The content of silymarin corresponds to:

– *sum of the contents of silicristin and silidianin* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 20 per cent to 45 per cent, calculated with reference to total silymarin;

– *sum of the contents of silibinin A and silibinin B* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 40 per cent to 65 per cent, calculated with reference to total silymarin;

– *sum of the contents of isosilibinin A and isosilibinin B* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 10 per cent to 20 per cent, calculated with reference to total silymarin.

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure, using one or more of the following solvents:

– ethyl acetate;

– acetone or mixture of acetone and water;

– ethanol or mixture of ethanol and water;

– methanol or mixture of methanol and water.

CHARACTERS

Appearance: yellowish-brown, amorphous powder.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.250 g of the extract to be examined in 5 ml of *methanol R*.

Reference solution. Dissolve 2 mg of *silibinin R* and 5 mg of *taxifolin R* in 10 ml of *methanol R*.

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

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

Application: 10 µl [or 8 µl] of the test solution and 10 µl [or 2 µl] of the reference solution, as bands.

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

Drying: at 100-105 °C.

Detection: spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min and examine in ultraviolet light at 365 nm.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other yellowish-green fluorescent zones may be present between the zones due to silibinin and taxifolin in the chromatogram obtained with the test solution.

Top of the plate	
Silibinin: a yellowish-green fluorescent zone	A yellowish-green fluorescent zone (silibinin)
——	——
Taxifolin: an orange fluorescent zone	An orange fluorescent zone (taxifolin)
	A yellowish-green fluorescent zone
——	——
	A fluorescent zone (starting line)
Reference solution	**Test solution**

TESTS

Loss on drying (*2.8.17*): maximum 5.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Dissolve 60.0 mg of the extract to be examined in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution. Dissolve a quantity of *milk thistle standardised dry extract CRS* corresponding to 10.0 mg (m_1 g) of silibinin in *methanol R* and dilute to 100.0 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*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per 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; if necessary, adjust the time periods of the gradient.

System suitability: reference solution:

– *resolution*: minimum 1.8 between the peaks due to silibinin A and silibinin B.

– the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *milk thistle standardised dry extract CRS*.

Locate the peaks due to silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B using the chromatogram supplied with *milk thistle standardised dry extract CRS*. In the chromatogram obtained with the test solution the peak due to silidianin may vary in size or be absent.

Calculate the percentage content of total silymarin, expressed as silibinin, using the following expression:

$$\frac{(F_1 + F_2 + F_3 + F_4 + F_5 + F_6) \times m_1}{(F_7 + F_8) \times m_2}$$

Calculate the percentage content of the sum of silicristin and silidianin, with reference to total silymarin, using the following expression:

$$\frac{(F_1 + F_2) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

Calculate the percentage content of the sum of silibinin A and silibinin B, with reference to total silymarin, using the following expression:

$$\frac{(F_3 + F_4) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

Calculate the percentage content of the sum of isosilibinin A and isosilibinin B, with reference to total silymarin, using the following expression:

$$\frac{(F_5 + F_6) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

F_1 = area of the peak due to silicristin in the chromatogram obtained with the test solution;

F_2 = area of the peak due to silidianin in the chromatogram obtained with the test solution;

F_3 = area of the peak due to silibinin A in the chromatogram obtained with the test solution;

F_4 = area of the peak due to silibinin B in the chromatogram obtained with the test solution;

F_5 = area of the peak due to isosilibinin A in the chromatogram obtained with the test solution;

F_6 = area of the peak due to isosilibinin B in the chromatogram obtained with the test solution;

F_7 = area of the peak due to silibinin A in the chromatogram obtained with the reference solution;

F_8 = area of the peak due to silibinin B in the chromatogram obtained with the reference solution;

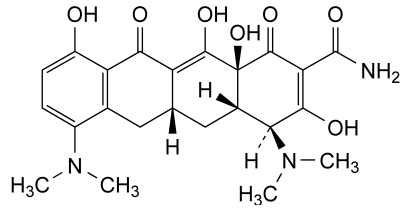
m_1 = mass of silibinin in the reference solution, in grams;

m_2 = mass of the extract to be examined, in grams.

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corrected 6.0

MINOCYCLINE HYDROCHLORIDE DIHYDRATE

Minocyclini hydrochloridum dihydricum



, HCl , 2 H_2O

$C_{23}H_{28}ClN_3O_7,2H_2O$ M_r 530.0
[13614-98-7]

DEFINITION

(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride dihydrate.

Semi-synthetic product derived from a fermentation product.

Content: 96.0 per cent to 102.5 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow, hygroscopic, crystalline powder.

Solubility: sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).