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IDENTIFICATION OF MILK THISTLE FRUITS AND EXTRACTS BY THIN-LAYER CHROMATOGRAPHY

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1. Introduction

At the present the characteristic ingredients existing in milk thistle and the preparations made therefrom are the flavolignans known as silymarin isomers. The method for the identification of these compounds described here corresponds to the Ph.Eur. monographs for the fruit and the standardized extract of milk thistle. The method consists on the thin-layer chromatography of the milk thistle sample and comparison with silybinin and taxifolin reference standard.

The method is suitable for the identification of fruits and dry extracts of milk thistle purchased and manufactured in Euromed. This assay can be used to identify the silymarin isomers: silybinin, isosilybinin, silychristin, silydianin and taxifolin.

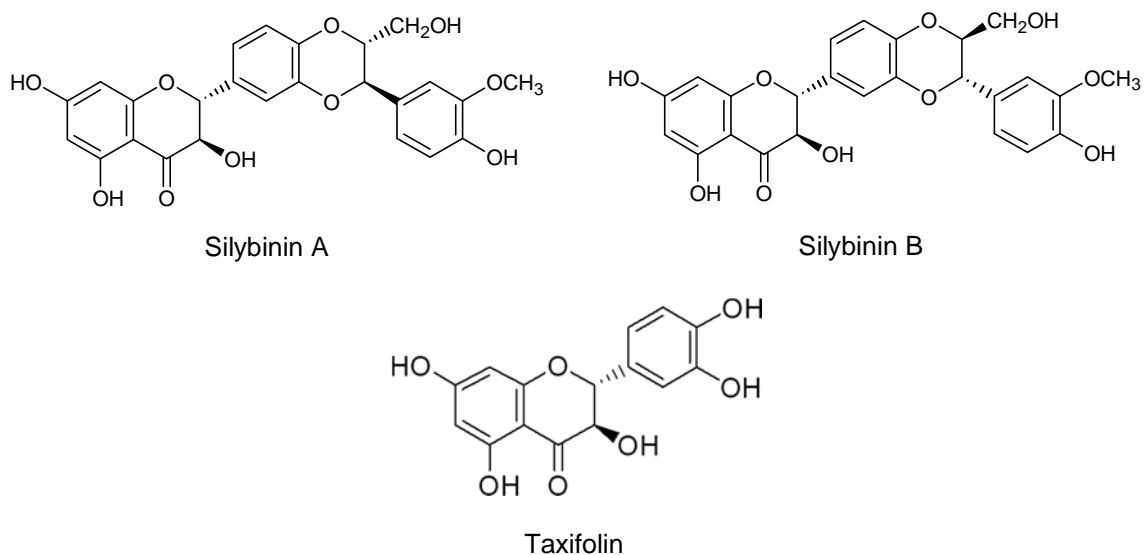
2. Reagents and equipment

2.1. Safety precautions

Consult the Material Safety Data Sheet (MSDS) for any used chemical that is unfamiliar. All chemicals should be considered hazardous (avoid direct physical contact). A short special information is given by the R- and S-chemical rules under instruction for handling (see point 2.3).

2.2. Reference standards (primary standards)

Silibinin and taxifolin reference standards are necessary for identity purposes. The structure formulas of these compounds are:



These reference standards are documented with a certificate of analysis, which assures its identity, purity and content.

2.3. Reagents and other materials

Substance	Instruction for handling	Grade of purity	Supplier / Art.-No
Methanol	EINECS-No.: 200-659-6 R Phrase: 11-23/24/25-39/ 23 /24 /25 S Phrase: 7-16-36/37-45 Hazard:H225-H301-H311-H331-H370 Precautionary:P210-P260-P280-P301 + P310-P307 + P311	HPLC grade	Scharlau (ME0306)
Water		HPLC grade	Purified through a water purification system (ELGA)
Acetone	EINECS-No.: 200-662-2 R Phrase: 23/24/25-39/23/24/25 S Phrase: 36/37-45 Hazard :H225-H319-H336 Precautionary :P210-P261-P305+ P351 + P338	HPLC grade	Scharlau (AC0310)
Methylene chloride	EINECS-No.: 200-838-9 R Phrase: 40 S Phrase: 23-24/25-36/37 Hazard : H320-H315-H302-H370-H336-H372-H350-H401-H411 Precautionary: P264-P280-P270-P260-P201-P202-P281-P273	Analytical grade	Scharlau (CL0334)
Polyethylene glycol	EINECS-No.: 203-473-3 R Phrase: - S Phrase: -	For synthesis	Panreac (162436)
Diphenylboric acid aminoethyl ester	EINECS-No.: 208-366-5 R-Phrases: 36/37/38 S-Phrases: 22 Hazard: H315, H319, H335 Precautionary: P261, P280, P305+P351+P338, P304+P340, P405, P501	For analysis	Alfa aesar (A16606)

Reagent solutions:

- Prepare a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol
- Prepare a 50 g/L solution of polyethylene glycol in methanol

2.4. Equipment

HPTLC plates Silica gel 60 F254 (100 mm x 100 mm or 200 mm x 100 mm) (Merck, 1.05628 or 1.05642)
Automatic TLC Sampler 4 (Camag) equipped with Camag Reprostar 3 lamps, a digital camera and a winCats software controller(Camag) for data acquisition and processing
Dipping equipment (Lothar Baron) working at high speed and time of 1 s
Calibrated analytical balance accurate to ± 0.01 mg (Mettler)
Milling equipment
Different Glassware
Different Volumetric Material
Paper filters
Water bath

3. Method of analysis

3.1. Information of the method

Homogeneous samples have to be taken in order to perform the analysis.

3.2. Sample preparation

3.2.1. Sample solution

3.2.1.1. Dry extract

- Dissolve 0.250 g of the extract to be examined in 5 mL of methanol.

3.2.1.2. Herbal drug

- To 1.0 g of powdered drug (500) add 10 mL of methanol.
- Heat under reflux in a water-bath at 70 °C for 5 min.
- Cool and filter.
- Evaporate the filtrate to dryness and dissolve the residue in 1.0 mL of methanol.

3.2.2. Standard solution

- Dissolve 2 mg of silibinin in 10 mL of methanol (reference solution A)
- Dissolve 5 mg of taxifolin in 10 mL of methanol (reference solution B)

3.3. TLC procedure

Stationary phase: HPTLC silica gel 60 F254 aluminum plates (100 mm x 100 mm or 200 mm x 100 mm)

Application volume: Dry extract: 8 µl of the sample solution
Herbal drug: 6 µL of the sample solution
Standard solution: Apply 2 µl of the standard solution

Mobile phase: Anhydrous formic acid/acetone/methylene chloride (8.5:16.5:75 V/V/V)

Development: 6 cm

Drying: at 100 – 105 °C

Derivatization: Treat the still-warm plate with a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol and subsequently treat with a 50 g/L solution of polyethylene glycol in methanol. Allow to dry for 30 min

Detection: examine under ultraviolet light at 365 nm

4. TLC evaluation

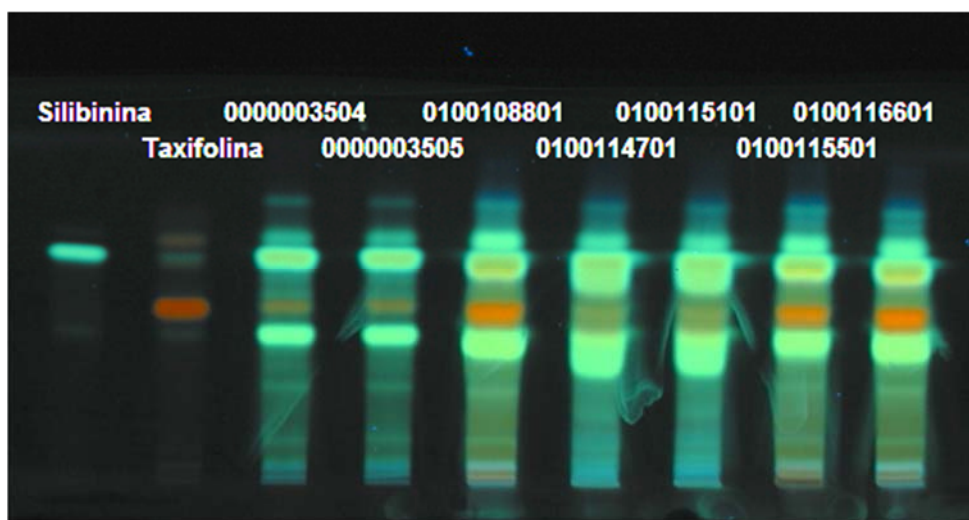
See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore,

- **Milk thistle fruit:** other orange and yellowish-green fluorescent zones may be present between the zones due to silibinin and taxifolin in the chromatogram obtained with the test solution.
- **Milk thistle dry extract:** 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 (silicristin) -----
Reference solution	Test solution

5. Typical chromatograms

The following chromatogram (reference standard, milk thistle fruit batches 000003504 and 000003505, and milk thistle dry extracts batches 0100108801, 0100114701, 0100115101, 0100115501 and 0100116601) is given as information and guidance only. It does not form a mandatory part of the SOP.



6. Bibliography

- *Milk-thistle fruit, Silybi mariani fructus*, Ph.Eur. 9.0, 01/2014:1860.
- *Milk thistle dry extract refined and standardised, Silybi mariani extractum siccum raffinatum et normatum*, Ph.Eur. 9.0, 01/2014:2071.
- EUROMED booklet of Silymarin Extract.