

0057 - Milk Thistle for Silymarius by HPLC

Botanical Name: *Silybum marianum* (L.) Gaertn

Common Names: Mary's thistle

Parts of Plant Used: Dried fruits

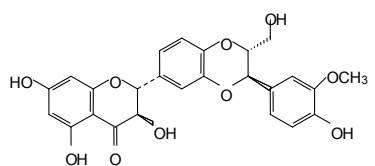
Uses: As an antihepatotoxic drug to treat alcoholic liver disease and drug-induced liver disease.

Modes of Action:

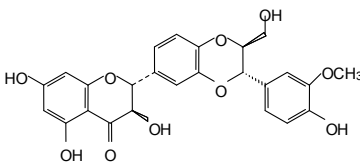
Various clinical trials have been performed on milk thistle that have demonstrated positive effects for indications such as cirrhosis and alcoholic liver diseases, hepatitis, and psychotropic-drug-induced liver damage. Silymarin-type flavonolignans are the bioactive components and they showed antioxidative, antifibrotic, anti-inflammatory, and antilipid activity as well as peroxidative, immunomodulating, and liver-regenerating effects. Silymarin can be used successfully in therapy to promote faster regeneration of diseased liver and silybinin was found to stimulate the activity of the DNA-dependent RNA-polymerase I, thus increasing the rate of synthesis of all cellular proteins. The increase of protein synthesis offers a good explanation for the liver-regenerating power of milk thistle.¹⁻⁴

Chemical Markers:

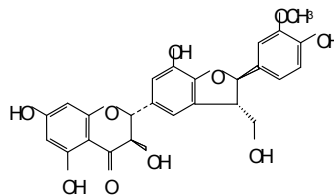
Milk thistle seed contains a group of compounds that includes flavonolignans, flavonoids, sterols, and fatty acids.^{5,6} Silymarins are the bioactive components in milk thistle and generally the milk thistle extract is expressed as silymarin percentage. Silymarins correspond to a group of flavonolignans and the major ones are silybin A, silybin B, isosilybin A, isosilybin B, silychristin, and silydianin. Due to the complexities of these structures, the stereochemistry of these compounds, especially the stereochemistry of silybin A and B, and isosilybin A and B was resolved only recently.^{7,8} Silymarins are used as marker compounds for quality control of milk thistle extract in the U.S. market.



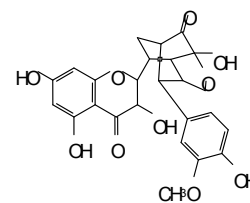
Silybin A



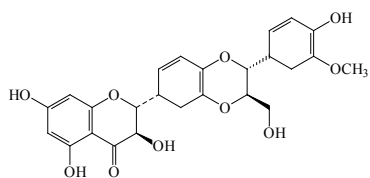
Silybin B



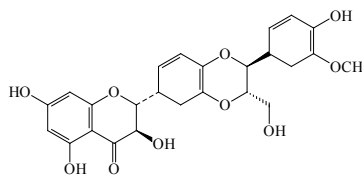
Silychristin



Silydianin



Isosilybin A



Isosilybin B

Methods of Analysis

The silymarin content in milk thistle has been analyzed by various methods including TLC, HPLC, UV-visible spectrophotometry, and capillary electrophoresis.⁹⁻¹¹ Currently, UV-visible spectrophotometric and HPLC methods are the most accepted for quality control of milk thistle extract. Although many HPLC methods have been published, few methods can separate silybin A from silybin B and isosilybin A from isosilybin B.

Method 1:

The method at www.chromadex.com was used. This method can be used to analyze seven compounds including silychristin, silydianin, silybin A, silybin B, isosilybin A, isosilybin B, and taxifolin.

Sample Preparation:

Weigh 500 mg of milk thistle extract into a 100-mL volumetric flask, add 25 mL of acetonitrile, sonicate for 15 minutes, cool to room temperature, and dilute to volume. Pipette 10 mL of this solution into a 50-mL volumetric flask and dilute to volume with acetonitrile.

Chromatography:

Column: Phenomenex Luna C18 (2), 5 μ m, 150 \times 4.6 mm.

Mobile phase: Solvent A = methanol–water–phosphoric acid (30:70:0.1 vol/vol/vol), solvent B = methanol–water–phosphoric acid (70:30:0.1 vol/vol/vol).

Gradient: 100%A to 100%B in 30 minutes, then keep at 100%B for 5 minutes.

Flow rate: 1.0 mL/minute

Injection volume: 5 μ L

Detection wavelength: 288 nm

Validation Data:

Not available

Method 2:

The method at www.nsfina.org was used.

Sample Preparation:

For fruits, accurately weigh about 10 g of finely ground milk thistle fruit. Transfer the powder into an extraction thimble within a continuous-extraction apparatus (250-mL round-bottom flask containing about 100 mL of hexane). Extract the powder for 4 hours and discard the extract. Transfer the defatted fruit powder to a continuous-extraction apparatus (250-mL round-bottom flask containing about 90 mL of methanol). Extract the powder for 8 hours, cool

down, and transfer to a 100-mL volumetric flask and dilute to volume with methanol. Dilute 1:25 with methanol and filter a portion into an HPLC vial.

For the extract, accurately weigh (± 0.1 mg) about 70 mg of extract into a 100-mL volumetric flask. Add 70 mL of methanol and sonicate for 20 minutes. Cool and dilute to volume with methanol.

Chromatography:

Column: YMC-Pack ODS-A C18, 5 μ m, 4.6 \times 150 mm or Phenomenex Luna C18 (2), 5 μ m, 150 \times 4.6 mm.

Mobile phase: Solvent A = methanol–water–phosphoric acid [Mix 200 mL of methanol with 800 mL of water. Mix 5 mL of phosphoric acid into 995 mL of the methanol–water (20:80)], solvent B = methanol–water–phosphoric acid [Mix 800 mL of methanol with 200 mL of water. Mix 5 mL of phosphoric acid into 995 mL of the methanol–water (80:20)].

Gradient:

Time (minutes)	%A	%B
0	85	15
5	85	15
20	55	45
40	55	45
41	85	15
55	85	15

Flow rate: 1.0 mL/minute

Injection volume: 10 μ L

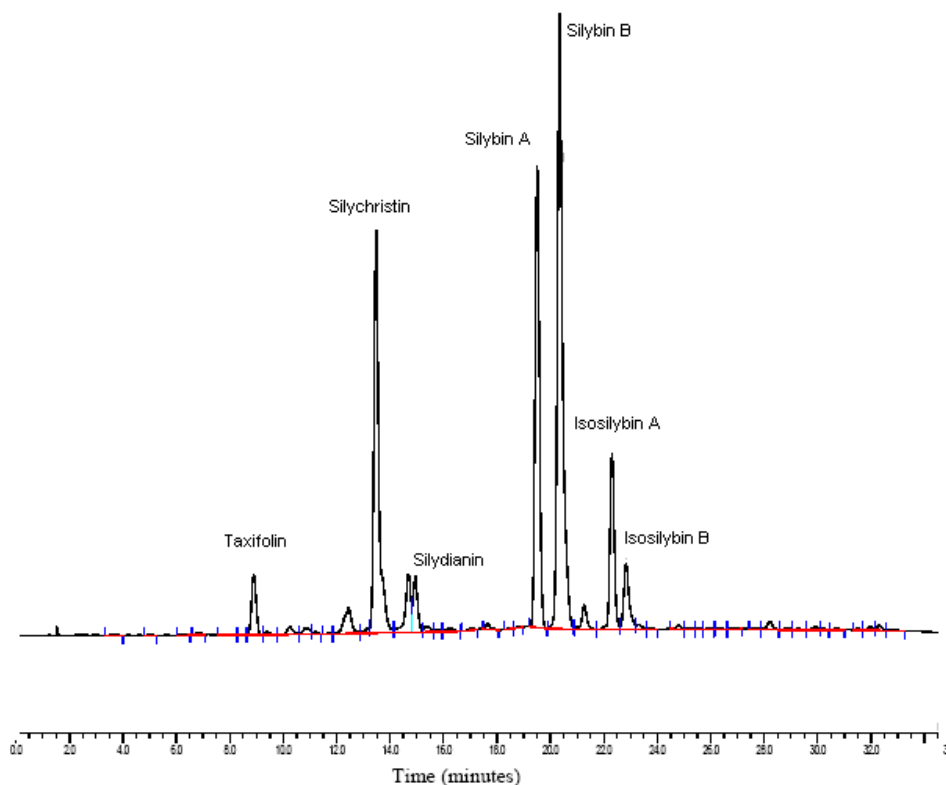
Column temperature: 40°C

Detection wavelength: 288 nm

Validation Data:

Not available, but this method is known to be a validated method.

Representative HPLC Chromatogram of Milk Thistle Extract Run by Method 1.



References:

1. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs*. 2001;61(14):2035–63.
2. Sonnenbichler J, Sonnenbichler I, Scalera F. Influence of the flavonolignan silybinin of milk thistle on hepatocytes and kidney cells. In: Lawson L, Bauer R, eds. *ACS symposium series 691: phytomedicines of Europe*. Washington, DC: American Chemical Society; 1998:263–77.
3. Luper S. A review of plants used in the treatment of liver disease: part 1. *Altern Med Rev*. 1998;3(6):410–21.
4. Leng-Peschlow E. Properties and medical use of flavonolignans (silymarin) from *Silybum marianum*. *Phytother Res*. 1996;10(Suppl. 1):S25–6.
5. Khafagy SM, Abdel Salam NA, Abdel HR, et al. Constituents of *Silybum marianum* (L.) Gaertn. (Compositae). *Scientia Pharmaceutica*. 1981;49(2):157–61.
6. Yin Q, Wang S, Nan J, et al. Analysis of fatty acids in *Silybum marianum* oil by GC/MS. *Yanbian Daxue Xuebao, Ziran Kexueban*. 1998;24(4):26–8.
7. Lee DY, Liu Y. Molecular structure and stereochemistry of silybin A, silybin B, isosilybin A, and isosilybin B, isolated from *Silybum marianum* (milk thistle). *J Nat Prod*. 2003;66(9):1171–4.
8. Kim NC, Graf TN, Sparacino CM, et al. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem*. 2003;1(10):1684–9.
9. Kvasnicka F, Biba B, Sevcik R, et al. Analysis of the active components of silymarin. *J Chromatogr A*. 2003;990(1-2):239–45.
10. Bilia AR, Bergonzi MC, Gallori S, et al. Stability of the constituents of calendula, milk-thistle and passionflower tinctures by LC-DAD and LC-MS. *J Pharm Biomed Anal*. 2002;30(3):613–24.
11. Quaglia MG, Bossu E, Donati E, et al. Determination of silymarin in the extract from the dried *Silybum marianum* fruits by high performance liquid chromatography and capillary electrophoresis. *J Pharm Biomed Anal*. 1999;19(3-4):435–42.