

Milk Thistle

Milk Thistle consists of the dried ripe fruit of *Silybum marianum* (L.) Gaertn. (Fam. Asteraceae), the pappus having been removed. It contains not less than 2.0% of silymarin, calculated as silybin ($C_{25}H_{22}O_{10}$), on the dried basis.

Packaging and storage— Preserve in well-closed containers, protected from light and moisture.

Labeling— The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

Botanic characteristics—

Macroscopic— The fruits (achenes) are elongated ovoid, slightly crooked, somewhat flattened, roughly 6 to 7 mm in length, up to 3 mm in width, and 1.5 mm in thickness, and have a projecting cartilaginous glossy yellowish edge on the upper surface and a grooved hilum at the base. The fruit coat is glossy brown-black or mat gray-brown with dark or pale gray streaks; it encloses the straight embryo having two thick, flattened cotyledons that contain fatty oil and aleurone granules.

Microscopic— The fruit wall epidermis consists of almost colorless palisade cells arranged at right angles to the surface. They have greatly thickened outer walls, into which the lumen continues for some distance in the form of a slit. Viewed from above under high magnification, the cells show only a slit-shaped lumen. They have thickened ridges that appear as nodular thickenings of the cell wall when viewed from above. The subepidermal layer of the fruit wall is made up of unligified thin-walled parenchymal cells and constitutes a pigment layer. Colorless cells and groups of cells alternate with pigment cells, the latter varying in number; this gives the fruit wall its mottled appearance. Next comes the fruit wall tissue, about 8 cell layers thick, with stippled parenchymal cells elongated in the longitudinal axis of the fruit. The cells of the innermost layer of the fruit wall may be collapsed; they contain large cigar-shaped or monoclinic calcium oxalate prisms. The seed coat epidermis is formed from large yellow palisade cells. The cells have a narrow lumen, somewhat expanded at each end of the cell, and the cell walls show conspicuous lamination. The subepidermal cells of the seed coat consist of peculiar stippled cells; their lignified cell membranes have prominent, close-set ridges or thickenings (“net cells”). Next to them is a single layer of cells having tough, somewhat swollen walls and lipophilic contents (endosperm residue). The embryo consists of thin-walled cells which, in addition to small glands, contain clumps of crystals and fat droplets.

Identification—

A: [Thin-Layer Chromatographic Identification Test](#) [201](#) —

Test solution— Use the Test solution, prepared as directed in the test for Content of silymarin.

Standard solution: 1.0 mg of [USP Silydianin RS](#) per mL, in methanol.

Developing solvent system: freshly prepared mixture of chloroform, acetone, and anhydrous formic acid (75:16.5:8.5).

Procedure— Proceed as directed in the chapter, except to dry the plate for 30 minutes in a current of cold air. Spray the plate with a solution of 2-aminoethyl diphenylborinate in methanol (1 in 100), allow to dry briefly, and then spray with a solution of polyethylene glycol 4000 in alcohol (5 in 100). An hour later, examine the plate under long-wavelength UV light: the chromatogram of the Test solution exhibits an intense green-blue fluorescent zone at an RF value of about 0.5 (presence of silybin); and a gray-blue spot at RF value of about 0.4, corresponding to a spot observed in the chromatogram of the Standard solution. The chromatogram of the Test solution may exhibit other colored zones: an intense green-blue zone at an RF value of about 0.25 (presence of silychristin) and a red-orange zone at an RF value of about 0.3 (presence of taxifolin).

B: The retention times of the peaks for silydianin, silychristin, silybin A, silybin B, isosilybin A, and isosilybin B in the chromatogram of the Test solution correspond to those in the chromatogram of the Milk thistle standard solution, as obtained in the test for Content of silymarin.

[Microbial enumeration](#) [2021](#) — The total bacterial count does not exceed 10,000 cfu per g, the total combined molds and yeasts count does not exceed 100 cfu per g, and it meets the requirements of the tests for the absence of Salmonella species and Escherichia coli and for absence of Staphylococcus aureus.

[Foreign organic matter](#) [561](#) : not more than 2.0%.

[Total ash](#) [561](#) : not more than 8.0%, determined on 1.0 g of finely powdered Milk Thistle.

[Pesticide residues](#) [561](#) : meets the requirements.

[Loss on drying](#) [731](#) — Dry 1.0 g of finely powdered Milk Thistle at 105 °C for 2 hours: it loses not more than 8.0% of its weight.

[Heavy metals](#) [231](#) : not more than 0.001%.

Content of silymarin—

Solution A— Use a filtered and degassed mixture of water, methanol, and phosphoric acid (80:20:0.5).

Solution B— Use a filtered and degassed mixture of methanol, water, and phosphoric acid (80:20:0.5).

Mobile phase— Use a variable mixture of Solution A and Solution B as directed in the Chromatographic system. Make adjustments if necessary.

Milk thistle standard solution— Dissolve an accurately weighed quantity of [USP Powdered Milk Thistle Extract RS](#) in methanol, sonicate for 20 minutes, and dilute with methanol to obtain a solution having a known concentration of about 0.7 mg of extract per mL. Transfer 1 mL of this solution to a 5-mL volumetric flask, and dilute with methanol to volume. Pass through a membrane filter having a 0.45- μm or finer porosity.

Silybin standard solutions— Dissolve an accurately weighed quantity of [USP Silybin RS](#) in methanol, and dilute with methanol to obtain solutions having known concentrations of about 0.20 mg per mL, 0.02 mg per mL, and 0.004 mg per mL, respectively. Pass through a membrane filter having a 0.45- μm or finer porosity.

Test solution— Transfer about 10 g of finely powdered Milk Thistle, accurately weighed, to an extraction thimble, and cover with a small cotton ball. Transfer the thimble to a continuous-extraction apparatus fitted with a 250-mL round-bottom flask containing 150 mL of solvent hexane, and heat the flask on a heating mantle for 4 hours. Following the extraction, separate the round-bottom flask containing solvent hexane extract from the extraction apparatus, and discard the solvent hexane solution. Remove the adherent solvent hexane from the extraction thimble by drying, and transfer the thimble to an extraction apparatus suitable for hot extraction and fitted with a 250-mL round-bottom flask containing 100 mL of ethyl acetate. [note—Adjust the volume of ethyl acetate, if necessary, to sustain a continuous extraction.] Heat the flask on a heating mantle to allow the solvent to reflux gently. After 8 hours, transfer the extract quantitatively into a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system— The liquid chromatograph is equipped with a 288-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1 and is maintained at a temperature of 40 . The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	85	15	isocratic
5–20	85→55	15→45	linear gradient
20–40	55	45	isocratic
40–41	55→85	45→15	linear gradient
41–55	85	15	equilibration

Chromatograph the Milk thistle standard solution, and record the peak responses as directed for Procedure: the chromatogram obtained is similar to the Reference Chromatogram provided with the [USP Powdered Milk Thistle Extract RS](#); the relative retention times are about 0.68 for silychristin, 0.73 for silydianin, 1.00 for silybin A, 1.05 for silybin B, 1.09 for dehydrosilybin, 1.15 for isosilybin A, and 1.19 for isosilybin B; the resolution factor, R, between silybin A and silybin B is not less than 1.0; the tailing factor is not less than 0.8 and not more than 2.0; and the relative standard deviation for the sum of peak responses due to silybin A and silybin B is not more than 2.0%.

Procedure— Separately inject equal volumes (about 10 µL) of the Milk thistle standard solution, each of the Silybin standard solutions, and the Test solution into the chromatograph, and record the chromatograms. Identify the peaks due to silychristin, silydianin, silybin A, silybin B, isosilybin A, and isosilybin B by comparison with the chromatogram of the Milk thistle standard solution, and measure the peak areas of the relevant peaks. Plot the areas of the sum of silybin A and silybin B peaks versus the concentration of [USP Silybin RS](#) in the Silybin standard solutions, and obtain a regression line for calibration. Separately calculate the percentage of each relevant component of silymarin as silybin (C₂₅H₂₂O₁₀) in the portion of Milk Thistle taken by the formula:

$$250C/W$$

in which C is the concentration, in mg per mL, of the relevant component in the Test solution as interpolated in the calibration graph; and W is the weight, in g, of the portion of Milk Thistle taken. Calculate the content of silymarin, in percentage, in the portion of Milk Thistle taken by adding the individual percentages.