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Powdered Milk Thistle

Change to read:

DEFINITION

Powdered Milk Thistle ▲ consists of the dried ripe fruit of *Silybum marianum* (L.) Gaertn. (Family Asteraceae) with the pappus removed, ▲ (USP 1-Aug-2022) reduced to a fine or very fine powder. It contains NLT 2.0% of silymarin, calculated as silybin ($C_{25}H_{22}O_{10}$), on the dried basis.

IDENTIFICATION

Delete the following:

▲ A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

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

Sample solution: Use the *Sample solution*, prepared as directed in the test for *Content of Silymarin*.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.25-mm layer of chromatographic silica gel, typically 20 cm long (TLC plates)

Application volume: 10 µL

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

Spray reagent A: 10-mg/mL solution of 2-aminoethyl diphenylborinate in methanol

Spray reagent B: 50-mg/mL solution of polyethylene glycol 4000 in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatograms until the solvent front has moved about three-fourths of the plate, and dry it for 30 min in a current of cold air. Spray the plate with *Spray reagent A*, allow to dry, and then spray with *Spray reagent B*. One hour later, examine the plate under long-wave UV light (365 nm).

Acceptance criteria: The chromatogram of the *Sample solution* exhibits an intense green-blue fluorescent zone at an R_F value of about 0.5 (presence of silybin) and a gray-blue spot at an R_F value of about 0.4, corresponding to that observed in the chromatogram of the *Standard solution*. The chromatogram of the *Sample solution* may exhibit other colored zones: an intense green-blue zone at an R_F value of about 0.25 (presence of silychristin) and a red-orange zone at an R_F value of about 0.3 (presence of taxifolin). ▲ (USP 1-Aug-2022)

Add the following:

▲ A. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#).

Standard solution A: 0.5 mg/mL of [USP Silydianin RS](#) and 0.1 mg/mL of [USP Silybin RS](#) in [methanol](#)

Standard solution B: 20 mg/mL of [USP Powdered Milk Thistle Extract RS](#) in [methanol](#). Sonicate for 10 min, and either centrifuge and use the clear supernatant or pass through a 0.45-µm syringe filter.

Sample solution: 100 mg/mL of Powdered Milk Thistle in [methanol](#). Sonicate for 10 min, and either centrifuge and use the clear supernatant or pass through a 0.45-µm syringe filter.

[NOTE—*Standard solutions* and the *Sample solution* are stable for 7 days at 4°.]

Chromatographic system

(See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\)](#), [Table 1](#).)

Application volume: 2 µL

Developing solvent system: [Toluene](#), ethyl formate, and [anhydrous formic acid](#) (40:50:5)

Derivatization reagent A: 5 mg/mL of [2-aminoethyl diphenylborinate](#) in [ethyl acetate](#)

Derivatization reagent B: 50 mg/mL of [polyethylene glycol 400](#) in [methylene chloride](#)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in a current of air for 5 min. Heat at 100° for 5 min and let the plate cool. Treat the plate first with *Derivatization reagent A* and then immediately with *Derivatization reagent B*, heat at 100° for 5 min, and examine under long-wave UV light.

System suitability: Under long-wave UV light, *Standard solution A* displays two light-blue bands in the middle third of the derivatized chromatogram: the lower band due to silydianin and the upper due to silybin. Under long-wave UV light, *Standard solution B* displays a distinct yellowish band due to silychristin in the bottom third of the derivatized chromatogram, among several bands of lower intensity. Two

prominent bands, corresponding to silydianin and silybin in *Standard solution A*, are bluish green, with an intense orange band due to taxifolin between them.

Acceptance criteria: Under long-wave UV light, the *Sample solution* displays a yellowish band in the bottom third of the derivatized chromatogram corresponding to silychristin in *Standard solution B*. In the middle third of the derivatized chromatogram, two bluish bands are observed, corresponding to silydianin and silybin in *Standard solution A* and *Standard solution B*. The orange taxifolin band between silydianin and silybin may be faint or not visible. One to several faint blue bands above silybin may be observed, typically not extending into the upper half of the derivatized chromatogram.▲ (USP 1-Aug-2022)

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Silymarin*.

Acceptance criteria: The retention times of the peaks for silydianin, silychristin, silybin A, silybin B, isosilybin A, and isosilybin B from the *Sample solution* correspond to those from the *Milk thistle standard solution*.

COMPOSITION

Change to read:

• CONTENT OF SILYMARIN

Solution A: Methanol, phosphoric acid, and water (20: 0.5: 80)

Solution B: Methanol, phosphoric acid, and water (80: 0.5: 20)

Mobile phase: See [Table 1](#).

Table 1

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

Milk thistle standard solution: 0.7 mg/mL of [USP Powdered Milk Thistle Extract RS](#) in methanol. Sonicate for 20 min to dissolve. Pass through a membrane filter of a 0.45-µm or finer pore size. ▲ (USP 1-Aug-2022)

Silybin standard solutions: 0.20, 0.02, and 0.004 mg/mL of [USP Silybin RS](#) in methanol. Pass through a membrane filter of a 0.45-µm or finer pore size.

Sample stock solution: Transfer 10 g of Powdered Milk Thistle 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 h. After the extraction, detach the round-bottom flask from the extraction apparatus and discard the solvent hexane. Dry the extraction thimble to remove residual solvent hexane, 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—If necessary, adjust the volume of ethyl acetate to sustain continuous extraction.] Heat the flask to achieve gentle reflux. After 8 h, transfer the extract quantitatively into a 100-mL volumetric flask and dilute with methanol to volume.

Sample solution: Dilute the *Sample stock solution* 1:25 with methanol.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 15-cm; 5-µm packing [L1](#)

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Milk thistle standard solution*

[NOTE—See [Table 2](#) for the relative retention times.]

Suitability requirements

Chromatogram similarity: The chromatogram of the *Milk thistle standard solution* is similar to the reference chromatogram provided with the lot of [USP Powdered Milk Thistle Extract RS](#) being used.