SHIMADZU

Application News

High Performance Liquid Chromatography

A Modified INA Method for the Analysis of Milk Thistle Using HPLC and Evaporative Light Scattering Detection

Introduction

One of the widely available nutraceutical products seen in supermarkets is a powdered form of Milk Thistle or *silybum marianum,* derived from the plant native to Mediterranean Europe. It has been used from ancient times as a liver tonic. The milky sap produced by Milk Thistle contains flavolignans, collectively known as the silymarin group. Growing consumer interest, combined with the low cost and growing availability of herbal supplements and remedies, has driven the need for standardization of these products. The Institute for Nutraceutical Advancement (INA) has developed a standardized method for the analysis of nutraceutical products, which involves an HPLC assay with UV detection.

As per INA method 115.00, the marker constituents, silymarin group flavolignans, of Milk Thistle are analyzed using ultraviolet detection at 288nm. Unfortunately, as is common with any spectroscopic detection method, the phytochemicals vary in their absorbance response at 288nm according to their individual molar absorptivities. Over the broad range of phytochemicals comprising the sample, the wavelength of maximum absorbance (λ_{max}) may vary considerably from one constituent to the next. This can result in a chromatogram that shows the compounds of interest, but does not offer a true indication of their relative abundance. Achieving a recommended wavelength for detection is always a compromise.

Shimadzu's Evaporative Light Scattering Detector (ELSD-LT) offers an alternative to spectroscopic detectors. With it, analyte response more accurately reflects the relative abundance of sample constituents. The ELSD-LT is not a spectroscopic detector, but instead makes a light scattering measurement of analyte particles after they have been dried of mobile phase through evaporation. It excels at the analysis of chromophoric and non-chromophoric compounds alike as its response is independent of the spectral properties of both the analyte and solvents. The ELSD-LT is also blind to gradients, producing stable gradient baselines.

Sample Preparation

As per INA Method 115.000, 70mg powdered Milk Thistle extract was placed into a 100mL volumetric flask. Approximately 70mL of methanol was added and the mixture was sonicated for 30 minutes with shaking. The mixture was diluted to 100mL with methanol, and then syringe filtered (0.45μm; Nylon) and placed into an autosampler vial for injection.

Analytical Conditions (as per INA Method 115.000)

Mobile Phase:	A: 1% Formic Acid in 80/20 Water/Methanol
	B: 1% Formic Acid in 20/80 Water/Methanol*
Gradient:	(Time, %B)(0,15)(5,15)(20,45)(40,45)(41,15)(55,15)
Column:	Shimadzu Premier C18, 5μm, 150 x 4.6mm**
Injection Volume:	10μL
Flow Rate:	1mL/min.
Column Temp:	40°C
Detector Settings:	Gain = 7; Temp = 80°C; Press. = 250kPa

*INA 115.000 calls for phosphoric acid for pH adjustment. Phosphoric acid is not suitable for use with Evaporative Light Scattering detection as it is a mineral acid and non-volatile. Formic acid is a volatile modifier and provides the necessary pH adjustment for selectivity.

** INA 115.000 calls for either a YMC-Pack ODS-A, 5μm, 150 x 4.6mm or a Phenomenex Luna C18(2), 5μm, 150 x 4.6mm, both hydrophobically end-capped, high-carbon load columns. The Shimadzu Premier C18, 5μm, 150 x 4.6mm column offers similar phase attributes and comparable selectivity.

Results

The chromatogram in Figure 1 compares the UV and ELSD profiles for Milk Thistle.

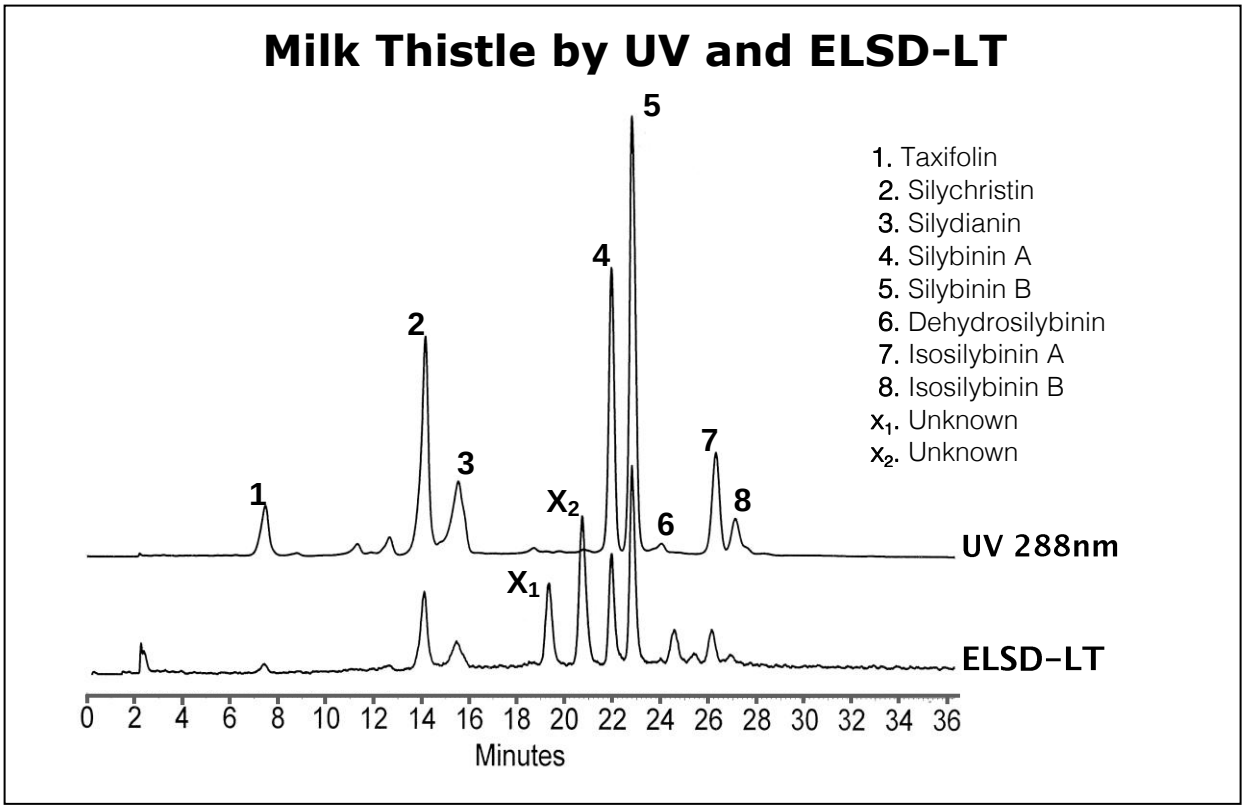

FIGURE 1

Interestingly, the ELSD profile for Milk Thistle reveals new peaks (x_1 and x_2) in the vicinity of the silymarin group and undetected by UV at 288nm. The retention times for x_1 and x_2 suggest structures that are similar to those of Silybinin A and B. Such findings illustrate the utility of Evaporative Light Scattering detection as a complementary detector for HPLC.

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