Fast Analysis of Silymarins in Milk Thistle Using the Agilent 1290 Infinity LC System and an Agilent ZORBAX Eclipse Plus C18 1.8 µm Column

Application Note

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Introduction

Using the Agilent 1290 Infinity LC System coupled with the Agilent ZORBAX Eclipse Plus 3 × 100 mm, 1.8 µm column not only reduces solvent consumption, but allows for greater peak resolution as well. This is illustrated by comparing analyses of silymarins in powdered milk thistle extract using the parameters outlined in the United States Pharmacopeia (USP) and the 1290 Infinity LC with the ZORBAX Eclipse Plus C18 column.

Milk Thistle Supplements

Milk thistle (*silybum marianum*) has been used for more than 2000 years to treat liver diseases and gall bladder problems. The milk thistle plant is native to the Mediterranean region and its seeds are harvested for their medicinal properties. The most active ingredient in milk thistle, silymarins, are believed to have powerful anti-inflammatory and antioxidant effects. These compounds are believed to protect the liver from various toxins and play a role in stimulating the growth of new liver cells.

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The chromatogram in Figure 1 shows a reduction of 3.8 times in analysis speed and solvent consumption when compared to the USP conditions shown in Figure 2. The USP assay uses a 150 × 4.6 mm, 5.0 µm C-18 column. This compares to the ZORBAX Eclipse Plus 100 × 3.0 mm, 1.8 µm column using the 1290 Infinity LC System. Both systems use the same mobile phase, flow rate, and temperature. The chromatogram in Figure 2 was provided by USP in the USP certificate of analysis for their milk thistle reference standard [1].

Milk Thistle Extraction Conditions

An extraction sample of 500 mg was dissolved in 100 mL of methanol with the aid of a 15-minute sonication step. Sample solutions were further diluted 1/10 in 50/50 methanol/water. The milk thistle extract was obtained from Pharmline, 41 Bridge St. Florida, NY 10921.

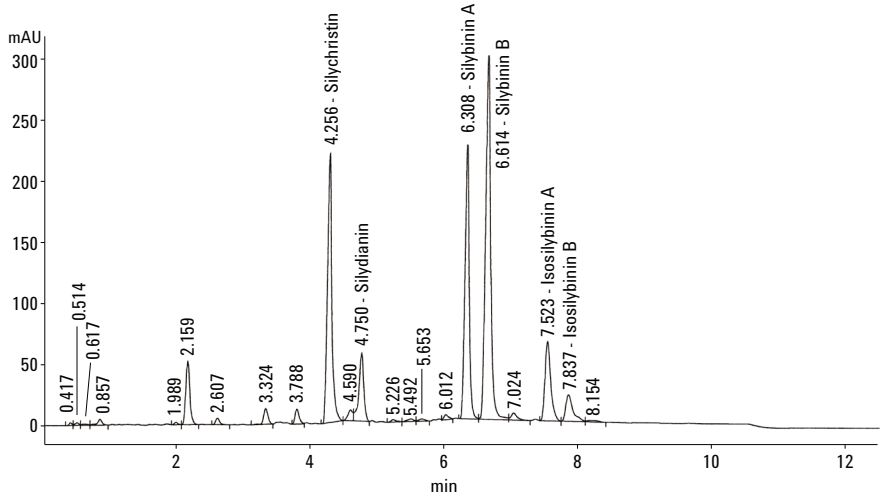

Figure 1. Silymarins in Milk Thistle Extract using the Agilent 1290 Infinity LC System with an Agilent ZORBAX Eclipse Plus C18, 3 × 100 mm, 1.8 µm column. The gradient in the USP monograph was scaled proportionally, for the reduced column size, using the Agilent Method Translator.

Instrument Parameters, Figure 1

Column	Agilent ZORBAX Eclipse Plus C18 RRHD, 100 × 3 mm, 1.8 µm, (p/n 959758-302)		
Temperature	40 °C		
Injection amount	5.0 µL		
Detection	UV, 288 nm		
Flow rate	1.0 mL/minute		
Mobile phase A	80% water/20% methanol/0.5% o-phosphoric acid		
Mobile phase B	20% water/80% methanol/0.5% o-phosphoric acid		
Gradient	Time (min)	% A	%B
	0	85	15
	1.42	85	15
	5.67	55	45
	11.34	55	45
	11.63	85	15
	14.46	85	15

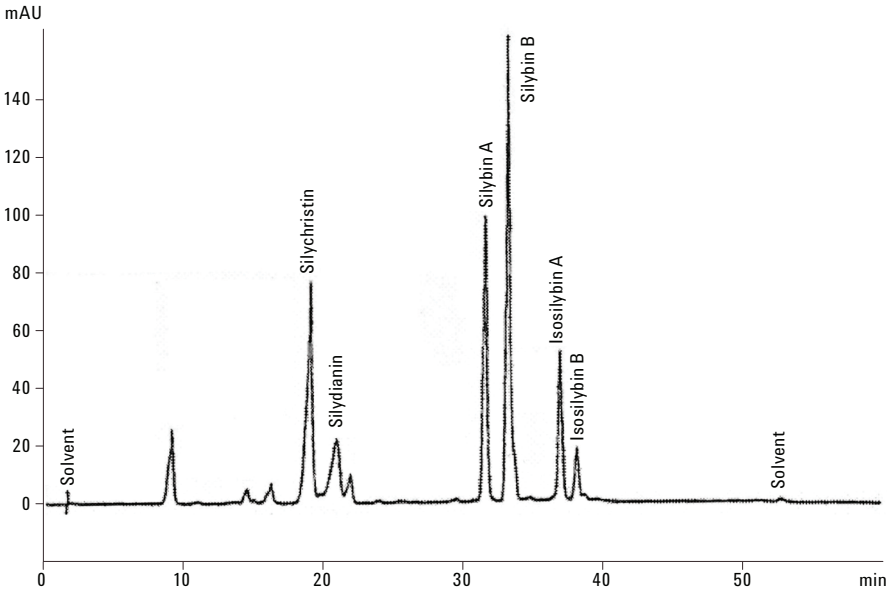

Figure 2. Representative chromatogram of USP reference material for Silymarins in milk thistle extract. 4.6 × 150 mm 5 µm C18, 55 minute run time. Source: USP Certificate of Analysis.

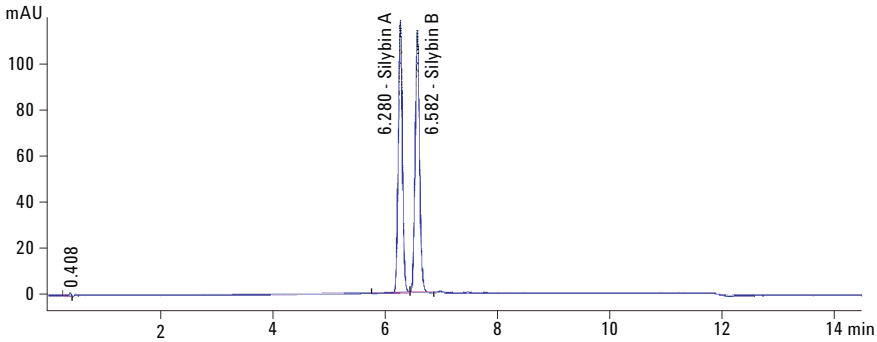

Figure 3. Silibinin reference standard Sigma-Aldrich #S0417 - Lot # BCBB4662. Calibration range: 9.60–480 µg/mL curve correlation: 0.99998. Operating conditions as shown for Figure 1.

Conclusion

In analyzing complex botanical extracts, the 1290 Infinity LC System in conjunction with smaller volume UHPLC 1.8-µm columns can speed up analysis time, thus saving cost in labor and solvent use. This is done without any loss in resolution.

Reference

1. Powdered Milk Thistle Extract Monograph, United States Pharmacopeia, USP35, NF30 p.1387.

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