Fast Analysis of Terpene Lactones in Ginkgo Biloba Extract Using the Agilent 1290 Infinity LC and Agilent Poroshell 120 SB-C18 2.7 µm Column

Application Note

Authors

Nick Severin
Jos. A. Schwabe
Green Bay, WI

Abstract

The use of the Agilent 1290 Infinity LC coupled with the Agilent Poroshell 120 SB-C18 3 × 100 mm, 2.7 µm column can not only reduce solvent consumption, but allows for faster run times. This is illustrated by comparing analyses of terpene lactones in ginkgo biloba extract using the parameters outlined in the United States Pharmacopeia (USP) and the 1290 Infinity LC with the Poroshell 120 SB-C18 column.

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Introduction

Ginkgo biloba extract is derived from the leaves and seeds of the ginkgo tree, one of the oldest living tree species in the world. The herb is used to improve memory, treat depression, and maintain healthy blood circulation. Terpene lactones in the extract inhibit platelet activating factors to improve blood circulation. The extracts are standardized to contain approximately 6% terpene lactones. Ginkgo biloba is regulated as a dietary supplement, not a drug.

Ginkgo Biloba extraction conditions

Gingo Biloba extract source
Schwabe Extracta GmbH, Germany, Lot 2900270.

Procedure
A 120-mg extract of sample was sonicated in 10 mL of a phosphate buffer. The sample solution was transferred to a chromatographic tube filled with siliceous earth (Merck, Extrelut NT20 column). To aid in the transfer, two additional 5-mL portions of the phosphate buffer were used. Allow the sample solution to be absorbed by the column contents, about 15 minutes, before eluting with 100 mL of ethyl acetate. The ethyl acetate elutant was evaporated under vacuum at 50 °C. The residue was dissolved in 20 mL of 50/50 methanol/water solution.

Phosphate buffer
A 1.19-g amount of dibasic sodium phosphate and 8.25 g of monobasic potassium phosphate were dissolved in 1,000 mL of water and pH adjusted to 5.8.

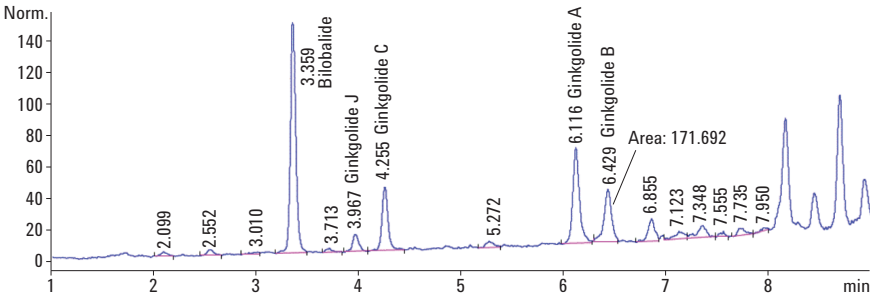
Table 1. Calibration Curves

Standard used	USP #1291559 – Lot # 10K042	
Sample	**Range**	**R^2**
Bilobalide	32.776–524.43 µg/mL	1.00000
Ginkgolide J	10.671–170.74 µg/mL	0.99994
Ginkgolide C	12.506–200.10 µg/mL	0.99985
Ginkgolide A	19.527–312-424 µg/mL	1.00000
Ginkgolide B	11.855–189.686 µg/mL	0.99997

All curves used quadratic equations

Results and Discussion

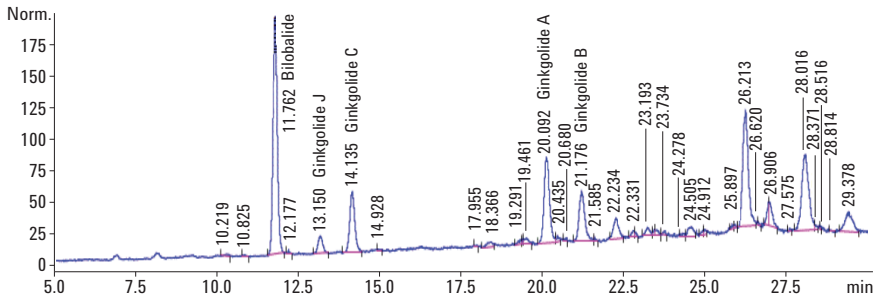
The chromatogram in Figure 1 shows a time reduction 4 times less than the USP conditions, shown in Figure 2, and an 8-fold solvent savings. The USP assay uses a 250 × 4.6 mm, 5.0 µm C-18 column. This compares to an Agilent Poroshell 120 SB-C18, 100 × 3 mm, 2.7 µm column using the 1290 Infinity LC. Both systems use the same mobile phase, detector settings (Agilent 385 Evaporative Light Scattering Detector (ELSD)), and temperature. The flow rate using the Poroshell column was half the flow rate of the USP method.



Instrument parameters	
Temperature	30 °C
Injection amount	3.0 µL
ELSD detection conditions	
Instrument	Agilent 385-ELSD Evaporative Light Scattering Detector, model #4261A
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Nitrogen gas flow rate	1.50 SLM

PMT gain	2.0		
Data rate	40 Hz		
Flow rate	0.50 mL/min		
Mobile phase A	Water		
Mobile phase B	Methanol		
Gradient	Time (min)	% A	%B
	0	75	25
	7.5	52	48
	9.5	10	90
	10.0	10	90
	10.5	75	25
	12.5	75	25

Figure 1. Terpene lactones in ginkgo biloba extract. Agilent 1290 Infinity LC with an Agilent Poroshell 120 SB-C18 2.7 µm column.



Instrument parameters	
Temperature	30 °C
Injection amount	15 µL
Flow rate	1.0 mL/min
ELSD detection conditions	
Instrument	Agilent 385-ELSD Evaporative Light Scattering Detector, model #4261A
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Nitrogen gas flow rate	1.50 SLM

PMT gain	2.0		
Data rate	40 Hz		
Flow rate	0.50 mL/min		
Mobile phase A	Water		
Mobile phase B	Methanol		
Gradient	Time (min)	% A	%B
	0	75	25
	23	52	48
	28	52	48
	30	25	75
	35	10	90
	40	75	25
	50	75	25

Figure 2. Terpene lactones in ginkgo biloba extract. USP method using Phenomenex Luna C-18 250 × 4.6 mm, 5.0 µm column.

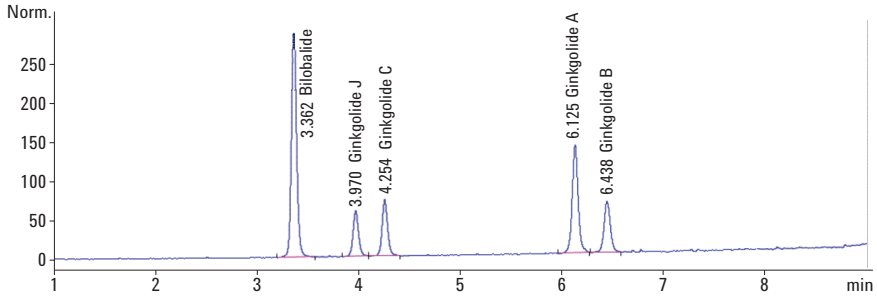


Figure 3. Terpene lactone reference standard chromatogram (Agilent Poroshell method). Standard used: USP #1291559 – Lot # 10K042.

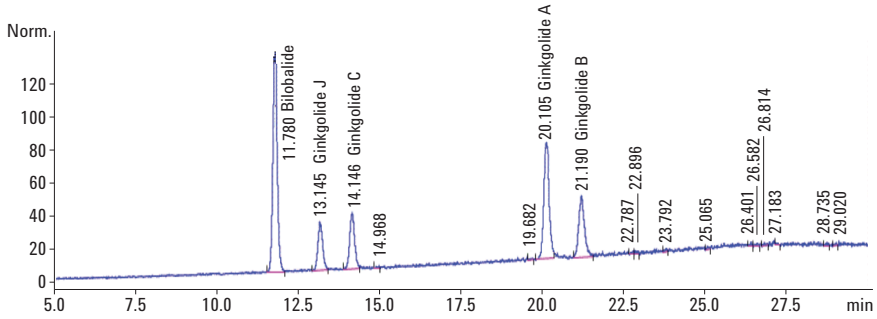


Figure 4. Terpene lactone reference standard chromatogram (USP method). Standard used: USP #1291559 – Lot # 10K042.

Conclusions

In analyzing complex botanical extracts, the Agilent 1290 Infinity LC in conjunction with Agilent Poroshell 120 SB-C18 columns can speed up analysis time, saving cost in labor and solvent use without any loss in resolution.

Reference

Ginkgo Extract Monograph, *United States Pharmacopeia*, USP36, NF31 p.1606.

For More Information

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