Fast Analysis of Triterpene Glycosides in Black Cohosh Using the Agilent 1290 Infinity LC System and an Agilent Poroshell 120 SB C-18 2.7 µm Column

Application Note

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Abstract

Using the Agilent 1290 Infinity LC System coupled with the Agilent Poroshell 120 SB C-18, 3 × 100 mm, 2.7 µm column can not only reduce solvent consumption, but allows for greater peak resolution as well. This is illustrated by comparing analyses of triterpene glycosides in black cohosh extract using the parameters outlined in the United States Pharmacopeia (USP) and the 1290 Infinity LC System with a Poroshell 120 SB C-18 column.

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Black Cohosh Supplements

Black Cohosh (*Actaea racemosa* and *Cimicifuga racemosa*) is a member of the buttercup family, native to North America. Extracts of black cohosh are made from its roots and rhizomes. The extracts are standardized to the amount of triterpene glycoside content using the 26-deoxyactein standard. Black cohosh extract is mainly used to treat menopausal symptoms. It is regulated as a dietary supplement, rather than as a drug.

Black Cohosh Extraction Conditions

Black cohosh extract source	Martin Bauer Group, Germany
Lot used	11018338

A 300 mg amount of sample extract was dissolved in 10 mL of methanol with the aid of a 20 minute sonication step.

Results and Discussion

The chromatogram in Figure 1A shows a time reduction 3.83 times better than the USP conditions shown in Figure 2A, and a solvent savings of 6.1 times. The USP assay uses a 250 × 4.6 mm, 5.0 µm C-18 column. This is compared to a Poroshell C-18, 100 × 3 mm, 2.7 µm column using the 1290 Infinity LC System. Both methods use the same mobile phase, detector settings (Agilent 385-Evaporative Light Scattering Detector (ELSD)) and column temperature.

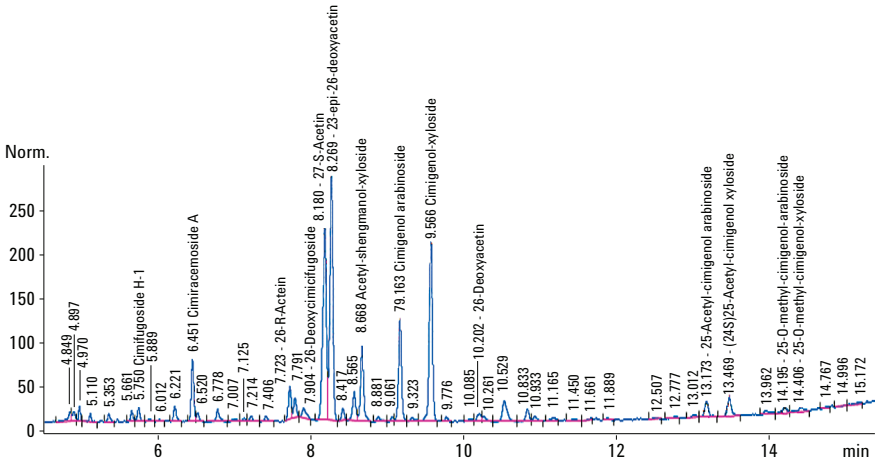

Figure 1A. Triterpene glycosides in black cohosh extract using an Agilent 1290 Infinity LC System with an Agilent Poroshell 120 SB C-18, 2.7 µm column.

Instrument parameters for Figures 1A and 1B

Column	Agilent Poroshell 12,0 SB C18, 3 × 100 mm, 2.7 µm, 35 °C
Flow rate	1.0 mL/minute
Injection volume	5.0 µL

ELSD detection conditions

Instrument	Agilent 385 Evaporative Light Scattering Detector, Model G4261A
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Nitrogen gas flow rate	1.50 SLM
PMT gain	2.0
Data rate	40 Hz
Mobile phase A	0.1% Formic acid in water
Mobile phase B	Acetonitrile

Gradient

Time (min)	% A	%B
0	80	20
2.19	80	20
4.10	68	32
15.03	36	64
17.77	5	95
18.77	5	95
19.13	80	20
22.20	80	20

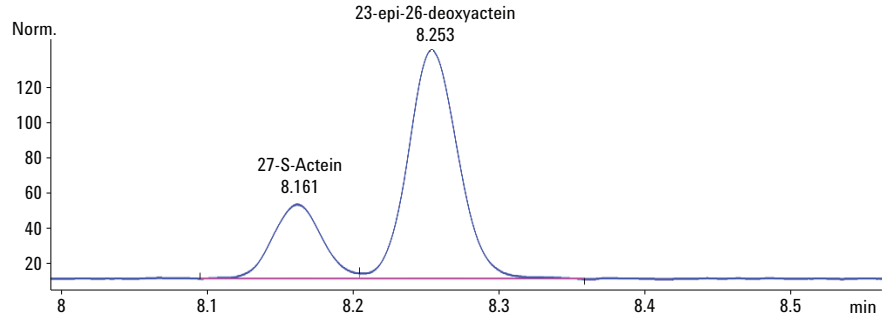

Figure 1B. Chromatogram of system suitability solution using an Agilent 1290 Infinity LC System and an Agilent Poroshell 120 SB C-18, 2.7 µm column. Sample description: 100 µg/mL solution of (27S) actein and 23-epi-26-deoxyactein standards.
Resolution factor 1.47
Acceptance criteria NLT 1.0

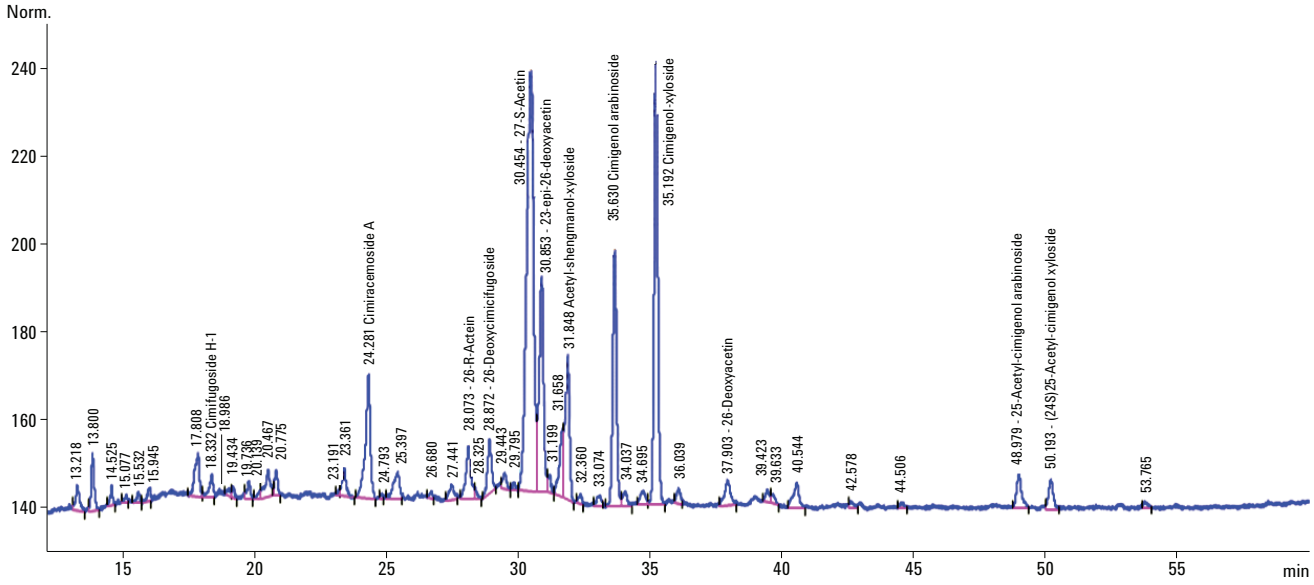

Figure 2A. Triterpene glycosides in black cohosh extract. USP method using a Phenomenex Luna C-18, 250 × 4.6 mm, 5.0 µm column.

Instrument parameters for Figures 2A and 2B

Column	Phenomenex Luna C-18, 250 × 4.6 mm, 5 µm, 35 °C
Flow rate	1.6 mL/minute
Injection volume	20 µL
ELSD detection conditions	
Instrument	Agilent 385 Evaporative Light Scattering Detector, Model G4261A
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Nitrogen gas flow rate	1.50 SLM
PMT gain	2.0
Data rate	40 Hz
Mobile phase A	0.1% Formic acid in water
Mobile phase B	Acetonitrile

Gradient

Time (min)	% A	%B
0	80	20
8	80	20
15	68	32
55	36	64
65	5	95
70	5	95
75	80	20
85	80	20

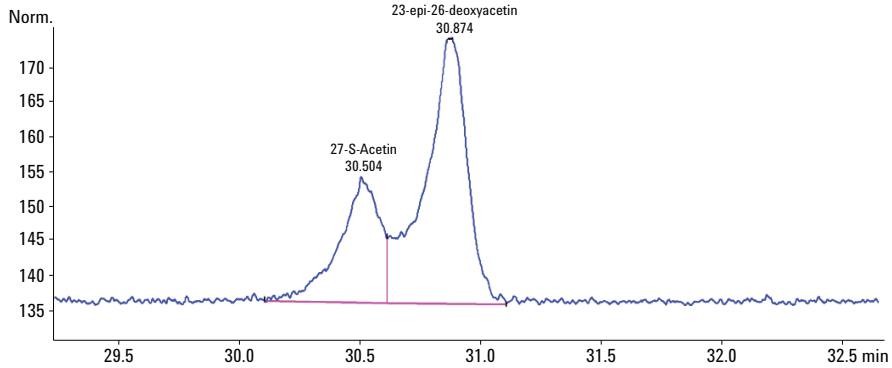

Figure 2B. Chromatogram of system suitability and a Luna 4.6 × 250 mm C18, 5um, column. 100 µg/mL solution of (27S) actein and 23-epi-26-deoxyactein standards.
Resolution factor 1.20
Acceptance criteria NLT 1.0 USP Method

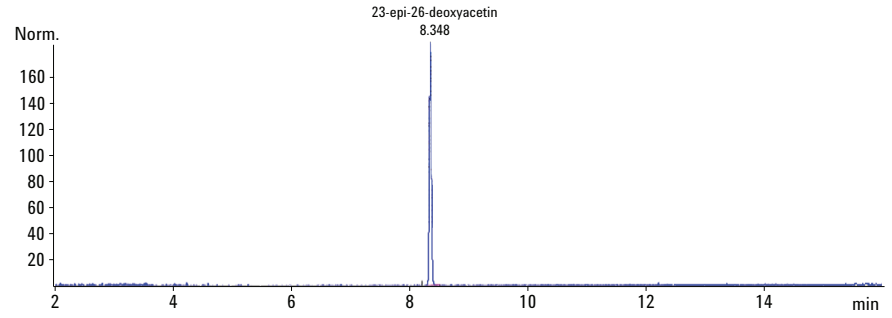

Figure 3. 23-epi-26-deoxyactein reference standard, chromadex #00004130, Lot # 4130-512
Calibration range 12.2–391.8 µg/mL
R^2 0.99999

Conclusion

In analyzing complex botanical extracts, the Agilent 1290 Infinity LC System, in conjunction with 3.0 mm id Agilent Poroshell 120 SB C-18, columns, can speed up analysis time, generating labor costs and solvent consumption. This is accomplished concurrently with improved resolution. The resolution factor between (27S)Actein and 23-epi-26-deoxyactein standard peaks using the USP method was 1.2 (Figure 2B) and the resolution factor for the Poroshell method was 1.47 (Figure 1B). With the current high cost of acetonitrile, a 6.1 times reduction in solvent consumption allows the analyst continue with acetonitrile in the method, avoiding the time-consuming redevelopment process, and avoid resolution changes and peak broadening noted with methanol mobile phases.

Reference

1. Black Cohosh Powder Extract Monograph, United States Pharmacopeia, USP35, NF30 p. 1210.

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