Determination of Triterpene Glycosides in *Cimicifuga racemosa* (Black Cohosh) by HPLC-CAD

CAN 113

Mark Roman
Tampa Bay Analytical Research, Inc., Largo, FL 33777

Introduction

Extracts of black cohosh (*Cimicifuga racemosa*) have been used since the 1950s to relieve symptoms of menopause, including hot flashes, and a number of clinical trials have supported this use. In addition, research has found that black cohosh may improve neurovegatative symptoms and psychological complaints associated with hormonal deficiencies in women, and may offer an alternative to traditional hormone therapy. Although the mechanism of action of black cohosh is not yet well understood, it is believed that the triterpene glycosides present in black cohosh (including 27-deoxyactein, actein, cimiracemoside F, and others) are bioactive.

Quantitation of the triterpene glycosides is challenging because many do not possess a chromophore that absorbs above 200 nm. As a result, high-performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD) has become the most accepted technique for the quantitation of the triterpene glycosides in black cohosh. This technique is used in the Institute for Nutraceutical Advancement (INA) Method 113.001.[1] However, ELSD suffers from poor sensitivity, highly non-linear calibration curves, and poor reproducibility.

The Thermo Scientific Dionex Corona™ CAD™ Charged Aerosol Detector offers improvement over ELSD in sensitivity, calibration linearity, and reproducibility. Here, the calibration curves and signal-to-noise ratios for ELSD and charged aerosol detection of the triterpene glycoside 27-deoxyactein are compared, and the separation and charged aerosol detection of triterpene glycosides in a black cohosh extract are shown.

Equipment

A Thermo Scientific Dionex Summit* HPLC system including:
- P680 pump
- ASI-100 autosampler
- TCC-100 column compartment

Dionex Corona CAD detector
Sedex 75 ELSD
Fused-Core® C18 HPLC column, 4.6 × 150 mm, 2.7 µm particle size
Sonication bath
Wrist-action shaker
Polytetrafluoroethylene (PTFE) syringe filters, 0.22 µm

**Company Note: The Dionex Summit system has been discontinued, so the company would recommend using a Thermo Scientific Dionex UltiMate™ 3000 system.*

Standard

27-Deoxyactein - Analytical Laboratories

Sample

Black cohosh powdered extract

Solvents and Reagents

Methanol
Water
Formic acid
Acetonitrile
Note: All solvents/reagents must be of HPLC-grade quality

Calibration Solutions Preparation

Weigh and transfer 5 mg (±0.5 mg) of 27-deoxyactein standard into a 50 mL volumetric flask. Dissolve the standard in methanol with sonication, and dilute to volume. Prepare serial dilutions from this stock standard solution in methanol as follows:

Calibration Solution	Volume of Stock Solution (mL)	Final Volume (mL)	Approx. Conc. of 27-Deoxyactein (µg/mL)
Stock #1	NA	NA	100
Stock #2	5	10	50
Stock #3	3	10	30
Stock #4	2	10	20
Stock #5	1	10	10

Sample Solution Preparation

Weigh ~300 mg of sample extract into a 50 mL volumetric flask. Add 40 mL of methanol and sonicate the flask for 15 min with occasional shaking. Sonication will naturally heat the sample, so allow to cool to room temperature. Fill the flask to volume with methanol and mix well. Filter a portion of the solution through a 0.2 µm PTFE syringe filter into an HPLC autosampler vial.

Chromatographic Conditions

Column:	Fused-Core C18 HPLC, 4.6 × 150 mm, 2.7 µm particle size
Mobile Phase:	A: 0.1% Formic acid in water
	B: Acetonitrile
Gradient:	30% B to 40% B in 12 min
	40% B to 60% B from 12–36 min
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Column Temp.:	35 °C
Detection:	Corona CAD or ELSD (N_2 pressure 2.3 bar, temperature 50 °C, gain 7)

Procedure

Inject each calibration solution, then follow with duplicate injections of the sample solution. Inject each calibration solution again after the sample solution injections.

Results and Discussion

Using relative retention times supplied by INA Method 113.001, 11 triterpene glycosides in the black cohosh extract sample were quantified against the 27-deoxyactein calibration solutions. A logarithmic calibration curve was required for the ELSD detector, whereas a linear calibration curve showed an acceptable correlation coefficient for the Corona CAD detector. Figure 1 shows the calibration curve on the Corona CAD detector. Figure 2 shows the calibration curve on the ELSD detector. The Corona CAD calibration curve was more linear than the ELSD calibration curve. The ELSD also demonstrated much higher variance between the calculated curve and measured peak areas at the beginning and the end of the calibration curve.

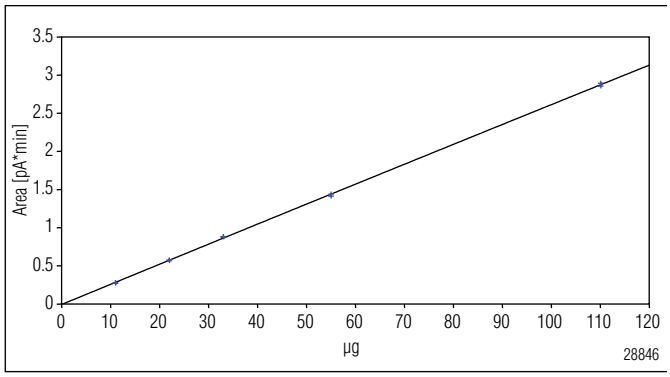

Figure 1. The 27-deoxyactein calibration curve using the Corona CAD detector.

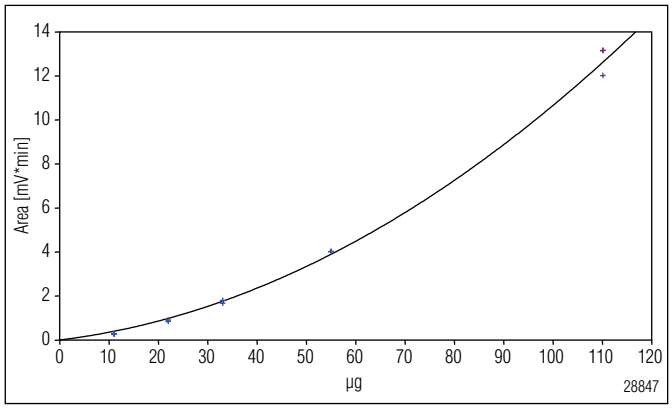

Figure 2. The 27-deoxyactein calibration curve using the ELSD detector.

Based on the signal-to-noise ratio, the Corona CAD detector yielded an improvement in sensitivity of approximately one order of magnitude, compared to the ELSD detector. Figure 3 shows overlay chromatograms of Calibration Solution #5 (~10 µg/mL 27-deoxyactein) obtained using both the Corona CAD and ELSD detectors.

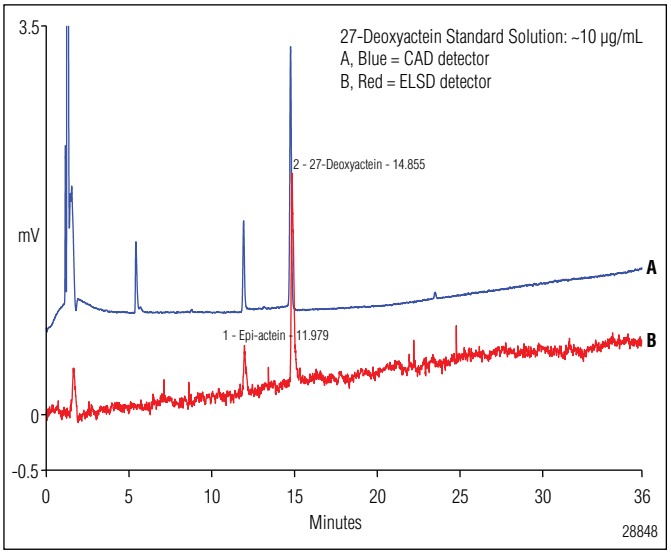

Figure 3. Overlay of chromatograms of Calibration Solution #5.

Figure 4 shows a chromatogram of the sample solution obtained using the Corona CAD detector. The total triterpene glycoside content was 3.27%.

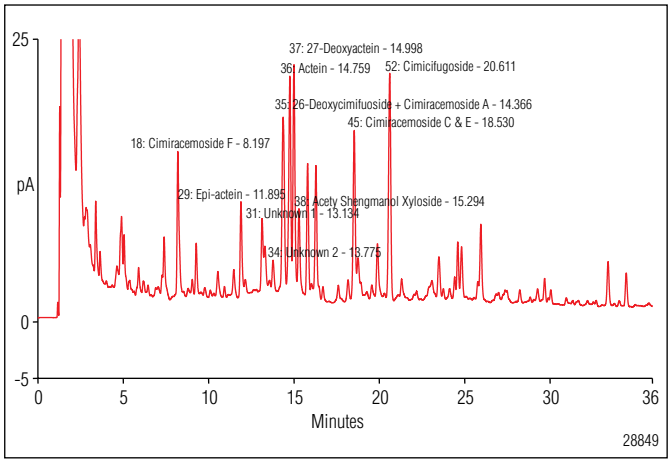

Figure 4. Black cohosh sample chromatogram using the Corona CAD detector.

Conclusion

Although evaporative light scattering detection has become the most accepted technique for the quantitation of the triterpene glycosides in black cohosh, the method suffers from poor sensitivity and highly nonlinear calibration curves. The Corona CAD detector calibration curve for 27-deoxyactein is more linear and provides a better fit to the data, and higher signal-to-noise ratios in the chromatograms indicate an order of magnitude improvement in detection levels.

Company Note: Charged aerosol detection is mass-based, provides nearly constant response regardless of the molecular structure of the analyte, and can often provide semi-quantitative data without standards. This is superior to UV or ELSD detectors since the ELSD requires specific analyte optimization parameters and the UV detector requires consistent chromophore response. However, organic solvent gradients can cause response variation due to changes in nebulizer efficiency, so the Inverse Gradient solution is recommended for increased accuracy when a calibration curve for one compound is used for quantitation of related analytes.

Reference

1. NSF International, Black Cohosh Assay by ELSD INA Method 113.001. http://www.nsf.org/business/ina/blackcohosh.asp?program=INA (accessed Jun 22, 2011).

This is a customer-submitted application note published as is.
No ISO data available for included figures.

Speed · Simplicity · Solutions

DIONEX

Dionex Products
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S./Canada (847) 295-7500

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.thermoscientific.com/dionex

LPN 2891 PDF 11/11