Novel, Universal Approach for the Measurement of Natural Products in a Variety of Botanicals and Supplements, Part 2

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Overview

Purpose: To evaluate the application of HPLC with a charged aerosol detector for the measurement of natural products and botanicals.

Methods: HPLC methods were developed for the analysis of black cohosh, ginkgo, ginseng, milk thistle, ashwagandha, and hoodia.

Results: HPLC with charged aerosol detection is sensitive (low ng levels on-column), has a wide dynamic range and minimal inter-analyte response variability. It is ideal for measuring analytes that lack a chromophore and offers superior performance over evaporative light scattering detection (ELSD) and refractive index (RI).

Introduction

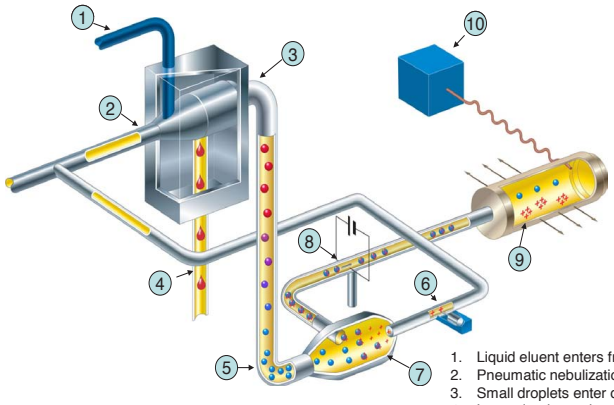
Botanicals contain a great diversity of compounds that exhibit wide variation in their physicochemical properties. Although no single analytical method is available to measure all potentially active components, HPLC with charged aerosol detection Is a nearly universal approach that nonselectively measures any nonvolatile and many semivolatile compounds; that is, charged aerosol detection does not require that analytes be ionizable (as required for mass spectrometry) or contain a chromophore (as required for UV spectrophotometry).

A number of isocratic and gradient HPLC/UHPLC methods with charged aerosol detection were developed and evaluated for the measurement of phytochemicals extracted from a variety of botanicals including: steroidal and pregnane glycosides from *Hoodia gordonii*; steroidal lactones from *Withania somnifera*; flavonolignans from milk thistle (*Silybum marianum*); triterpene glycosides from black cohosh (*Cimicifuga racemosa*); and ginsenosides from ginseng (*Panax ginseng).*

Analytes showed consistent response independent of chemical structure (typically < 10% variability between compounds corrected for gradient elution). All methods had a wide dynamic range (~four orders of magnitude), good sensitivity (typically low ng levels of detection), and excellent reproducibility (RSDs typically < 2%) even at low detection levels. Comparative data from ELSD and UV detection will also be discussed.

The charged aerosol detector is a sensitive, mass-based detector, especially well-suited for the determination of any nonvolatile analyte independent of chemical characteristics. As shown in Figure 1, the detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. The charge is then measured by a highly sensitive electrometer, providing reproducible, nanogram-level sensitivity. This technology has greater sensitivity and precision than ELSD and RI, and it is simpler to operate than a mass spectrometer (MS).

FIGURE 1. Schematic showing how charged aerosol detection works.



1. Liquid eluent enters from HPLC system
2. Pneumatic nebulization occurs
3. Small droplets enter drying tube
4. Large droplets exit to drain
5. Dried particles enter mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and charge is transferred
8. High mobility species are removed
9. Charge is measured by a highly sensitive electrometer
10. Signal transferred to chromatographic software

Methods

Liquid Chromatography

HPLC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 RS system with:
- Diode Array Detector DAD-3000RS and a
- Corona™ ultra RS™ Charged Aerosol Detector:
Nebulizer Temperature: 25–35 °C
Power function: 1.00
Data collection rate: 20 Hz

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software, 7.1 SR1

Black Cohosh

Column: Fused-Core C18 HPLC Column, 4.6 × 150 mm, 2.7 µm
Column Temp: 35 °C
Flow Rate: 1.0 mL/min
Mobile Phase A: 0.1% Formic acid in water
Mobile Phase B: Acetonitrile
Gradient: 30% B to 40% B in 12 min; 40% B to 60% B from 12–36 min
Injection Vol.: 10 µL
Additional Detection: ELSD (N_2 pressure 2.3 bar, 50 °C, gain 7)
Sample Prep.: Weigh 300 mg of sample extract into a 50 mL volumetric flask. Add 40 mL methanol and sonicate for 15 min with occasional shaking. Cool to room temperature and bring to volume with methanol. Filter through a 0.2 µm PTFE syringe filter into an HPLC autosampler vial.

Ginkgo Biloba

Column: Thermo Scientific™ Accucore™ C8, 4.6 × 150 mm; 2.6 µm
Column Temp.: 45 °C
Flow Rate: 2.0 mL/min
Mobile Phase A: Deionized water
Mobile Phase B: Acetonitrile, Fisher Scientific™ Optima LCMS
Gradient: 20%B to 50%B in 6 min; to 95%B in 10 min; hold 10 min.
Injection Vol.: 5 µL
Sample Prep.: Sonicate 1 g powder from commercially available health supplement in 10 mL methanol for 15 min. Centrifuge at 13,000 rpm for 10 min. Transfer supernatant to a glass HPLC autosampler vial.

Ginseng

Column: Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp: 30 °C
Flow Rate: 0.67 mL/min
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient: 15% B to 35% B in 30 minutes
Injection Vol.: 20 µL
Sample Prep.: Weigh 400 mg of sample into a 100 mL volumetric flask. Add 15 mL methanol and sonicate for 10 min with occasional shaking. Add 60 mL of water and sonicate for an additional 10 min. Cool to room temperature and bring to volume with water. Filter through a 0.2 µm PVDF syringe filter into an HPLC autosampler vial.

Milk Thistle

Column: Thermo Scientific™ Acclaim™ RSLC 120 C18 Column, 2.1 × 100 mm, 2.2 µm
Column Temp: 40 °C
Flow Rate: 0.50 mL/min
Mobile Phase A: 3 mM ammonium formate, 0.3% formic acid in water:MeOH 80:20 v:v
Mobile Phase B: 3 mM ammonium formate, 0.3% formic acid in water:MeOH 20:80 v:v
Gradient: 15% B to 45% B in 4.4 min.
Injection Vol.: 1 µL
Sample Prep.: Weigh 70 mg of powdered milk thistle extract into a 250 mL amber glass bottle. Add 100 mL methanol and sonicate for 20 min. Centrifuge an aliquot at 5000 rpm for 10 min. Transfer supernatant to a glass HPLC autosampler vial.

Ashwagandha

Column: Accucore C8, 4.6 × 150 mm; 2.6 µm
Column Temp.: 45 °C
Flow Rate: 2.0 mL/min
Mobile Phase A: Deionized water
Mobile Phase B: Acetonitrile, Optima LCMS
Gradient: 20%B to 50%B in 6 min; to 95%B in 10 min; hold 10 min.
Injection Vol.: 1 µL
Sample Prep.: Sonicate 625 mg powder from commercially available capsule in 10 mL ethanol for 30 min. Centrifuge extract at 13,000 rpm for 10 min and transfer supernatant to a glass HPLC autosampler vial.

Hoodia

Column:	Accucore C8, 4.6 × 150 mm; 2.6 µm
Column Temp.:	40 °C
Flow Rate:	2.0 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Optima LCMS
Gradient:	40%B to 47%B in 2.5 min; to 95%B in 15 min; hold 10 min.
Injection Vol.:	10 µL
Sample Prep.:	Place 50 mg powder from commercially available capsule into a 2 mL centrifuge tube, add 1 mL methanol, vortex for 10 min. Transfer supernatant to a 5 mL volumetric flask. Repeat two times with fresh methanol. Combine extracts and bring to volume with methanol. Centrifuge at 13,000 rpm for 10 min and transfer supernatant to a glass HPLC autosampler vial.

Results and Discussion

Extracts of black cohosh have been used since the 1950s to relieve symptoms of menopause. The active ingredients are believed to be related to the content of triterpene glycosides present in black cohosh, including 27-deoxyactein, actein, cimiracemoside F, and others. Many of the triterpene glycosides do not possess chromophores above 200 nm. HPLC-ELSD is the most common method for quantitation of triterpene glycosides in black cohosh products. However, ELSD suffers from poor sensitivity (Figure 2), and has a very non-linear response (Figure 3). Charged aerosol detection overcomes these issues, offers better reproducibility and can easily measure low-abundant analytes in a black cohosh extract (Figure 4).

FIGURE 2. Comparison of charged aerosol detection vs. ELSD for determination of triterpene glycosides in black cohosh (27-deoxyactein standard solution: 11 µg/mL).

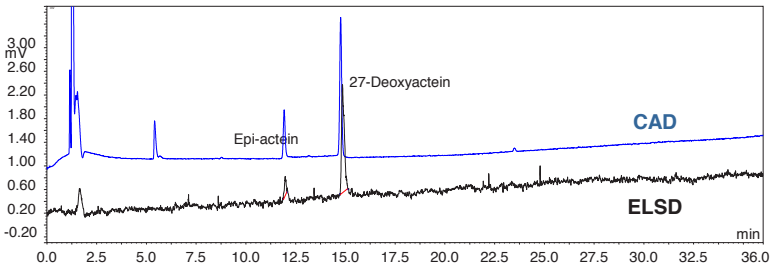


FIGURE 3. Comparison of charged aerosol detection vs. ELSD calibration curves for 27-deoxyactein standard.

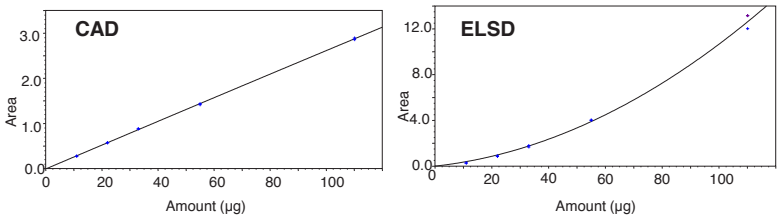
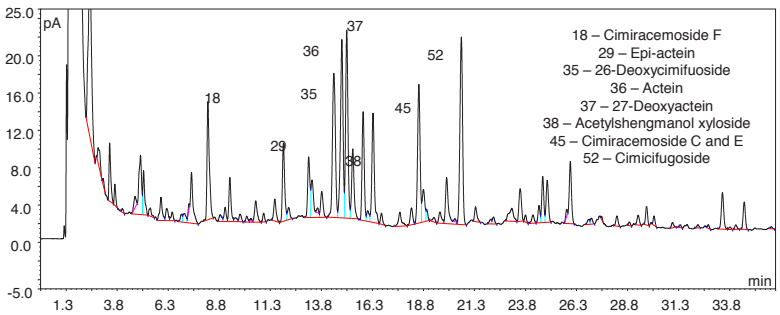
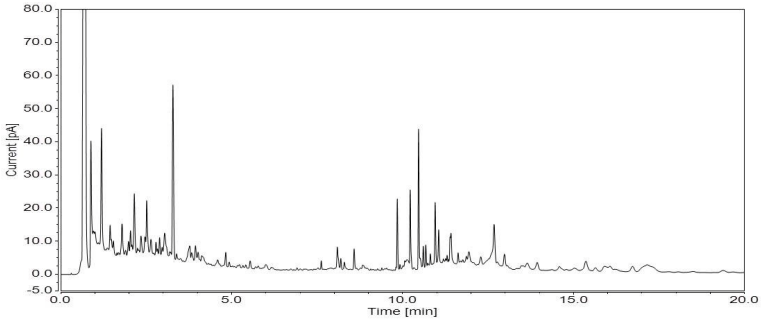


FIGURE 4. Determination of triterpene glycosides in black cohosh extract.



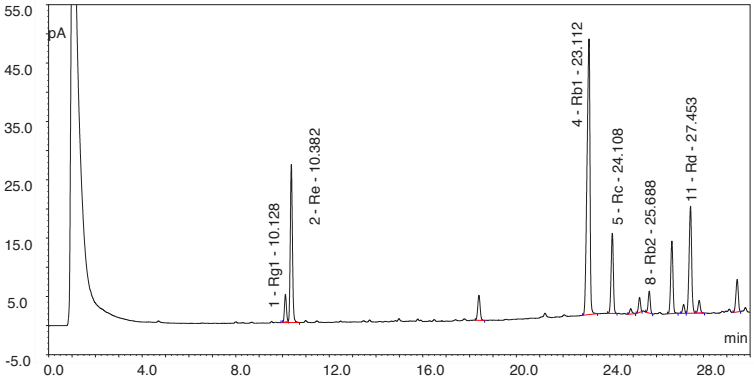
Ginkgo biloba is thought to possess nootropic activity, and is taken to improve memory and enhance concentration. Sesquiterpenoid bilobalide and numerous diterpenoid ginkgolides are believed to be the active ingredients. As seen in Figure 5, charged aerosol detection is able to detect numerous non-volatile compounds in a *Ginko biloba* extract.

FIGURE 5. HPLC-Charged aerosol analysis of *Ginkgo biloba* extract



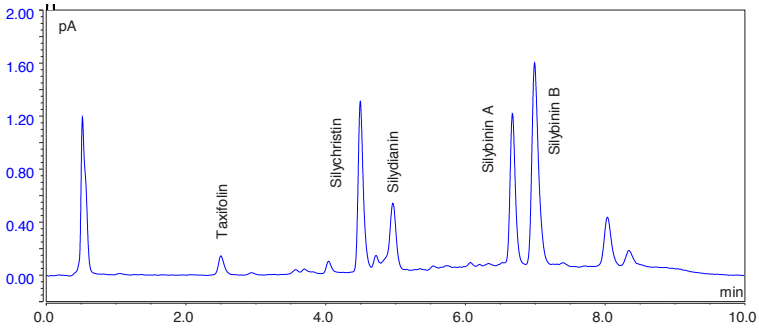
Asian Ginseng (*Panax ginseng*) has traditionally been used as a tonic to reduce the effects of stress, counteract fatigue, and increase stamina. The main bioactive ingredients found in *Panax ginseng,* and a related species *Panax quinquefolius* (American ginseng) are triterpene saponins, commonly referred to as ginsenosides. There are seven major ginsenosides present in *Panax ginseng*: the protopanaxatriols (Rg1, Re and Rf), and protopanaxadiols (Rb1, Rc, Rb2 and Rd). Compared to HPLC with low-wavelength UV detection (203 nm), HPLC with charged aerosol detection not only improves the baseline slope seen with gradient elution, but also offers improved sensitivity (Figure 6).

FIGURE 6. HPLC-Charged aerosol analysis of ginseng extract



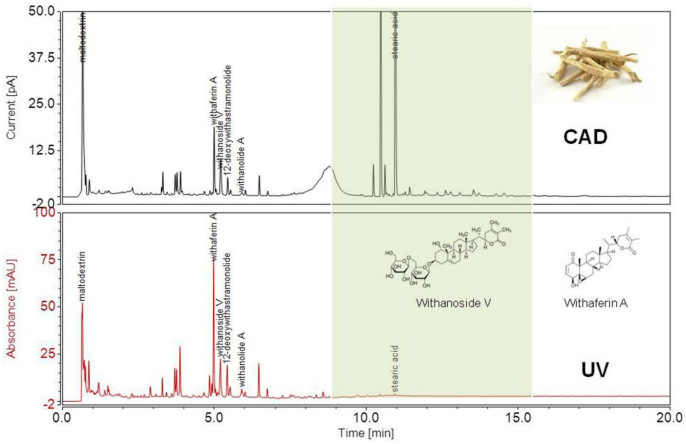
Evidence suggests that milk thistle extracts may both prevent and repair damage to the liver from toxic chemicals and medications and may be of use in the treatment of mushroom poisoning. The active ingredients are thought to be numerous flavonolignans including silybinin, isosilybinin, silycristin and silydianin (Figure 7).

FIGURE 7. HPLC-Charged aerosol analysis of milk thistle extract.



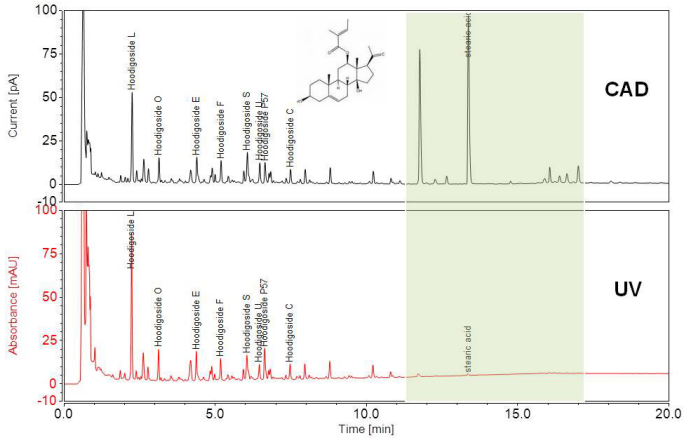
Withania somnifera is a woody shrub in the nightshade family (Solanacea) native to India. Known as ashwagandha, it is used in Ayurvedic medicine as a strengthening tonic and to cool the body, typically by drinking a cup of hot milk containing the powdered root. Purported active compounds include steroidal lactones isolated from the root, some of which are well resolved by HPLC, as depicted in Figure 8. The figure shows that charged aerosol detection detects several compounds not readily seen by UV at 230 nm.

FIGURE 8. Comparison between charged aerosol detection and UV absorbance detection for HPLC separation of Ashwagandha extract.



Hoodigosides are oxypregnane steroidal glycosides abundant in *Hoodia gordonii* and related plants native to the deserts of southwestern Africa. This plant was used traditionally to ease hunger during long hunting expeditions and enjoys wide use today in dietary supplements purported to aid in appetite suppression and weight loss. Figure 9 demonstrates the superior sensitivity of charged aerosol detection for several late-eluting components of a commercial Hoodia extract.

FIGURE 9. Comparison between charged aerosol detection and UV absorbance detection for HPLC separation of a Hoodia extract.



Conclusion

Charged aerosol detection offers numerous analytical improvements over ELSD, RI and low-wavelength UV and is of particular use to the analysis of natural products and botanicals separated by modern HPLC methods. Charged aerosol detection:

- Measures any non-volatile and many semi-volatile species
- Response is independent of chemical structure
- Allows estimation of analyte amounts even when external standards are not available
- Has excellent sensitivity, reproducibility and a dynamic range of over four orders of magnitude

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