Improved Universal Approach to Measure Natural Products in a Variety of Botanical and Supplements

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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, caralluma, bacopa, 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.

Introduction

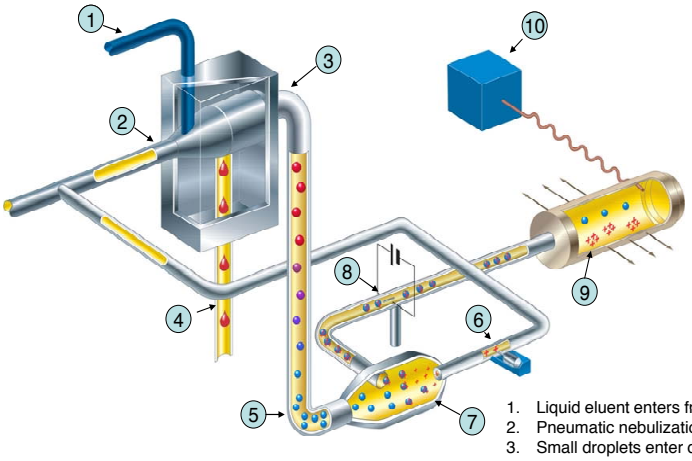
Botanicals contain a great diversity of compounds that exhibit wide variation in their physicochemical properties. Although no single analytical method is available to measure all potentially active components, HPLC with charged aerosol detection is a nearly universal approach that nonselectively measures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

Presented are several HPLC/UHPLC methods with charged aerosol detection that have been improved to increase speed and sensitivity. The improved methods were evaluated for the measurement of phytochemicals extracted from a variety of botanical and herbal supplements including: triterpene glycosides from black cohosh (*Cimicifuga racemosa*) and *Bacopa monnieri*; oxypregnane glycosides from *Caralluma fimbriata;* flavonolignans from milk thistle (*Silybum marianum*); steroidal lactones from Ashwagandha (*Withania somnifera*teroidal and oxypregnane glycosides from *Hoodia gordonii*.

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 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
- Thermo Scientific™ Dionex™ Corona™ Veo™ Charged Aerosol Detector:
  Evaporation Temperature: 35 or 50 °C
  Power function: 1.00
  Data collection rate: 2 Hz

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software, 7.2

Black Cohosh

Column:	Thermo Scientific™ Accucore™ C18 column, 2.1 × 150 mm, 2.6 µm
Column Temp:	40 °C
Flow Rate:	0.5 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Fisher Scientific™ Optima LCMS
Gradient:	30% B to 40% B in 12 min; 40% to 60% B from 12–30 min; 60% - 95%B from 30 to 40 min.
Injection Vol.:	2 µL
Sample Prep.:	Sonicate 300 mg of sample in 40 mL methanol in 50 mL vol. flask for 15 min. Cool to room temperature and bring to volume with methanol. Centrifuge at 13,000 rpm for 15 min. Transfer supernatant to an amber glass HPLC autosampler vial.

Caralluma

Column:	Accucore C8 column, 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:	40%B to 47%B in 2.5 min; to 95%B in 12.5 min; hold 10 min
Injection Vol.:	10 µL
Sample Prep.:	Sonicate 1 tablet from commercial health supplement in 20 mL methanol for 15 min. Centrifuge at 13,000 rpm for 10 min. Transfer supernatant to a glass HPLC autosampler vial.

Bacopa

Column:	Accucore column C18, 2.1 × 150 mm; 2.6 µm
Column Temp.:	45 °C
Flow Rate:	0.50 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Fisher Scientific™ Optima LCMS
Gradient:	28%B
Injection Vol.:	1 µL
Sample Prep.:	Standard mixture dissolved in 25:75 (v/v) water:acetonitrile.

Milk Thistle

Column:	Thermo Scientific™ Acclaim™ 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.:	2 µL
Sample Prep.:	Sonicate 70 mg of dry milk thistle extract in 100 mL methanol in a 250 mL amber glass bottle for 20 min. Centrifuge an aliquot at 5000 rpm for 10 min. Transfer supernatant to a glass HPLC vial.

Ashwagandha

Column:	Accucore C8 column, 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 commercial 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.

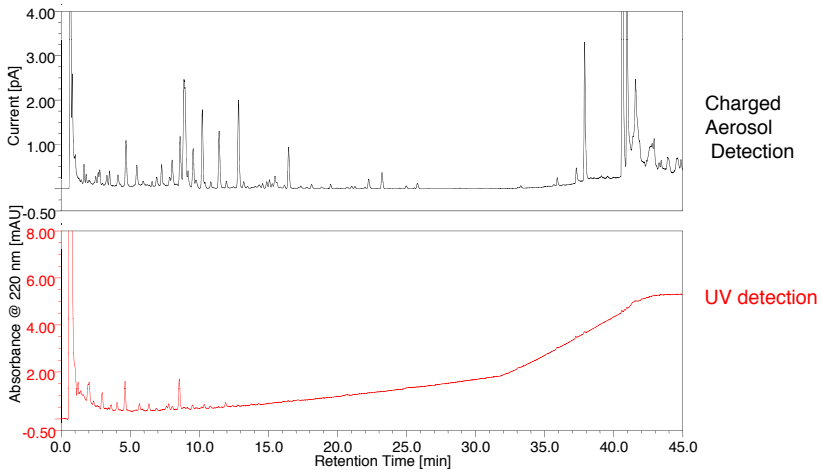
Hoodia

Column:	Accucore C8 column, 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

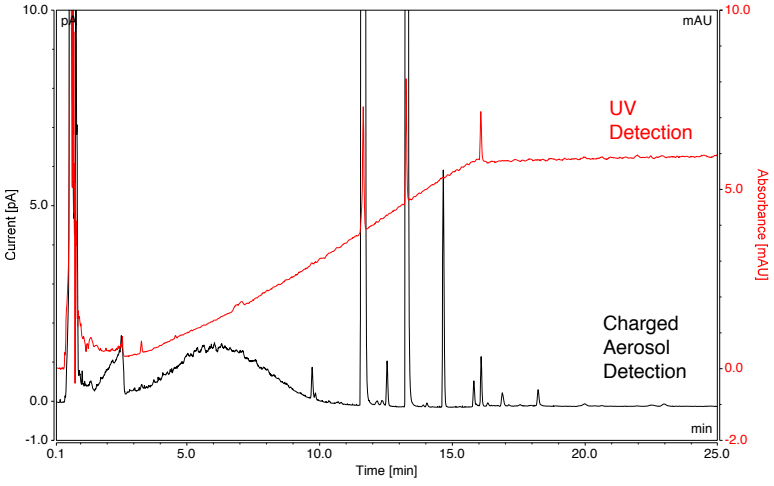
Extracts of black cohosh have been used since the 1950s to relieve symptoms of menopause. The active ingredients are believed to include triterpene glycosides including 27-deoxyactein, actein, cimiracemoside F, and others. Many of the triterpene glycosides do not possess chromophores above 200 nm. Charged aerosol detection easily measures low-abundant analytes in a black cohosh extract (Figure 2).

FIGURE 2. Comparison of charged aerosol detection to UV detection at 220 nm for determination of triterpene glycosides in black cohosh extract.



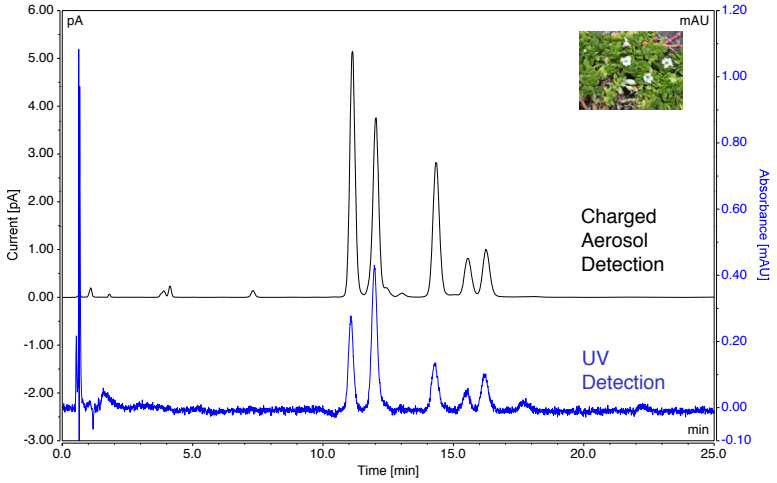
The edible cactus *Caralluma fimbriata* is used throughout India both as a food and to suppress hunger and enhance endurance. The active ingredients are believed to include oxypregnane glycosides. As with the triterpenoids in black cohosh, many of the oxypregnane glycosides are not well detected by low-wavelength UV absorbance. Charged aerosol detection detects the analytes with higher sensitivity than UV while also showing less baseline rise due to the mobile phase gradient (Figure 3).

FIGURE 3. HPLC-Charged aerosol analysis of oxypregnane glycosides in *Caralluma fimbriata* leaf extract.



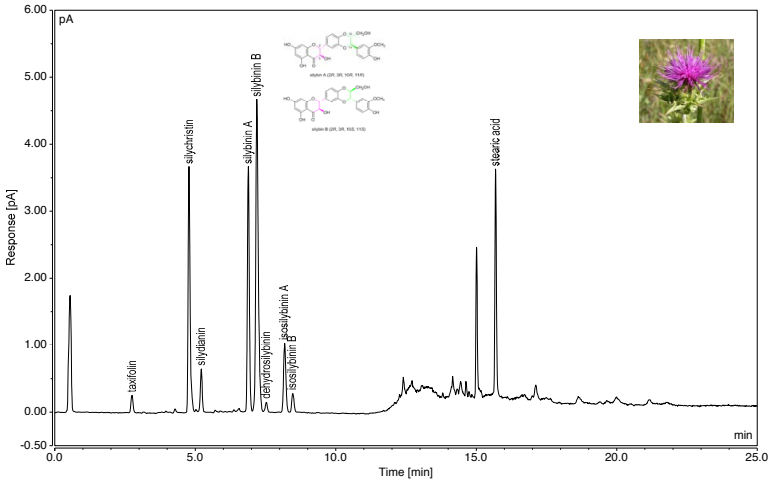
In Ayervedic medicine Brahmi (*Bacopa monnieri*) is purported to enhance mind power (Medhya effect) and improve all aspects of mental functioning, including comprehension, memory and recollection. Among the many bioactive ingredients found in *Bacopa monnieri* are the triterpene saponins separated in Figure 4, including Bacoside A3, Bacopaside II, Bacopaside X and Bacopasaponin C. Compared to HPLC with low-wavelength UV detection (220 nm), HPLC with charged aerosol detection not only improves the baseline slope seen with gradient elution, but also offers improved sensitivity.

FIGURE 4. HPLC-Charged aerosol analysis of *Bacopa monnieri* extract



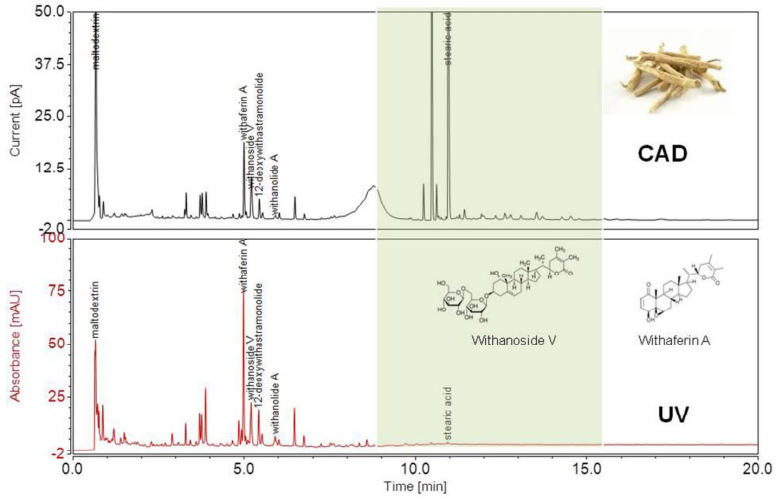
Evidence suggests that milk thistle extracts may both prevent and repair damage to the liver from toxic chemicals and medications and may be of use in the treatment of mushroom poisoning. The active ingredients are thought to be numerous flavonolignans including silybinin, isosilybinin, silychristin and silydianin (Figure 5). Note that the gradient program is extended for several minutes to elute stearic acid and other late eluting formulation components. These are poorly detected by UV absorbance but should be removed from the column to ensure good long term performance of the HPLC method.

FIGURE 5. HPLC-Charged aerosol analysis of milk thistle extract.



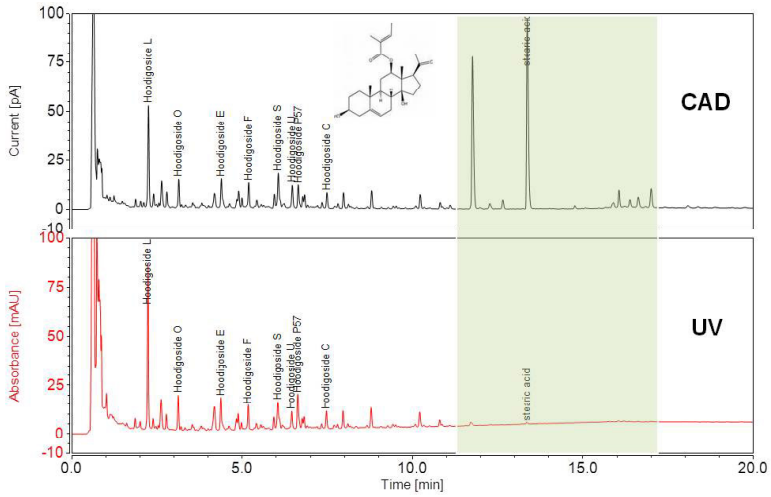
Withania somnifera is a woody shrub in the nightshade family (Solanacea) native to India. Known as ashwagandha, it is used in Ayurvedic medicine as a strengthening tonic and to cool the body, typically by drinking a cup of hot milk containing the powdered root. Purported active compounds include steroidal lactones isolated from the root, some of which are well resolved by HPLC, as depicted in Figure 6. The figure shows that charged aerosol detection detects several compounds not readily seen by UV at 230 nm.

FIGURE 6. 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 7 demonstrates the superior sensitivity of charged aerosol detection for several late-eluting components of a commercial Hoodia extract.

FIGURE 7. 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 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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