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

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Overview

Purpose: To evaluate the application of HPLC with charged aerosol detection to the measurement of natural products and botanicals.

Methods: Several methods were developed for the analysis of black cohosh, ginkgo, ginseng, milk thistle, gotu kola and stevia.

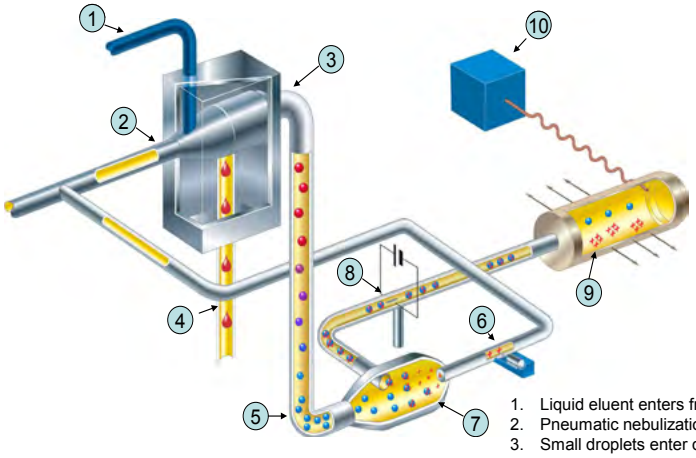
Results: HPLC with charged aerosol detection is sensitive (low ng levels on-column), has a wide dynamic range and minimal inter-analyte response variability. It is ideal for measuring analytes that lack a chromophore and offers superior performance over evaporative light scattering detection (ELSD) and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

Introduction

Botanicals contain a great diversity of compounds that can show extreme variation in their physicochemical properties. Analysis of potentially active components can be challenging as not all contain a chromophore or can be ionized, thereby limiting the use of UV absorbance and mass spectrometry, respectively. HPLC with charged aerosol detection is a sensitive, universal (nonselective) approach that can measure any nonvolatile and many semi-volatile compounds. A number of isocratic and gradient HPLC with the Thermo Scientific Dionex Corona Charged Aerosol Detector (CAD™) methods were developed and evaluated for the measurement of phytochemicals extracted from a variety of botanicals including: triterpene glycosides from black cohosh (*Cimicifuga racemosa*); ginkgolides and bilobalides from ginkgo (*Ginkgo biloba*); ginsenosides from ginseng (*Panax ginseng*); silibinins in milk thistle (*Silybum marianum*); ursane and oleanane triterpenes from gotu kola (*Centella asiatica*); and diterpene glycosides from stevia (*Stevia rebaudiana*). 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 ELS 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 refractive index (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

Black Cohosh
Column: Fused-Core C18 HPLC Column, 4.6 × 150 mm, 2.7 µm
Column Temp: 35 ºC
Flow Rate: 1.0 mL/min
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
Injection Vol.: 10 µL
Detection: Charged aerosol detection or evaporative light scattering detection (N_2 pressure 2.3 bar, temperature 50 ºC, gain 7)
Sample Prep.: About 300 mg of sample extract was weighed into a 50 mL volumetric flask. 40 mL of methanol was added and the flask was sonicated for 15 minutes with occasional shaking. After cooling to room temperature, the flask was filled to volume with methanol and mixed well. A portion of the solution was filtered through a 0.2 µm PTFE syringe filter into an HPLC autosampler vial.

Ginkgo Biloba
Column: Capcell PAK® MG 100 C18, 4.6 × 250 mm; 5 µm
Column Temperature: ambient
Flow Rate: 1.0 mL/min
Mobile Phase A: 5% acetonitrile in 0.1% trifluoroacetic acid
Mobile Phase B: 70% acetonitrile in 0.1% trifluoroacetic acid
Gradient: Time, % A: 0, 100; 30, 25; 35, 25; 40, 100
Injection Vol.: 10 µL
Sample Prep.: Sample (1 g powder from commercially available health supplement) was sonicated in 10 mL methanol for 30 min. The extract was centrifuged (9,000 rpm) for 5 mins and the supernatant was passed through a 0.2 µm nylon filter by centrifugation (9,000 rpm – 2 min).

Ginseng
Column: Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp: 30 ºC
Flow Rate: 0.67 mL/min
Mobile Phase: A – water; B – acetonitrile
Gradient: 15% B to 35% B in 30 minutes
Injection Vol.: 20 µL
Sample Prep.: About 400 mg of sample extract was weighed and transferred into a 100 mL volumetric flask. 15 mL of methanol was added and the flask was sonicated for 10 min with occasional shaking. About 60 mL of water was added, and the flask was sonicated for an additional 10 min. After cooling to room temperature, the flask is filled to volume with water and mixed well. A portion of the solution is filtered 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 ºC
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.: About 70 mg of powdered milk thistle extract was weighed and transferred into a 250 mL amber glass bottle. 100 mL of methanol was added and the flask was sonicated for 20 minutes. A portion of the solution was transferred to a 1.5 mL polypropylene centrifuge tube and centrifuged at 5000 x *g* for 10 min. The supernatant was transferred into an HPLC autosampler vial.

Gotu Kola
Column: Fused-Core C18 HPLC Column, 3.0 × 100 mm, 2.7 µm
Column Temp: 35 ºC
Flow Rate: 0.64 mL/min
Mobile Phase: A – 0.1% formic acid in Water; B – acetonitrile
Gradient: 18% B to 22% B in 8 min; 22% B to 45% B from 8 min to 17 min; 45% B to 80% B from 17 min to 23 min
Injection Vol.: 5 µL
Sample prep.: About 250 mg of sample extract was weighed and transferred into a 100 mL volumetric flask. About 70 mL of methanol was added and the flask was sonicated for 10 min with occasional shaking. After cooling to room temperature, the flask was filled to volume with methanol and mixed well. A portion of the solution is filtered through a 0.2 µm PTFE syringe filter into an HPLC autosampler vial.

Stevia
HPLC
Column: Capcell PAK C18 AQ 4.6 × 250 mm, 5 µm
Column Temperature: 50 °C
Flow Rate: 1.0 mL/min
Mobile Phase A: deionized Water (DI), acetonitrile, trifluoroacetic acid (TFA) (95:5:0.1)
Mobile Phase B: DI:acetonitrile (5:95)
Gradient: 0 – 90% B in 30 min. Hold for 5 min and return to starting conditions.
Injection Vol.: 10 µL
UHPLC
Column: Acclaim™ RSLC Polar Advantage II 2.1 × 250 mm, 2.2 µm
Column Temperature: 40 °C
Flow Rate: 0.7 mL / min
Mobile Phase A: deionized Water (DI) + 0.1% formic acid
Mobile Phase B: acetonitrile + 0.1% formic acid
Gradient: 5% to 60% B in 9 min hold 1 min and return to 5% B
Injection Vol.: 5 µL
Sample Prep.: Commercially available stevia extract powder was dissolved in deionized water (0.9 mg/mL)

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).

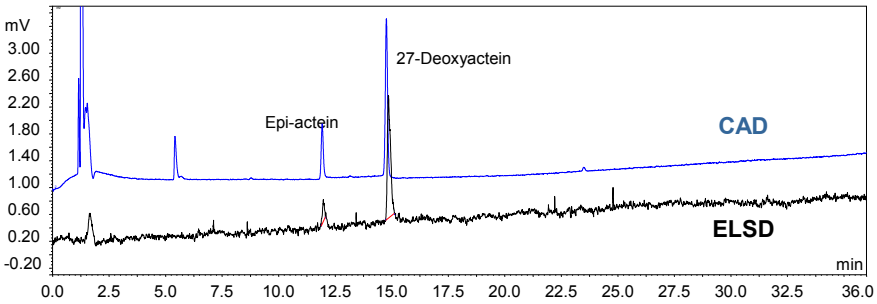


Figure 3. Comparison of charged aerosol detection vs. ELSD calibration curves for 27-deoxyactein standard.

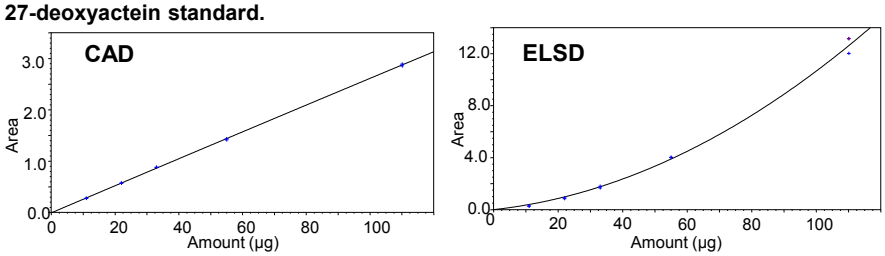
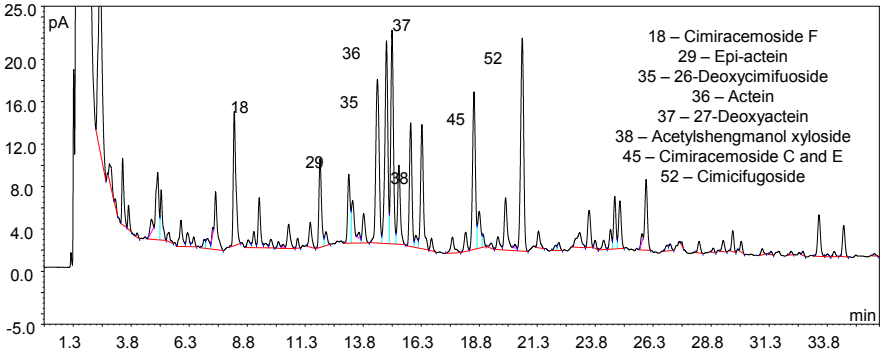
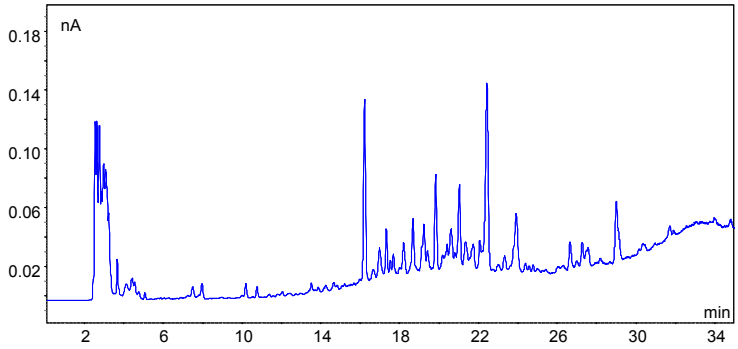


Figure 4. Determination of triterpene glycosides in black cohosh extract.



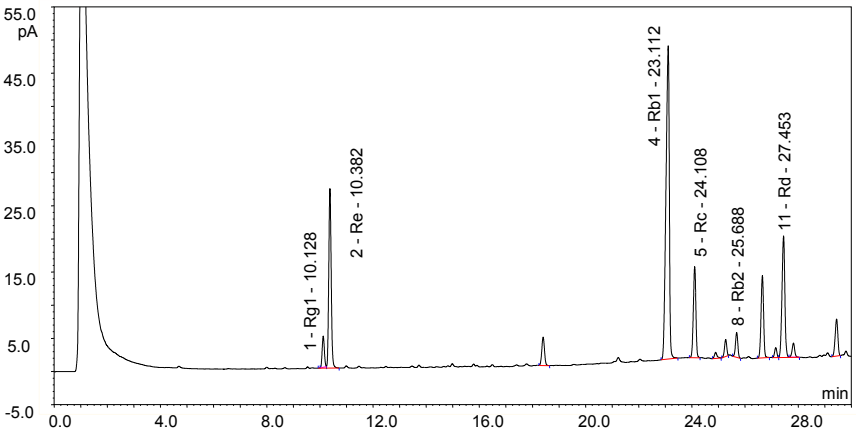
Ginkgo biloba is thought to possess nootropic activity, and is taken to improve memory and enhance concentration. The active ingredients are believed to be the sesquiterpenoid bilobalide and numerous diterpenoid ginkgolides. Although not labeled in Figure 5, these compounds elute between 3 and 14 minutes.

Figure 5. Analysis of *Ginkgo biloba* extract.



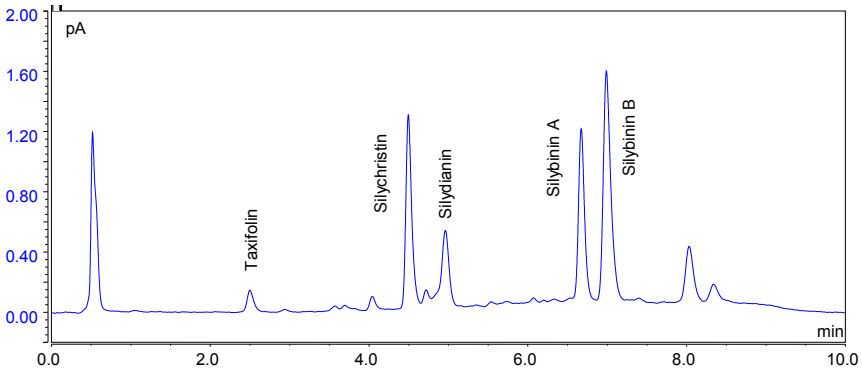
Asian Ginseng (*Panax ginseng*) has traditionally been used as a tonic to reduce the effects of stress, counteract fatigue, and increase stamina. The main bioactive ingredients found in *Panax ginseng,* and a related species *Panax quinquefolius* (American ginseng) are triterpene saponins, commonly referred to as ginsenosides. There are 7 major ginsenosides present in *Panax ginseng*: the protopanaxatriols (Rg1, Re and Rf), and protopanaxadiols (Rb1, Rc, Rb2 and Rd). HPLC with low-wavelength UV detection (203 nm) is most commonly used but suffers from poor sensitivity. HPLC with charged aerosol detection not only improves the baseline slope seen with gradient elution, but also offers improved sensitivity (Figure 6).

Figure 6. Analysis of ginseng extract.



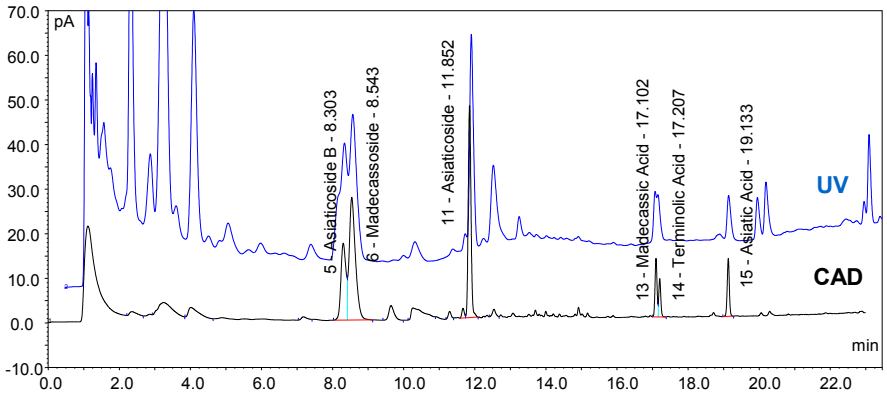
Evidence suggests that milk thistle extracts may both prevent and repair damage to the liver from toxic chemicals and medications and may be of use in the treatment of mushroom poisoning. The active ingredients are thought to be numerous flavonolignans including silybinin, isosilybinin, silycristin and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

Figure 7. Analysis of milk thistle extract.



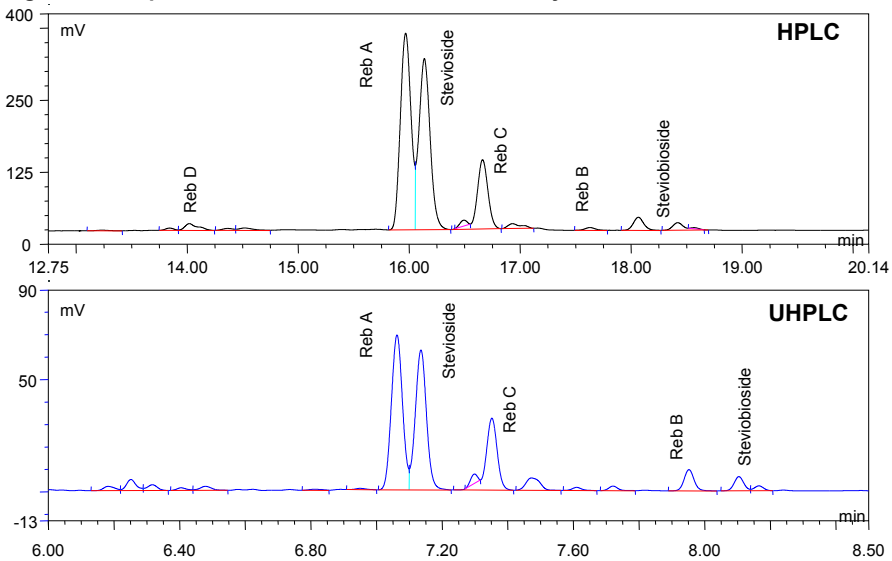
Centella asiatica (commonly called gotu kola) is a small herbaceous annual plant that is native to India, Sri Lanka, northern Australia, and other parts of Asia and the western Pacific. It is used as a medicinal herb in Ayurvedic medicine as well as traditional Chinese medicine for a wide variety of conditions, such as improving memory, blood flow, as a wound-healing agent, and as a topical application for skin conditions such as ulcers, wounds, and eczema. The chemical compounds of interest in gotu kola are usually considered to be the ursane- and oleanane-type triterpenes and triterpene glycosides. Although low-wavelength UV can be used to measure these compounds, it suffers from sensitivity and baseline issues – these can be readily overcome by the CAD (Figure 8). Interestingly, this figure also shows that the number of potential interferences are reduced when using the CAD, probably as the sample has a number of volatile compounds that absorb low-wavelength UV.

Figure 8. Comparison of UV and charged aerosol detection response for gotu kola extract.



Stevia rebaudiana, commonly known as stevia, is currently being used as a low calorie natural sweetener. The active ingredients include the aglycone diterpene known as steviol and numerous glycosides including stevioside and the rebaudiosides. Although low-wavelength UV was initially used, it suffered from the numerous issues described above. There are a number of HPLC with charged aerosol detection methods in the literature. Here we show the improved chromatographic resolution and significantly reduced analysis time using an UHPLC method.(Figure 9).

Figure 9. Comparison of HPLC and RSLC for the analysis of a stevia 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. The CAD:

- Measures any non-volatile and many semi-volatile species.
- Response is little affected by chemical structure.
- Allows estimation of analyte amounts even when external standards are not available.
- Has excellent sensitivity, reproducibility and a dynamic range of over 4 orders of magnitude,

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