

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

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Key Words

Natural products, botanicals, supplements, charged aerosol detection, phytochemicals, HPLC

Goal

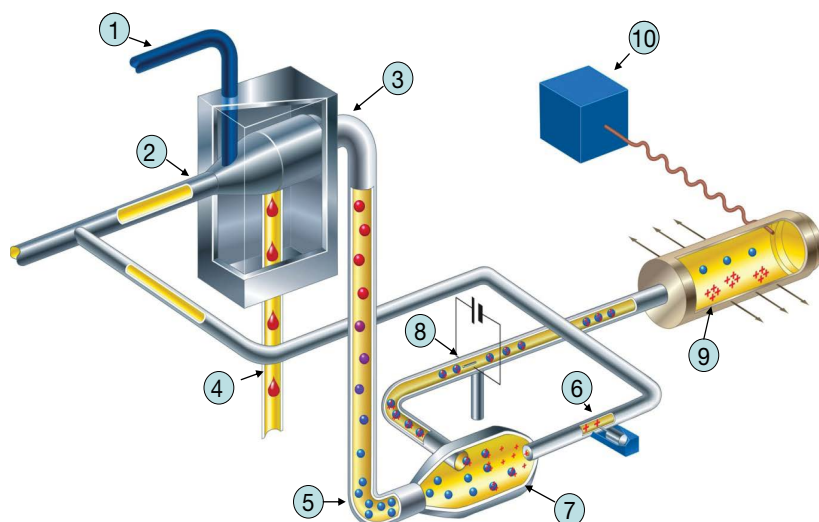
To evaluate the application of HPLC with charged aerosol detection to the measurement of natural products and botanicals.

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. This mass-based detector is 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 an aerosol. The mobile phase evaporates in the drying tube, leaving analyte particles that 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 evaporative light scattering (ELS) or refractive index (RI) detection, and it is simpler to operate than a mass spectrometer (MS).



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

Figure 1. Schematic showing how charged aerosol detection works

A number of isocratic and gradient HPLC methods with the Thermo Scientific Dionex Corona ultra RS Charged Aerosol Detector 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 evaporative light scattering (ELS) and UV detection will also be discussed.

Experimental

Several methods were developed for the analysis of black cohosh, ginkgo, ginseng, milk thistle, gotu kola, and stevia with the charged aerosol detector. The sample preparation method and experimental conditions used for each botanical are described below.

Black Cohosh

Approximately 300 mg of sample extract was weighed into a 50 mL volumetric flask. Then, 40 mL of methanol was added and the flask was sonicated for 15 minutes with occasional shaking. After being cooled 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.

Column:	Fused-core C18 HPLC; 4.6 × 150 mm, 2.7 µm particle size
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 minutes 40% B to 60% B from 12–36 minutes
Injection Volume:	10 µL
Detection:	Charged aerosol detection or evaporative light scattering detection (N ₂ pressure 2.3 bar, temperature 50 °C, gain 7)

Ginkgo Biloba

The sample (1 g powder from a commercially available health supplement) was sonicated in 10 mL methanol for 30 minutes. The extract was centrifuged (9,000 rpm) for 5 minutes and the supernatant was passed through a 0.2 µm nylon filter by centrifugation (9,000 rpm for 2 minutes).

Column:	C18, 4.6 × 250 mm, 5 µm particle size
Column Temp.:	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 Volume:	10 µL

Ginseng

Approximately 400 mg of sample extract was weighed and transferred into a 100 mL volumetric flask. Then, 15 mL of methanol was added and the flask was sonicated for 10 minutes with occasional shaking. Approximately 60 mL of water was added and the flask was sonicated for an additional 10 minutes. After being cooled to room temperature, the flask was filled to volume with water and mixed well. A portion of the solution was filtered through a 0.2 µm PVDF syringe filter into an HPLC autosampler vial.

Column:	Fused-core C18 HPLC; 3.0 × 100 mm, 2.7 µm particle size
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 Volume:	20 µL

Milk Thistle

Approximately 70 mg of powdered milk thistle extract was weighed and transferred into a 250 mL amber glass bottle. Then, 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 × g for 10 minutes. The supernatant was transferred into an HPLC autosampler vial.

Column:	Thermo Scientific Acclaim RSLC 120 C18; 2.1 × 100 mm, 2.2 µm particle size
Column Temp.:	40 °C
Flow Rate:	0.50 mL/min
Mobile Phase A:	3 mM ammonium formate, 0.3% formic acid in water / methanol (80:20 v/v)
Mobile Phase B:	3 mM ammonium formate, 0.3% formic acid in water / methanol (20:80 v/v)
Gradient:	15% B to 45% B in 4.4 minutes
Injection Volume:	1 µL

Gotu Kola

Approximately 250 mg of sample extract was weighed and transferred into a 100 mL volumetric flask. Then, approximately 70 mL of methanol was added, and the flask was sonicated for 10 minutes with occasional shaking. After being cooled 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.

Column:	Fused-core C18 HPLC; 3.0 \times 100 mm, 2.7 μm particle size
Column Temp.:	35 $^{\circ}\text{C}$
Flow Rate:	0.64 mL/min
Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	Acetonitrile
Gradient:	18% B to 22% B in 8 minutes; 22% B to 45% B from 8 minutes to 17 minutes; 45% B to 80% B from 17 minutes to 23 minutes
Injection Volume:	5 μL

Stevia

Commercially available stevia extract powder was dissolved in deionized water (0.9 mg/mL).

HPLC

Column:	C18 AQ, 4.6 \times 250 mm, 5 μm particle size
Column Temp.:	50 $^{\circ}\text{C}$
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% to 90% B in 30 minutes. Hold for 5 minutes and return to starting conditions.
Injection Volume:	10 μL

UHPLC

Column:	Acclaim [™] RSLC Polar Advantage II; 2.1 \times 250 mm, 2.2 μm particle size
Column Temp.:	40 $^{\circ}\text{C}$
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 minutes. Hold 1 minute and return to 5% B.
Injection Volume:	5 μL

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, cimracemoside 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). The Corona[™] ultra RS[™] Charged Aerosol Detector, which is also non-linear, offers a linear response for this compound in the concentration range used. The Corona ultra RS detector offers additional functionality that provides linear calibration curves.

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 identified in Figure 5, these compounds elute between 3 and 14 minutes.

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). 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).

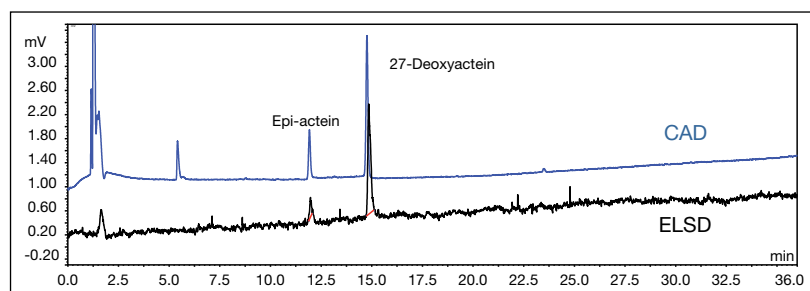


Figure 2. Comparison of charged aerosol detection vs. ELSD for determination of triterpene glycosides in black cohosh (27-deoxyactein standard solution: 11 $\mu\text{g}/\text{mL}$)

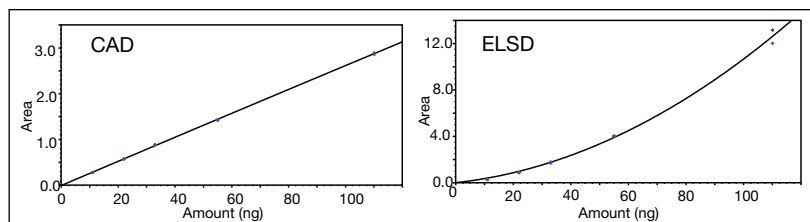


Figure 3. Comparison of charged aerosol detection vs. ELSD calibration curves for 27-deoxyactein standard

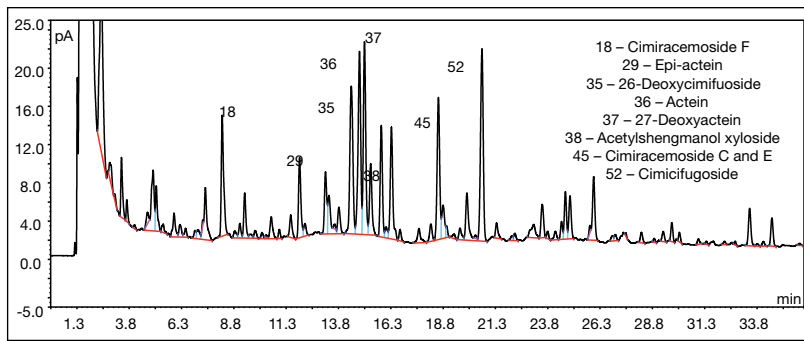


Figure 4. Determination of triterpene glycosides in black cohosh extract

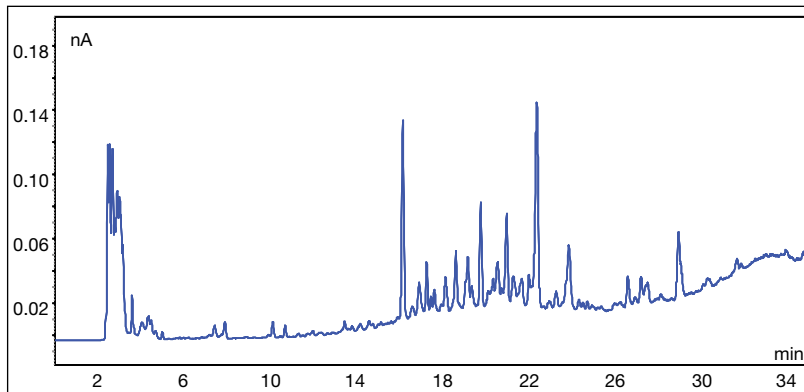


Figure 5. Analysis of *Ginkgo biloba* extract

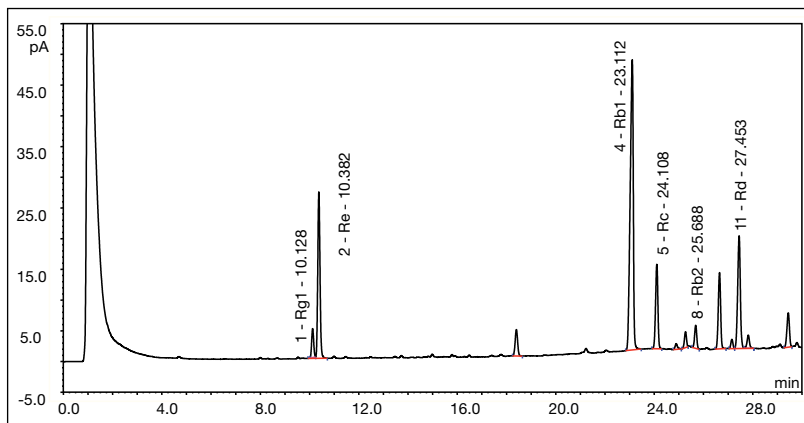


Figure 6. Analysis of ginseng extract

Milk thistle extracts are purported to both prevent and repair damage to the liver caused by 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 7).

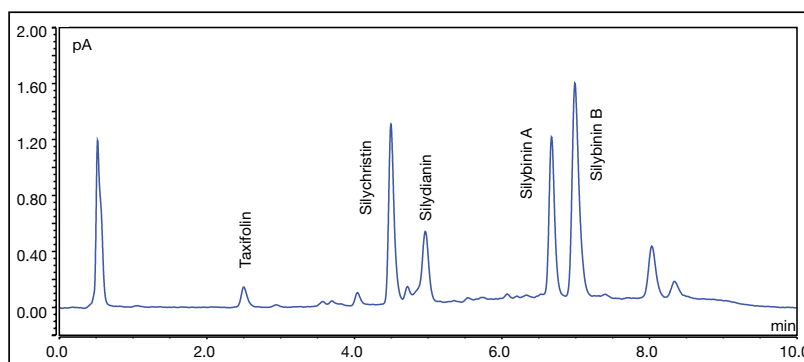


Figure 7. Analysis of milk thistle extract

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 and 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 include 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 using charged aerosol detection (Figure 8). Interestingly, this figure also shows that the number of potential interferences are reduced when using the Corona Charged Aerosol Detector, probably as the sample has a number of volatile compounds that absorb low-wavelength UV.

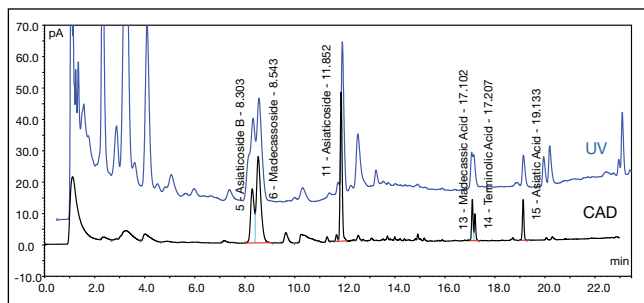


Figure 8. Comparison of UV and charged aerosol detection response for gotu kola extract

Stevia rebaudiana, commonly known as stevia, is currently being used as a low-calorie natural sweetener. The active ingredients include the aglycone diterpene steviol and numerous glycosides, including stevioside and the rebaudiosides. As found with the other chemistries described above, charged aerosol detection showed numerous advantages over low-wavelength UV. In this example, the use of UHPLC allowed a 60% reduction in analysis time and a marked improvement in chromatographic resolution (Figure 9).

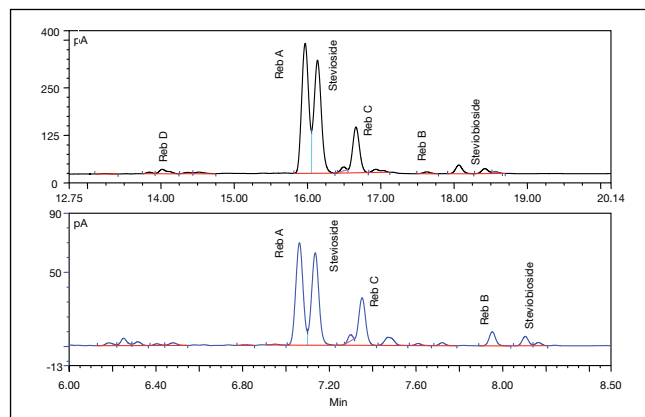


Figure 9. Comparison of HPLC (upper) and RSLC (lower) for the analysis of a stevia extract

Conclusion

HPLC with charged aerosol detection is sensitive (low ng levels on-column) and has a wide dynamic range and minimal inter-analyte response variability. It is ideal for measuring analytes that lack a chromophore and is of particular use for the analysis of natural products and botanicals. It offers numerous analytical improvements over ELSD, RI and low-wavelength UV.

Charged aerosol detection has the following advantages:

- Measures any non-volatile and many semi-volatile species.
- Has a response that 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 four orders of magnitude.

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