Determination of the Composition of Natural Products by HPLC with Charged Aerosol Detection

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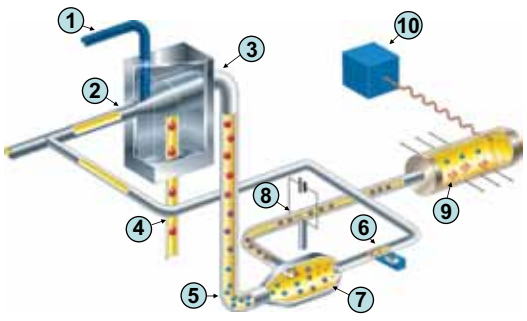
Introduction

Natural products contain a great diversity of compounds that can show extreme variation in their physicochemical properties. Analysis of 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. Charged aerosol detection is a sensitive, universal (nonselective) approach that can measure any nonvolatile and many semivolatile compounds. A number of isocratic and gradient HPLC-Thermo Scientific Dionex Corona™ CAD™ Charged Aerosol Detector methods have been developed and evaluated for the measurement of analytes from a variety of natural products, including black cohosh, ginkgo biloba, ginseng, soy, and stevia. Analytes showed consistent response independent of chemical structure (typically < 10% variability between compounds). 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.

FIGURE 1. Charged aerosol detection principle.



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. A secondary gas stream passes over corona needle
7. Charged gas collides with particles and charge is transfered
8. High mobility species are removed by an ion trap
9. Remaining charged particles pass to a collector where the charge is measured by a very sensitive electrometer
10. Signal transferred to chromatographic software

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Black Cohosh

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

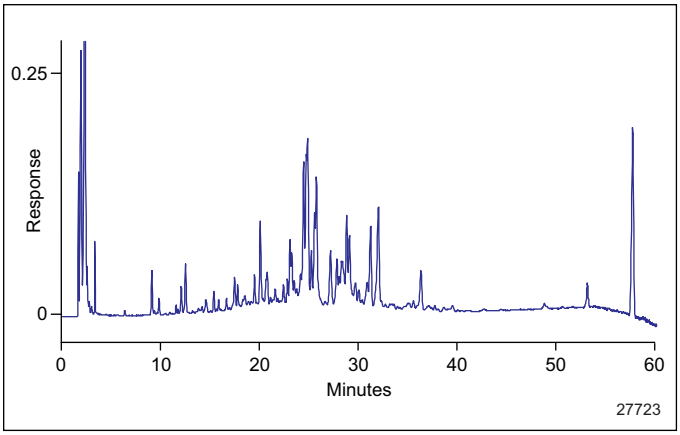
HPLC Parameters

Mobile Phase A:	0.1% Formic acid					
Mobile Phase B:	100% Acetonitrile					
Gradient:	Time (min)	0	60	65	66	80
	% A	80	0	0	80	80
	% B	20	100	100	20	20
Flow Rate:	1.0 mL/min					
Column:	Shiseido MG 100 C18, 4.6 × 250 mm, 5 µm					
Column Temp.:	Ambient					
Injection Volume:	10 µL					

Sample Preparation

The sample (1 g powder from capsule) was sonicated in 10 mL methanol for 30 min. The mixture was then passed through a Centrex™ 0.2 µm nylon filter by centrifugation.

FIGURE 2. Separation of metabolites in black cohosh.



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Ginseng

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

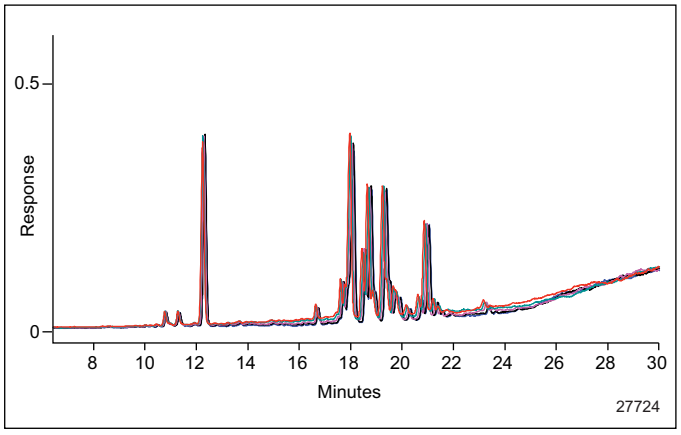
HPLC Parameters

Mobile Phase A:	Water				
Mobile Phase B:	Acetonitrile				
Gradient:	Time (min)	0	30.00	30.01	35.00
	% A	80	40	80	80
	% B	20	60	20	20
Flow Rate:	0.8 mL/min				
Column:	Shiseido MG 100 C18, 4.6 × 250 mm, 5 µm				
Column Temp.:	Ambient				
Injection Volume:	10 µL				

Sample Preparation

The sample (1 g powder from capsule) was sonicated in 10 mL methanol for 30 min. The mixture was then passed through a Centrex 0.2 µm nylon filter by centrifugation.

FIGURE 3. Reproducibility of ginseng analysis (five replicates).



Ginkgo Biloba

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

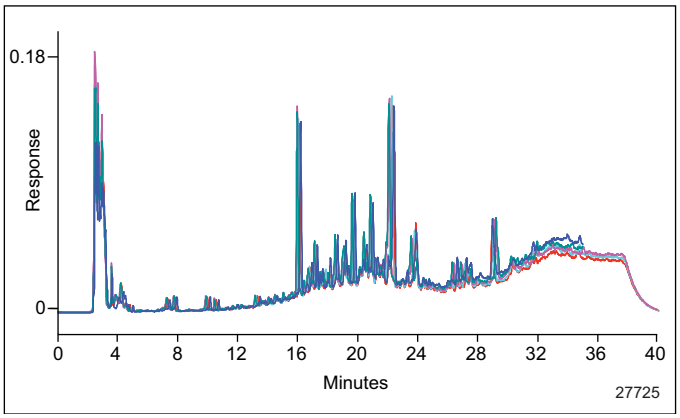
HPLC Parameters

Mobile Phase A:	5% Acetonitrile in 0.1% trifluoroacetic acid				
Mobile Phase B:	70% Acetonitrile in 0.1% trifluoroacetic acid				
Gradient:	Time (min)	0	30	35	40
	% A	100	25	25	100
	% B	0	75	75	0
Flow Rate:	1.0 mL/min				
Column:	Shiseido MG 100 C18, 4.6 × 250 mm, 5 µm				
Column Temp.:	Ambient				
Injection Volume:	10 µL				

Sample Preparation

The sample (1 g powder from capsule) was sonicated in 10 mL methanol for 30 min. The mixture was then passed through a Centrex 0.2 µm nylon filter by centrifugation.

FIGURE 4. Reproducibility of ginko biloba analysis (five replicates).



Soy Saponins

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

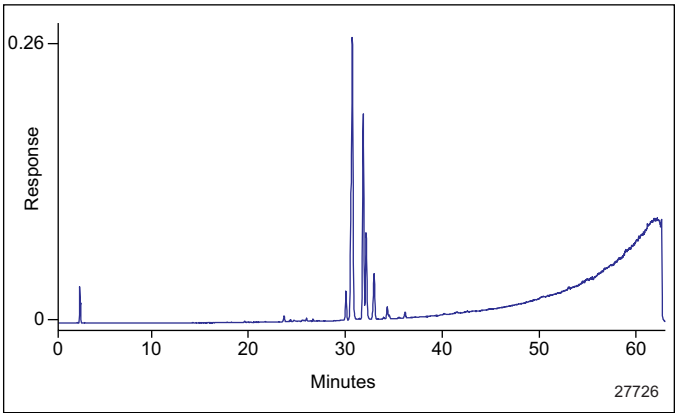
HPLC Parameters

Mobile Phase A:	0.1% Trifluoroacetic acid			
Mobile Phase B:	100 % Acetonitrile			
Gradient:	Time (min)	0	60	65
	% A	90	10	90
	% B	10	90	10
Flow Rate:	1.0 mL/min			
Column:	Shiseido MG 100 C18, 4.6 × 250 mm, 5 µm			
Column Temp.:	Ambient			
Injection Volume:	10 µL			

Sample Preparation

The sample (50 mg powder) was dissolved in 25 mL water in a 50 mL volumetric flask. The mixture was sonicated for 10–15 min. Ethanol (24 mL) was then added and the now warm solution was cooled. The solution was brought to 50 mL by the addition of ethanol. The solution was then passed through a Centrex 0.2 µm nylon filter by centrifugation.

FIGURE 5. Separation and detection of soy saponins.



Phytoesterogens—Isoflavones

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

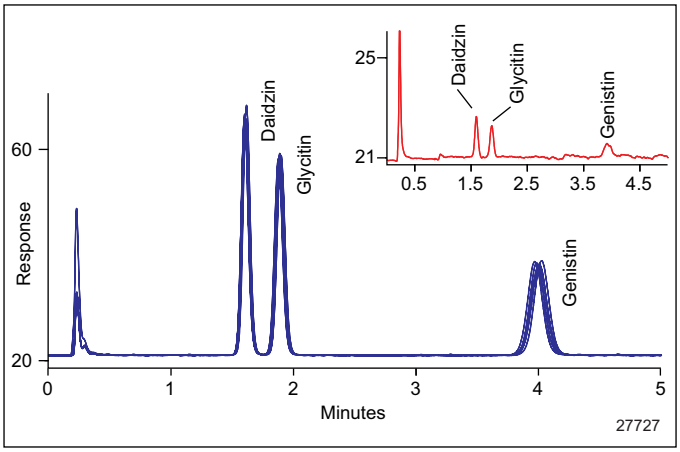
HPLC Parameters

Mobile Phase:	15% (v/v) Acetonitrile in 0.1% (v/v) acetic acid
Flow Rate:	1.5 mL/min
Column:	Shiseido MG 100 C18, 4.6 × 35 mm, 3 µm
Column Temp.:	Ambient
Injection Volume:	10 µL

Sample Preparation

Standards (1 mg/mL) were prepared in DMSO. Dilutions were made in mobile phase

FIGURE 6. Phytoestrogen standards (200 ng each on column, five replicate injections). Inset: 10 ng injections of each standard.



Phytoestrogens—Coumestans and Mammalian Lignans

Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

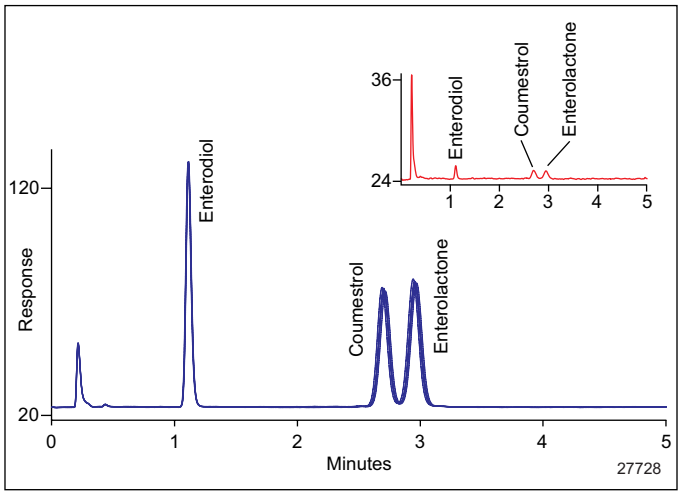
HPLC Parameters

Mobile Phase:	30% (v/v) Acetonitrile in 0.1% (v/v) acetic acid
Flow Rate:	1.5 mL/min
Column:	Shiseido MG 100 C18, 4.6 × 35 mm, 3 µm
Column Temp.:	Ambient
Injection Volume:	10 µL

Sample Preparation

Standards (1 mg/mL) were prepared in DMSO. Dilutions were made in 20% acetonitrile.

FIGURE 7. Phytoestrogen standards (200 ng each on column, five replicate injections). Inset: 5 ng injections of each standard.



Stevia

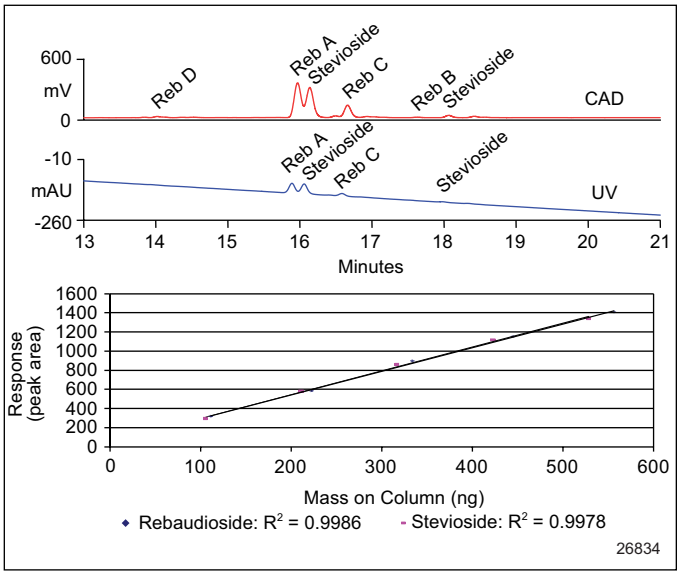
Corona Detector Parameters

Gas:	35 psi using nitrogen generator
Filter:	None
Range:	100 pA

HPLC Parameters

Mobile Phase A:	DI water, acetonitrile, TFA (95:5:0.1)
Mobile Phase B:	Acetonitrile, DI water (95:5)
Gradient:	Time (min) 0 3 30 35 40 45 % A 95 95 10 10 95 95 % B 5 5 90 90 5 5
Flow Rate:	1.0 mL/min
Column:	Shiseido MG 100 C18, 4.6 × 250 mm, 5 µm
Column Temp.:	Ambient
Injection Volume:	10 µL
UV Detection:	210 nm

FIGURE 8. Selected portion of chromatogram of SweetLeaf® Stevia Extract at ~860 ng on column run with UV at 210 nm and the Charged Aerosol Detector in series (top). Overlay of response curves for rebaudioside A (Reb A) and stevioside from ~500 to 100 ng on column each (bottom). Average of 3 injections, each fit to a linear regression.



Detector	Linear Correlation Coefficients	
	Rebaudioside A	Stevioside
CAD	0.9986	0.9978
UV at 210	0.9992	0.9994

Detector	Limit of Detection, s/n = 3 (mass on column)		
	Rebaudioside A	Stevioside	Isosteviol
CAD	4 ng	4 ng	60 ng
UV at 210	65 ng	65 ng	> 900 ng

Conclusions

The need for universal HPLC detection in analytical laboratories is widespread. While several detection technologies (e.g., low wavelength UV, refractive index, evaporative light scattering, chemiluminescent nitrogen detectors) are currently being used, there is significant room for improvement in performance characteristics (i.e., sensitivity, dynamic range, consistency of response factors, and gradient or solvent compatibility). The Corona CAD detector. was developed to help address the many challenges of universal detection. This novel technology offers many benefits to analytical scientists, including universal detection of nonvolatile analytes with response independent of chemical properties, a wide dynamic response range, high sensitivity, and good precision. These characteristics, along with reliability and simple operation, make this a superior detector for the measurement of numerous natural products.

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