The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods and Beverages - Part 2: Targeted Analyses

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Overview

Purpose: Use of a spectro-electro array platrform is a powerful approach for the measurement of a wide variety of natural products in botanicals, foods and beverages. Its use for the targeted measurement of specific compounds in a variety of matrices is examined.

Methods: Gradient HPLC with diode-array detection and electrochemical array detection were used.

Results: This approach enabled the separation and quantitation of 49 different phytochemicals commonly found in a number of herbs, spices and beverages. With the Thermo Scientific Dionex CoulArray detector, it is possible to sum the electrochemical response of each analyte across the electrochemical array. The sum of the response of all the analytes in a sample is a measure of the antioxidant capacity of the sample.

Introduction

Plant secondary metabolites show great structural diversity and a wide variability in relative abundance. No one analytical method exists that is capable of separating and detecting all of these compounds simultaneously. Rather, individual chemistries are used to target specific compounds or groups of compounds that possess similar chemical structures. For example, gradient reversed phase HPLC using a C18 column and diode array detection is most often the method of choice for the measurement of polyphenols when present at relatively high abundance. The spectro-electro array combines the universality of diode array detection (DAD) with the selectivity and sensitivity of coulometric electrode array detection. In this poster, a gradient HPLC spectro-electro array approach is used to resolve and quantify specific polyphenols in crude extracts of a variety of natural products supplements (e.g., ginseng, black cohosh, St. John's wort, and ginkgo), beverages (e.g., black tea, green tea, whisky and bourbon), culinary herbs (e.g., oregano, rosemary, sage and thyme) and spices (clove and nutmeg). The relative abundance and lability (ease of oxidation on the coulometric electrochemical array) of the individual analytes can be used to estimate the antioxidant capacity of the sample. This is important not only to the consumer (who may be taking supplements for their antioxidant content and purported health benefits) but also to the food industry where antioxidant activity may retard oxidative degradation of nutrients.

Methods

Liquid Chromatography

Pump:	Thermo Scientific Dionex LPG-3400BM with SR-3000 Solvent Rack
Autosampler:	Thermo Scientific Dionex WPS-3000TBSL
UV Detector:	Thermo Scientific Dionex DAD-3000RS Diode Array Detector Channel 1: 218 nm Channel 2: 240 nm Channel 3: 254 nm Channel 3: 275 nm
EC Detector:	Thermo Scientific Dionex CoulArray Detector with Thermal Organizer
EC Parameters:	16 channel array from 0 to +900 mV in 60 mV increments
Mobile Phase A:	20 mM monobasic sodium phosphate, 3% acetonitrile, 0.2% tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM monobasic sodium phosphate, 50% acetonitrile, 10% tetrahydrofuran, pH 3.45
Mobile Phase C:	90% methanol
Gradient:	0-2 min: 2%B/3%C. 30 min: 97%B/3%C, 45 min 97% B/3%C. Curve 7 (concave).
Analytical Column:	Thermo Scientific Acclaim 120, C18, 3 × 150 mm, 3 μm
Flow Rate:	0.65 mL/min
Injection:	10 or 20 μL

Data Analysis

Data were analyzed using Thermo Scientific Dionex Chromeleon Chromatography Data System 6.8 (SR9) and CoulArray™ software 3.1.

Standard Preparation

Stock standards, depending on solubility, were prepared in ethanol, methanol or methanol/water solutions at 1000 μg/mL or 100 μg/mL. Working standards were prepared at 0.2, 0.5 and 1.0 μg/mL in 5% methanol containing 0.02% ascorbic acid.

Sample Preparation

Supplements, culinary herbs, and spices were prepared for analysis by extracting 100 mg of the material with 20 mL of methanol. The samples were sonicated for 30 min. and subsequently centrifuged to obtain a clear solution. The solution was diluted 5x with a preservative solution (10% methanol containing 0.2% ascorbic acid with 0.02% EDTA) for injection into the HPLC system. Wine samples were diluted 50x and beer samples diluted 2x with the preservative solution. Tea was prepared by steeping 0.5 g of tea with 75 mL of boiling water for 15 min. This solution was diluted 10x with the preservative solution. Beverage samples were analyzed directly without processing.

Results and Discussion

There continues to be considerable interest in the potential health benefits of phenolic and polyphenolic compounds present in a number of botanical supplements, foods, and beverages. For example, rosemary, thyme, sage, and wine are purported to have medicinal value. Many of these compounds have antioxidant properties and, as shown in numerous animal studies, may be protective against inflammation, cancer, and cardiovascular disease. While most of these compounds can be measured by HPLC with UV or DAD, UV spectra are often indistinguishable from each other. In complex matrices such as botanical supplements, foods, and beverages, analyte co-elutions are common, making identification and quantitation of many compounds difficult. HPLC with coulometric electrode array detection uses multiple sensors that can be optimized to overcome the issue of chromatographic co-elution. Easily oxidized compounds can be selectively detected at upstream, low-potential sensors while compounds that require a higher potential to oxidize respond at downstream higher-potential sensors. This approach extends the number of analytes that can be measured simultaneously and provides qualitative information. In addition to improved selectivity, coulometric electrode array detection is in most cases more sensitive and has a wider linear range than UV detection. However, EC detection is not universal. The combination of UV and EC detection in the Spectro-Electro Array platform extends the range of compounds that can be detected simultaneously. The theory behind this platform and its analytical merits are discussed in Part 1.

Table 1. Analyte identity (UV1-UV6 are analytes that have strong UV but weak EC response).

Peak No.	Compound	Peak No.	Compound
1	Gallic acid	23	Rutin
2	4-Hydroxybenzyl alcohol	24	Ethyl vanillin
3	p-Aminobenzoic acid	UV3	Methoxybenzaldehyde
4	3,4-Dihydroxybenzoic acid	25	4-Hydroxycoumarin
5	Gentisic acid	26	Hesperidin
6	2-Hydroxybenzyl alcohol	27	Naringin
7	4-Hydroxybenzoic acid	28	Rosemarinic acid
8	Chlorogenic acid	29	Fisetin
9	p-Hydroxyphenyl acetic acid	30	Myricetin
10	Catechin	31	Trans-resveratrol
11	Vanillic acid	UV4	Cinnamic acid
12	4-Hydroxybenzaldehyde	32	Luteolin
13	Syringic acid	33	Cis-resveratrol
14	Caffeic acid	34	Quercetin
15	Vanillin	UV5	Apigenin
16	Syringaldehyde	35	Kaempferol
17	Umbelliferone	36	Isorhamnetin
18	p-Coumaric acid	37	Eugenol
UV1	3,4-Dimethoxybenzoic acid	38	Isoxanthohumol
19	Salicylic acid	UV6	Chrysin
20	Sinapic acid	39	Cavarcrol
21	Ferulic acid	40	Thymol
22	Ellagic acid	41	Carnosol
UV2	Coumarin	42	Xanthohumol
		43	Carnosic acid

The list of targeted analytes measured for this study are presented in Table 1. Table 2 shows the amount of these analytes in a variety of beverages and the dried herb oregano. Analyte levels are in good agreement with previous publications. Few of these compounds were found in extracts of ginseng, black cohosh, and ginkgo. The purported active phytochemicals in these supplements are not phenols and polyphenols, but include triterpene glycosides such as 27-deoxyactein, actein, and cimiracemoside (black cohosh), the triterpene saponin ginsenosides (ginseng) and the sesquiterpenoid bilobalide and diterpenoid ginkgolides (gingko) instead. These phytochemicals often lack strong chromophores, thus limiting the use of UV detection and many cannot be measured by EC detection. However, as these compounds are not volatile, they can be readily measured with the Thermo Scientific Dionex Corona Charged Aerosol Detector.

Table 2, Abundance of different analytes in a variety of beverages and oregano herb. * Cabernet Sauvignon, 2008, Argentina.

Compound	Green Tea mg/g	Black Tea mg/g	*Wine mg/L	Scottish Whisky mg/L	American Bourbon mg/L	Oregano mg/g
4-hydroxybenaldehyde					0.05	0.02
4-hydroxybenzoic acid					0.17	0.04
4-hydroxybenzyl alcohol				0.06	0.06	0.05
4-hydroxycoumarin				0.04		0.98
Caffeic acid			8.0			0.05
Carnosol				0.28	0.34	
Catechin	3.73	3.0	37			0.76
Cavarcrol						2.79
Chlorogenic						0.25
Dihydroxybenzoic acid				0.11	0.34	0.07
Ellagic acid			52.0			
Epicatechin	50.8	9.3	19.0			
Epicatechingallate	65.3	40.6				
Epigallocatechin	49.2	2.5				
Epigallocatechingallate	180	31.3				
Ethyl vanillin				0.05	0.12	
Eugenol				0.05	0.05	
Ferulic acid			1.0	0.03	0.20	0.10
Fisetin					0.06	0.04
Gallic acid			57.0	0.11	0.10	1.05
Gallocatechin	18.8	3.2				
Gallocatechingallate	5.9	7.0				
Hesperidin					0.12	0.04
Isorhamnetin						0.10
Kaempferol				0.04		0.04
Luteolin				0.07	1.02	0.23
Myricetin			11.0			0.11
Naringenin					0.48	0.35
p-coumaric acid			8.5		0.03	0.03
Quercetin						
Rosemarinic acid					0.10	1.98
Salicylic acid					0.86	0.23
Sinapic acid			2.0		3.64	0.17
Syringaldehyde				2.27		
Syringic acid			19.2			0.05
Thymol						0.13
Umbelliferone				0.20	0.53	0.06
Vanillic acid			6.3	0.15	1.49	0.03
Vanillin				0.65		

Examples of samples differing in complexity are presented in Figures 1−3 that compare UV with EC's capabilities. Saint John's Wort (*Hypericum perforatum*) is reported to be effective in the treatment of moderate depression in a number of clinical trials. The two major polyphenols in Saint John's Wort, hypericin and pseudohypericin, are easily measured using both UV and ECD (Figure 1, ~18 mins). Nutmeg is particularly abundant in Eugenol (Figure 2, Peak 37). Although this methoxyphenol is an hepatotoxin, it also has antimicrobial and anticarcinogenic properties. Rosemary (Figure 3) is particularly abundant in carnosic acid (Peak 43), a potent antioxidant and anticarcinogen, typically making up 1.5 to 2.5% of the dried leaf. As can be seen in all examples, EC detection is much more sensitive than UV detection.

Analyte oxidation across the electrode array can be used as an indicator of compound lability, with the more easily oxidized compounds reacting at the earlier (upstream electrodes) and the more stable compounds reacting at the later (downstream) electrodes. Analyte lability is also a reflection of antioxidant activity—the more easily oxidized the compound, the more potent it is as an antioxidant. Data from the CoulArray detector can be used in two ways. First, the summation of all analyte peaks in the chromatogram give an indication of the total antioxidant capacity of the sample. Second, summation of analyte response on each channel enables the total antioxidant capacity of the sample to be broken down by contribution of each class of antioxidant (a rank ordering of antioxidant contribution to the total antioxidant capacity of the sample). The antioxidant capacity of a sample obtained from the CoulArray detector was equivalent to data obtained using an oxygen radical antioxidant capacity (ORAC) assay.[2, 3]

FIGURE 1. Saint John's Wort.

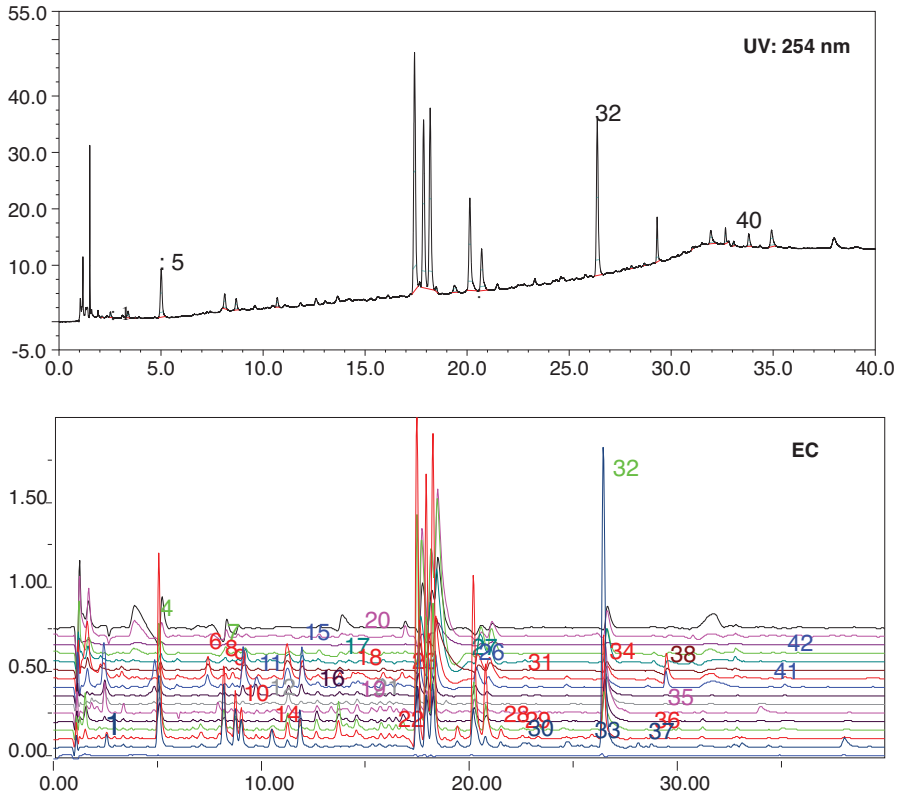


FIGURE 2: Nutmeg.

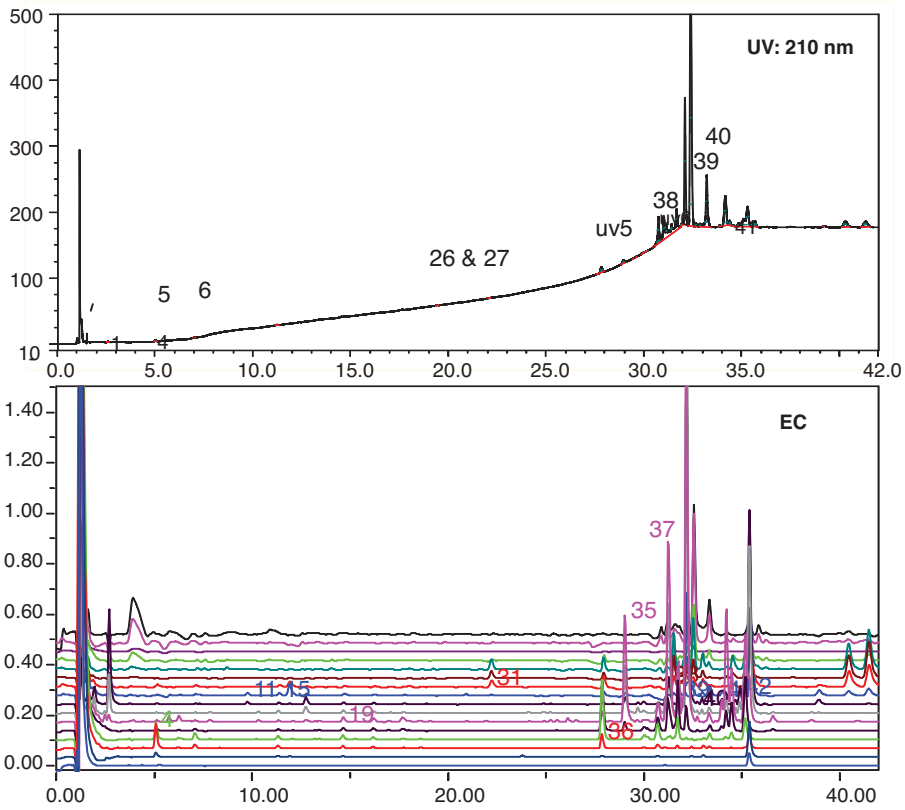
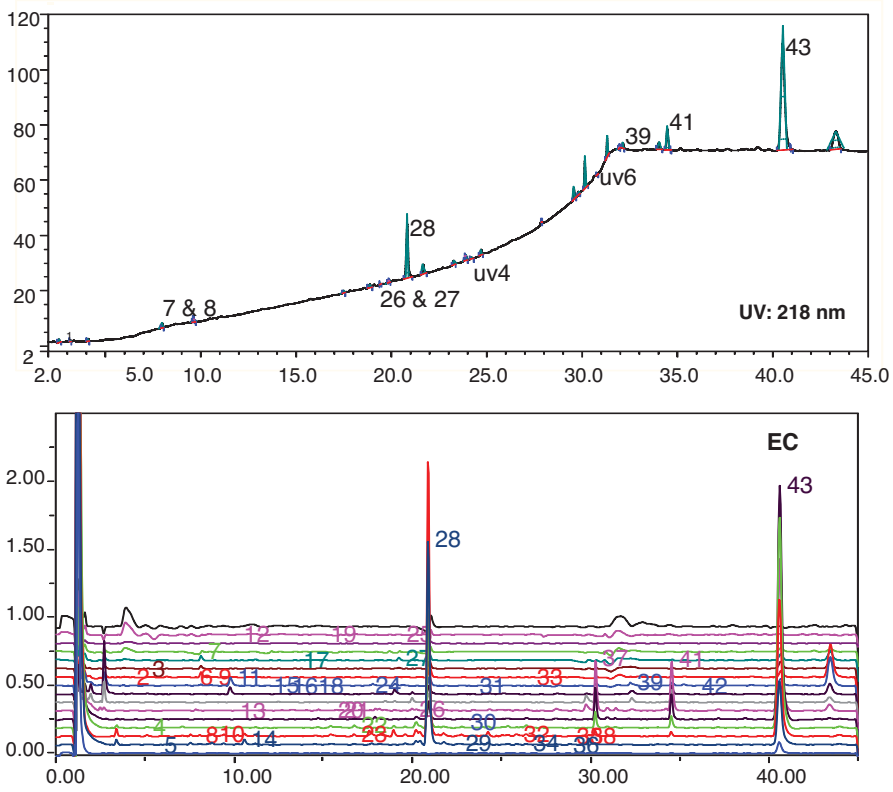


FIGURE 3: Rosemary.



Conclusion

• A multi-analyte targeted technique was developed using a spectro-electro array for the determination of phenols, phenolic acids and polyphenols in a variety of samples, including botanicals and beverages.
• The CoulArray detector uses unique 3D voltammetric resolution to offer better compound separation than traditional spectrometric techniques.
• The sensitivity of electrochemical detection surpassed that of the UV detector, therefore allowing for a more complete characterization of the trace levels of compounds in samples. In another poster, this technique, in conjunction with pattern recognition techniques for fingerprint identification of various products, is presented.
• This approach can also be used to measure the antioxidant capacity of the sample and the contribution of individual groups of antioxidants.

References

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