The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods and Beverages - Part 1: Theory and Concepts

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Overview

Purpose: A gradient HPLC UV Diode Array (DAD) and multichannel electrochemical (EC) detection method was developed and evaluated for the determination of phenols, phenolic acids and polyphenols in botanicals, supplements, foods, and beverages.

Method: Gradient HPLC with DAD and multi-channel EC detection were used.

Results: The method enables the separation and quantitation of 49 different compounds commonly found in botanicals, supplements, foods, and beverages.

Introduction

Plants contain an amazingly diverse range of secondary metabolites, many of which are purported to offer health benefits. The challenge for the analytical chemist wanting to measure these compounds is two-fold: first, to develop an assay that can accurately discriminate between compounds that often have similar physicochemical characteristics; second, to analyze such compounds in complicated matrices including botanicals, supplements, foods and beverages. Gradient HPLC with diode array detection (DAD) is often used for the determination of natural products. However, this approach often suffers from a lack of specificity as compounds with similar structures may co-elute chromatographically and cannot be easily deconvoluted spectrally. Furthermore, this technique lacks sensitivity limiting its use for the study of natural products metabolism in animals and humans. Alternately, coulometric electrode array detection is selective and is able to distinguish between subtle changes in chemical structure with sub-picogram limits of detection. The Thermo Scientific Dionex CoulArray detector can be used to examine natural product absorption and metabolism. The combination of DAD and coulometric electrochemical array detection extends the range of compounds measured by either technology alone. The concept behind the spectro-electro array will be discussed and the power of this approach will be illustrated using a global method capable of measuring ~ 50 analytes simultaneously.

Method

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 Module.
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
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 and Processing

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

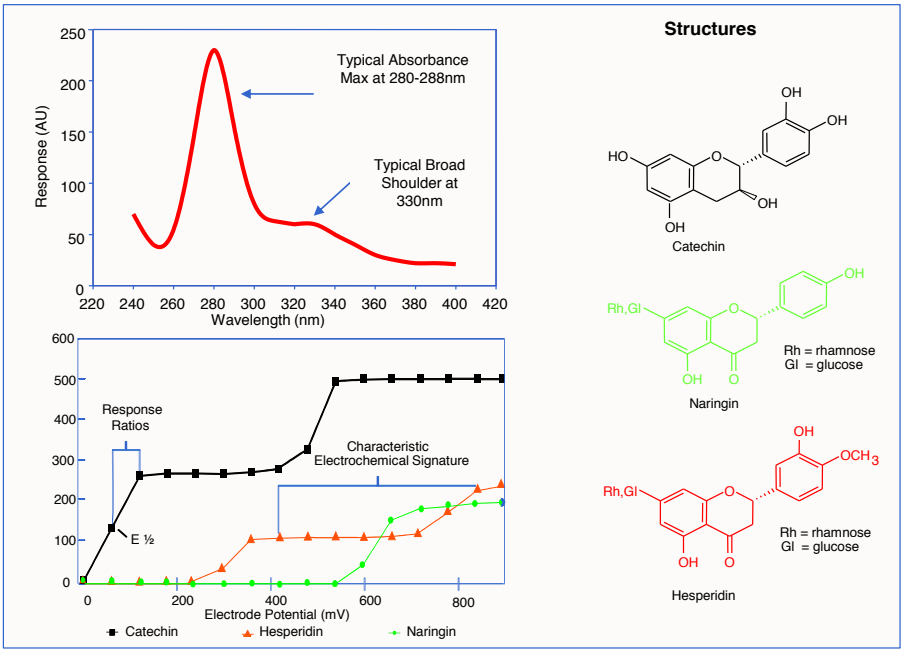
Standard Preparation

Stock standards were prepared, depending upon solubility, 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.

Results and Discussion

Electrochemical detection is renowned for being both highly sensitive and selective, whereas UV detection is much less sensitive but more universal in nature. The platform described here (spectro-electro array) makes use of the benefits of both detection formats and is used to extend the range of compounds that can be measured by either detector alone. The unique resolution of the coulometric electrode array comes from the high sensitivity of the flow-through porous graphite working electrodes. When these electrodes are used in series in an array, compounds are resolved based on their voltammetric behavior. Compounds detected by the CoulArray detector are resolved based both on their chromatographic and voltammetric (redox) behavior. Furthermore, these parameters can be used to identify and authenticate a compound. Electrochemical detection is incredibly sensitive to small changes in chemical structure, UV detection much less so. Figure 1 demonstrates that voltammetric data from the CoulArray detector and spectral data from the DAD are complementary. Spectral data is primarily dependent on the backbone of the chemical structure. For example, flavonones all have similar absorbance as shown in the top left of Figure 1. This can be an issue if these compounds co-elute. Electrochemcial detection, on the other hand, is extremely sensitive to the type, degree, and position of the substitution on the backbone of the chemical structure. Shown on the bottom left of the figure are the current-voltage curves (hydrodynamic voltammograms) for three comounds: catechin, with a catechol ring, is the most labile oxidixing at 100 mV; hesperidin oxidizes at ~400 mV; while naringin, containing a phenol ring, oxidizes at ~650 mV. Such structural/voltammetric relationships are used by the CoulArray detector to identify and authenticate analytes and to resolve co-eluting peaks. Spectral data are used when a phytochemical is not EC active.

Figure 1. Comparison of the spectral and electrochemical properties of selected flavonoids.



The wide variety of compounds that were measured in this study, along with their retention times and dominant electrochemical channel are listed in Table 1. It should be noted that there are six compounds designated UV1-UV6. These compounds show poor response by EC detection but are readily detectable by UV. Figure 2 shows their chromatographic separation and detection by UV at 218 nm and 254 nm, while Figure 3 shows their separation and response on the CoulArray detector. The CoulArray detector readily enables the resolution of co-eluting compounds, and response to gradient is minimal.

Table 1. Standard mixture - identification, retention time and array channel.

Peak No.	Compound	Retention Time minutes	Dominant EC Channel
1	Gallic acid	2.5	2
2	4-Hydroxybenzyl alcohol	4.3	10
3	p-Aminobenzoic acid	4.9	11
4	3,4-Dihydroxybenzoic acid	5.0	4
5	Gentisic acid	5.7	2
6	2-Hydroxybenzyl alcohol	7.5	10
7	4-Hydroxybenzoic acid	8.2	13
8	Chlorogenic acid	8.3	3
9	p-Hydroxyphenyl acetic acid	8.6	10
10	Catechin	9.0	3
11	Vanillic acid	9.7	9
12	4-Hydroxybenzaldehyde	10.1	15
13	Syringic acid	10.3	6
14	Caffeic acid	10.5	2
15	Vanillin	11.9	9
16	Syringealdehyde	12.8	9
17	Umbelliferone	13.6	11
18	p-Coumaric acid	14.4	9
UV1	3,4-Dimethoxybenzoic acid	14.7	n/a
19	Salicylic acid	15.0	15
20	Sinapic acid	15.4	6
21	Ferulic acid	15.8	6
22	Ellagic acid	16.4	4
UV2	Coumarin	16.7	n/a
23	Rutin	16.8	3
24	Ethyl vanillin	17.4	9
UV3	Methoxybenzaldehyde	18.7	n/a
25	4-Hydroxycoumarin	19.5	15
26	Hesperidin	20.0	8
27	Naringin	20.1	13
28	Rosemarinic acid	20.9	3
29	Fisetin	22.3	2
30	Myricetin	22.9	1
31	Trans-resveratrol	23.4	9
UV4	Cinnamic acid	24.2	n/a
32	Luteolin	25.9	3
33	Cis-resveratrol	26.5	10
34	Quercetin	26.5	2
UV5	Apigenin	27.6	n/a
35	Kaempferol	28.8	3
36	Isorhamnetin	29.0	2
37	Eugenol	29.2	6
38	Isoxanthohumol	29.6	9
UV6	Chrysin	30.8	n/a
39	Cavarcrol	32.3	9
40	Thymol	32.6	9
41	Carnosol	34.7	6
42	Xanthohumol	35.7	8
43	Carnosic acid	38.7	4

An example of the ability of the spectro-electro array to resolve co-eluting compounds is presented in Figure 4. The upper chromatogram shows the UV response at 254 nm and the co-elution of chlorogenic and 4-hydroxybenzoic acids. The lower chromatogram is from the CoulArray detector and shows that even though these two compounds co-elute, they can be resolved voltammetrically, with chlorogenic acid responding on channel 3 (red tracing at 120 mV) and 4-hydroxybenzoic acid responding on channel 13 (green tracing at 780 mV). Only selected channels are shown for visual clarity.

FIGURE 2. UV detection of separation of Standard mixture at 218 and 254 nm.

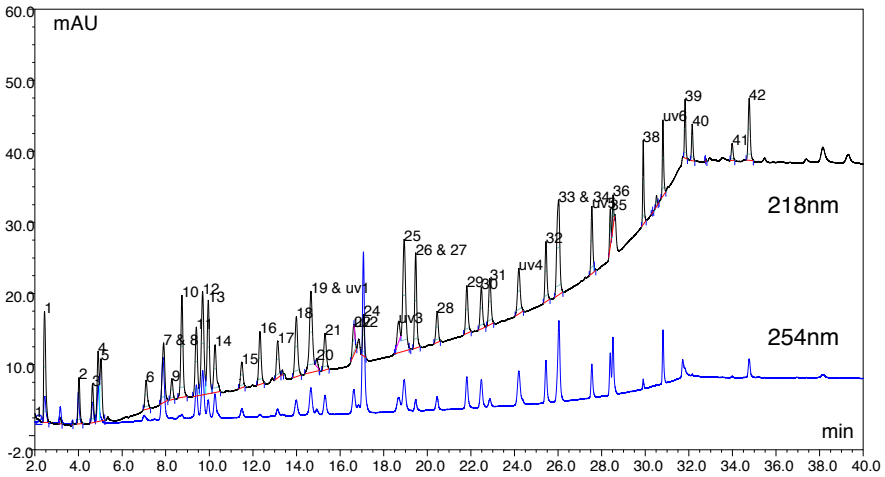


FIGURE 3. 16 channel coulometric electrochemical array detection of separation of Standard mixture

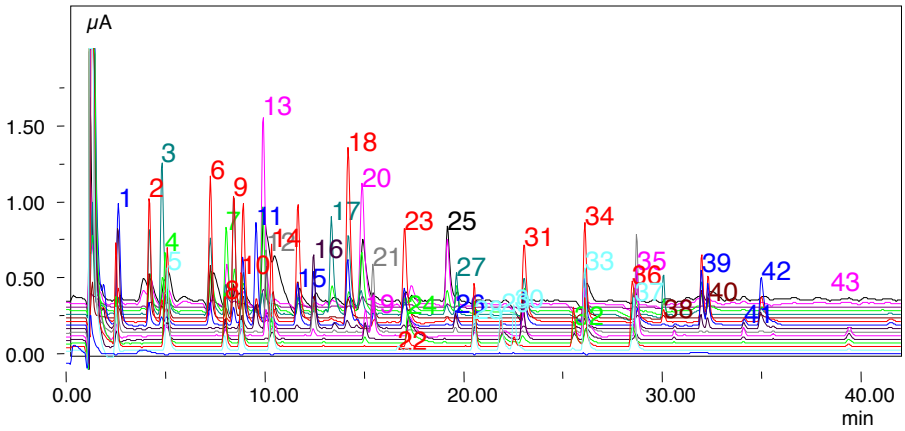
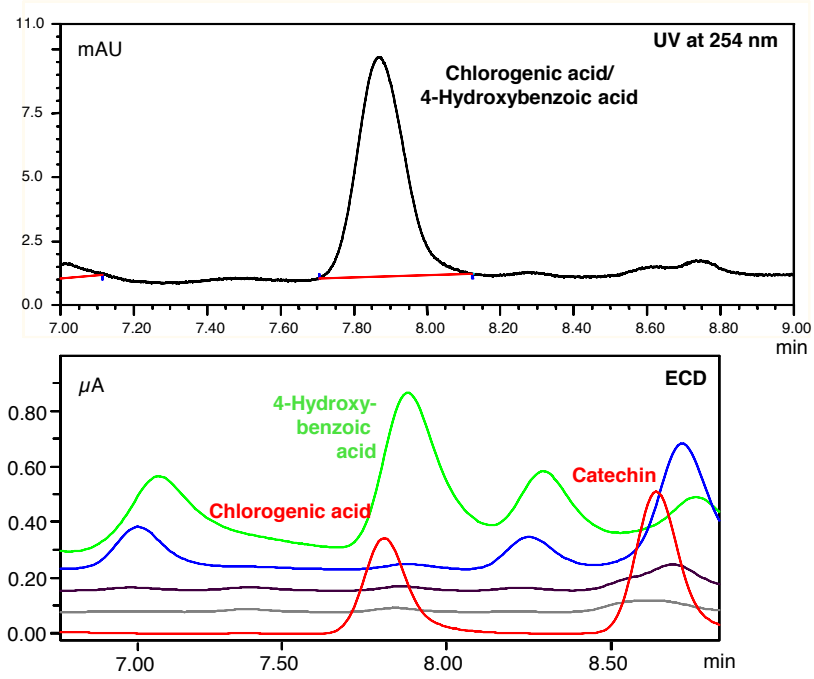


FIGURE 4: Detection of chlorogenic and 4-hydroxybenzoic acid. Top: UV - shows co-elution. Bottom: EC array – shows full resolution.



The limits of detection were typically 10-50 pg on-column by ECD and 100–500 pg by UV. The limits of quantification were 200–1000 pg (on-column) by ECD and 500-5000 pg by UV. Response range was over seven orders of magnitude by ECD and five by UV. Typical R^2 values were ~0.99 or better for all compounds. Examples of calibration curves of selected compounds are given in Figure 5. Average intra-day retention time precision for all analytes averaged 0.55% RSD over a 10 day period, with a range of 0.30–1.22%.

FIGURE 5. Typical response curves for representative analytes.

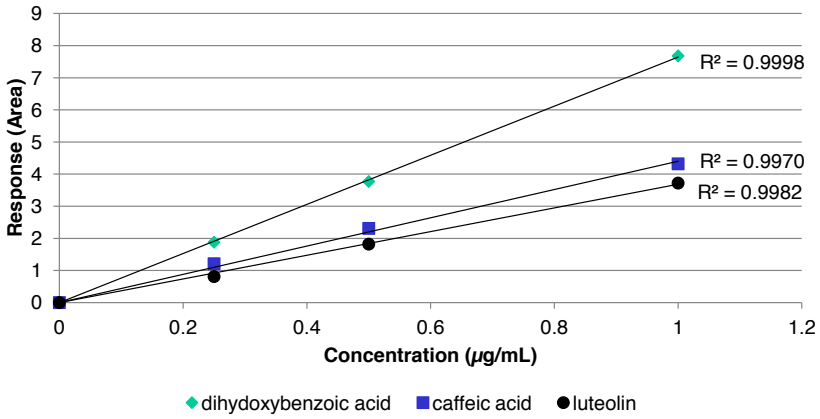


FIGURE 6. The spectro-electro array system used in this study.

Conclusion

A multi-analyte method for the determination of phenols, phenolic acids, and polyphenols was developed that combined UV Diode Array detection with multi-channel electrochemical detection.

- The combination of the two detection techniques extends the range of compounds that can be detected by either technology alone.
- Analyte co-elutions are readily identified and resolved.
- This approach has application to both targeted and metabolomic studies.
- The method demonstrates two important and unique features of the CoulArray detector: First, the CoulArray is the only EC detector fully compatible with gradient elution chromatography. Second, the CoulArray utilizes signal autoranging technology to permit simultaneous measurement of analytes with disparate concentrations thus eliminating manual gain adjustments or re-analysis of samples.

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