

# Application of HPLC with Photodiode Array and Coulometric Electrochemical Array Detection to the Study of Natural Products, Botanicals, and Mammalian Tissues: From Targeted Analyses to Metabolomics

Ian Acworth, Paul Ullucci, John Waraska, and Paul Gamache  
Thermo Fisher Scientific, Chelmsford, MA, USA

## Abstract

The potential health benefits of polyphenolic compounds present in fruits, vegetables, and natural products continues to be of great interest. Of the numerous polyphenols present in plant materials, the most widely studied include flavanols, flavonols, and phytoestrogens. The biological effects of these compounds, as shown by numerous in vitro and animal studies, suggest that they may be protective against cancer, inflammation, cardiovascular disease, and other diseases. Although there have been great strides in understanding the occurrence and the bioavailability and biological activities of some of these compounds, their in vivo effects in humans is an active area of research. Analytical methods capable of measuring low levels of these compounds and their metabolites with a high degree of specificity in biological tissues are therefore needed.

Polyphenols are electrochemically active and can be measured using HPLC with coulometric electrode array detection. This technique utilizes multiple electrochemical sensors that can be optimized for more than one chemical class. Easily oxidized compounds can be selectively detected at upstream, low-potential sensors, while higher oxidizing compounds respond at downstream, higher-potential sensors. Consequently, this approach is both extremely sensitive (fg levels) and provides qualitative information (voltammetric behavior) for analyte identification. It also offers a wide linear range and is the only electrochemical approach that is fully compatible with gradient elution. Presented here are examples of its use for targeted analysis of polyphenols (e.g., catechins, isoflavones) in a number of animal and human tissues (e.g., urine, plasma, and brain tissue) and its use for generation of global metabolite patterns to study the contents of commercially available natural product supplements.

## Background to Coulometric Array Detection

Unlike most spectrophotometric approaches, electrochemical detection is very sensitive to variation in chemical structure. For example, although the three

structurally related polyphenols (Figure 1) have similar absorbance spectra (Figure 2), their electrochemical responses (voltammetric behavior) are markedly different (Figure 3). As shown in Figure 3, catechols (e.g., catechin) oxidize at lower potentials than methoxycatechols (e.g., Hesperidin), with phenols (e.g., Naringin) requiring the highest potentials. The oxidation potential of a polyphenol is dependent upon:

- Type of substitution (e.g., hydroxy, methoxy)
- Degree of substitution (e.g., phenol, catechol)
- Position relative to other groups (e.g., *ortho*, *para*, and *meta*)

In general, oxidation is as follows: from easiest (lowest oxidation potential) to hardest (highest oxidation potential):

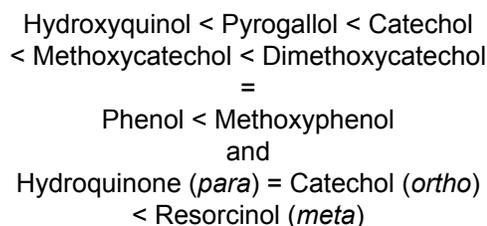
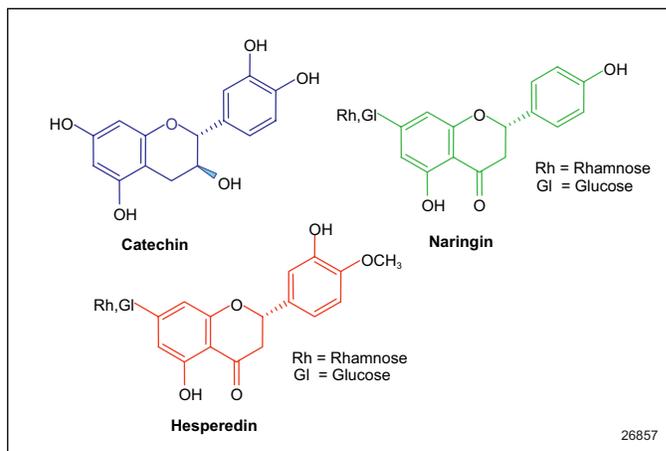
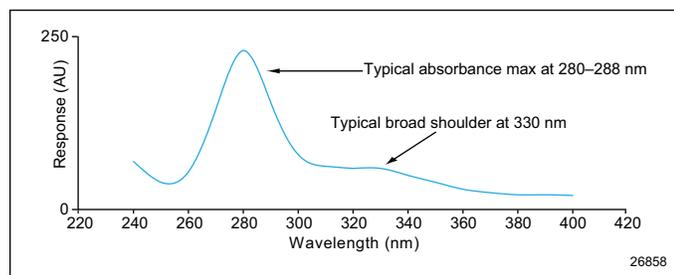


FIGURE 1. Three structurally related polyphenols.

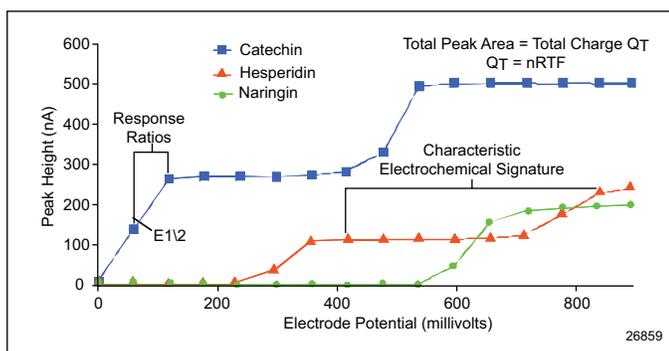


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**FIGURE 2. Absorbance spectra of the structurally related polyphenols.**



**FIGURE 3. Electrochemical responses (voltammetric behavior) of the structurally related polyphenols.**



The Thermo Scientific Dionex CoulArray™ Coulometric Array Detector uses a series of highly efficient flow-through graphite electrodes (coulometric array) to measure polyphenols. With this approach, coeluting compounds (compounds with the same retention time) can be resolved voltammetrically. As shown in Figure 4, although catechin elutes at the same time as 4-hydroxyphenylacetic acid (4HPLA), these compounds are resolved voltammetrically, as catechin (a catechol) oxidizes at lower potentials (upstream electrodes) and 4HPLA (a phenol) oxidizes at higher potentials (downstream electrodes). Furthermore, in addition to resolution between coeluting compounds, the response of an analyte across adjacent sensors is indicative of chemical structure, and can be used to qualify (identify) the analyte in an analogous fashion to spectra generated by photodiode array (PDA) detectors. The characteristics of the Dionex CoulArray and PDA detectors are summarized in Table 1.

**FIGURE 4. Polyphenol measurement with the Dionex CoulArray Detector.**

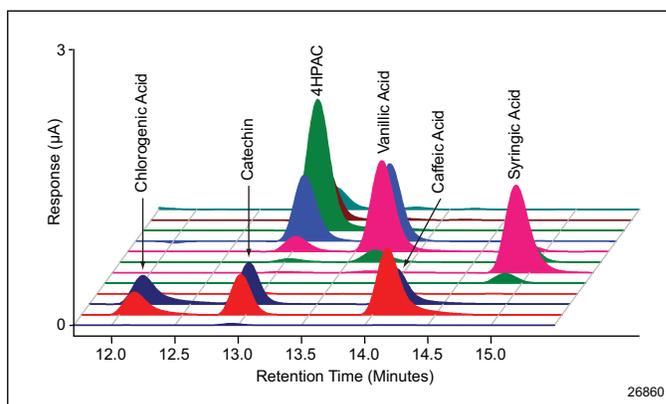


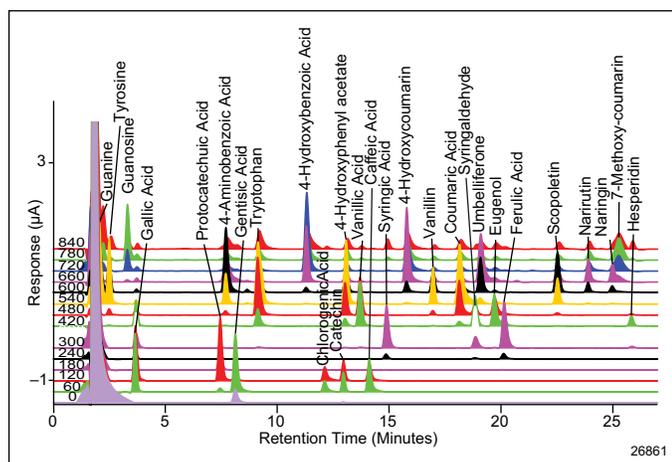
Table 1. Characteristics of CoulArray and PDA Detectors	
Dionex CoulArray Coulometric Array Detector	PDA Detector
Series of flow-through graphite electrodes	Series of photodiodes
Chemical basis of detection	Physical basis of detection
Characterize analytes based on voltammetric behavior	Characterize analytes based on spectral behavior
Highly selective	Nonselective
Sensitive to small changes in chemical structure	Less sensitive to changes in chemical structure
Clean, simple chromatograms as small changes in chemical structure can appear at different potentials	Chromatograms may be complex as small changes in chemical structure may not affect absorbance spectrum
Extreme sensitivity (fg to pg on column)	Less sensitive (ng to µg on column)
Good linear response range (fg to µg on column)	Poor linear response range (ng to µg on column)
Output direct	Often requires complex math to render interpretable
Perfectly coeluting peaks may be resolved providing their half-wave potentials differ by 60 mV	Perfectly coeluting peaks may be difficult to resolve

## Introduction

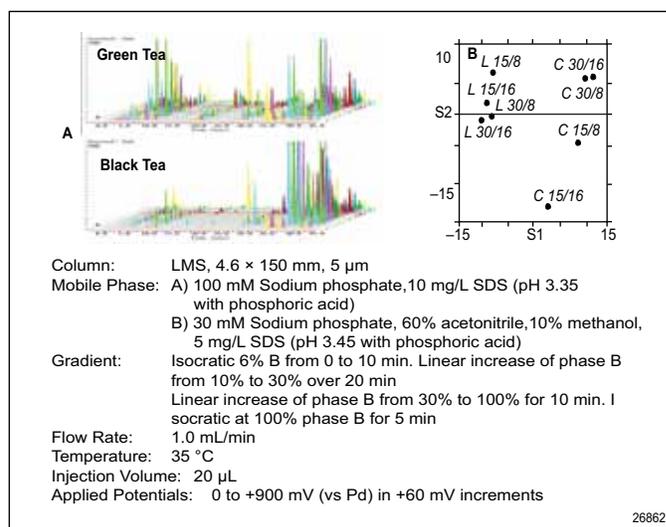
There continues to be a great deal of interest in the potential health benefits of polyphenolic compounds present in fruits and vegetables. The most widely studied compounds include catechins, flavonols, and phytoestrogens. The biological effects of these compounds, as shown by numerous *in vitro* and animal studies, suggest that they may be protective against cancer, cardiovascular, inflammatory, and other diseases.

Polyphenols are electrochemically active and can be measured by HPLC with electrochemical detection. HPLC with coulometric array electrochemical detection (Dionex CoulArray Detector) uses multiple sensors that can be optimized for more than one chemical class. Easily oxidized compounds can be selectively detected at upstream, low-potential sensors, while higher oxidizing compounds respond at downstream higher-potential sensors. This extends the number of analytes that can be measured simultaneously and also provides qualitative information. The Dionex CoulArray Detector is highly sensitive, selective, and has a wide linear range. It can be used to either target specific analytes in natural products and animal samples, or to generate metabolite patterns that can be used to evaluate product quality, study pharmacokinetics in animals and humans, and to examine potential health benefits of foods and supplements in animal models of disease.

**FIGURE 5. Gradient chromatogram using Dionex CoulArray Detector showing separations of standards (10  $\mu$ L of a 1 ppm mixture).**

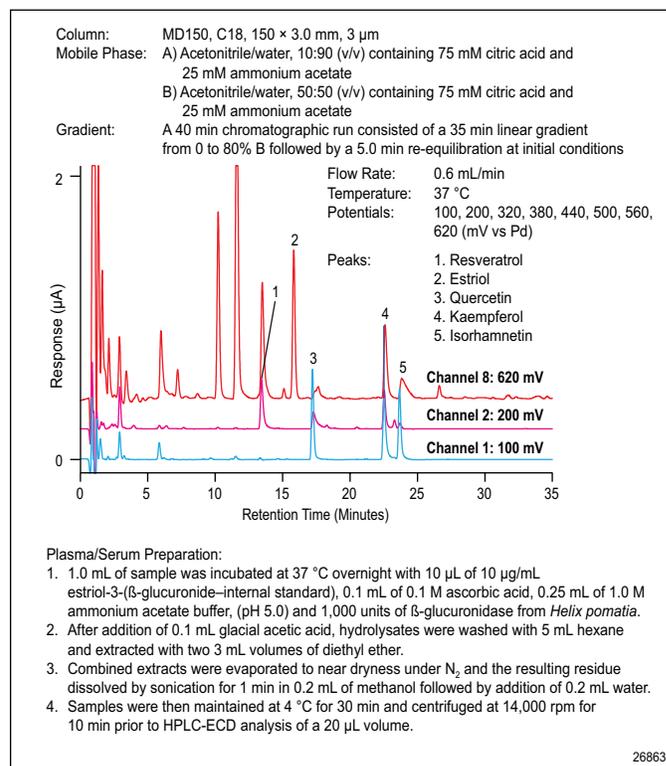


**FIGURE 6. (A) Gradient chromatograms using Dionex CoulArray Detector showing metabolite profiling of green and black tea samples. (B) Principal component pattern recognition of tea samples: (L) domestic, (C) foreign with extraction time/heating time in minutes shown in italics.**

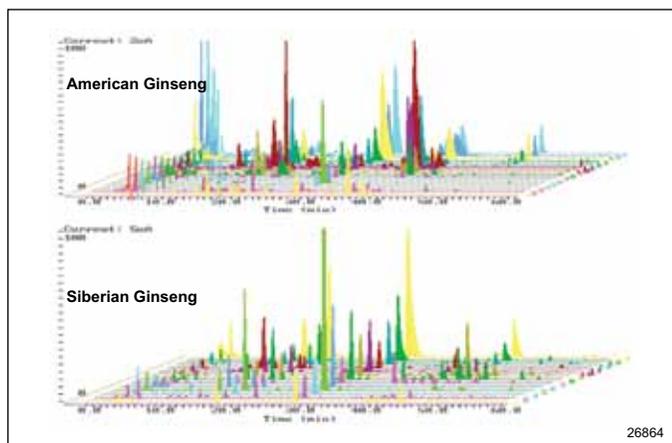


## Polyphenols: Metabolic Profiling and Pattern Recognition

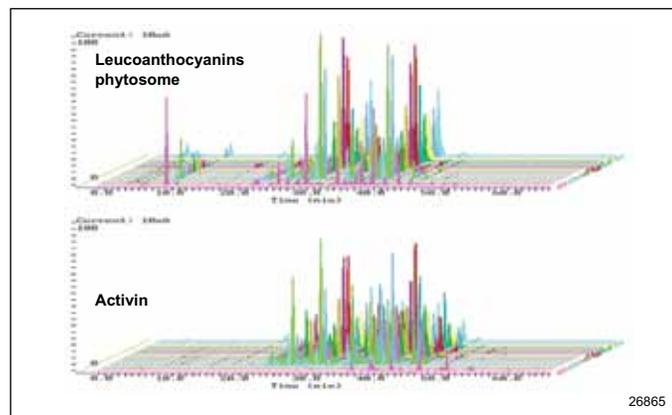
**FIGURE 7. Spiked human plasma (500 ng/mL). For simplicity, only three channels are shown.**



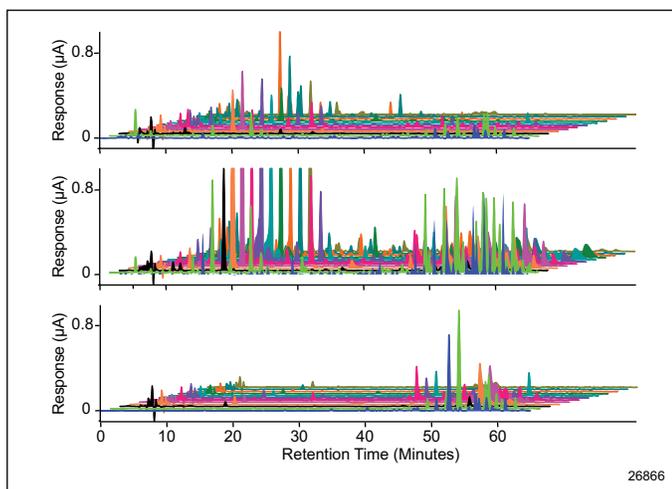
**FIGURE 8. Gradient chromatogram using Dionex CoulArray Detector showing metabolite profiling of ginseng extracts.**



**FIGURE 9. Gradient chromatogram using Dionex CoulArray Detector showing metabolite profiling of grape seed extracts.**

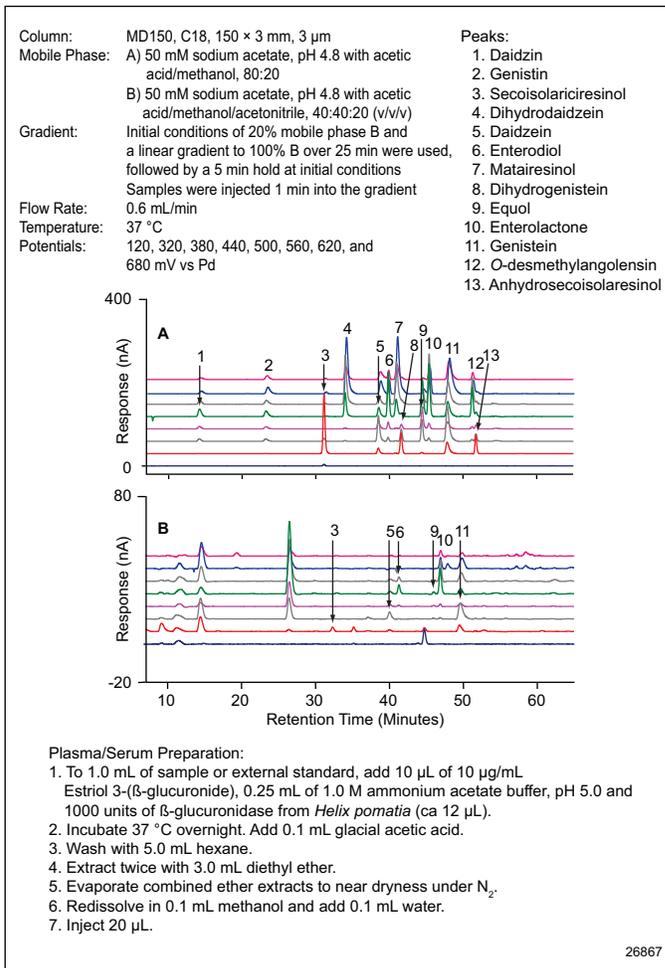


**FIGURE 10. Gradient chromatogram using Dionex CoulArray Detector showing metabolite profiling of extracts of three different ginkgo biloba brands.**

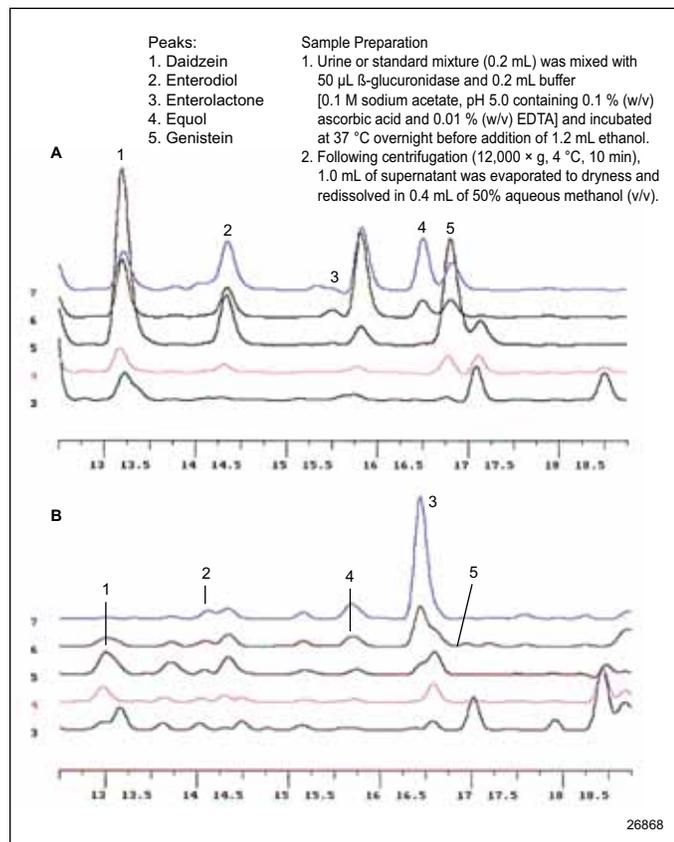


## Isoflavonic Phytoestrogens: Human Tissues

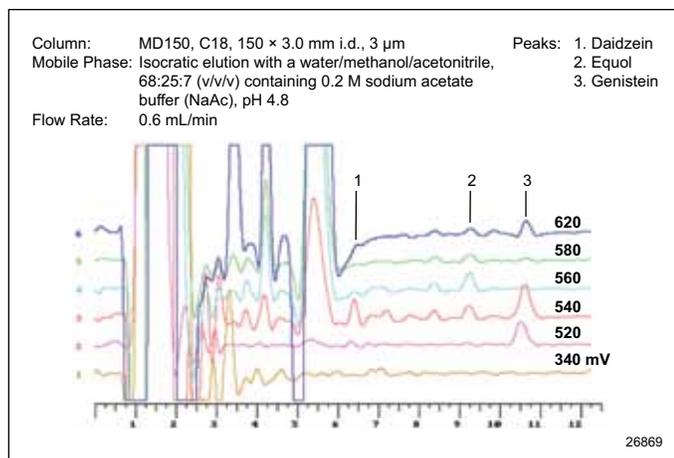
**FIGURE 11. Chromatograms using Dionex CoulArray Detector of (A) phytoestrogen standards and (B) plasma extract. Courtesy of Drs. Nurmi and Adlercreutz.**



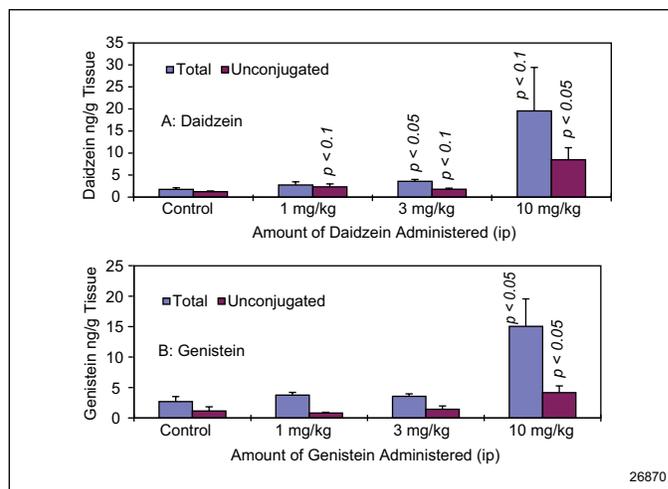
**FIGURE 12. Chromatograms using Dionex CoulArray Detector of total urinary lignans and phytoestrogens in women consuming soy supplements. (A) Women consuming Boston Diet with soy protein isolate, presented at 1  $\mu$ A full scale; (B) Women consuming Boston Diet alone, presented at 100 nA full scale.**



**FIGURE 13. Chromatogram using Dionex CoulArray Detector of an acetone/ethanol extract of hydrolyzed Sprague-Dawley rat plasma. Levels found were 96 (378), 210 (868) and 46 (170) ng/mL (nM) daidzein, equol, and genistein, respectively (ca 1–2.5 ng on column).**

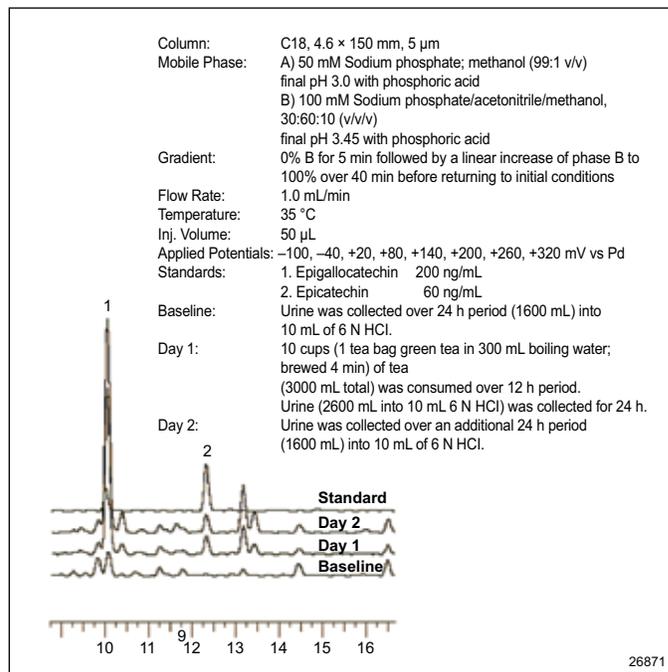


**FIGURE 14. Levels (mean and standard error [SEM], ng/g) of daidzein (DE) and genistein (GE) found in brain cortical homogenates of male Sprague-Dawley rats. A) Four animals in each group were given vehicle 1, 3, or 10 mg/kg DE in 1% acacia ip. B) Four animals in each group were given vehicle 1, 3, or 10 mg/kg GE in 1% acacia ip. P values from single-tailed Student's *t* test are shown in italics. Cerebral cortical tissue levels in controls of free/conjugated DE and GE were 1.22/0.55 and 1.15/1.54 ng/g tissue. Hydrolysis was achieved using  $\beta$ -glucuronidase from *Helix pomatia* using estriol 3-( $\beta$ -glucuronide) as the internal standard.**



## Catechins in Human Urine

**FIGURE 15. Chromatogram of human urine showing the presence of EGC and EC. Only channel 2 (-40 mV) is shown for clarity.**



## Discussion

- HPLC with Dionex CoulArray Coulometric Array detection is an extremely powerful analytical approach. It offers high sensitivity (fg level), a wide dynamic and linear range (fg to µg), excellent selectivity, and provides qualitative information (voltammetric characterization). Furthermore, it is the only electrochemical detector that can be used routinely with gradient elution.
- The technique shown here can be used to measure specific compounds (e.g., resveratrol, epicatechin, etc.) in plant and animal tissues.
- This method can be used to generate metabolite patterns (metabolomics) of up to several hundred compounds. Such patterns can be interrogated using principal component analysis (pattern recognition) and can be used to study:
  - Product authentication
  - Product composition
  - Product adulteration
  - Product stability
  - Competitive information
  - Gut microflora metabolism
  - ADME
  - Effects of polyphenols on metabolism and disease state

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