Deconvolution of Curry Powder Constituents Using HPLC-ECD

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Key Words
Curry powder, Coriander, Cumin, Flavanoids, Polyphenols, Phenolics, HPLC-ECD

Goal

To develop a robust and sensitive HPLC-electrochemical detection (ECD) method for separating non-volatile phenols, flavnoids and related compounds in curry powder.

Introduction

Understanding the dynamics of a mixture of complex materials poses difficult analytical problems. The most common analytical approach to solving this problem is to assay for a single constituent of each of the components. However, this method gives no indication of the contribution of the complexity of the components to the overall taste and consistency of the mixture. Another draw back to this approach is that there is no indication of the accuracy of the assigned identity of the individual component, a serious problem with complex samples. Various techniques have been tried to alleviate these problems. More complex mapping techniques utilizing multiple compounds have been tried but this exacerbates the problem of peak identity. To address peak identity issues PDA detection with HPLC has been tried. Although the spectral information is useful in determining peak identity and purity, the large number of spectra generated and the complex math required to deconvolute these spectra require extensive post-run processing. Both time and extensive user intervention make this approach impractical.

The use of HPLC coupled with coulometric array detection offers a new approach to deal with these problems.[1] This robust technique is both sensitive and selective, producing three-dimensional chromatograms, or analyte patterns, for each sample. An analyte is qualified both by its retention time and voltammetric behavior across the array. The latter is referred to as a "ratio accuracy" and is used as an indicator of analyte authenticity or to identify possible co-elutions. The inherent selectivity of the coulometric array detector simplifies the process by focusing on phenols, flavonoids and related compounds that are significant components of the hedonistic character of spices. As a result compounds directly responsible for the taste are selected for analysis. This simplifies the process by focusing on compounds providing relevant information. The uncertainty of the identity of the analytes is minimized through the use of ratio accuracy information. Thus an orthogonal data set is produced, that can be further analyzed by the Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector software to deconvolute the samples.

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Materials and Methods

The gradient analytical system consisted of two pumps, an autosampler, a thermostatic chamber and a 16-channel CoulArray Coulometric Array detector.

LC Conditions

Column	C18, 4.6 × 150 mm, 5 µm
Mobile Phase A	50mM Sodium phosphate: methanol (99:1 v/v) final pH 3.0 with phosphoric acid
Mobile Phase B	100mM Sodium phosphate: acetonitrile: methanol (30:60:10 v/v/v) final pH 3.45 with phosphoric acid
Gradient Conditions	0% B for 5 min followed by a linear increase of phase B to 100% over 40 min. Hold 100%B 5 min before returning to initial conditions
Flow Rate	1.0 mL/min
Temperature	35 °C
Injection Volume	20 µL

Detector Conditions

Detector	Model 5600A, CoulArray
Applied potentials	-100 to +800 mV in 60 mV increments (vs. Pd)

Sample Preparation

Spices were individually sonicated in 50% methanol (1:5 w/v) and then vortexed for 3 min. The slurry was centrifuged at 9000 rpm for 5 min. The supernatant was then passed through a 0.2 µm filter by centrifugation. The filtrate was then diluted 1:10 in 50% methanol prior to analysis.

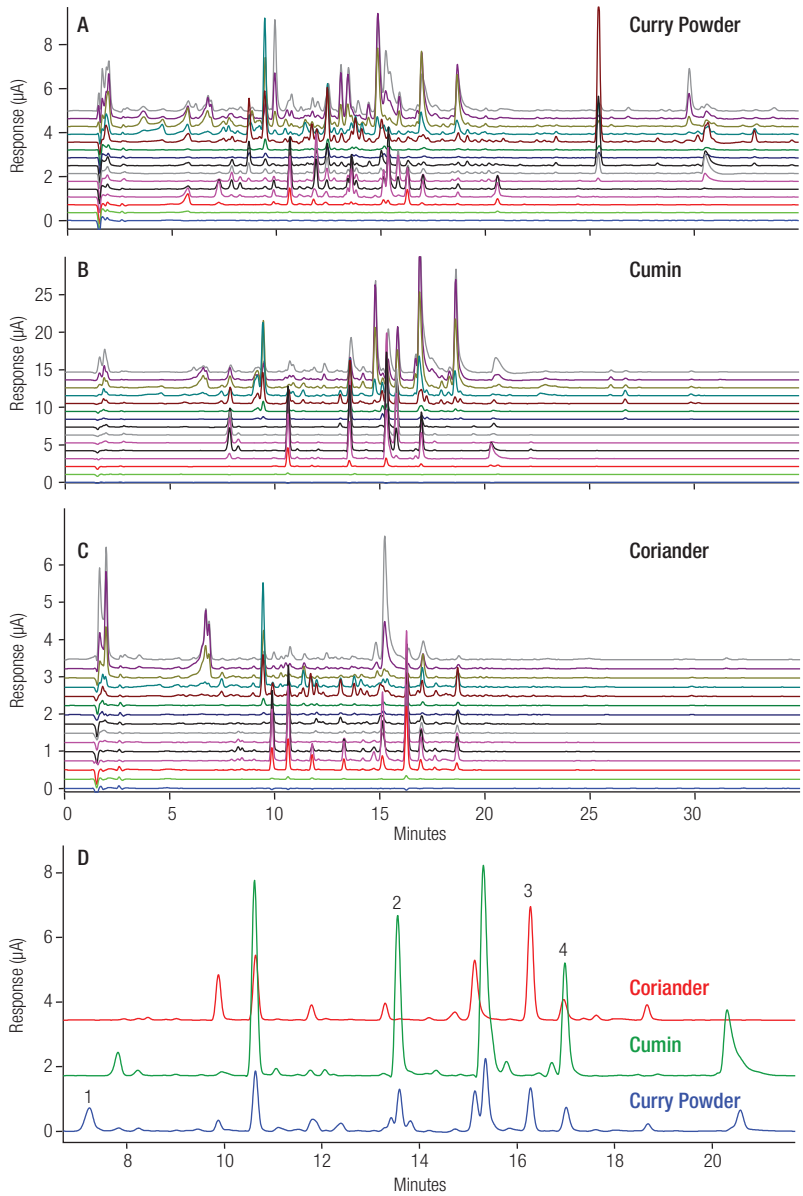

Figure 1. A - Chromatogram of curry powder mixture extract (10 mA full scale). B - Chromatogram of cumin extract (30 mA full scale). C - Chromatogram of coriander extract (7 mA full scale). D - Comparison of single channel from chromatograms A, B, and C (8 mA full scale). 1 - Peak coming from other constituents of curry powder mixture. 2 - Peak coming from cumin. 3 - Peak coming from coriander. 4 - Peak common to both cumin and coriander.

Results and Discussion

Characteristic chromatograms obtained for curry powder and two of its constituents, coriander and cumin, are presented in Figure 1. Although no attempt was made to identify individual analytes many would be expected to be flavonoids, other polyphenols, and phenolics.[2]

In this example 3D data sets describing the sample and its components are generated. This is done in real time. The data can then be analyzed in a number of ways. Simple subtraction can result in information about residual components not related to individual compounds. This provides information about ingredient inter-relationships, degradation or contamination. Alternatively, by selecting specific array channels the source of specific components can be identified back to component even if the identity is unknown. Such patterns can be used to assess consistency in the ratios of starting materials. Finally, patterns of components in the mixture can be further analyzed using external pattern recognition software.

References

1. Acworth, I. N., and Gamache, P. G. . Coulometric array detection with HPLC and its application to the food and natural products industries. *American Lab.*, **1997**, *5*.
2. Gamache, P. H., Ryan, E., and Acworth, I. N. . Analysis of phenolic and flavonoid compounds in juice beverages using HPLC with coulometric array detection. *J. Chromatogr.*, **1993**, *635*, 143-150.

Ordering Information

Description	Part Number
HPG-3400RS Biocompatible Binary Rapid Separation Pump with two solvent selector valves	5040.0046
WPS-3000TBRS Biocompatible Rapid Separation Thermostatted Autosampler	5841.0020
CoulArray, Model 5600A - 16 channel	70-4334
CoulArray Organizer with Temp. Control	70-4340T
Accessory Kit, CoulArray Detector to Thermo Scientific™ Dionex™ UltiMate™ 3000 System	70-9191

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