Computer-Aided Development of a Reversed-Phase HPLC Separation of Acids in Coffee

Application Note 228-207

Food

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Abstract
This application note describes the use of Hewlett-Packard's Interactive Computer-Aided Optimization Software (ICOS) for HPLC separations for the development of a reversed-phase separation. The elution strength search module of the software was used to determine the concentrations of two binary solvent blends that would elute the sample in a predetermined time. The ternary solvent space between the binary blends was then explored using the lattice search module of the software. Finally, the retention modeling module was applied to predict future optimization steps.

Introduction
Green coffee beans have no desirable flavor or aroma of their own; these aspects of coffee are derived from compositional changes that occur in the bean during the roasting process. Among the coffee components that undergo the greatest compositional change during roasting are the chlorogenic acid isomers. These isomers, a group of phenolic acids found in coffee and other plant materials, are commonly referred to simply as "chlorogenic acid". The major component of chlorogenic acid has been designated 5-cafeoyl-quinic acid by IUPAC (1976) nomenclature.

Destruction of chlorogenic acid occurs in a progressive and extensive manner during the roasting process, with losses sometimes exceeding 90%\(^1\). The degree of roast may have an influence on the final beverage flavor because the individual isomers appear to have different sensory qualities and are not necessarily destroyed at the same rate\(^2\).

Several analytical methods have been developed that monitor roast severity by comparing the relative loss of chlorogenic acid to that of caffeine, a component of coffee found to be essentially stable to the roasting process, including an HPLC separation that correlates the 5-cafeoyl-quinic acid/caffeine ratio to the degree of coffee roast\(^3,4\).

HPLC offers a simple, fast, analytical method capable of separating and quantitating the 3-, 4-, and 5-cafeoyl-quinic acid isomers of chlorogenic acid.

Experimental Conditions

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<tr>
<th>Chromatographic Conditions</th>
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<tr>
<td>Column: 0DS Hypersil 100 x 2.1 mm, 5 μm particle size (HP p/n 79516SI-S52)</td>
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<tr>
<td>Mobile phase: 20-mm citric acid buffer, pH 2.25, ACN, MeOH: variable organic modifier ratio</td>
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<td>Flow rate: 0.45 mL/min</td>
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<td>Column temperature: 40°C</td>
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<td>Injection volume: 15 μL</td>
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<td>Detector: Diode array, at 290 and 320 nm with 8 nm bandwidth; reference at 420 nm with 20 nm bandwidth</td>
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Instrumentation
Chromatography was conducted using an HP 1090 HPLC liquid chromatograph equipped with a ternary DR5 solvent delivery system, an automatic sampler, an automatic injector, and a diode array detector.
Data were acquired using an HP 7999A/Pascal HPLC ChemStation with HP 79998A Operating Software, version 5.22, and the HP 79979A Interactive Computer Optimization Software (ICOS) for HPLC separations, revision 1.0.

Reagents
Citric acid was purchased from Fisher Scientific (Fair Lawn, NJ). All chromatography solvents were obtained from Burdick and Jackson (a division of Baxter Health Care, Muskegon, MI). Deionized HPLC-grade water was obtained using a Hewlett-Packard model 661A water purifier. Chlorogenic acid, caffeine, and 5-hydroxymethylfurfural were obtained from Sigma Chemical (St. Louis, MO).

Sample and Standard Preparation
The chlorogenic acid isomers were extracted from a coffee matrix by adding 0.50 g of coffee to 25 ml of boiling HPLC-grade water and diluting to 50 ml with water. Six ml of the extract were then diluted to 50 ml with water and an aliquot injected for chromatography. The chlorogenic acid isomers were prepared by adding several drops of concentrated ammonium hydroxide (Fisher Scientific) to an aqueous solution of the chlorogenic acid standard. The caffeine and 5-hydroxymethylfurfural standards were dissolved in HPLC-grade methanol for chromatography.

Results and Discussion
A low pH buffer was chosen for initial starting conditions, and the elution strength search module of the software was used to determine an elution concentration for acetonitrile.
Starting with a concentration sufficiently high to elute all analytes in a short time period, the software automatically and iteratively reduced the acetonitrile concentration to fulfill a preset analysis time requirement of 12 minutes. The final concentration of acetonitrile determined in this fashion was 4%.

Isoeluotropic theory was then used to calculate a comparable methanol elution concentration.

Although either binary solvent system, buffer:acetonitrile:ACN or buffer:methanol:MeOH, provided sufficient separation and resolution of all components in the sample mixture, the two organic binary mixtures were incorporated into a solvent lattice search in one dimension with two limits. This search moved from a binary buffer ACN mix through ternary mixes of buffer:ACN:MeOH to a binary system of buffer:MeOH in four steps (analyses). Figure 1 is an example of a typical ICOS lattice search report. The report shows the result of the first step of the lattice search defined above.

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**Figure 1.** Plot and report for a single analysis in a lattice search.
During this search, spectral information was acquired from 195.5 to 421.5 nm using a diode array detector. The lattice search module of the software offers the user the option of reporting chromatographic and spectral information automatically as displayed in figure 2. Spectra that have been acquired at the inflection points and the apex were normalized and overlaid, and a peak purity factor was calculated. The spectral information and peak purity factor are presented for each peak of interest in separate windows below the chromatogram. For example, peak 1, which is not as well resolved as the other peaks, has a peak purity factor of 974.35. A peak with a purity factor less than 990 is typically considered impure.

A retention model generated from the data obtained during the lattice search is shown in the upper part of figure 3. The analyses incorporated into the retention model are marked by asterisks on the right side of the retention model window; the two binary limit analyses are also indicated by percentage composition given as text, and the two ternary steps are indicated by asterisks alone.

Figure 2. Plot of chromatographic signal and peak spectra for purity analysis.

Figure 3. Retention model based on three analyses.
The retention model automatically calculates separation criteria. "Rs, min" gives the resolution of the least separated pair. "Rs, rp" the relative resolution product, describes how evenly the chromatographic peaks are distributed. Both measures are emphasized in the two simulated chromatograms displayed below the retention model in figure 3.

The top chromatogram (95.7% 20 mm citric acid buffer pH 2.25/0.6% MeOH/ 3.7% ACN) shows a simulated separation that should provide the best resolution of the least separated pair of peaks in the chromatogram. In the lower chromatogram, the predicted separation that would provide the best "Rs, rp" value for this analysis (92.0% 20 mm citric acid buffer, pH 2.25/8.0% MeOH) is displayed.

Separation of the five components in this sample can be obtained using either a binary buffer/ACN or buffer/MeOH mixture; however, a solvent lattice search was done to examine the use of a ternary solvent system. A retention model was created based on the lattice search data base with intent to further optimize the separation. In this instance, however, the separation criteria "Rs, min" and "Rs, rp" indicate that a simple binary blend provides adequate separation of all five peaks and that moving to a ternary solvent system does not enhance separation.

If an appropriate separation had not been identified through a simple lattice search, the retention modeling portion of the software could have been redefined iteratively to aid the chromatographer in optimizing the separation in a minimum number of analyses (see Product Brief, publication no. 12-5091-0325E).

Conclusion

ICOS solvent optimization software can be used to develop a reversed-phase HPLC separation of acid components extracted from coffee in a minimum number of analyses.

References


