APPLICATION NOTE

LIQUID CHROMATOGRAPHY

Increased Throughput for the Determination of Synthetic Dyes in Beverages Using HRes Fast-LC Technology

Introduction

It is widely recognized that we "eat with our eyes" as well as our palates. The color of food has a great impact on consumer's ultimate perception of food's quality and flavor. The use of colorants in food has become pervasive, not only in highly-processed foods such as cereals and frozen desserts, but also in seemingly natural foods such as oranges and fish. Color additives are used for decorative purposes or to enhance product identity. They are also used to minimize variations in natural color and provide color stability on the shelf.

Certified synthetic colorings are regulated in the USA by the Federal Food, Drug, and Cosmetic Act (FD&C). In the European Union and many other countries, regulations are set according to the international Codex Alimentarius, set forth by the Food and Agricultural Organization of the UN and the World Health Organization (WHO). Because of these regulations, and the fact that color influences consumer purchasing decisions, dye content is a tightly-controlled food-product characteristic. Liquid chromatography is a widely accepted QA/QC technique for determining the dye content of foods.

In this study, an HRes fast-LC method was developed for the determination of typical dyes found in aperitif beverages, and was compared with a conventional chromatographic method. Two aperitif beverages were analyzed using the HRes fast-LC method. This application note demonstrates how HRes fast-LC using sub-3 µm particle column technology can be used to accelerate dye QA/QC methods. The use of photo diode array (PDA) detection in combination with HRes technology provides added confidence in peak identification for method development in complex beverage formulations.

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Experimental

A PerkinElmer® Series 275 HRes™ PDA LC System comprised of a Series 275 binary high-pressure (10,000 psi) blending pump, vacuum degasser, a high-throughput Series 275 autosampler with high-pressure (>10,000 psi) injector valve, a column oven and a Series 275 photo diode array (PDA) detector fitted with a high-efficiency 2.4-µL flow cell, was used for HRes fast-LC chromatography. A PerkinElmer Series 200 binary micro pump, vacuum degasser, column oven, conventional injection valve and Series 200EP photo diode array detector were used for conventional chromatography for comparative purposes.

Both conventional 5-µm and HRes 1.9-µm particle-size columns were compared using the conditions in Table 1.

A standard mixture of EU dyes (Table 2) was prepared containing 30 ppm of each dye dissolved in 95% methanol and 5% formic acid. Aperitif beverages were injected neat, without any sample preparation.

Results

Gradient chromatograms using both conventional and HRes methodology are compared in Figure 1 (Page 3). The retention time for E131 dye using conventional chromatography was 17.8 minutes, while its retention time using HRes technology was 5.4 minutes. An improvement in throughput of over 3x was achieved using HRes methodology.

Table 1. Chromatographic Conditions for HRes and Conventional LC Methods.

	Conventional			**HRes**			
Column	Brownlee™ HRes Aqueous DB-C18 25-cm length x 4.6-mm I.D. 5-µm			Brownlee Aqueous DB-C18 10-cm length x 2.1-mm I.D. 1.9 µm			
Operating Pressure	2500 psi (172 bar)			6830 psi (470 bar)			
Gradient	Step	Time (min)	Acetonitrile (%)	NaAcetate (20 mM) (%)	Step	Time (min)	Acetonitrile (%)	NaAcetate (20 mM) (%)
	0	5.0	5	95.0	0	3.0	0	100
	1	20.0	40.0	60.0	1	1.0	5.5	95.0
					2	2.0	35.0	65.0
					3	2.0	45.0	55.0
					4	1.0	50.0	50.0
					5	1.0	50.0	50.0
Flow	1.5 mL/min			0.55 mL/min			
Temperature	25 ˚C			55 ˚C			
Detector Wavelengths	Spectra scanned from 190 nm to 700 nm Chromatograms recorded at 277 nm			Spectra scanned from 190 nm to 700 nm Chromatograms recorded at 277 nm			
Detector Flow Cell	10 mm, 15 µL			6 mm, 2.4 µL			
Injection Volume	50 µL			5 µL			

Table 2. EU Dyes Determined.

EU Code	**Dye Name**
E102	Tartrazine (FD&C Yellow 5)
E104	Quinoline Yellow
E110	Sunset Yellow FCF, Orange Yellow S, FD&C Yellow 6
E120	Cochineal, Carminic Acid, Carmines, Natural Red 4
E122	Carmoisine, Azorubine
E123	Amaranth (FD&C Red 2)
E129	Allura Red AC (FD&C Red 40)
E131	Patent Blue V
E133	Brilliant Blue FCF (FD&C Blue 1)

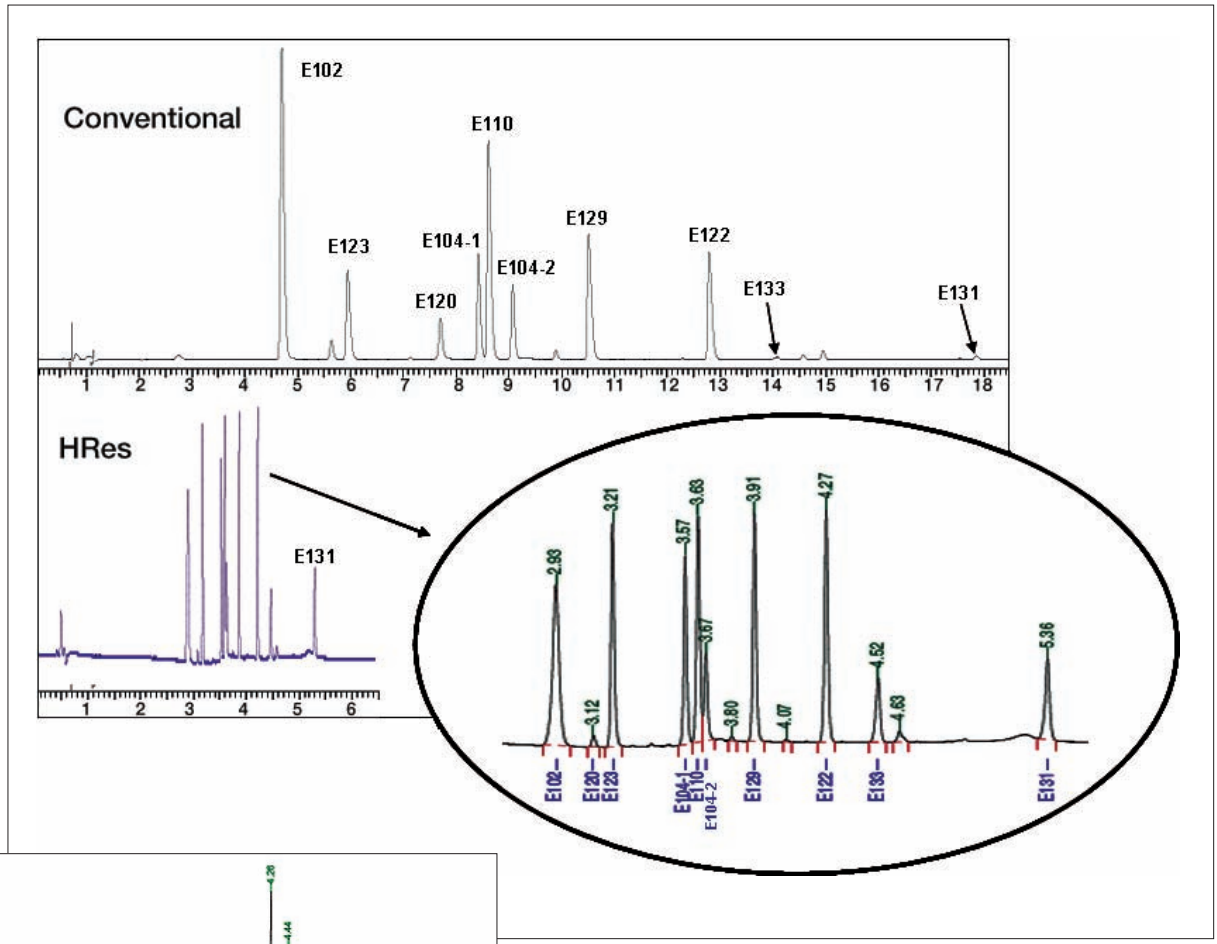

Figure 1. Throughput for food-dye determinations is improved by over 3x using HRes methodology.

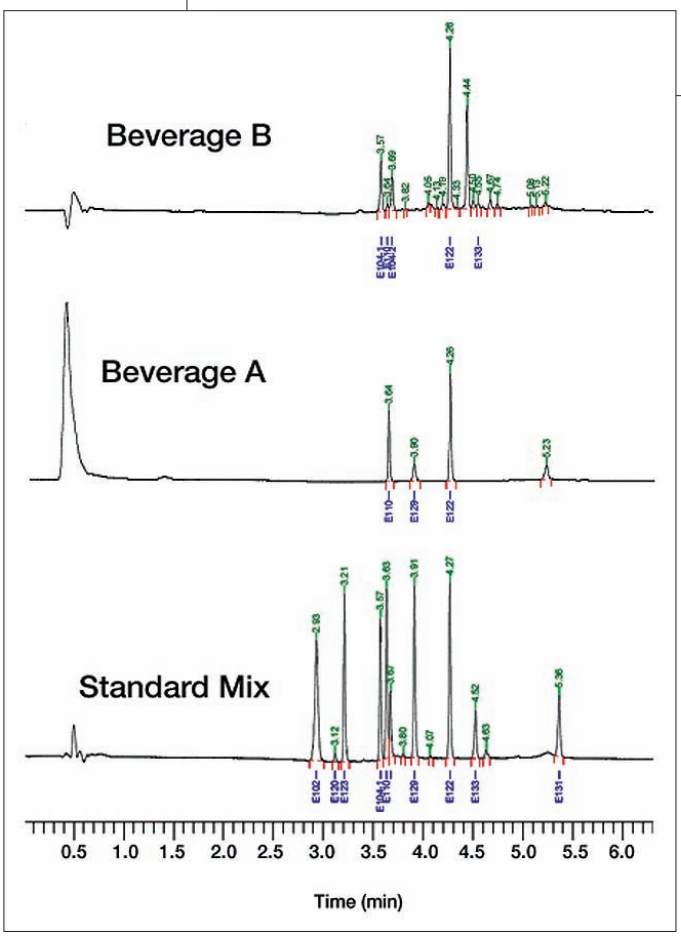

Figure 2. Comparison of dye profiles for non-alcoholic (Beverage A) and alcoholic (Beverage B) aperitifs with a standard mixture of synthetic dyes.

It is also clear from this figure that excellent separation efficiency for the dyes is maintained, even while accelerating the chromatographic throughout by over 3x.

Chromatograms for two aperitif samples are shown in Figure 2. Beverage B is an alcoholic aperitif and is known to contain numerous natural colorants as well as synthetic dyes. Beverage A is a non-alcoholic aperitif and has a much simpler dye profile.

A major advantage of the PDA detector is the ability to identify chromatographic peaks based on their spectra. This provides added confidence over utilizing only retention time for identification, especially when analyzing complex samples.

As an example, the spectra shown in Figures 3 and 4 illustrate the power of using UV/Vis spectra for confirmation of peak identification in complex beverage samples. Comparison of the UV/Vis spectral profile for the chromatographic peak for E122 in the Standard Mix (retention time 4.27 min) with that of the peak in Beverage A having the same retention time, provides powerful evidence for the identification of E122 in Beverage A. Furthermore, a comparison of the UV/Vis spectrum of the same peak in Beverage A with a known library peak for EU dye E122 shown in Figure 4, is further confirmation of the presence of E122 in Beverage A.

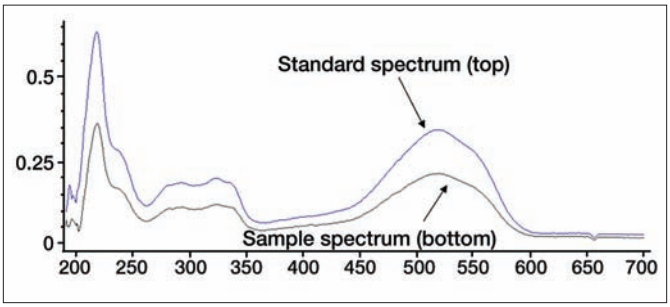

Figure 3. UV/Vis spectral comparison of unknown peak in Beverage A at 4.26-min retention time with that of E122 peak in Standard Mix at same retention time.

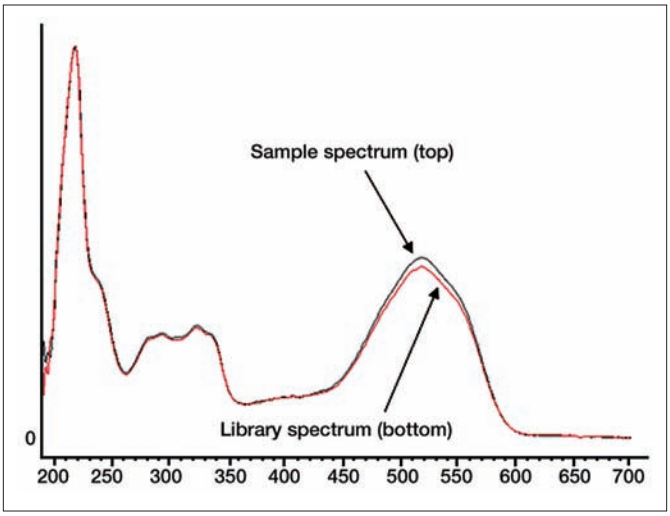

Figure 4. Comparison of sample spectrum from chromatogram of Beverage A at 4.26-min retention time with reference-library spectrum of EU dye E122.

Conclusions

Continuous improvement in product quality and increased consumer awareness of product safety are driving enhancements in food QA/QC methodologies. Global sourcing of food products and concern with maintaining a high level of quality and food safety while utilizing ingredient systems originating abroad, is driving regulators to harmonize regulations worldwide and strengthen capabilities for food-product analysis.

HRes fast-LC technology can be used to accelerate throughput in the determination of synthetic dyes in beverage products. The use of PDA detection in the LC determination of dyes in beverages provides increased confidence in component identification especially in complex product formulations. This is invaluable for method development.

The Series 275 HRes PDA LC System is built upon proven PerkinElmer micro-binary pump technology, and can be used for both conventional and HRes methodology. Methods can be developed across a wide range of operating pressures, greatly expanding separation capabilities of the LC laboratory. This flexibility facilitates method transfer and the adoption of faster HRes LC methods, without having to abandon proven, reliable conventional methods in the same lab. Higher productivity can be achieved without sacrificing separation efficiency and simple, turnkey HRes methods such as the one illustrated here can be developed and implemented readily.

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