APPLICATION NOTE

UHPLC

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Analysis of Color Additives in Beverages with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Food colors have a great impact on consumers' perception of food quality. That explains why the use of color additives or dyes in food has become pervasive, not only in highly processed food such as cereals and frozen dessert, but also in seemingly natural food such as dairy products. Dyes are used to intensify the color of food products and make them look tempting. They are also used to minimize color variation, and to prolong color stability on shelf. There are instances however, where dyes are used unscrupulously to mask the poor quality of food products. In the U.S., the Food and Drug Administration's (FDA) data shows an alarming five-fold increase in consumption of dyes since 1955.

Color additives in food products have practically no nutritional value. Some color additives are of natural origin and are generally safe but most are synthesized from petroleum and have the potential of tainting food supplies. A commonly used dye in the U.S. called sunset yellow has carcinogenic impurities such as sudan I. In vitro studies suggest that brilliant blue, another widely used dye has the potential for neurotoxicity. A study by Schab (2004) and another study by McCann (2007) suggest that mixtures of dyes cause hyperactivity and other behavioral problems in some children. In 2010, acting on these concerns, European countries mandated a warning label stating that food containing dyes may have adverse effect on activity and attention in children.

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All around the world, toxicological considerations are prompting regulatory agencies to lower the acceptable level of dyes in comestible products. These different regulations are continuously harmonized by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) in order to promote food safety and trade. In the U.S. certified synthetic colorings (FD&C colors) are regulated by the FDA. In Europe, their regulation is under the European Commission's directives governing food dyes. In all these countries there is a push by consumer protection agencies to ban their use altogether.

This application note presents a fast and robust HPLC method for the determination of dyes in beverages (Table 1). Method conditions and performance data including precision and linearity are presented. A popular orange soda is analyzed and the type and amount of dyes used are confirmed.

Table 1. **Dyes analyzed.**

Dyes name	U.S. Code	EU Code
Amaranth	FD&C Red # 2[1]	E123
Indigo Carmine	FD&C Blue # 2	E132
Sunset Yellow	FD&C Yellow # 6	E110
New Coccine	Red # 18	E124
Eosin Y	Acid Red # 87	
Erythrosin B	FD&C Red # 3	E127
Phloxine B	Acid Red # 92	
Rose Bengal	Acid Red # 94	

[1] *Banned in food in the USA.*

Experimental

A 0.1 mg/mL stock standard solution was prepared by diluting the appropriate net weight of the eight dyes with methanol followed by 15 min. sonication. From the stock standard four calibration levels were prepared by dilution with water (Table 2). The calibration curve and the repeatability were evaluated with three injections per level. The solutions were thoroughly mixed and filtered with a 0.2 µm nylon membrane prior to testing. The orange soda was filtered and injected directly.

Table 2. **Calibration preparation.**

	Level 1	Level 2	Level 3	Level 4*
Stock Std. (mL)	2.5	1.25	0.6	–
Total Vol. (mL)	10	10	10	10
Conc. (µg/mL)	25	13	6	3

**Level 4 is a direct 1:1 dilution from level 3.*

Table 3. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar™ FX UHPLC
Setting:	50 µL loop, partial loop injection mode 350 µL mixer volume injection 4 µL; flush solvent: methanol
PDA Detector:	Scanned from 190-700 nm, analytical wavelength 254 nm
	Reference 400 nm, bandwidth 10 nm
HPLC Column:	PerkinElmer Brownlee™ analytical C-18, 150 x 4.6 mm, 5 µm at 55 °C (Cat. No. N9303513)
Mobile Phase:	A: 20 mM ammonium acetate
	B: 80:20 acetonitrile:methanol

Time (min)	Flow rate (mL/min)	B %	Curve
8	1.2	5-60	1
2	1.2	60	1

	Three minutes equilibration after injection.
Software:	Chromera® version 3.0
Sampling Rate:	5 pts/sec

Results and Discussion

The separation was achieved using a PerkinElmer Brownlee analytical C-18, 5 µm, 150 x 4.6 mm column. The optimal flow rate was 1.2 mL/min and the run time was about ten minutes with a back pressure of approximately 3000 PSI (207 bar). All eight components were well resolved with resolution ranging from 2.7 to 9.4. The calibration curve demonstrates a coefficient of determination not less than the cutoff of 0.999. The repeatability was good with %RSD values ranging from 1.0 to 1.9 along the four levels of calibration. The tailing was excellent with value not more than 1.4 (cutoff value is 2.0). Representative chromatograms of the standard solutions are shown in Figures 1 and 2; a representative chromatogram of the orange soda analyzed is presented in Figure 3. In Figure 4 the color additive in the orange soda is confirmed using the spectral library. The calibration curve and the performance of the method are presented in Figure 5 and Table 4.

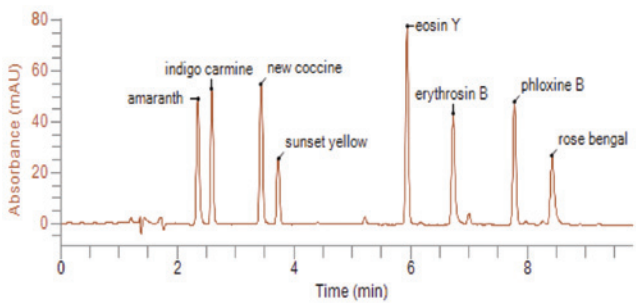

Figure 1. Chromatogram from the analysis of a 25 µg/mL standard solution.

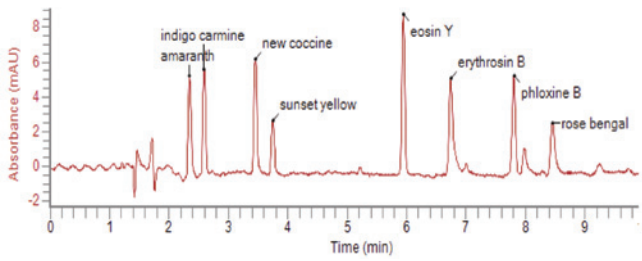

Figure 2. Chromatogram from the analysis of a 3 µg/mL standard solution.

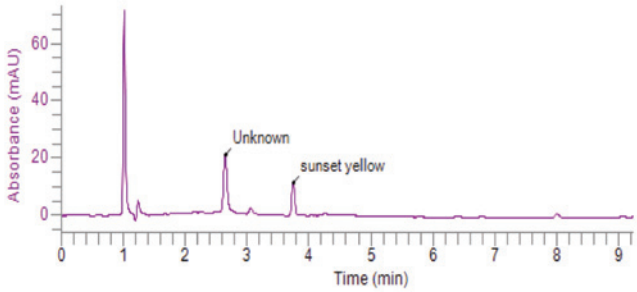

Figure 3. Chromatogram from the analysis of a popular orange soda.

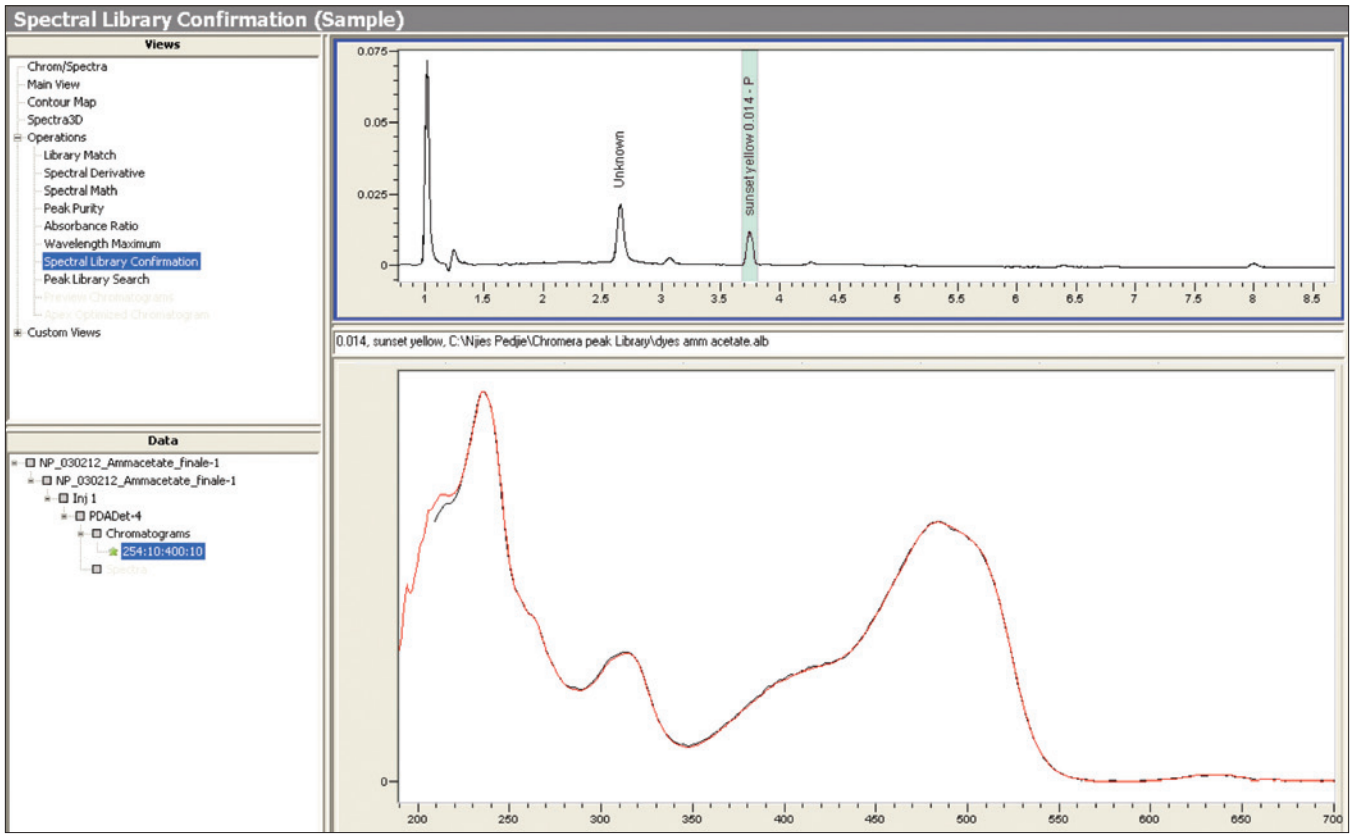

Figure 4. Peak identification in sample using Chromera spectral library

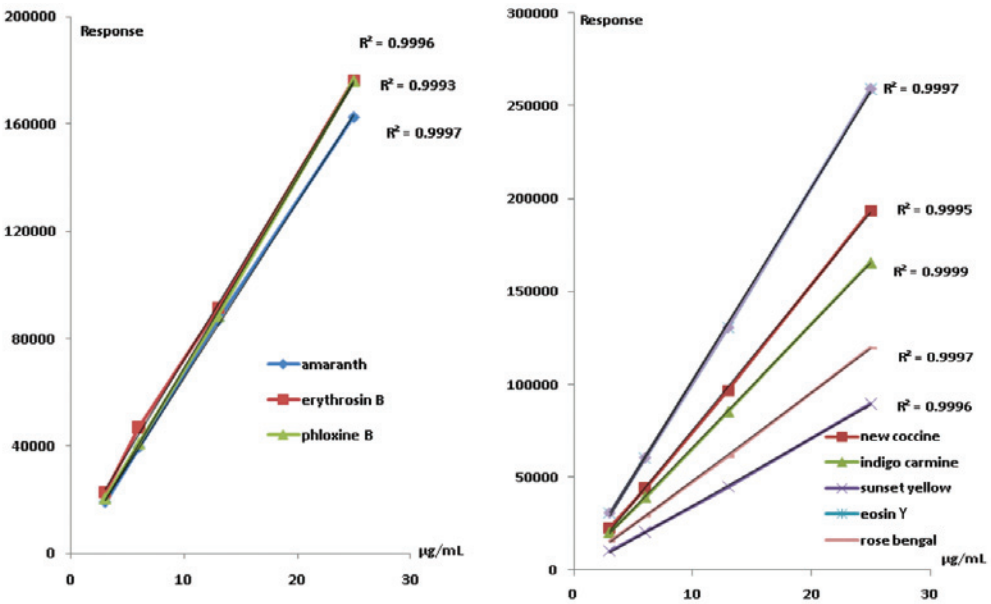

Figure 5. Calibration curves.

Table 4. **Method performance.**

Compound	Repeatability %RSD (n = 9)	Resolution	Tailing (n = 9)	Soda (µg/mL)
Amaranth	1.2	–	1.4	ND
Indigo Carmine	1.1	2.7	1.2	ND
New Coccine	1.1	9.4	1.2	ND
Sunset Yellow	1.6	3.1	1.1	11.0
Eosin Y	1.4	8.2	1.1	ND
Erythrosin B	1.0	7.9	1.4	ND
Phloxine B	1.7	8.6	1.1	ND
Rose Bengal	1.9	3.8	1.3	ND
Average	1.4	6.2	1.3	

Conclusion

Increased awareness of food safety is driving the improvement in quality control methodologies. In a world where global sourcing of food products is becoming the norm, concern about the type and quantity of color additives in food products is prompting regulators to harmonize regulations worldwide and is forcing the food industry to adopt stringent requirements. The method in this study, with outstanding performance and a calibration curve encompassing the concentrations at which dyes are typically used, subscribes to that effort. All the eight color additives analyzed were resolved within ten min. with resolution between consecutive peaks not less than the cutoff value of 1.5. The method was shown to be precise and linear with %RSD less than 2%, and r^2 not less than 0.999. The popular orange soda analyzed has 11.0 µg/mL of sunset yellow, far less than the 20 µg/mL maximum amount allowed in drinks in the European countries.

PerkinElmer's Flexar FX 15 pump fitted with durable pistons is capable of generating at each stroke a pressure up to 18000 PSI. A low dispersion injection valve combined with a carefully designed mixer capable of delivering a very precise and constant gradient result in very reproducible peaks during analysis. The wide flow range capability (0.01 - 5.0 mL/min.) allows the use of a traditional HPLC column as well as columns specifically designed for UHPLC. The low dispersion PDA detector provides a rugged and accurate detection over a range of 190 nm to 700 nm wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, peak purity, spectra 3D and contour maps. Among these features, the spectral library function showcased in this application note is a powerful tool used to confirm the identity of components in the sample. It adds certainty to the results as it is known that components with the same relative retention time are not necessarily the same.

References

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6. Food Dyes A Rainbow of Risks. Center for Science in the Public Interest cspinet.org/new/pdf/food-dyes-rainbow-of-risks; retrieved Jan. 19, 2012.

Note: this application note is subject to change without prior notice.

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