Fast Separation of Synthetic/Artificial Food Colors on Agilent Poroshell 120 EC-C18

Application Note

Food Testing & Agriculture

Author

Rongjie Fu
Agilent Technologies (Shanghai) Co. Ltd

Abstract

Many synthetic or artificial colorants (tartrazine, red 33, sunset yellow, and so forth) are used in food and beverages to improve product appearance. These compounds can be readily separated by reversed-phase liquid chromatography. A new Agilent Poroshell 120 EC-C18 column was used to separate 11 food colorants using a gradient method with an acetate buffer/methanol mobile phase. This method allows a rapid separation of the colorants in 9 minutes. It is suitable for many samples and was applied here to the analysis of these colorants in beverages and cakes.

Agilent Technologies

Introduction

Most synthetic or artificial food colorings are water soluble, making them ideal for analysis by HPLC with reversed-phase columns. These compounds are generally safe, but there are some possible harmful effects, such as allergic reactions and hyperactivity in children; so their use is restricted in some countries. Because of these restrictions, the quantity of these compounds in food quality control is becoming more important, as is the need to prove that foods using the colorants meet international quality control standards. These compounds are used in food to give them a colorful, attractive appearance. The structures of 11 colorants, mostly azo dyes, are shown in Table 1.

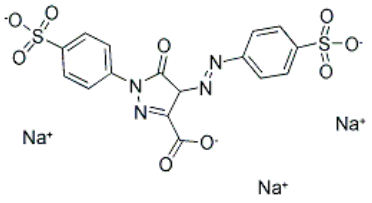
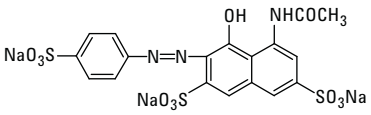
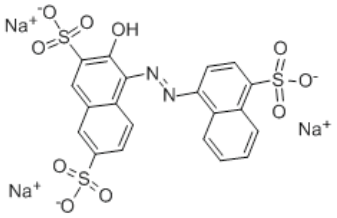
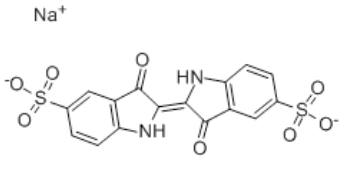
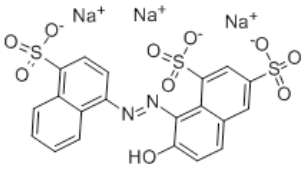
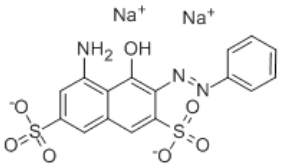
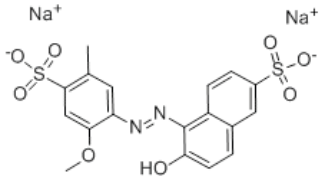
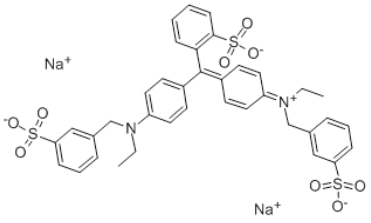
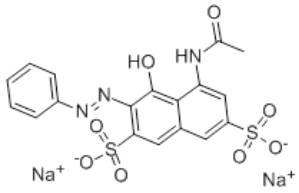
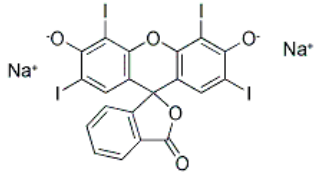
No.	Name	CAS	Structure
1	Tartrazine	1934-21-0	
2	New red	220658-76-4	
3	Amaranth	915-67-3	
4	Indigo carmine	860-22-0	
5	Ponceau 4R	2611-82-7	
7	Red 33	3567-66-6	
8	Allura red	25956-17-6	
9	Erioglaucine	3844-45-9	
10	Azophloxine	3734-67-6	
11	Erythrosine	49oC6-10-3	

Table 1. Details of food colorants in this study.

A previous application note described a method developed using Agilent TC-C18(2) and HC-C18(2) columns. The method applied a mobile phase comprised of phosphate buffer at pH 7.0 with methanol [1]. In this work, we focus on developing a method for rapidly separating more colorants in beverages and cakes using Agilent Poroshell 120 EC-C18.

Experimental

The HPLC analysis was performed with the Agilent 1290 Infinity LC system, including an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity Autosampler (G4226A), an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and an Agilent Infinity Diode Array Detector (G4212A).

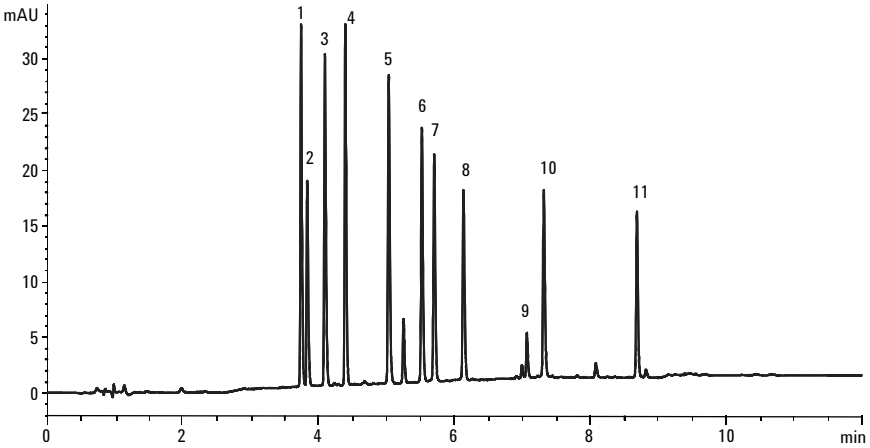
Conditions

Column:	Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)	
Mobile phase:	A, 10 mM acetate B, methanol	
Injection volume:	2 µL	
Flow rate:	1.0 mL/min	
Gradient:	Min	% B
	0	5
	1.5	5
	8	75
	12	75
Stop time:	12 minutes, post run 2 minutes	
Temperature:	25 °C	
Wavelength:	254 nm	

The samples were purchased in a local market. Mango juice was diluted 1/10 with water and filtered with 0.2 µm regenerated cellulose membrane filters (p/n 5064-8222) before injection. Cake samples were prepared by weighing 5 g and adding to 40 mL water with a drop of ammonium hydroxide. The samples were then ultrasonicated at 60 - 80 °C for 30 minutes. The samples were increased to 50 mL with methanol and then filtered using 0.2 µm regenerated cellulose membrane filters (p/n 5064-8222) before injection.

Results and Discussion

The colorant compounds shown in Table 1 are polar, water-soluble compounds containing sulfonic acid groups, increasing their solubility in water. In fact, many of these compounds are used in the salt form in food. In a previous work, 7 of these colorants were well separated using a traditional 5 µm TC-C18(2) column with a phosphate buffer and a gradient starting with 10% methanol. Resolution and peak shape are best at mid pH with a phosphate buffer. However, more and more users apply LC/MS to analyze these colorants in very complex food samples. To be compatible with MS detectors, a 10 mM acetate buffer was used. With a highly efficient, superficially porous Poroshell 120 EC-C18,



Peaks

1. Tartrazine
2. New red
3. Amaranth
4. Indigo carmine
5. Ponceau 4R
6. Sunset yellow
7. Red 33
8. Allura red
9. Erioglaucine
10. Azophloxine
11. Erythrosine

Figure 1. Separation of 11 colorant standards using an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column.

3.0 × 100 mm, 2.7 µm column, 11 synthetic colorants were well separated in only 9 minutes (Figure 1).

Synthetic or artificial food colorants exist in many common beverages and foods, such as fruit-flavored drinks and sodas, cakes, and candy. Colorants in a mango juice and two different types of cake were analyzed. The chromatogram of mango juice is shown in Figure 2. Based on the standard, the first known peak, tartrazine, could be resolved completely from the unknown peak before it, and the amount of tartrazine could be measured.

Cake samples were more complex than mango juice because many polar compounds were extracted with water. To separate colorants from these polar compounds, a gradient starting with low percentage of organic phase was used. Figures 3 and 4 show separations of cake samples, with many peaks in both. The compounds could be identified through retention times of standards. However, for complex samples such as these, other compounds co-eluted with the colorants, which made it difficult to identify the exact colorants. This problem was resolved with an Agilent Infinity Diode Array Detector (G4212A), where the spectra of the peaks in the

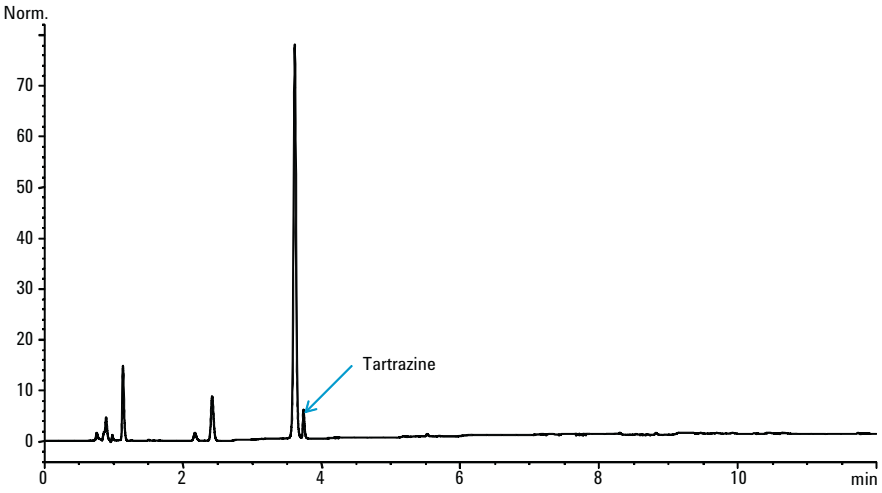


Figure 2. Separation of mango juice with an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column.

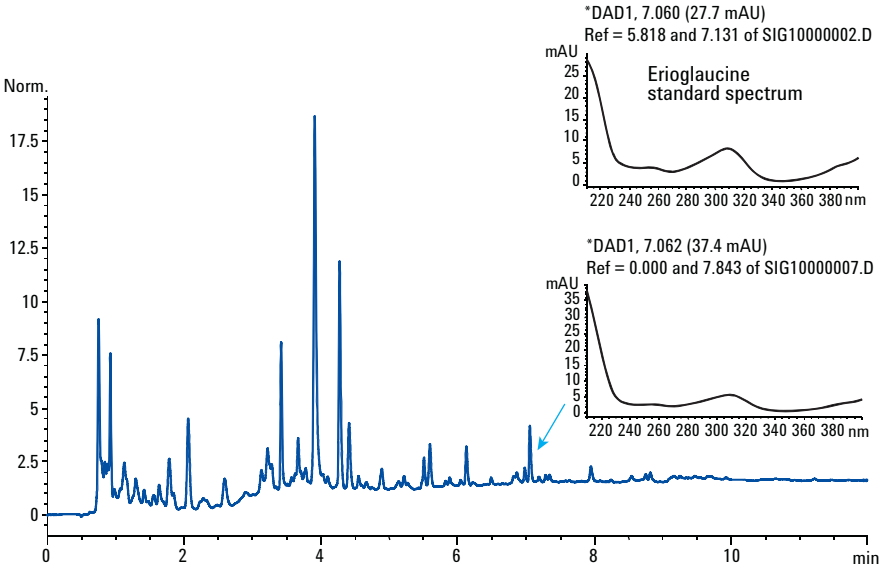


Figure 3. Separation of food colorants in cake sample 1 on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column.

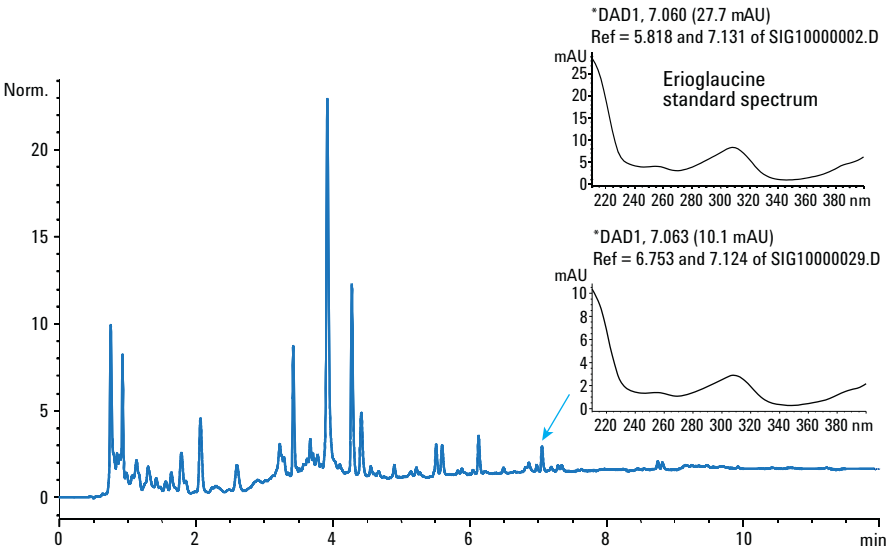


Figure 4. Separation of food colorants in cake sample 2 on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column.

sample were matched with those of standards. In both cake samples, the colorant erioglaucine was identified based on the standard retention time coupled with its spectrum.

Conclusions

Using a simple gradient method, many common synthetic colorants can be separated on the Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm column. This method allows rapid separation and screening for many artificial food colorants. In addition, the mass-spectrum-friendly acetate buffer allows the method to be easily transferred to an HPLC/MS method.

Reference

1. Rongjie Fu. "The Separation of Seven Synthetic/Artificial Food Colors on Agilent HC(2)/TC(2) Reversed Phase columns." Agilent Technologies, Inc., Publication 5989-8307EN (2008).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
July 17, 2012
5991-0816EN

Agilent Technologies