

# Fast HPLC Analysis of Dyes in Foods and Beverages

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## INTRODUCTION

Dyes have many applications in the food and beverage industries, such as being used to make food more appealing, hide defects, or to strengthen consumer perception of the association between color and flavor. For example, lime flavor is associated with the color green and thus, lime soft drinks are often colored with green food dye. Despite the existence of many dyes, only a few have been approved for use in foods and beverages. The U.S. FDA permits seven artificial colorings in food: Brilliant Blue FCF (FD&C Blue 1), Indigotine (FD&C Blue 2), Sunset Yellow FCF (FD&C Yellow 6), Tartrazine (FD&C Yellow 5), Allura Red AC (FD&C Red 40), Fast Green FCF (FD&C Green 3), and Erythrosine (FD&C Red 3).

Reversed-phase chromatography is an excellent technique for the analysis of dyes. Many dyes are hydrophobic, readily soluble in reversed-phase eluents, and have strong visible and UV absorbance properties. This application note (AN) demonstrates fast separation of 10 dyes in less than 5 min using an Acclaim® PA2 (3 µm) column in a 3 × 75 mm format.

The Acclaim PA2 column is ideal for resolving mixtures of compounds with a wide range of hydrophobicities, including very polar compounds. This method was used to determine the quantity of food dyes in six soft drinks and a gelatin dessert. The combination of an UltiMate® 3000 Rapid Separation LC (RSLC) system and an Acclaim PA2 column is suitable for the fast analysis of food and beverage samples that have both approved and illegal dyes.

## EQUIPMENT

Dionex UltiMate 3000 RSLC system consisting of:

- SRD-3600 Solvent rack with integrated vacuum degasser

- HPG-3400RS Binary gradient pump with 400 µL static mixer kit

- WPS-3000RS Split loop sampler with 100 µL sample loop

- TCC-3000RS Thermostatted column compartment

- DAD-3000RS Diode array detector, 5 µL flow cell

Chromeleon® Chromatography Data System,  
Version 6.80 SR7

## REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistivity or better

Acetonitrile (CH<sub>3</sub>CN), LAB-SCAN

Di-ammonium hydrogen phosphate ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>), Ajax

8 N Potassium hydroxide solution (KOH), KANTO

Tartrazine (C<sub>16</sub>H<sub>9</sub>N<sub>4</sub>Na<sub>3</sub>O<sub>9</sub>S<sub>2</sub>), Fluka

Amaranth (C<sub>20</sub>H<sub>11</sub>N<sub>2</sub>Na<sub>3</sub>O<sub>10</sub>S<sub>3</sub>), Fluka

Indigo Carmine (C<sub>16</sub>H<sub>8</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>S<sub>2</sub>), Fluka

New Coccine (C<sub>20</sub>H<sub>11</sub>N<sub>2</sub>Na<sub>3</sub>O<sub>10</sub>S<sub>3</sub>), Fluka

Sunset Yellow FCF (C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>7</sub>S<sub>2</sub>), Fluka

Fast Green FCF (C<sub>37</sub>H<sub>34</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>10</sub>S<sub>3</sub>), Fluka

Eosin Y (C<sub>20</sub>H<sub>6</sub>Br<sub>4</sub>Na<sub>2</sub>O<sub>5</sub>), Fluka

Erythrosin B (C<sub>20</sub>H<sub>6</sub>I<sub>4</sub>Na<sub>2</sub>O<sub>5</sub>), Fluka

Phloxine B (C<sub>20</sub>H<sub>2</sub>Br<sub>4</sub>C<sub>14</sub>Na<sub>2</sub>O<sub>5</sub>), Fluka

Bengal Rose B (C<sub>20</sub>H<sub>2</sub>Cl<sub>4</sub>I<sub>4</sub>Na<sub>2</sub>O<sub>5</sub>), Fluka

Brilliant Blue (C<sub>37</sub>H<sub>34</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>9</sub>S<sub>3</sub>), Fluka

## CHROMATOGRAPHIC CONDITIONS

Column: Acclaim PA2, 3 μm, 3 × 75 mm (P/N 066277)

Mobile Phase: A) 20 mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> pH 8.8  
B) 50% 20 mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> pH 8.8 in CH<sub>3</sub>CN (v/v)

Flow Rate: 0.709 mL/min

Gradient: 12% B from –3 to 0.00 min, ramp to 100% B in 3.5 min, hold 100% B for 1.0 min and return to 12% B in 0.1 min.

Column Temp.: 30 °C

Inj. Volume: 3 μL

Detection: UV 254 nm and wavelength scanning 200–800 nm

System

Backpressure: 2100 psi

## PREPARATION OF SOLUTIONS AND REAGENTS

### Eluent A [20 mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> pH 8.8]

Weigh 2.64 g di-ammonium hydrogen orthophosphate in a 250 mL beaker. After dissolving with deionized water (used for all eluent and sample preparation), transfer to a 1 L volumetric flask. Add 850 μL of 8 N sodium hydroxide, dilute to 1 L with water, and mix. Filter with a 0.45 μm filter before use.

### Eluent B [50% 20 mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> pH 8.8 in CH<sub>3</sub>CN]

Mix eluent A and CH<sub>3</sub>CN 1:1. Filter with a 0.45 μm filter before use.

## Standard Solutions

All 1000 mg/L stock standard solutions were prepared separately in water and used to prepare four mixtures of the 10 dyes that were the working standards for method calibration. The dye concentrations in the working standard solutions are shown in Table 3.

## Sample Preparation

All samples were purchased from a supermarket in Bangkok, Thailand.

Table 1. Samples		
Sample	Designation	Color
Electrolyte sports drink	1	Yellow
	2	Yellow
	3	Orange
Carbonated drink	1	Orange
	2	Orange
	3	Green
Gelatin dessert	1	Red

The electrolyte sports drinks were filtered with a 0.45 μm filter before analysis. The carbonated drinks were placed in an ultrasonic bath for 5 min to degas, then diluted with water (1:2 for sample 1 and 1:1 for samples 2 and 3), and filtered with a 0.45 μm filter. Then, half a gram of the gelatin dessert was placed in a 25 mL beaker, mixed with 7 mL water, and placed in hot water for 5 min or until it completely dissolved. After cooling to room temperature, the sample was transferred to a 10 mL volumetric flask, and diluted to 10 mL with water.

## RESULTS AND DISCUSSION

Figure 1 shows the separation of a mixture of 10 dyes, including the US FDA-permitted food dyes Tartrazine, Sunset Yellow, Fast Green, and Erythrosine, in less than 5 min. This separation uses an ammonium phosphate/acetonitrile eluent at pH 8.8, a pH value that would pose a problem for most silica-based, reversed-phase columns. The Acclaim PA2 column is tolerant of this high pH. The separation is achieved in less than 5 min by using a 3  $\mu$ m particle size and a 3  $\times$  75 mm column format.

When the food dye Brilliant Blue was added to the standard mixture, complete resolution between Fast Green and Brilliant Blue was not achieved. Though few food samples will contain both dyes, a method for the fast separation of the 10 dyes (listed in Figure 1) and Brilliant Blue using the Acclaim PA column is presented in Figure 2. Similar to the Acclaim PA2 column, the PA column provides a polar-embedded phase that can be used to separate compounds with a wide range of hydrophobicities. Figure 2 shows that Brilliant Blue is resolved from Fast Green (peaks 6 and 7). Because the Acclaim PA column does not have the high pH tolerance of the PA2 column, the eluent pH was lowered to 7.3.

The separation on the Acclaim PA column is also less than 5 min because it uses the 3  $\mu$ m particle size resin and the 3  $\times$  75 mm column format. The eluents used in both the PA and PA2 separations are compatible with MS detection. In both figures, the dyes are detected at 254 nm. Given that both these dyes absorb at other wavelengths, a more selective wavelength can be chosen for each dye. The PA2 separation was used for the rest of the analysis but both methods can be used to analyze the samples. Because the last compound elutes at about 0.5 min earlier on the PA2 column and the resolution between peaks 2 and 3 is better, it is possible to make the PA2 separation slightly faster than the PA separation using the 3  $\times$  75 mm column format, but this was not evaluated.

Spectral scanning was used for the analysis of the standard mix (Figure 1). Table 2 displays the match and PPI values from the spectral scanning. The high match values suggest that the peaks are pure and the peak spectra were loaded in the spectral library for use in identifying dye peaks in samples. Table 2 also displays resolution values, with no resolution values less than 2.8.

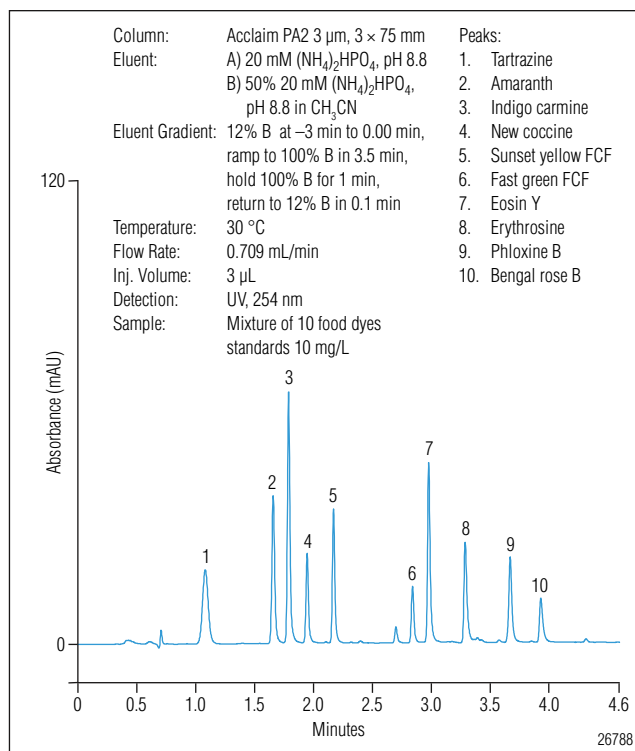


Figure 1. Chromatogram of the standard mixture of 10 dyes.

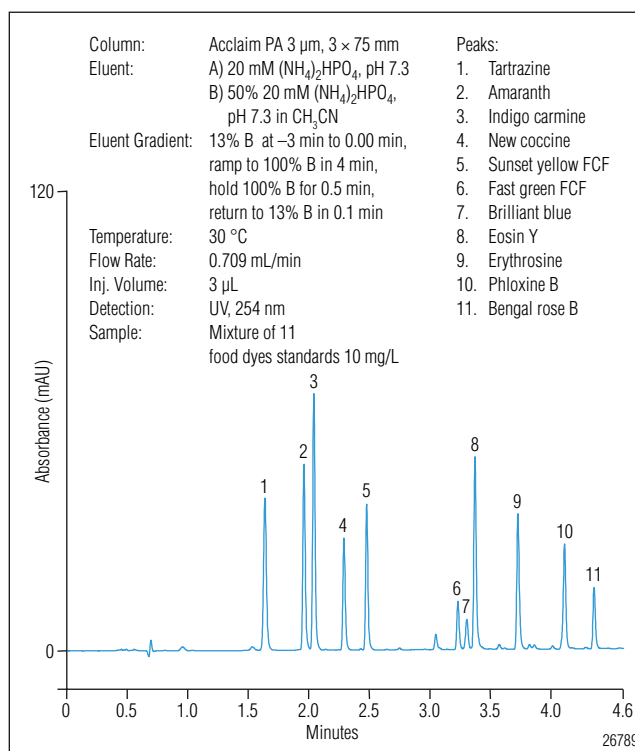


Figure 2. Chromatogram of the standard mixture of 11 dyes.

**Table 2. Resolution and Peak Purity Results**

Component Name	Resolution (USP)	Match	%RSD Match	PPI (nm)	%RSD PPI
Tartrazine	8.69	998	3.55	335.8	1.05
Amaranth	3.26	1000	0.10	318.0	0.03
Indigo Carmine	4.18	999	3.19	360.9	0.88
New Coccine	6.12	1000	0.52	324.5	0.16
Sunset Yellow FCF	5.78	1000	0.25	328.7	0.08
Fast Green FCF	3.53	1000	0.56	563.6	0.10
Eosin Y	7.41	1000	0.32	440.7	0.07
Erythrosin	2.83	1000	0.15	441.8	0.03
Phloxine B	5.46	1000	0.74	437.8	0.17
Bengal Rose B	8.05	1000	0.56	439.2	0.13

### METHOD CALIBRATION

Prior to sample analysis, the method was calibrated for each of the 10 dyes between 1 and 30 mg/L. Four concentrations, 1, 10, 20, and 30 mg/L, were used and the curves were forced through the origin. Table 3 shows that for this range, calibration for each of the 10 dyes was linear.

**Table 3. Standard Calibration Results**

Compound	Calibration Standard Concentration (mg/L)				Cal. Type	Coeff. Det × 100%	Slope
	Level 1	Level 2	Level 3	Level 4			
Tartrazine	1	10	20	30	Lin	99.9970	0.1090
Amaranth	1	10	20	30	Lin	99.9973	0.1067
Indigo Carmine	1	10	20	30	Lin	99.9383	0.1692
New Coccine	1	10	20	30	Lin	99.9969	0.0574
Sunset Yellow FCF	1	10	20	30	Lin	99.9933	0.0811
Fast Green FCF	1	10	20	30	Lin	99.9959	0.0361
Eosin Y	1	10	20	30	Lin	99.9989	0.1197
Erythrosin	1	10	20	30	Lin	99.9991	0.0756
Phloxine B	1	10	20	30	Lin	99.9979	0.0667
Bengal Rose B	1	10	20	30	Lin	99.9921	0.0375

### SAMPLE ANALYSIS

Seven samples were purchased from a local supermarket for analysis. Three different electrolyte sports drinks, two yellow and one orange, were analyzed (Table 1). Three carbonated drinks, two orange and one green, were also analyzed. The seventh sample was a red gelatin dessert. All samples were labeled to contain a dye, but none listed the dye or dyes used. Figures 3–9 show

the chromatography for each sample. Using the spectral library and retention time, the two yellow sports drinks were found to contain Tartrazine (Figures 3 and 4). The samples were found to have similar concentrations of the dye (Table 4). The same approach identified the allowed food dye Sunset Yellow FCF in the orange sports drink (Figure 5). The dye was found in both orange carbonated drinks (Figures 6 and 7). The green carbonated drink contained two dyes, Tartrazine and Fast Green FCF (Figure 8). More importantly, the red dye New Coccine was found in the red gelatin dessert (Figure 9). This dye is banned for use in foods in the United States and some other countries.

All samples were spiked with the standard or standards identified by spectral matching and retention time to assess the accuracy of the determination. Table 4 shows the concentration of each dye in each sample, the spectral match, and the results of the spiking studies. Good recoveries were observed in each sample, suggesting that the method is accurate. Method reproducibility was evaluated by making five injections of each sample and each spiked sample, and concentrations of dyes in each sample were determined. Table 5 shows that the method has good short-term reproducibility.

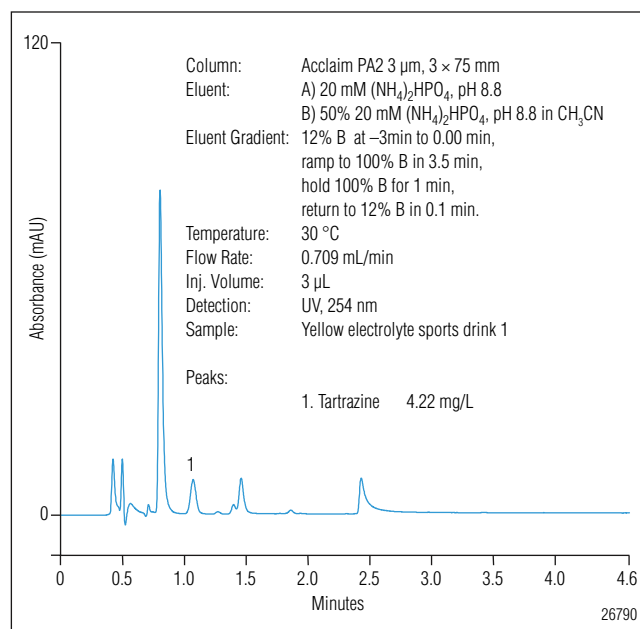


Figure 3. Chromatogram of electrolyte sports drink 1.

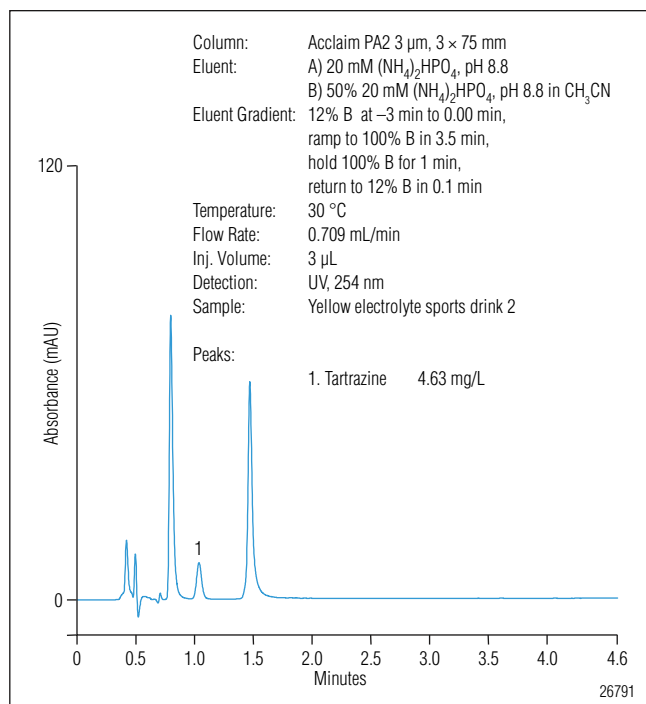


Figure 4. Chromatogram of electrolyte sports drink 2.

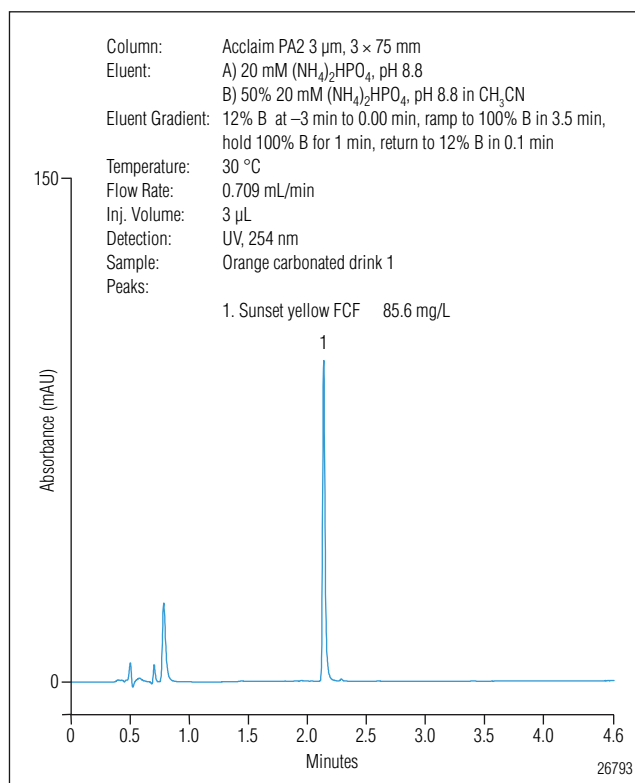


Figure 6. Chromatogram of carbonated drink 1.

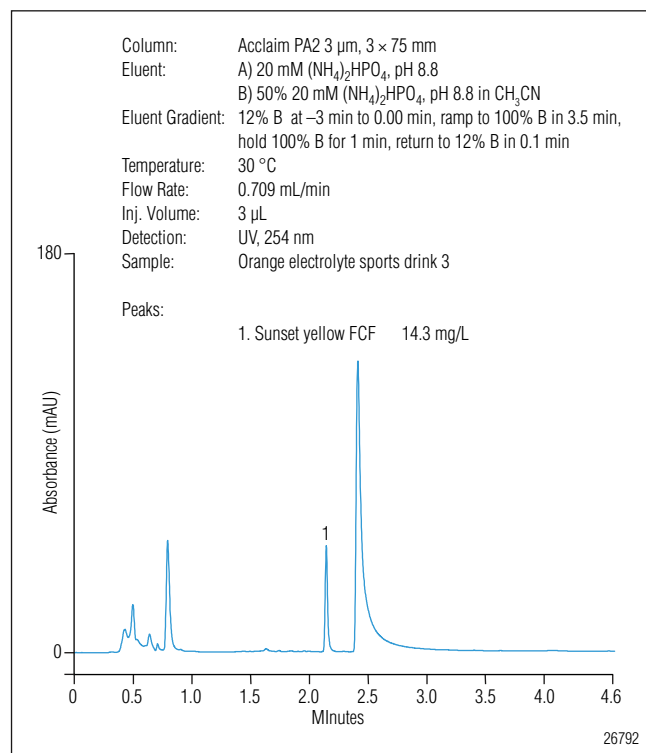


Figure 5. Chromatogram of electrolyte sports drink 3.

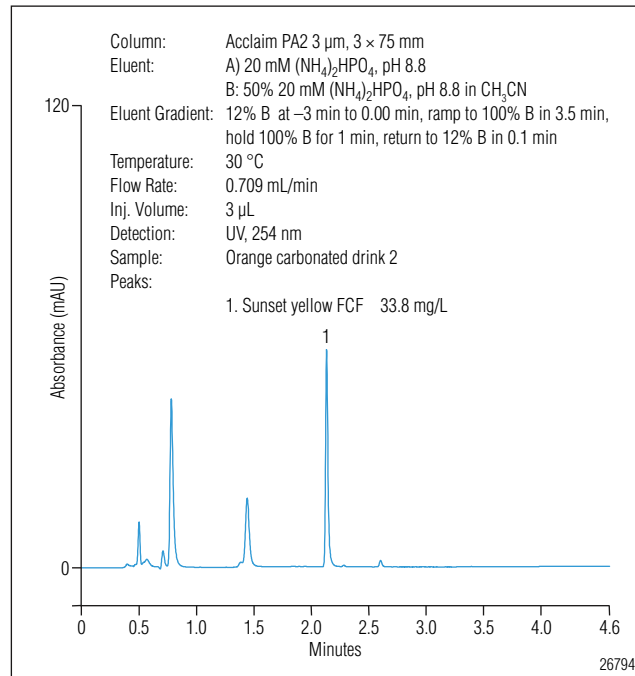


Figure 7. Chromatogram of carbonated drink 2.

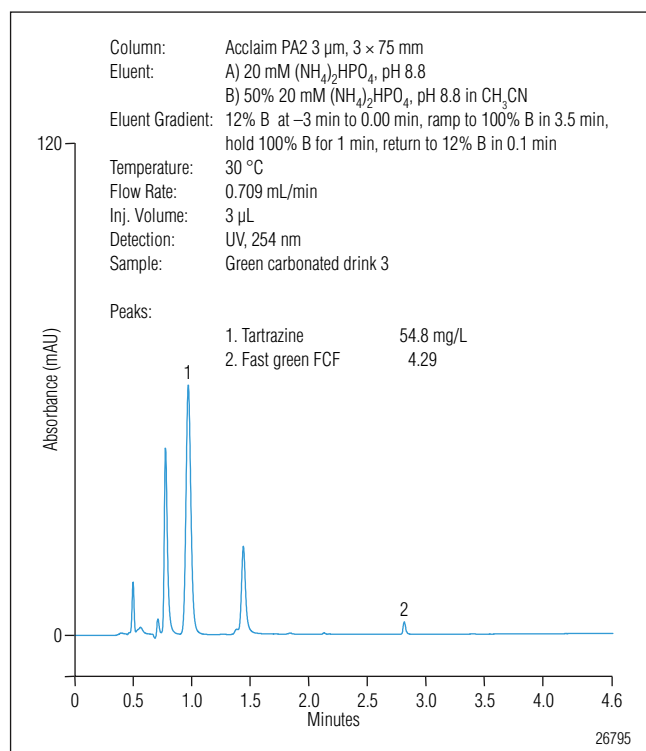


Figure 8. Chromatogram of carbonated drink 3.

### FASTER ANALYSIS

While the method presented in this application note is fast, it is possible to make it faster. Figure 10 shows that by switching to a shorter column with a smaller particle size, it is possible to reduce the separation time from

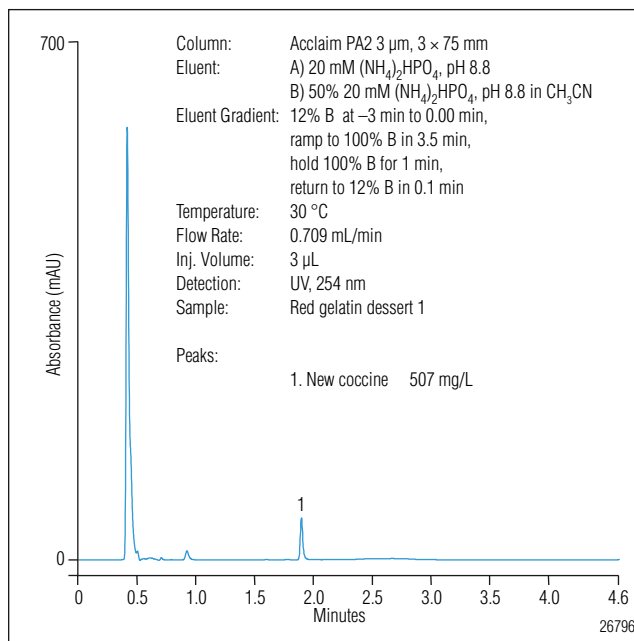


Figure 9. Chromatogram of the red gelatin dessert sample.

4.5 min to 2.5 min while still resolving all 10 dyes. To accomplish this, the injection volume was reduced to 1  $\mu\text{L}$ , the 400  $\mu\text{L}$  static mixer was replaced with a 200  $\mu\text{L}$  static mixer (P/N 6040.5150), and the flow cell was changed to a semi-micro 2.5  $\mu\text{L}$  flow cell. The data collection rate was also set to 25 Hz and the response time to 0.5 s. The backpressure of this separation was 3150 psi.

Table 4. Sample and Recovery Results

Sample	Color	Dye Spiked into the Sample	Spiked Conc. (mg/L) Added to Sample	Average Dye Concentration in Sample (mg/L)	Average Dye Concentration in Spiked Sample (mg/L)	%Recovery	Peak Purity Match	Match with Spectra Library
Electrolyte sports drink 1	Yellow	Tartrazine	1	4.22	5.18	96.0	997	996
Electrolyte sports drink 2	Yellow	Tartrazine	1	4.63	5.57	94.0	999	996
Electrolyte sports drink 3	Orange	Sunset yellow FCF	4	14.3	18.3	100	1000	1000
Carbonated drink 1	Orange	Sunset yellow FCF	10	85.6	93.8	82	1000	1000
Carbonated drink 2	Orange	Sunset yellow FCF	10	33.8	43.3	95.0	1000	1000
Carbonated drink 3	Green	Tartrazine	10	54.8	63.1	83.0	1000	997
Carbonated drink 3		Fast Green FCF	2	4.29	6.12	91.5	1000	999
Gelatin dessert	Red	New Coccine	40	507	545	95.0	1000	1000

**Table 5. Reproducibility of Five Injections of Samples and Spiked Samples**

Sample	Color	Dyes Found in Samples and Spiked Samples	Concentrations Found in Samples and Spiked Samples (mg/L)					RSD
			Injection #					
			1	2	3	4	5	
Electrolyte sports drink 1	Yellow	Tartrazine in sample	4.20	4.27	4.21	4.21	4.21	0.72
		Tartrazine in spiked sample	5.19	5.16	5.20	5.17	5.17	0.31
Electrolyte sports drink 2	Yellow	Tartrazine in sample	4.62	4.64	4.63	4.62	4.62	0.17
		Tartrazine in spiked sample	5.58	5.56	5.56	5.57	5.56	0.17
Electrolyte sports drink 3	Orange	Sunset yellow FCF in sample	14.3	14.3	14.3	14.3	14.3	0.13
		Sunset yellow FCF in spiked sample	18.3	18.3	18.3	18.3	18.3	0.11
Carbonated drink 1	Orange	Sunset yellow FCF in sample	85.6	85.7	85.6	85.7	85.5	0.10
		Sunset yellow FCF in spiked sample	93.7	93.8	94.0	93.7	93.8	0.14
Carbonated drink 2	Orange	Sunset yellow FCF in sample	33.8	33.8	33.7	33.7	33.8	0.13
		Sunset yellow FCF in spiked sample	43.3	43.3	43.3	43.2	43.3	0.10
Carbonated drink 3	Green	Tartrazine in sample	54.8	54.8	54.8	54.7	54.8	0.10
		Tartrazine in spiked sample	63.0	63.1	63.0	63.3	63.1	0.19
		Fast Green FCF in sample	4.29	4.33	4.32	4.27	4.23	0.95
		Fast Green FCF in spiked sample	6.09	6.14	6.12	6.15	6.12	0.39
Gelatin dessert	Red	New Coccine in sample	507	506	507	507	507	0.08
		New Coccine in spiked sample	546	546	544	543	545	0.23

Note: The results were multiplied by the appropriate dilution factor in the Chromeleon software.

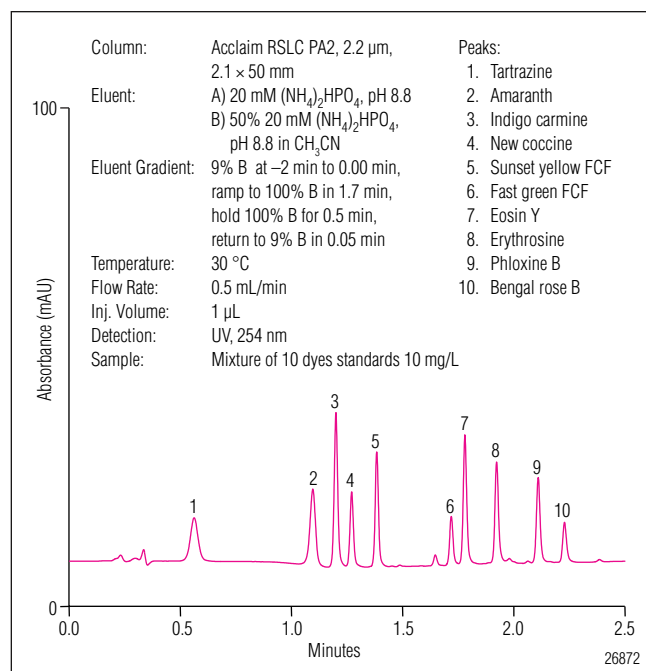


Figure 10. Faster separation of the 10 dyes standard.

## CONCLUSION

This application note presents a fast HPLC method (< 5 min) for the accurate determination of dyes in food and beverage samples. This method can be used to quantify permitted dyes and identify illegal dyes in food and beverage samples.

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