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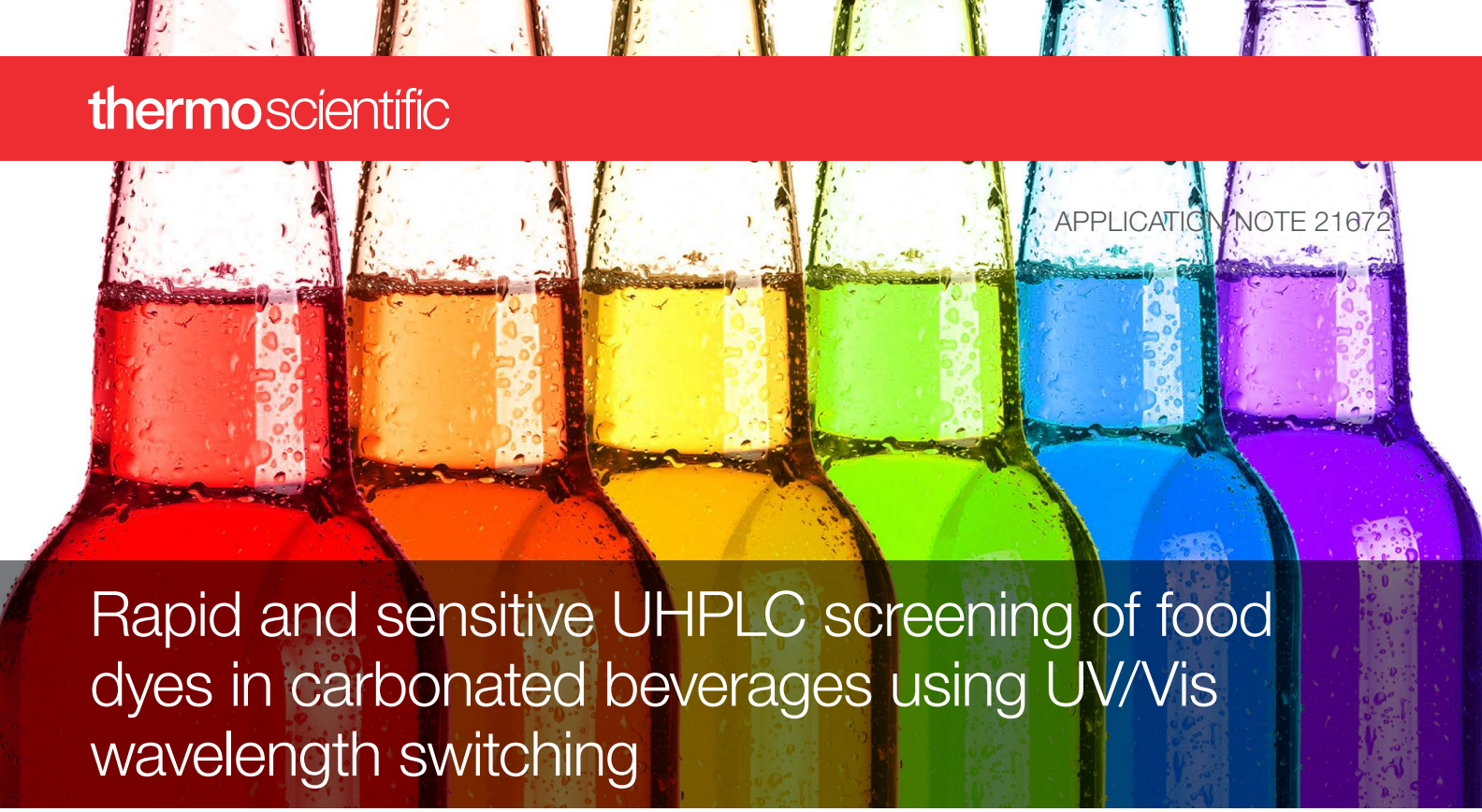
APPLICATION NOTE 21672



Rapid and sensitive UHPLC screening of food dyes in carbonated beverages using UV/Vis wavelength switching

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Keywords

Vanquish Flex, Hypersil GOLD VANQUISH C18, Rapid Analysis, Food Dyes, Beverage Analysis, UHPLC, Tartrazine, Amaranth, Indigo Carmine, New Coccine, Sunset Yellow FCF, Fast Green FCF, Eosin Y, Erythrosine, Phloxine B, Bengal Rose B

Goal

- To demonstrate the capability of the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 column and Thermo Scientific™ Vanquish™ Flex Binary UHPLC system combination for the rapid separation of dyes in carbonated beverages with excellent linearity, reproducibility, and recoveries.
- To show the capability of the Vanquish Flex Binary UHPLC system to support fast UHPLC methods with excellent performance.
- To demonstrate the capability of the Vanquish Flex Binary UHPLC system to enhance method sensitivity with the use of wavelength switching.

Introduction

The analysis of food dyes in carbonated beverages is important as many of these dyes are either controlled substances or are being phased out in certain countries due to their reported adverse health effects. Being able to identify and quantify food dyes in beverages quickly and with high sensitivity is therefore important.

Reversed-phase chromatography is an excellent technique for the analysis of dyes. Many dyes are readily soluble in reversed-phase eluents and have strong visible and UV absorbance properties. This method demonstrates the

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separation of 10 contentious dyes that can be found in soft drinks by UHPLC with UV detection with and without wavelength switching to maximize method sensitivity.

The Hypersil GOLD VANQUISH 1.9 µm column family includes high-performance, reversed-phase columns with excellent resolution, efficiency, and sensitivity. These columns feature a patented surface chemistry that incorporates endcapped, ultra-pure silica-based columns providing significant reduction in peak tailing while retaining C18 selectivity. The Vanquish Flex Binary UHPLC system allows the user the method speed expected from a binary high-pressure mixing pump.

Experimental

Consumables and apparatus

- Hypersil GOLD VANQUISH C18 column, 50 mm × 2.1 mm x 1.9 µm (P/N 25002-052130-V)
- 18 MΩ water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific Ammonium phosphate bibasic (P/N 10509263)
- Fisher Scientific Potassium hydroxide pellets (P/N 10575355)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)
- Thermo Scientific™ 30 mm Target 2™ 0.45 µm Nylon Syringe Filter (P/N F2500-1)

All standards were purchased from a reputable supplier.

Instrumentation

Analyses were performed using a Vanquish Flex Binary UHPLC System consisting of:

- Binary Pump F (P/N VF-P10-A-01)
- System Base Vanquish Flex (P/N VF-S01-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ LightPipe™ flow cell, 2 µL, 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ vial identification system (P/N 60180-VT-100)

Software

Thermo Scientific™ Chromeleon™ 7.2 SR4 MUb (8525)

Standard preparation

Solutions of the 10 dyes shown in Table 2 were prepared by dissolving the solid compound in water to produce 1 mg/mL primary solutions. A mixed working standard solution containing all compounds was then prepared in deionized water at 100 µg/mL.

Linearity preparation

Mixed calibration standards were prepared in water covering the concentration range of 1–30 µg/mL to assess method linearity.

Sample preparation

The carbonated sports drinks were decanted into a suitable container and placed in an ultrasonic bath for 5 minutes to degas, diluted 1 in 10 volumetrically with water, then filtered through a 0.45 µm nylon syringe filter. Aliquots (950 µL) of each diluted sample were spiked with 50 µL of the mixed working standard solution. Samples were prepared as above, however, these were spiked with the same amount of deionized water instead of the mixed working standard. Vial labelling was supported by the Virtuoso vial identification system.

All samples were sourced from a local supermarket (Table 1).

Table 1. Sample identification.

Sample	Drink	Color
Carbonated drinks	1	Bright Yellow
	2	Bright Orange
	3	Bright Red

UHPLC conditions

HPLC column:	Hypersil Gold VANQUISH C18 50 mm × 2.1 mm × 1.9 µm		
Mobile phase A:	20 mM $(NH_4)_2HPO_4$, pH 8.8 in water adjusted with 8N NaOH		
Mobile phase B:	20 mM $(NH_4)_2HPO_4$, pH 8.8 / acetonitrile (50:50 v/v)		
Gradient conditions:	Time (min)	A%	B%
	-3.0	95.0	5.0
	0.0	95.0	5.0
	1.5	0.0	100.0
	2.2	0.0	100.0
	2.3	95.0	5.0
Flow rate:	0.55 mL/min		
Column temperature:	40 °C (still air mode)		
Preheater temperature:	40 °C		
Injection volume:	1 µL		
UV detection:	254 nm and 3D Field		
Backpressure:	Approximately 340 bar maximum		
Gradient mixer:	200 µL		

Results and discussion

Full resolution of all 10 dyes (>1.5 EP resolution) was achieved in approximately two minutes on the Vanquish Flex Binary UHPLC system using a Hypersil GOLD VANQUISH C18 column (Figure 1).

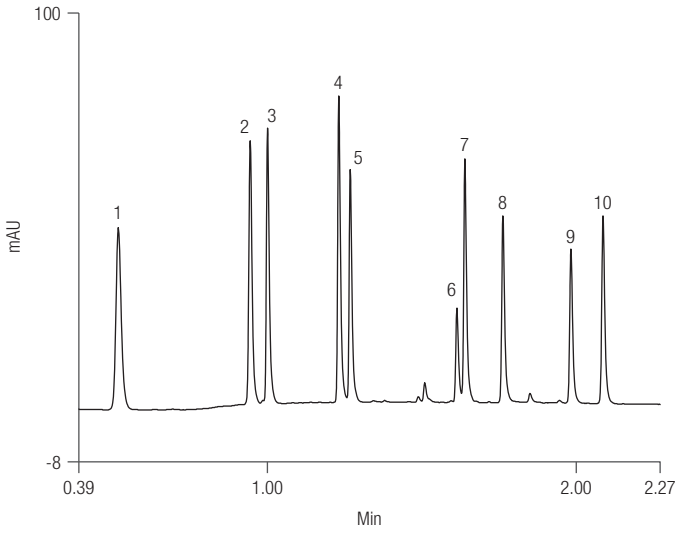

Figure 1. Chromatogram showing the separation of 10 dyes in a 10 µg/mL calibration standard on the Vanquish Flex Binary UHPLC system.

Method reproducibility

Excellent method reproducibility for retention time, peak area, and peak width at half height was achieved for all dyes (n=6) at 5 µg/mL (Table 2).

Table 2. Comparison of % RSD of retention time (RT), peak area, and peak width at half height for 10 dyes in a 5 µg/mL mixed calibration standard.

Compound	Peak Number	% RSD (n=6)		
		RT	Area	Width at Half Height
Tartrazine	1	0.240	0.126	0.299
Amaranth	2	0.084	0.307	0.507
Indigo carmine	3	0.044	1.958	0.500
New coccine	4	0.033	0.197	0.116
Sunset yellow	5	0.032	0.170	0.156
Fast green FCF	6	0.022	0.143	0.130
Eosin Y	7	0.030	0.237	0.063
Erythrosine	8	0.020	0.311	0.154
Phloxine B	9	0.023	0.170	0.107
Bengal rose	10	0.030	0.313	0.140

Method linearity

All calibration curve R^2 values were found to be between 0.9997 and 1.0000 (Figure 2).

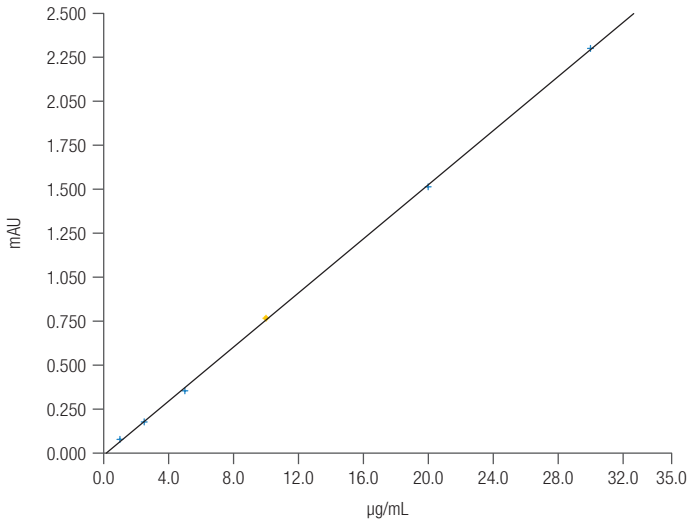

Figure 2. Correlation coefficient (R^2) for indigo carmine is 0.9997.

Retention time comparison of the chromatographic peaks in the three soft drinks found that two of them contained the dyes new coccine and sunset yellow FCF (Table 3, Figure 3). This result is of interest to food testing laboratories when screening for non-approved food colorings as new coccine is only approved for use in certain geographical markets.

Table 3. Concentration of dyes in µg/mL found in soft drinks.

Drink	Color	Dye Concentration µg/mL	
		New Coccine	Sunset Yellow FCF
1	Bright Yellow	1.9	79.8
2	Bright Orange	2.8	97.7

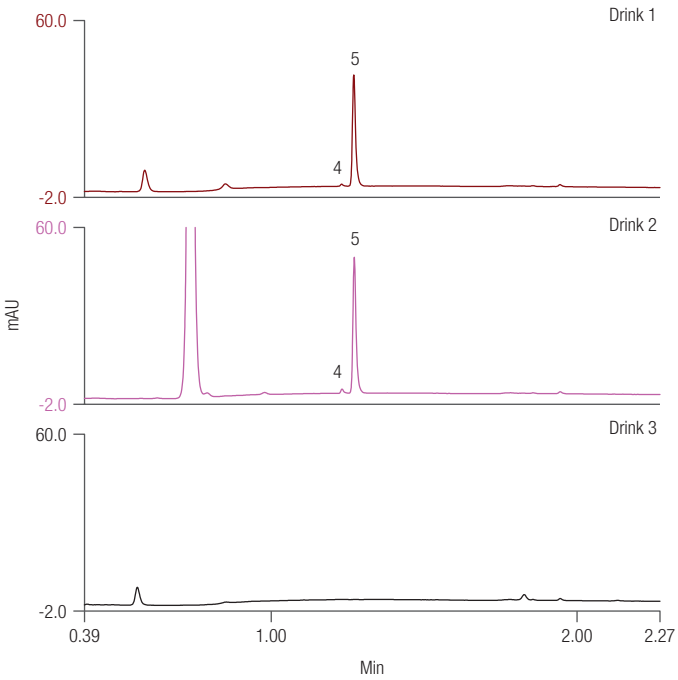

Figure 3. Chromatogram of three carbonated drinks.

Excellent spiked recoveries were observed in each sample for all 10 dyes. All recoveries were ≥80% with the majority being ≥95%, which shows the method has minimal matrix interference (Figure 4).

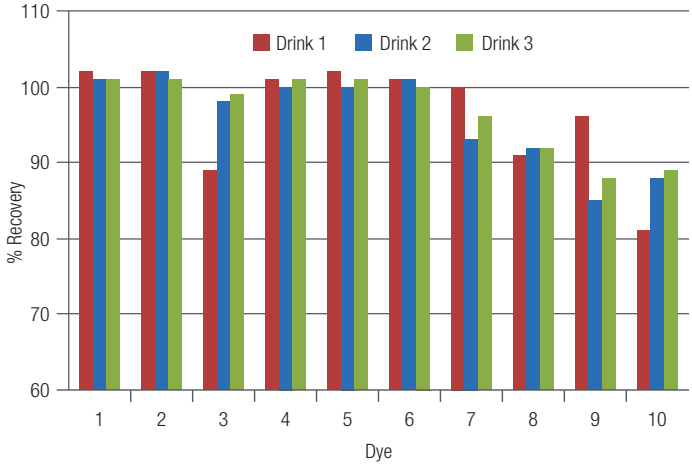

Figure 4. % recoveries of 10 dyes from three sample solutions.

Wavelength switching

To improve method sensitivity, a feature of the software called wavelength switching was utilized. By using data from 3D field analysis, the λ_{MAX} for each compound was determined (Figure 5, bottom trace). The difference in peak height seen in the chromatogram is a result of selecting a more highly absorbing wavelength of light specific to a particular component's λ_{MAX}, leading to a significant increase in response.

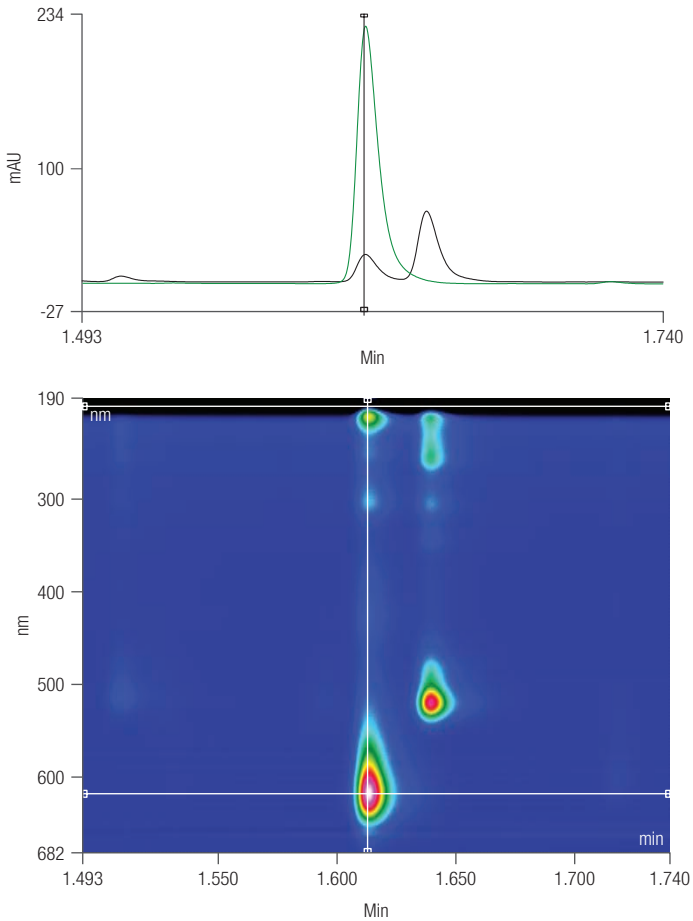

Figure 5. Chromatogram of a 10 µg/mL mixed calibration standard (top) using 3D field and corresponding contour plot (bottom), with Fast green FCF highlighted in the cross-hairs.

The optimum λ_{MAX} values were determined for all components and relative time windows encompassing the peaks were chosen. Table 4 was created and used within the method for UV/Vis switching; all other method parameters remained the same.

Table 4. Showing UV/Vis selected wavelengths and corresponding retention time windows.

Time (min)	Wavelength (nm)
0.000	426
0.690	217
0.980	287
1.125	217
1.250	235
1.425	620
1.628	520
1.700	529
1.875	544
2.050	554

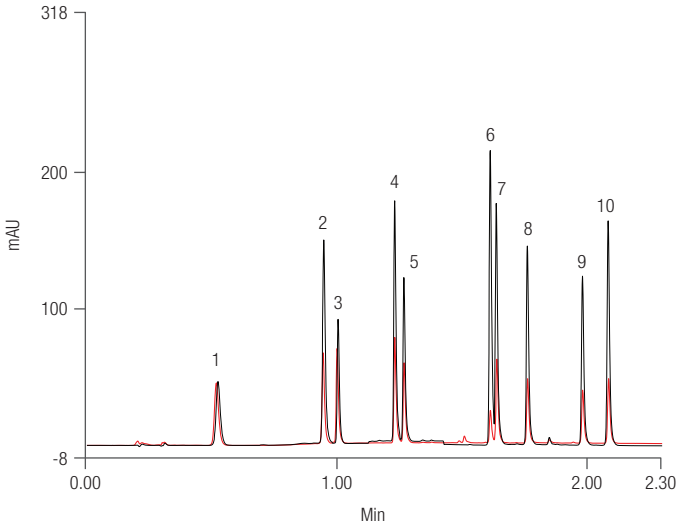

Figure 6. Chromatogram showing the separation of 10 dyes on the Vanquish Flex Binary UHPLC system, with wavelength switching (black trace) and without switching (red trace).

The peak heights and response were significantly higher for many components using wavelength switching (Figure 7). A significant enhancement in method sensitivity can be achieved by utilizing this feature of the software, which can increase the signal-to-noise ratio by a factor of nine in the case of fast green FCF.

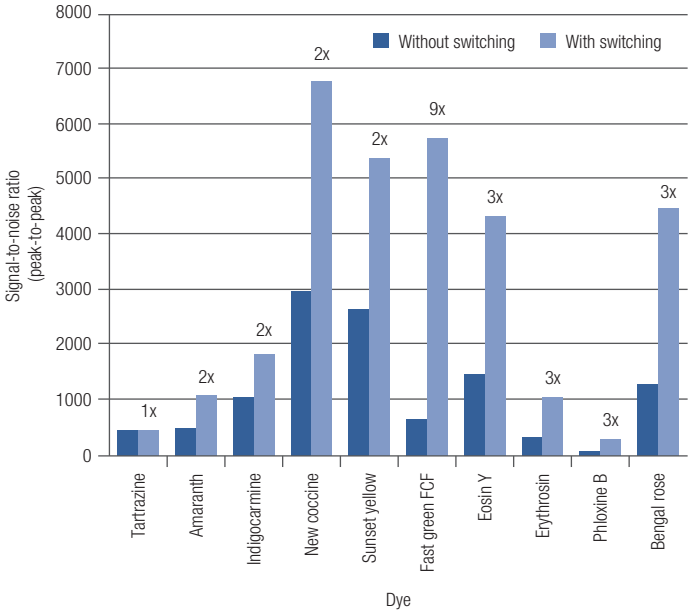

Figure 7. Method sensitivity enhancement for 10 dyes in a 10 µg/mL mixed calibration standard with wavelength switching.

Conclusions

This application demonstrates the following:

- The capability of the Hypersil GOLD VANQUISH C18 column and Vanquish Flex Binary UHPLC system combination for the rapid separation of dyes in carbonated beverages with excellent linearity, reproducibility, and recoveries.
- The capability of the Vanquish Flex Binary UHPLC system to provide excellent retention time precision through new pump features.
- The use of wavelength switching on the Vanquish Flex Binary UHPLC system enhances method sensitivity by up to an order of magnitude.

Find out more at **thermofisher.com/VANQUISHcolumn**

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