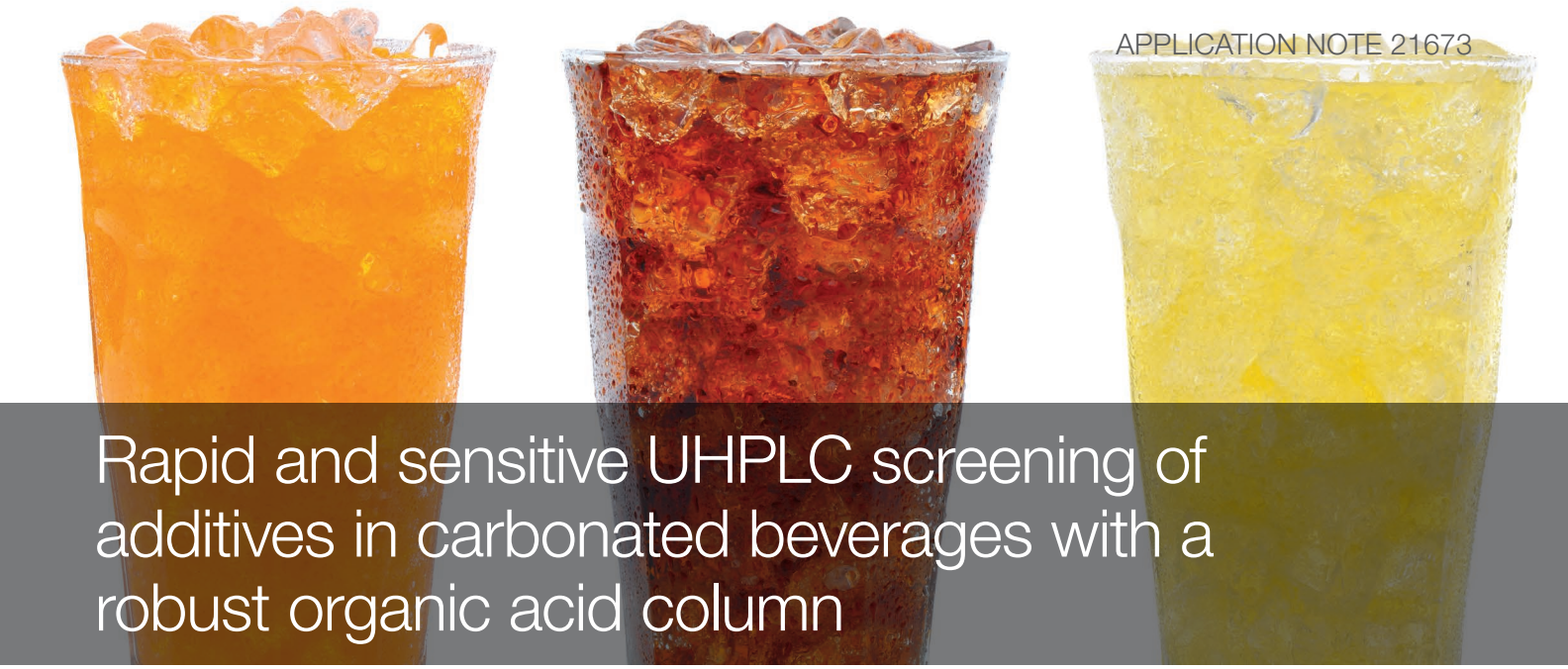
thermo scientific



APPLICATION NOTE 21673

Rapid and sensitive UHPLC screening of additives in carbonated beverages with a robust organic acid column

Authors

Aaron Lamb and Brian King,
Thermo Fisher Scientific,
Runcorn, UK

Keywords

Vanquish Flex, Acclaim Organic Acid, OA, Rapid Analysis, Food Additives, Beverage Analysis, UHPLC

Goal

- To demonstrate the capability of the Thermo Scientific™ Acclaim™ Organic Acid (OA) column and the Thermo Scientific™ Vanquish™ Flex Binary UHPLC system in combination for the rapid separation of additives in carbonated beverages with excellent linearity, reproducibility, and recoveries.
- To show the capability of the Vanquish Flex Binary system to provide excellent retention time precision.

Introduction

The analysis of food additives in carbonated beverages is important as many additives are controlled as part of the formulations of these beverages. In addition, analysis may detect possible counterfeiting of branded products. Being able to identify and quantify food additives in beverages quickly and with high sensitivity is therefore important.

Reversed-phase chromatography is an excellent technique for the analysis of food additives. Many food additives are readily soluble in reversed-phase eluents and have strong visible and UV absorbance properties in the region of 210 nm to 280 nm. This method demonstrates the separation of seven common food additives that can be found in soft drinks by UHPLC with UV detection.

ThermoFisher SCIENTIFIC

The Acclaim Organic Acid (OA) column used can operate in high salt and low pH mobile phases. Acclaim OA columns were developed for the reversed-phase separation of hydrophilic aliphatic and aromatic organic acids with UV detection. The Acclaim OA columns are use-tested to ensure optimum, controlled separations. This column uses a patented polar embedded bonding chemistry that offers stable bonding, which is very resistant to hydrolysis. The Acclaim OA column was chosen due to this ability to work with low pH and high salt buffer conditions.

The Vanquish Flex Binary UHPLC system allows the user the method speed expected from a binary high-pressure mixing pump. A shallow gradient was used to give elution in less than six minutes with time including re-equilibration of 10 minutes. The critical resolution between sorbate and benzoate had been found to limit the application in isocratic mode but steps were taken to establish an Rs value of >2.5 for this method.

Experimental

Consumables and apparatus

- Acclaim Organic Acid column, 150 mm x 2.1 mm x 3 µm (P/N 070087)
- 18 MΩ•cm water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific Potassium phosphate dibasic (P/N BP3631)
- Fisher Scientific Methanesulfonic acid (MSA) (P/N 125612500)
- 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 UHPLC Binary 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-VT100)

Software

Thermo Scientific™ Chromeleon™ 7.2 SR4 MUb (8525)

Sample preparation

Solutions of the seven food additives shown in Table 1 were prepared by dissolving the solid compound in water to produce primary solutions. A mixed stock solution containing all compounds at a level of 20 times the top linearity standards was then prepared in 50 mL of deionized water at the levels below (Table 1).

Table 1. Primary solutions and mixed stock standard.

Analyte	Primary Solutions (mg/L)	Mixed Stock Standard (mg/L)
Citrate	120,000	12,000
Acesulfame	40,000	4000
Saccharin	12,000	1200
Caffeine	20,000	400
Aspartame	6,000	1200
Sorbate	20,000	2000
Benzoate	20,000	2000

Linearity preparation

Mixed calibration standards were prepared in water from the mixed stock standard covering the concentration range shown in µg/mL to assess method linearity.

Table 2. Concentrations of mixed working standard solutions.

Analyte	Concentration (µg/mL)								
	#1	#2	#3	#4	#5	#6	#7	#8	#9
Citrate	60.0	75.0	100.0	120.0	150.0	200.0	300.0	600.0	
Acesulfame	20.0	25.0	33.3	40.0	50.0	66.7	100.0	200.0	
Saccharin	6.0	7.5	10.0	12.0	15.0	20.0	30.0	60.0	
Caffeine	2.0	2.5	3.3	4.0	5.0	6.7	10.0	20.0	70.0
Aspartame	6.0	7.5	10.0	12.0	15.0	20.0	30.0	60.0	
Sorbate	10.0	12.5	16.7	20.0	25.0	33.3	50.0	100.0	
Benzoate	10.0	12.5	16.7	20.0	25.0	33.3	50.0	100.0	

Sample preparation

The carbonated sports drinks were placed in an ultrasonic bath for five minutes to degas, diluted 1 in 10 or 1 in 20 in water, then filtered through a 0.45 µm nylon syringe filter. Spiked and unspiked sample solutions were then prepared at the concentration level of linearity standard three and these were used to assess analyte recoveries. Vial labelling was supported by the Virtuoso vial identification system.

All samples were sourced from a local supermarket (Table 3).

Table 3. Sample identification.

Sample	Drink	Type
Carbonated drinks	A	Cola
	B	Orange Soda
	C	Energy Drink

UHPLC conditions

HPLC column:	Acclaim Organic Acid, 3 µm × 150 mm × 2.1 mm		
Mobile phase A:	100 mM KH_2PO_4 pH 3 adjusted with MSA		
Mobile phase B:	Acetonitrile		
Gradient conditions:	Time (min)	A%	B%
	0	95	5
	5	75	25
	7	75	25
	7.1	95	5
	10	End	
Flow rate:	0.6 mL/min		
Column temperature:	50 °C (still air mode)		
Preheater temperature:	50 °C		
Injection volume:	5 µL		
UV detection:	210 nm		
Backpressure:	Approximately 340 bar maximum		
Mobile phase mixer:	50 µL capillary + 150 µL static		

Results and discussion

Full resolution of all seven food additives (>1.5 EP resolution) was achieved within approximately six minutes on the Vanquish Flex Binary system, using the Acclaim OA column (Figure 1).

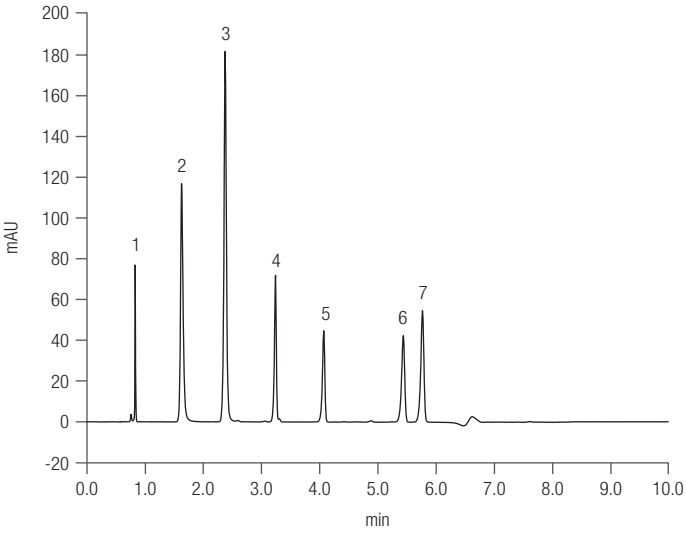


Figure 1. Chromatogram showing the separation of seven food additives in a linearity calibration standard on the Vanquish Flex Binary system.

Method reproducibility

Excellent method reproducibility for retention time and peak area was achieved for all food additives (n=9) at level 3 calibration (Table 4).

Table 4. Comparison of % RSD of retention time (RT) and peak area for seven food additives in a mixed calibration standard.

Compound	Peak Number	% RSD	
		RT	Area
Citrate	1	0.056	0.225
Acesulfame	2	0.080	0.104
Saccharin	3	0.023	0.215
Caffeine	4	0.032	0.166
Aspartame	5	0.020	0.410
Sorbate	6	0.010	0.459
Benzoate	7	0.012	0.518

Method linearity

Six calibration curves R^2 values were found to be between 0.9991 and 0.9999 (Figure 2, Table 5). Citrate was lower with 0.9953 and retained linearity between Level #1 and #6.

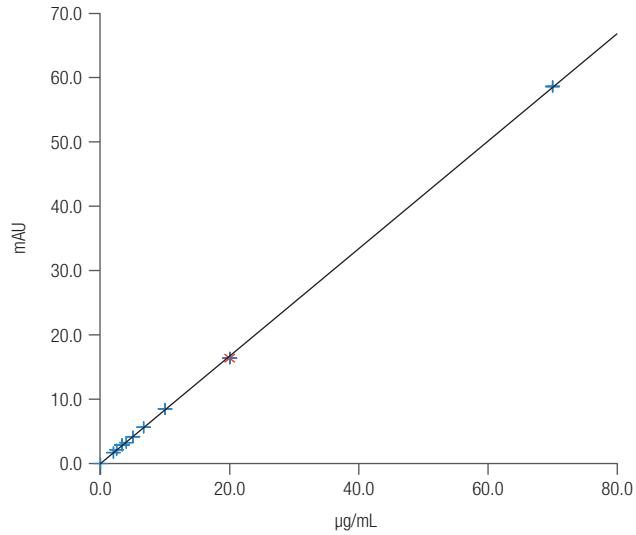


Figure 2. Correlation coefficient (R^2) for caffeine is 0.9999.

Table 5. Calibration data.

Peak Number	Peak Name	Ret.Time (min)	Correlation Coefficient R^2	Lower Linearity Std. (µg/mL)	Higher Linearity Std. (µg/mL)
1	Citrate	0.831	0.9957	60	200
2	Acesulfame	1.630	0.9995	20	200
3	Saccharin	2.378	0.9995	6	60
4	Caffeine	3.242	0.9999	2	20
5	Aspartame	4.070	0.9991	6	60
6	Sorbate	5.440	0.9994	10	100
7	Benzoate	5.771	0.9995	10	100
Maximum			0.9999		
Minimum			0.9957		

Retention time comparison of the chromatographic peaks in the three soft drinks identified citrate, acelsulfame, caffeine, and aspartame as identified additives in diluted samples (Table 6, Figure 3).

Table 6. Concentrations of food additives in µg/mL found in soft drinks adjusted for dilution.

Peak Number	Diluted	Composition (µg/mL)		
		Brand A (Dilution x20)	Brand B (Dilution x20)	Brand C (Dilution x10)
1	Citrate	63	3251	2076
2	Acesulfame	ND	123	ND
3	Saccharin	ND	ND	ND
4	Caffeine	94	ND	121
5	Aspartame	ND	ND	ND
6	Sorbate	ND	216	136
7	Benzoate	ND	ND	ND

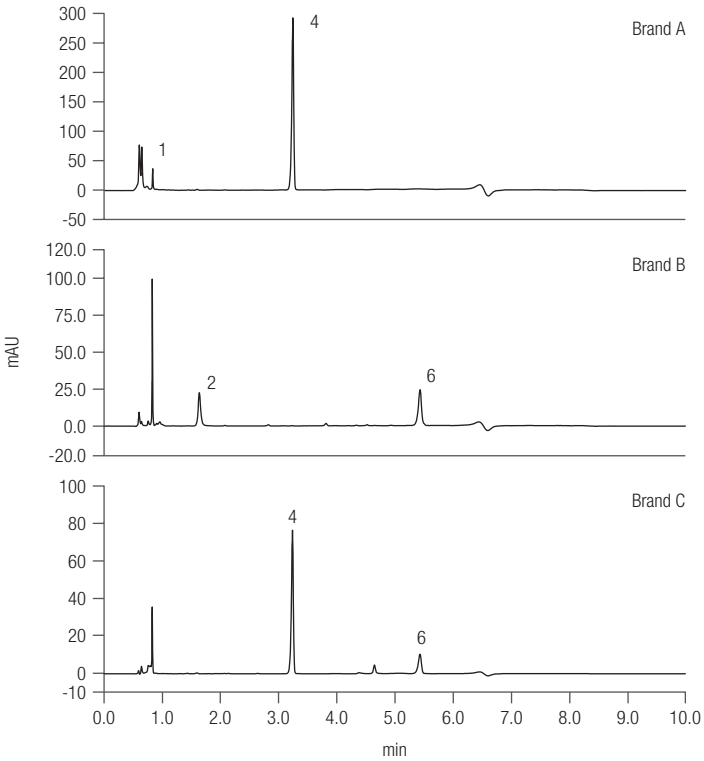


Figure 3. Chromatograms of three carbonated drinks.

Method recovery

Excellent spiked recoveries were observed in each sample for all seven food additives. All recoveries were ≥93% with the majority being ≥98 % which shows the method has minimal matrix interference. The citrate recovery was impacted by its early elution (Figure 4).

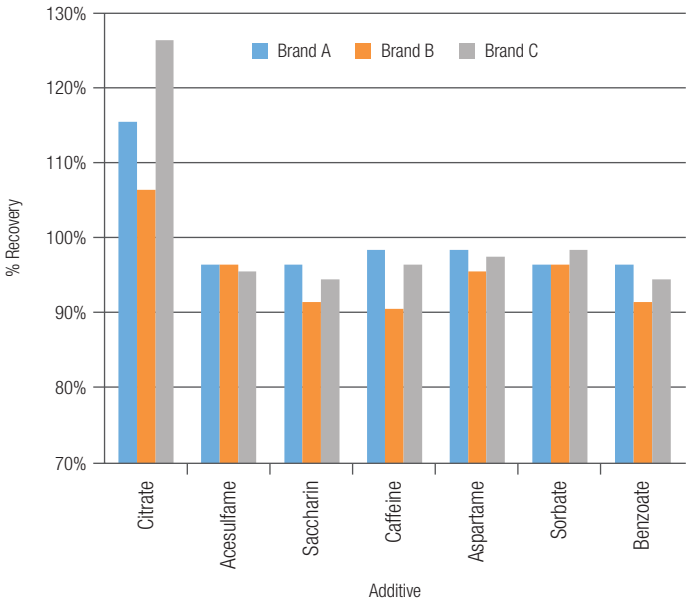


Figure 4. % recoveries of seven food additives from three sample solutions.

Conclusions

This application demonstrates the following:

- The capability of the Acclaim OA column and Vanquish Flex Binary UHPLC system in combination to give rapid separation of food additives in carbonated beverage with excellent linearity, reproducibility, and recoveries.
- The capability of the Vanquish Flex Binary system to provide excellent retention time precision through its injection control and binary pump proportioning with innovative mixing units.

Find out more at **thermofisher.com/LC-columns**

ThermoFisher SCIENTIFIC