Food and beverages

Rapid and sensitive quantitative method for screening of additives in beverages

Authors

Jérôme Cotton[1], Jean-Charles Hagnere[1], Elodie Thiel[1], Charles Kentzinger[1], Sonal Sharma[2], Tristan Bernard[2], Maryline Carvalho[3]

[1]Nestlé Quality Assurance Center, Vittel, France

[2]Thermo Fisher Scientific, Villebon-sur-Yvette, France

[3]Thermo Fisher Scientific, Customer Solution Centre, Villebon-sur-Yvette, France

Keywords

Vanquish Binary Pump Flex, Vanquish Charged Aerosol Detector, Vanquish Diode Array Detector, Chromeleon CDS, Hypersil GOLD C18 column, quality control, beverages, additives, sweeteners, preservatives, caffeine

Workflow benefits

- Simplifies additives control workflow using a single quantitative screening analysis
- Maximizes lab responsiveness and throughput with global method approach
- Reduces sample handling time and risk of preparation errors using Thermo Scientific™ Vanquish™ Split Sampler programming for automatic calibration curves generation
- Improves confidence in results with automation

Goal

- To develop a unique analytical method enabling the simultaneous determination of additives from different functional classes in beverages
- To demonstrate the method accuracy and precision by testing multiple soft drink types and running an interlaboratory proficiency test

Introduction

Additives analysis in the food industry is essential to ensure product quality and verify that food and beverage products comply with regulations and labelling.

High performance liquid chromatography (HPLC) is widely employed as an analytical technique in food control laboratories and is commonly used with cost-effective optical detection. While many food additives have strong ultraviolet (UV) and visible absorbance properties, some others are missing chromophores and require alternative detection to be identified and measured. The Thermo Scientific™ Charged Aerosol Detector (CAD) delivers universal detection of non- and many semi-volatile compounds, independently of the analytes' chemical structure, making it an ideal complementary detector for routine control.

A new HPLC analytical strategy combining the advantages of specific diode array detection and universal charged aerosol detection was evaluated to determine multi-class food additives content in a variety of soft drinks samples. Ten of the most common additives covering three main classes (sweeteners, preservatives, stimulants) were targeted.

Experimental

Instrumentation

Analyses were performed using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system consisting of:

- System Base Vanquish Horizon/Flex (P/N VF-S01-A-02)
- Vanquish Binary Pump F (P/N VF-P10-A-01)
- 150 µL Static Mixer, Vanquish H/F Pumps (P/N 6044.5110)
- Vanquish Split Sampler FT (P/N VF-A10-A-02)
- Sample loop, 25 µL, biocompatible (P/N 6850.1911)
- Vanquish Column Compartment H (P/N VH-C10-A-03)
- Passive Pre-heater, MP35N, 1 µL, 0.10 × 530 mm (P/N 6732.0174)
- Vanquish Diode Array Detector FG (P/N VF-D11-A-01)
- Flow cell, path length 10 mm (13 µL, SST) (P/N 6083.0510)
- Vanquish Charged Aerosol Detector F (P/N VF-D20-A)
- Thermo Scientific™ Hypersil GOLD™ C18 HPLC Column 4.6 × 250 mm, 5 µm (P/N 25005-254630)
- Vanquish Column ID Tag (P/N 6732.0610)

Standards and samples preparation

Standards

Individual solutions of the ten additives were prepared by dissolving the neat powder to produce primary solutions at the concentration level shown in Table 1. Compound solubility challenges were observed, and solvent composition was optimized for each target compound.

Table 1. Stock standard solutions preparation

Compound name	CAS number	Class	Primary solution concentration (g/L)	Solvent
Acesulfame (ACE)	33665-90-6	Sweetener	20	H_2O
Aspartame (ASP)	22839-47-0	Sweetener	1	H_2O + 0,1% FA
Benzoate (BEN)	766-76-7	Preservative	20	H_2O
Caffeine (CAF)	58-08-2	Stimulant	5	H_2O
Cyclamate (CYC)	100-88-9	Sweetener	20	H_2O
Neohesperidine dihydrochalcone (NHDC)	20702-77-6	Sweetener	20	MeOH + 1% FA
Neotame (NEOT)	165450-17-9	Sweetener	20	MeOH
Saccharin (SAC)	81-07-2	Sweetener	20	H_2O + 1% 10N NAOH
Sorbate (SOR)	72138-88-6	Preservative	20	H_2O
Sucralose (SUC)	56038-13-2	Sweetener	20	H_2O

FA: formic acid; H_2O: water; MeOH: methanol; NaOH: sodium hydroxide

Even if all targeted compounds could be analyzed in a single analysis, dedicated sample batches (with a subset of the targeted compounds) were routinely used to reflect the most frequent sample analysis demand. Therefore, three mixed stock standard solutions were prepared with all compounds at 1 g/L in water (Table 2). All sweeteners were included in one single mixture (mix1) except for aspartame which required specific acidified conditions (mix3), while all preservatives were included in another separate mixture together with caffeine (mix2).

Table 2. Subset mixture composition description

Stock standard solution composition (1 g/L in water)	
mix1	Acesulfame K (ACE)
	Cyclamate (CYC)
	Neohesperidin dihydrochalcone (NHDC)
	Neotame (NEOT)
	Saccharin (SAC)
	Sucralose (SUC)
mix2	Benzoate (BEN)
	Caffeine (CAF)
	Sorbate (SOR)
mix3	Aspartame (ASP)

To minimize sample handling errors and improve lab productivity, the calibration curves and check standard levels were set up by injecting variable volumes of mixed standards solutions prepared at 100 mg/L (Calibration level 8) (Tables 3 and 4).

Table 3. Calibration levels

Calibration level	1	2	3	4	5	6	7	8
Injection volume (µL)	0.20	0.40	1.00	2.00	4.00	10.00	16.00	20.00
Concentration (mg/L)	1	2	5	10	20	50	80	100

Table 4. Check standard levels

Check standards level	QC1	QC2	QC3
Injection volume (µL)	0.20	0.40	10.00
Concentration (mg/L)	1	2	50

Samples

A panel of diverse beverage types from various commercial brands were tested: energy drinks, sweetened drinks, carbonated drinks, teas, and infusions.

Samples were centrifuged and 5-times diluted in water before injection. Acidified conditions (0.1% formic acid) were required for aspartame determination. Carbonated drinks were placed in ultrasonic bath for 10 minutes before filtration and dilution.

Proficiency test samples

Two proficiency test samples, part of a LGC AXIO Proficiency Testing Quality in Beverages Scheme[1], were tested. PT-BV-516 and PT-BV-517 were selected as test material for carbonated drinks (degassed) and dilutable and ready to drink matrices, respectively.

Analytical parameters

Chromatographic conditions		
Column	Hypersil GOLD C18 (4.6 × 250 mm, 5 µm)	
Mobile phase A	H_2O, 10 mM ammonium acetate	
Mobile phase B	MeOH	
Run time	20 min	
Flow rate	1.0 mL/min	
Column temperature	40 °C (forced air), passive preheater	
Autosampler temperature	5 °C	
Sample injection volume	20 µL	
Detection settings		
Diode array detector	Wavelength 1: 244 nm	Bandwidth: 4 nm
	Wavelength 2: 230 nm	Bandwidth: 4 nm
	Wavelength 3: 200 nm	Bandwidth: 4 nm
	Wavelength 4: 205 nm	Bandwidth: 4 nm
	Data collection rate: 2.0 Hz	Response time: 2 s
	3D UV spectrum:	190–450 nm
	Bunch width: 4 nm	Slit width: Wide
Charged aerosol detector	Evaporation temperature: 35 °C	
	Data collection rate: 2.0 Hz	
	Filter: 5	
	Power function: 1	

Chromatography Data System

Data acquisition and processing were performed using Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS), an efficient and compliant software to manage all analytical processes, from instrument control to final report generation.

Results and discussion

Linearity and LOQ

All targeted additives were eluted within 12 minutes as shown in Figure 1. Charged aerosol detection was selected for cyclamate, neotame, and sucralose quantification, while all other additives were measured by UV detection.

All additives showed good linearity and excellent curve fit, all with a coefficient of determination $R^2>0.9975$.

The calibration residual errors for all ten additives were within the acceptance criteria set to ±20% at the limit of quantification and ±10% for all other calibration levels (Table 5). As examples, Figure 2 shows the details for caffeine (stimulant), sorbate (preservatives), aspartame and neotame (sweeteners).

Two quality control levels, namely QC Low and QC High, corresponding to limit of quantification and mid-concentration range at 50 mg/L, respectively, were injected systematically before and after the batch of samples. Acceptance criteria for QC Low was ±20% maximum deviation and ±10% for QC High. Table 6 summarizes quality control deviation in % for all additives. After matrix samples analysis, QC Low deviation was below 15% and QC High was below 6% for all target compounds.

Calibration curves were generated by injecting variable volumes of mixed stock solutions, ranging from 0.2 to 20 µL injection volumes (Table 3). Autosampler accuracy was systematically verified by including in each sequence a quality control sample at 50 mg/L prepared manually. Acceptance criteria was set to ±10%. Table 7 shows quality control calculated amount deviation for each sweetener compound.

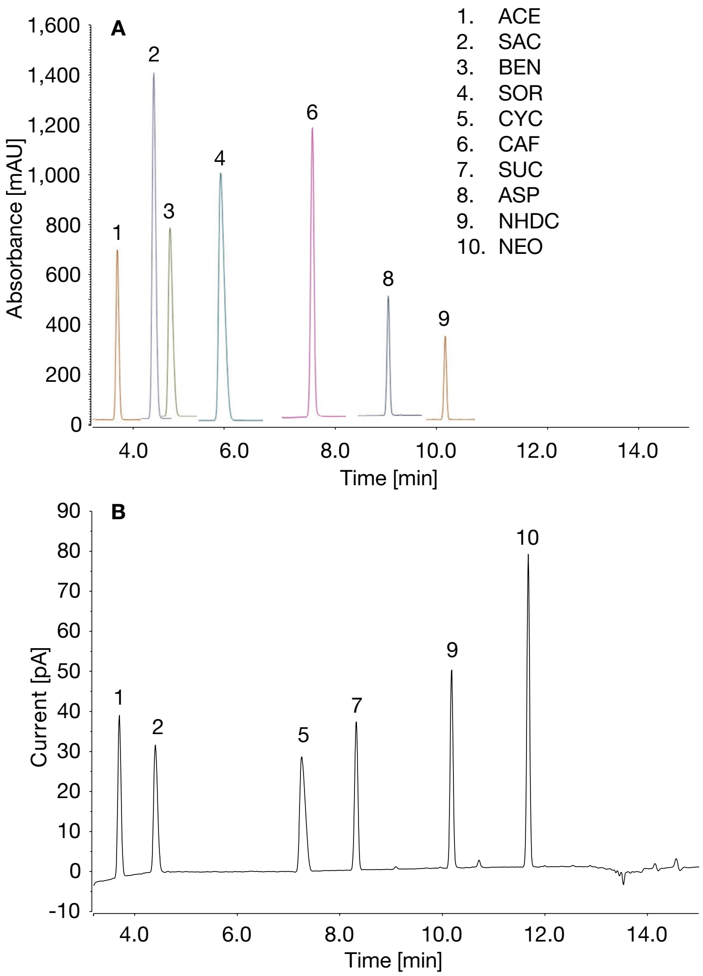

Figure 1. UV (A) and CAD (B) chromatogram profiles centered on the elution range of all targeted additives for the standard at 50 mg/L

Table 5. Calibration results summary

Compound	RT (min)	Detection channel	Concentration range (mg/L)	Calibration type	R^2	LOQ system (mg/L)	LOQ method (mg/L)
ACE	3.68	UV-Vis, 230 nm	2–100	Quadratic, 1/X	1.0000	2	10
ASP	9.09	UV-Vis, 200 nm	2–100	Quadratic, 1/X	1.0000	2	10
BEN	4.73	UV-Vis, 200 nm	1–100	Quadratic, 1/X	1.0000	1	5
CAF	7.55	UV-Vis, 205 nm	1–100	Quadratic, 1/X	1.0000	1	5
CYC	7.28	CAD	2–100	Quadratic, 1/X	0.9987	2	10
NHDC	10.17	UV-Vis, 230 nm	2–100	Quadratic, 1/X	1.0000	2	10
NEOT	11.65	CAD	2–100	Quadratic, 1/X	0.9991	2	10
SAC	4.42	UV-Vis, 200 nm	2–100	Quadratic, 1/X	0.9999	2	10
SOR	5.73	UV-Vis, 244 nm	1–100	Quadratic, 1/X	1.0000	1	5
SUC	8.31	CAD	2–100	Quadratic, 1/X	0.9976	2	10

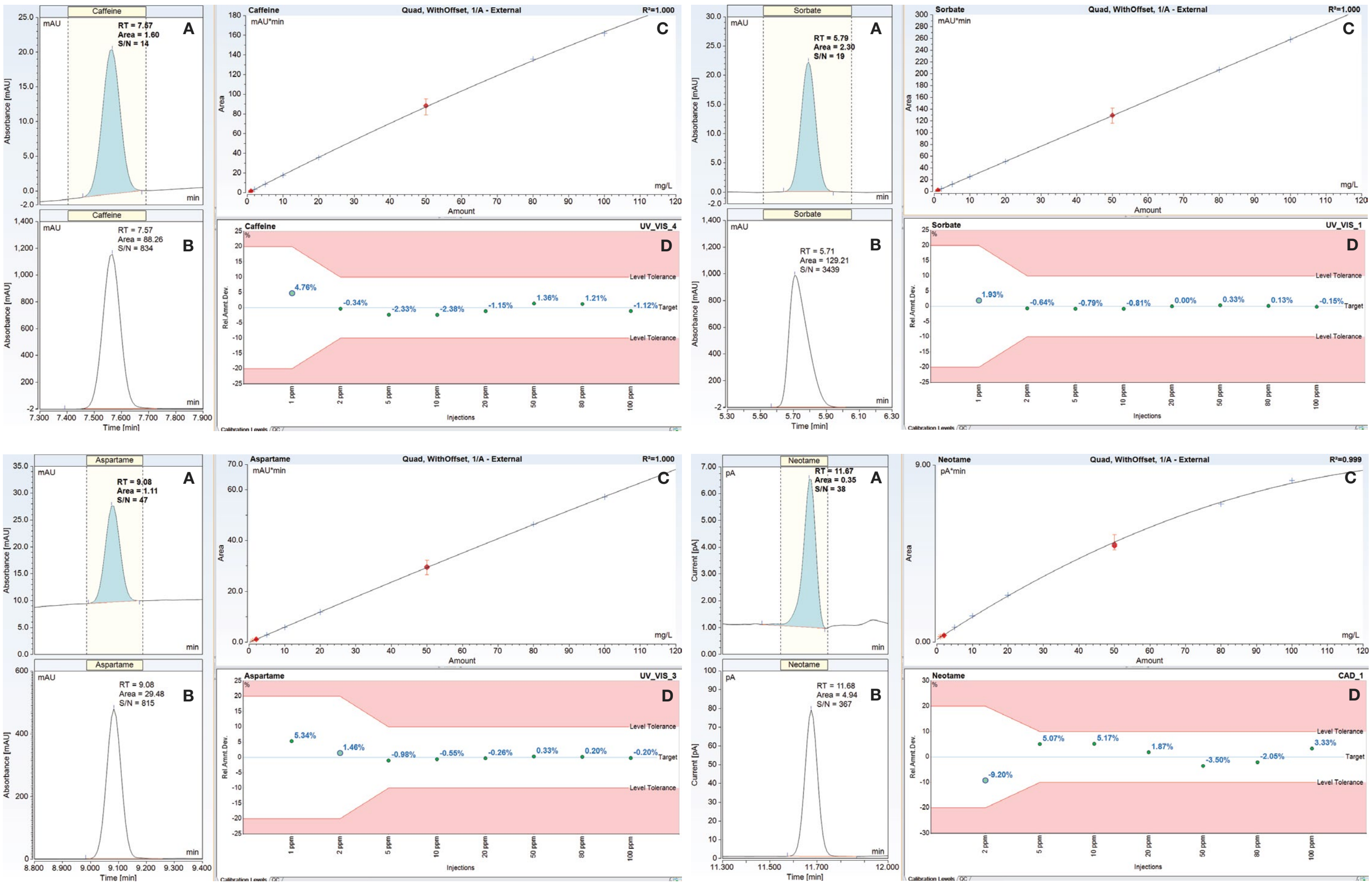

Figure 2. Results page customization in Chromeleon CDS interface for quick data review and straightforward calibration curve validation. Focus on automatic peak integration at the limit of quantification (A) and 50 ppb (B), calibration curve plot with levels and quality control points (C) and calibration residual errors chart (D) for caffeine (stimulant), sorbate (preservatives), aspartame and neotame (sweeteners).

Table 6. Quality control deviation in % for all additives

Compound	QC start deviation (%)		QC end deviation (%)	
	Low	High	Low	High
ACE	0.72	0.12	1.22	0.12
ASP	1.74	0.61	1.45	-0.03
BEN	-4.05	-0.10	1.12	-0.08
CAF	3.36	1.45	3.47	1.38
CYC	-10.34	-3.74	-8.44	-3.16
NHDC	0.78	0.09	0.94	0.01
NEOT	-7.97	-4.62	-11.79	-4.86
SAC	2.23	1.29	2.35	1.37
SOR	1.65	0.43	1.02	0.46
SUC	-13.75	-5.81	-14.88	-5.50

Table 7. Calculated amount deviation in % for each sweetener compound

Compound	ACE	ASP	CYC	NEOH	NEOT	SAC	SUC
QC deviation (%)	1.94	1.08	-1.55	1.58	-2.59	3.44	-4.42

Bias was below 5% for all additives which demonstrates the highest injection precision of the Vanquish Split Sampler with very small injection volumes without any tradeoff on robustness. This automated procedure was preferred to minimize the risks of solution preparation error and to save labor time.

Method accuracy and precision evaluation

Method accuracy and precision were evaluated through multiple approaches described in the following sections.

Caffeine content measurement comparison with commercial labelling claims

Caffeine was measured in diverse commercial beverages, and results were compared with the labelling claims (Table 8).

Bias was below 5% deviation for all drink types tested, which demonstrates that the method is accurate for caffeine content determination.

Large spike-and-recovery study for all compounds' classes

A spike-and-recovery experiment was carried out to evaluate the method accuracy. Various sample types were selected to evaluate the effect of complex matrices on the measurement of all targeted additives. Pre-spiked (blank) and post-spiked samples were analyzed, and recoveries were calculated based on both measurements (Table 9).

Excellent average spiked recoveries were observed for all ten food additives, which demonstrates that the method is not significantly affected by potential matrix effects and is safe for highly complex matrix analysis like tea samples. RSDs were all below 5%. Average recoveries were within 93–103% for all compounds.

Table 8. Comparison of caffeine content measurements against labeling claim in commercial drinks

Sample ID	Sample type	Assigned value (mg/L)	Result value (mg/L)	Bias (%)
Brand I	Cola	97	95	-2.6
Brand J	Energy drink	320	307	-4.1
Brand K	Energy drink	310	321	3.5
Brand L	Energy drink	320	318	-0.7
Brand M	Energy drink	320	314	-1.9
Brand N	Energy drink	200	202	1.1
Brand O	Energy drink	320	330	3.0
Brand P	Cola	101	96	-4.7

Table 9. Spiked recoveries results for sweeteners class

		Recovery (%)									
Sample ID	Sample type	ACE	ASP	CYC	NHDC	NEOT	SAC	SUC	BEN	SOR	CAF
Brand A	Sweetened drink	101	102	99	101	94	102	97	105	106	108
Brand B	Sweetened drink	92	107	89	90	95	93	87	99	100	100
Brand C	Tea/Infusion	100	95	94	99	97	100	94	95	96	96
Brand D	Tea/Infusion	100	106	92	99	96	101	95	98	100	101
Brand E	Tea/Infusion	97	96	91	96	94	98	91	96	97	98
Brand F	Sweetened drink	102	108	98	101	97	102	92	98	97	98
Brand G	Sweetened drink	99	106	94	98	93	101	92	94	94	96
	Mean	**99**	**103**	**94**	**98**	**95**	**100**	**93**	**98**	**98**	**100**
	SD	0.03	0.05	0.04	0.04	0.02	0.03	0.03	0.04	0.04	0.04
	RSD (%)	**3**	**5**	**4**	**4**	**2**	**3**	**4**	**4**	**4**	**4**

Proficiency testing program

Proficiency tests were conducted on two matrix types (Table 10) covering eight parameters of interest.

The results for all additives included in the interlaboratory program test were within the acceptable range with z-scores below 2 and demonstrated the method's accuracy, precision, and reliability.

Table 10. Summary of measured content (mg/L) of additives tested in carbonated drink proficiency test sample as well as dilutable and ready to drink proficiency test sample and resulting Z-scores

	PT-BV-516 Carbonated drinks (degassed)				PT-BV-517 Dilutable and Ready to drink			
Compound name	Assigned value (mg/L)	Result (mg/L)	Z-score	Conclusion	Assigned value (mg/L)	Result (mg/L)	Z-score	Conclusion
ACE	112.3	**114.6**	**0.41**	Passed	65.3	**69.0**	**0.74**	Passed
ASP	381.5	**383.3**	**0.09**	Passed	262.1	**267.1**	**0.38**	Passed
BEN	120.3	**119.7**	**-0.10**	Passed	129.8	**128.6**	**-0.18**	Passed
CAF	223.8	**227.9**	**0.37**	Passed	283.0	**287.8**	**0.34**	Passed
CYC	148.0	**143.3**	**-0.64**	Passed	227.6	**228.1**	**0.04**	Passed
SAC	69.0	**71.1**	**0.20**	Passed	78.7	**83.7**	**0.42**	Passed
SOR	124.2	**125.7**	**0.25**	Passed	46.0	**46.0**	**0.00**	Passed
SUC	73.8	**77.4**	**0.72**	Passed	103.7	**103.7**	**0.00**	Passed

Conclusion

A unique analytical method was successfully tested for determination of the ten most commonly used food additives in soft drinks composition.

Method accuracy was demonstrated with passing proficiency tests successfully.

Compared to mass spectrometry, the Vanquish Charged Aerosol Detector is an attractive tool option for routine screening analysis of food additives in beverages as it requires lower capital investment and operational costs while providing sensitive detection capabilities. The CAD is critical for those additives that cannot be detected by UV due to lack of chromophore, thus expanding the scope of additives that can be detected in a single injection. Furthermore, the CAD is user-friendly with simplified operation and maintenance, requiring less technical expertise.

Reference

1 LGC AXIO Proficiency Testing Quality in Beverages Scheme (QBS)

Learn more at **thermofisher.com/hplc**