Determination of Sweeteners, Preservatives, and Caffeine in Various Food and Consumer Products Using the Agilent 1290 Infinity II LC

Application Note

Food Testing and Agriculture

Authors

Gerd Vanhoenacker, Mieke Steenbeke, Koen Sandra, Frank David, Pat Sandra
Research Institute for Chromatography
President Kennedypark 26
B-8500 Kortrijk
Belgium

Udo Huber
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The Agilent 1290 Infinity II LC was used to analyze additives such as sweeteners, preservatives, and caffeine in various food products, beverages, and consumer toothpaste. The developed method facilitates accurate and sensitive determination of nine additives using a diode array detector (DAD) and an evaporative light scattering detector (ELSD) placed in series. After the DAD, a valve was installed to remove nonretained solutes in reversed-phase LC, which could increase the noise level of the ELSD. Total analysis time, including re-equilibration, was below 10 minutes with minimal sample preparation. Method performance was evaluated with standard solutions as well as with a series of real samples.

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Introduction

Additives are added to food and beverages for several purposes. Preservatives such as benzoic acid and sorbic acid are added to extend the shelf life of products, sweeteners are added to replace sugars, while stimulants such as caffeine may be added to increase alertness. Addition of all of these ingredients are subject to regulations, and need to be controlled in food, beverage, and consumer products.

Caffeine is a common additive in soft and energy drinks. It can help the consumer feel less tired or stay awake, and has little or no nutritional value. The amount of caffeine in beverages can vary considerably, but due to its activity and possible interaction with medication, maximum levels are enforced.

Enhancing preservation of food and consumer products by adding chemical preservatives is common practice, and has become even more important because people consume more processed food. Benzoic acid and sorbic acid are antimicrobial agents that control mold, yeast, and fungal growth. Toxicological levels of benzoic and sorbic acid are quite high, and they are easily degraded in the environment. Maximum levels for their application in nutritional products have been established, and are controlled by international food laws.

The increased need for low-calorie and sugar-free products available in the food, beverage, and even pharmaceutical market has boosted the global use of artificial and natural sweeteners. These products are introduced to reduce or eliminate sugar intake, and are useful in the prevention or treatment of obesity and complications thereof. They are also of value to diabetics. The use of sweeteners is regulated, and maximum daily intake levels have been set.

This Application Note presents a method to analyze a selection of common additives with the Agilent 1290 Infinity II LC. The sample preparation is very simple: detection was performed with both a diode array detector (DAD) and an evaporative light scattering detector (ELSD) since some of the additives are UV-transparent. A valve was used to divert the flow from the DAD to the waste (instead of ELSD) during the first part of the analysis. This greatly enhanced the ELSD baseline and signal. The method performance was evaluated, and its applicability illustrated with a set of samples.

Experimental

Instrumentation

An Agilent 1290 Infinity II LC was used.

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with 2-position/6-port valve, 1200 bar (G4231B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent 1290 Infinity II Evaporative Light Scattering Detector (G7102A)

The 2-position/6-port valve was placed in the flow path between the DAD and the ELSD. Flow coming from the DAD entered the valve and could be diverted to waste or to the ELSD. The position of the valve was switched during the analysis, and flow was sent to the ELSD 1 minute after injection.

Method parameters

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Mobile phase	A) 0.08 % formic acid and 0.25 % triethylamine in water/methanol (99/1) B) Acetonitrile/methanol (1/1)
Flow rate	0.6 mL/min
Gradient	0–4 minutes, 5–40 %B 4–6 minutes, 40–90 %B 6–7.5 minutes, 90 %B 7.5–9.5 minutes, 5 %B
Temperature	25 °C
Injection	2 µL, with needle wash (flush port, 3 seconds, water/methanol 1/1)
Diode array detection	DAD, 10 Hz Wavelength, bandwidth with reference (wavelength, bandwidth): (235, 5) with reference (380, 40) (285, 5) with reference (360, 100)
ELSD	Evaporator temperature 35 °C Nebulizer temperature 35 °C Gas flow rate 1.4 SLM Data rate 80 Hz Smoothing 5 PMT gain 2
Valve	0–1 minutes, DAD to waste 1–7.5 minutes, DAD to ELSD 7.5–9.5 minutes, DAD to waste

Standard solutions

The standard solution contained the following compounds: acesulfame-K, saccharin, caffeine, benzoic acid, sorbic acid, cyclamate, sucralose, aspartame, and stevia. Each compound in the standard solution had the same concentration. Standard solutions of 10 to 600 ppm were prepared in water.

Sample preparation

- The caffeinated soft drinks (cola of different types) were degassed by sonication and filtered with an Agilent Captiva regenerated cellulose filter (pore size 0.45 µm, p/n 5190-5109).
- A 5-mL amount of the milk products, liquid yoghurt and fruit yoghurt, were mixed with 5 mL of methanol. The samples were then centrifuged (5 minutes at 12,500 rpm), and the upper phase was filtered with an Agilent Captiva regenerated cellulose filter (pore size 0.45 µm).
- To 1 g of marmalade, peppermint candy, and toothpaste, 10 mL of water was added, and the sample was placed in an ultrasonic bath for 10 minutes. Afterwards, the aqueous solution was filtered with an Agilent Captiva regenerated cellulose filter (pore size 0.45 µm).

Results and Discussion

Table 1 lists the target additives. The corresponding classification, abbreviation, CAS number, and E number are given for each compound. The detection was performed with DAD and ELSD. The corresponding signal for each compound is indicated.

The rationale of using two detectors in series, namely UV and ELSD, with a valve in between, is as follows. The ELSD detects nonvolatile analytes, and does not rely on the optical properties (chromophore) of a compound.

This means that with the exception of the volatile sorbic and benzoic acids, which contain a chromophore enabling UV detection, all targets were detected with ELSD. However, beverages such as soft drinks contain nonvolatile but highly polar compounds such as sugars and salts. Therefore, they elute in reversed-phase LC with nearly no retention at the very beginning of the sample run.

The influence of these polar substances on the ELSD signal is significant, and a noisy baseline is obtained that slowly decreases over nearly the entire run. This makes the detection of the analytes of interest with ELSD troublesome. When these polar compounds are diverted to waste by the valve, they will not enter the ELSD, and the noise in the chromatogram will be minimalized. The use of the valve, whereby the flow is diverted to the ELSD after 1 minute of analysis, has a significant influence on the ELSD signal, as illustrated in Figure 1, showing the difference between an analysis with and without the valve activated.

Table 1. Compounds of interest.

Group	Analyte	Abbreviation	CAS number	E number	Detection
Sweeteners	Acesulfame-K	ACE	55589-62-3	E950	DAD: 235 nm ELSD
	Aspartame	ASP	22839-47-0	E951	ELSD
	Cyclamate	CYC	139-05-9	E952	ELSD
	Saccharine	SAC	81-07-2	E954	DAD: 235 nm ELSD
	Stevia	STE	Mixture of steviolglycosides*	E960	ELSD
	Sucralose	SUC	56038-13-2	E955	ELSD
Preservatives	Benzoic acid	BA	65-85-0	E210	DAD: 235 nm
	Sorbic acid	SA	110-44-1	E200	DAD: 235 nm
Stimulants	Caffein	CAF	58-08-2		DAD: 285 nm ELSD

*Under the LC conditions applied, only one peak was obtained for the glycosides.

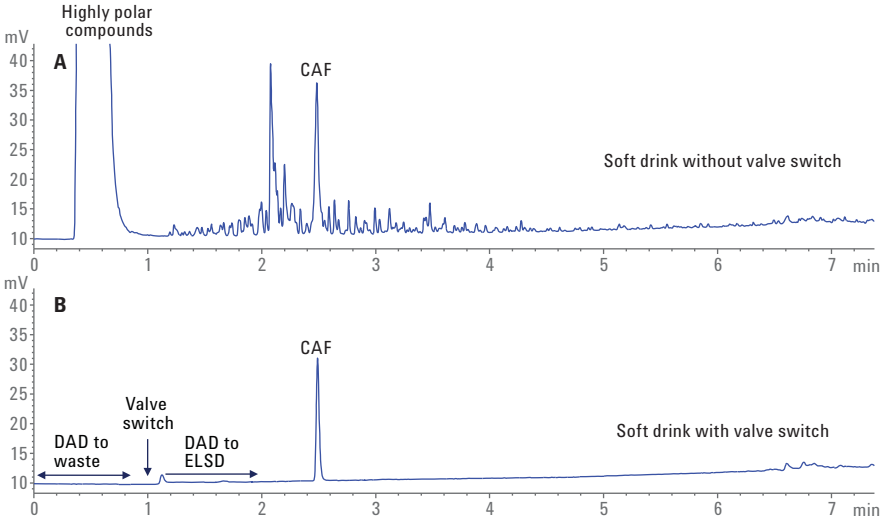

Figure 1. The influence of a valve switch on the ELSD signal for a soft drink sample.

Figure 2 shows the results for a standard solution (100 ppm of each compound). All compounds present in the standard solution are separated from each other. At the DAD signal of 235 nm, acesulfame-K, saccharin, caffeine, benzoic acid, and sorbic acid are visible. At the DAD signal of 285 nm, caffeine has a much higher response compared to the DAD signal at 235 nm. Cyclamate, sucralose, aspartame, and stevia were not detected by UV. Acesulfame-K, saccharin, cyclamate, caffeine, sucralose, aspartame, and stevia were detected with the ELSD due to their nonvolatile characteristic. The optimal signal for each of the compounds was selected for quantitative purposes. Table 1 shows the selected signals.

Calibration for each compound was carried out on 10 levels using standard solutions at 10 to 600 ppm. Quadratic curves were applied for the ELSD signal. Standard solutions of 20 and 200 ppm were measured six consecutive times to check injection precision. The RSDs of the standards were less than 5 % for ELSD at 20 ppm, and less than 2 % for DAD at both levels and for ELSD at 200 ppm. Table 2 summarizes the results.

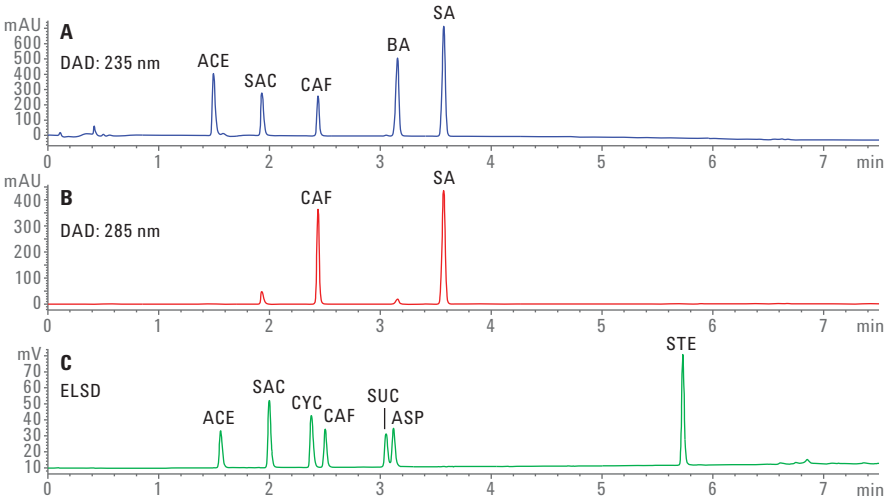

Figure 2. Result for the analysis of the 100 ppm standard solution.

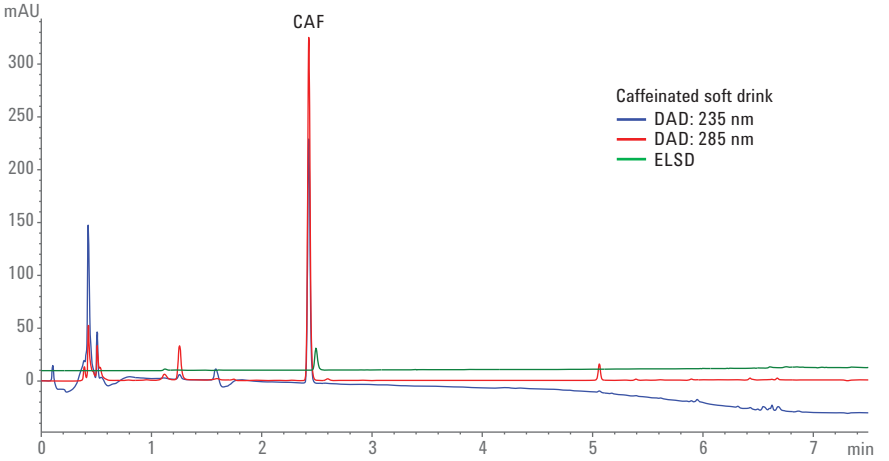

Figure 3. Overlay of the various signals for the caffeinated soft drink.

Table 2. Repeatability for standard solution 20 and 200 ppm (n=6) and correlation of the calibration between 10 and 600 ppm.

		DAD			ELSD		
Analyte	λ	%RSD 20 ppm	%RSD 200 ppm	R^2	%RSD 20 ppm	%RSD 200 ppm	R^2
ACE-K	235 nm	1.26	0.13	1.0000	4.28	1.20	0.9994
ASP					4.40	0.89	0.9991
CYC					2.96	1.26	0.9987
SAC		1.84	0.12	0.9999	3.50	1.16	0.9983
STE					3.63	1.97	0.9982
SUC					3.12	1.27	0.9992
BA		0.06	0.27	0.9999			
SA		0.14	0.13	0.9990			
CAF	285 nm	1.28	0.28	0.9996	2.06	1.16	0.9999

Five different soft drinks were analyzed. Figure 3 shows the results for a caffeinated soft drink without artificial sweeteners (normally contains about 10 g sugar per 100 mL).

One of the sweetened soft drinks (Figure 4) contains the natural sweetener stevia; steviolglycosides are 200 to 300 times sweeter than sugar. To have the same degree of sweetness, less stevia must be added than sugar.

In the diet versions of the caffeinated soft drinks (Figure 5 and 6), the sugar is replaced by acesulfame-K and aspartame. In soft drink 1 (Figure 5) there is approximately three times more acesulfame-K as in soft drink 2 (Figure 6).

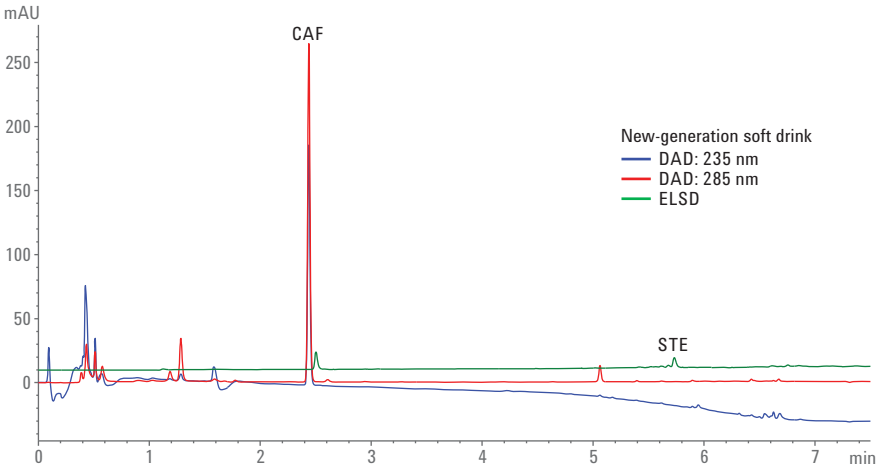

Figure 4. Overlay of the various signals for the naturally sweetened soft drink.

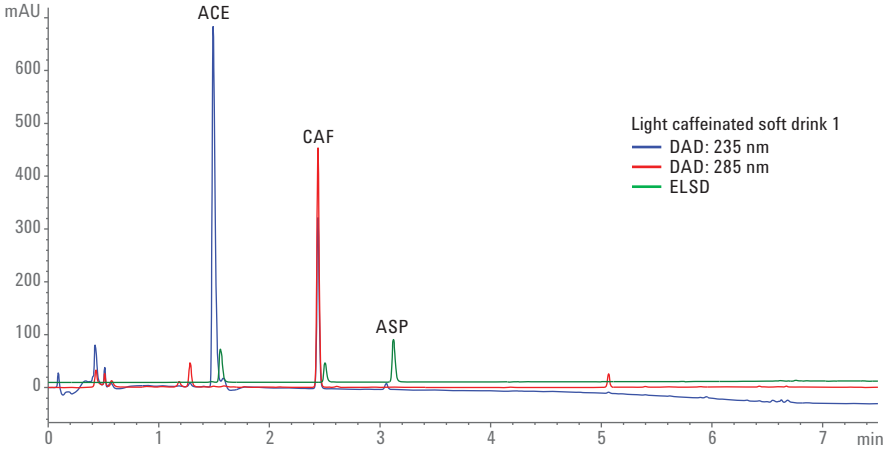

Figure 5. Overlay of the various signals for the diet caffeinated soft drink 1.

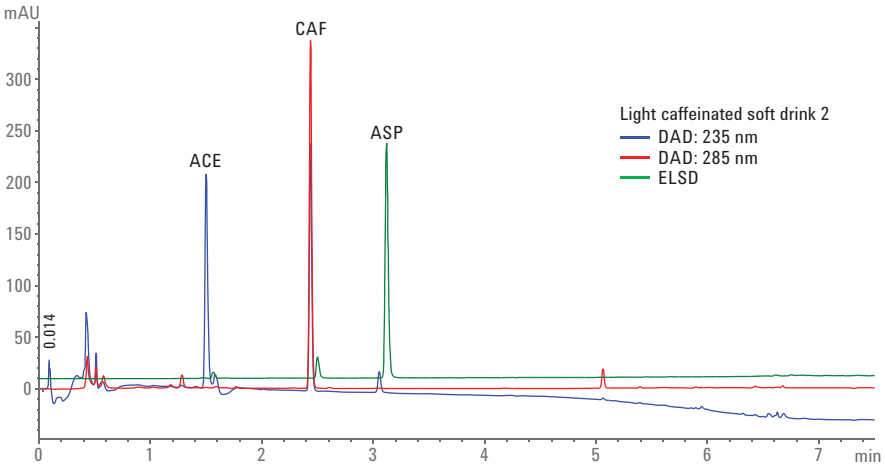

Figure 6. Overlay of the various signals for the diet caffeinated soft drink 2.

The diet noncaffeinated soft drink (Figure 7) has, on average, the same amount of acesulfame-K and aspartame as the diet caffeinated soft drink in Figure 5, but it does not contain any caffeine.

The preservative benzoic acid was found in the two analyzed dairy products: fruit yoghurt (Figure 8) and liquid yoghurt (Figure 9).

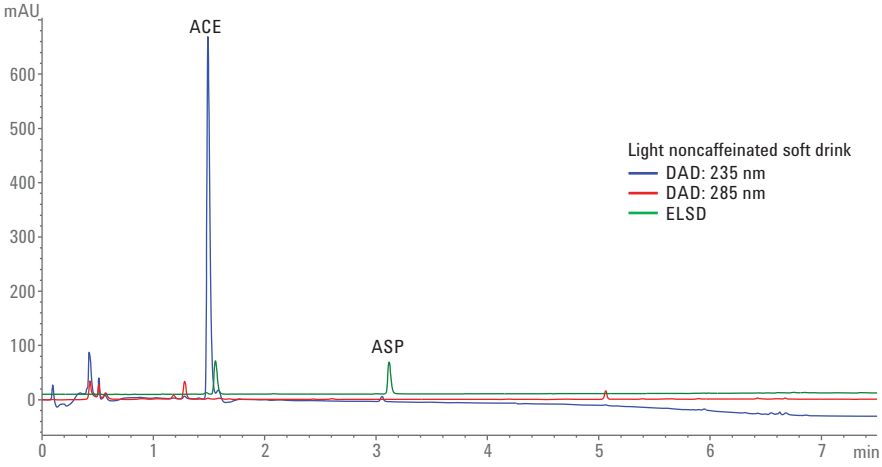

Figure 7. Overlay of the various signals for the diet noncaffeinated soft drink.

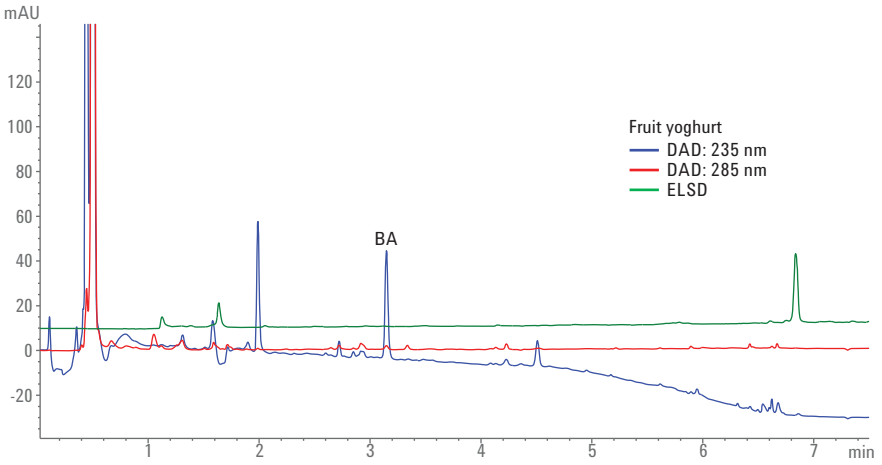

Figure 8. Overlay of the various signals for the fruit yoghurt.

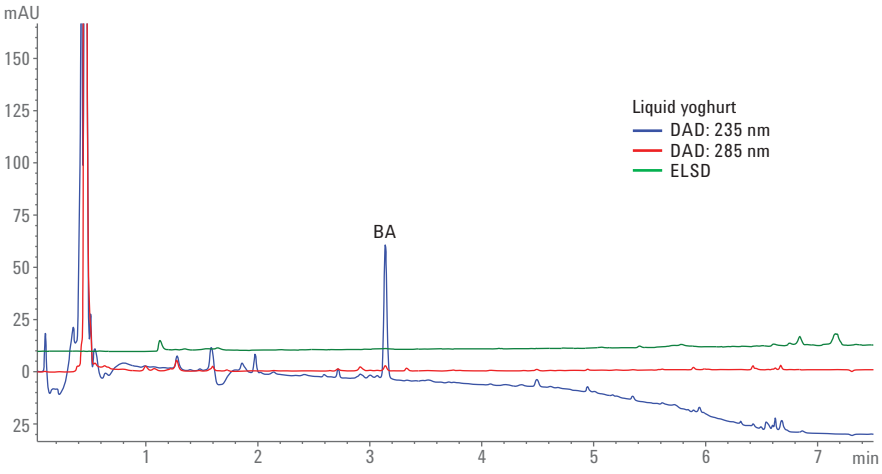

Figure 9. Overlay of the various signals for the liquid yoghurt.

Figure 10 shows the results of the analysis of marmalade in which sorbic acid was found. The peppermint candy contained acesulfame-K and sucralose (Figure 11), and saccharine and benzoic acid were detected in toothpaste (Figure 12).

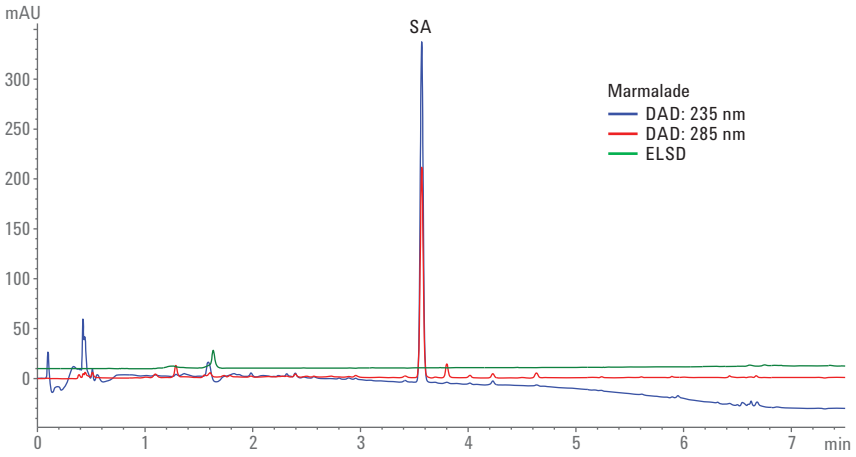

Figure 10. Overlay of the various signals for the marmalade.

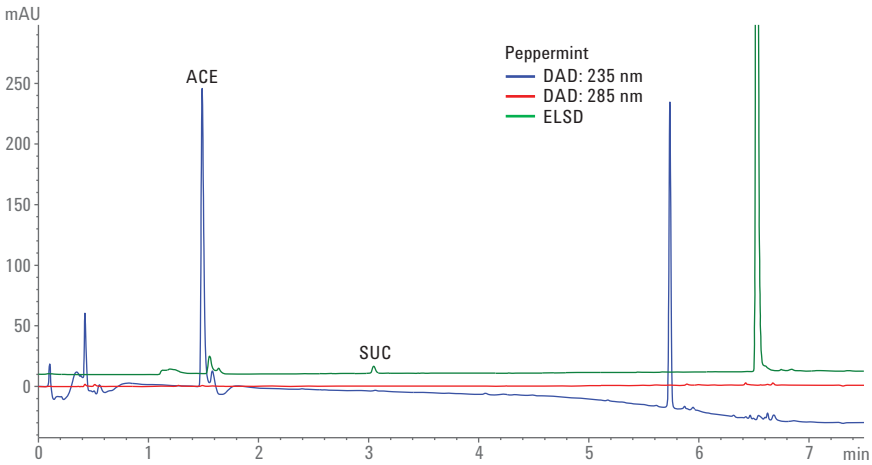

Figure 11. Overlay of the various signals for the peppermint.

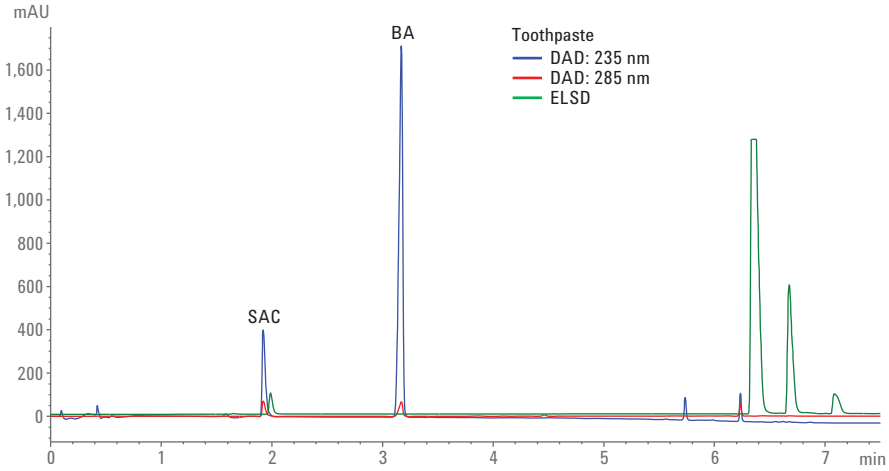

Figure 12. Overlay of the various signals for the tooth paste.

Tables 3 and 4 summarize the quantitative results for all samples. The concentration of the additives for each sample was determined. Each sample was also spiked at 100 ppm and the recovery was calculated. Recoveries were satisfactory, with most results showing almost 100 % of the theoretical value. All values were between 74 % and 126.5 %. Considering the minimal sample preparation that was carried out on these samples, this is more than acceptable. Additional cleanup could result in even better values for some samples.

Table 3. Concentrations (ppm) of the additives detected in the various samples.

	DAD					ELSD						
	235 nm	235 nm	235 nm	235 nm	285 nm							
	ACE-K	SAC	BA	SA	CAF	ACE-K	SAC	CYC	CAF	SUC	ASP	STE
Caffeinated soft drink					93.4				91.9			
Naturally sweetened soft drink					71.4				71.7			28.1
Diet caffeinated soft drink 1	174.2				127.1	186.1			125.9		191.5	
Diet caffeinated soft drink 2	48.1				93.2	44.5			91.1		341.2	
Diet noncaffeinated soft drink	169.8					182.3					163.4	
Fruit yoghurt			6.8									
Liquid yoghurt			9.7									
Marmalade				43.6								
Peppermint	52.9					74.0				46.9		
Toothpaste		169.2	399.8				176.5					

Table 4. Recovery (%) of the additives detected in the various samples.

	DAD					ELSD						
	235 nm	235 nm	235 nm	235 nm	285 nm							
	ACE-K	SAC	BA	SA	CAF	ACE-K	SAC	CYC	CAF	SUC	ASP	STE
Caffeinated soft drink	93.2	93.6	91.7	93.4	94.0	95.1	86.2	90.6	95.1	92.3	92.2	81.3
Naturally sweetened soft drink	94.3	98.5	96.3	98.5	100.00	112.9	93.5	95.8	98.7	97.4	97.4	95.7
Diet caffeinated soft drink 1	91.0	98.6	96.4	98.8	97.1	96.4	92.3	95.8	98.9	103.3	101.7	86.9
Diet caffeinated soft drink 2	94.8	97.2	95.7	97.2	100.9	109.7	92.1	93.9	103.5	96.5	91.5	90.0
Diet noncaffeinated soft drink	91.6	99.0	96.5	98.8	98.9	89.2	93.00	95.6	99.6	96.5	94.1	84.9
Fruit yoghurt	101.2	97.9	104.3	104.8	111.3	116.9	94.7	110.8	120.1	111.4	115.3	100.2
Liquid yoghurt	99.6	90.5	99.1	102.7	110.5	113.6	87.4	106.6	115.2	116.6	126.4	117.3
Marmalade	97.4	102.1	99.0	105.4	98.9	123.7	97.6	100.3	102.8	103.6	101.8	94.5
Peppermint	95.9	98.4	98.4	99.7	96.9	103.3	94.6	98.6	98.1	103.7	103.4	89.8
Toothpaste	95.8	90.8	74.1	102.7	100.2	97.8	88.6	108.1	105.7	112.2	75.7	98.0

Conclusions

A method was developed to analyze a selection of common additives with the Agilent 1290 Infinity II LC. Detection was done with a DAD and an ELSD in series. The first part of the effluent, containing the most polar sample constituents, was diverted to waste after passage through the DAD. This way, the ELSD baseline was not affected by these polar nonvolatile compounds, and detection and accurate quantification of the target analytes could be performed. The method was tested on a selection of illustrative samples to demonstrate its applicability. Even with minimal sample preparation, recovery of all additives was acceptable.

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