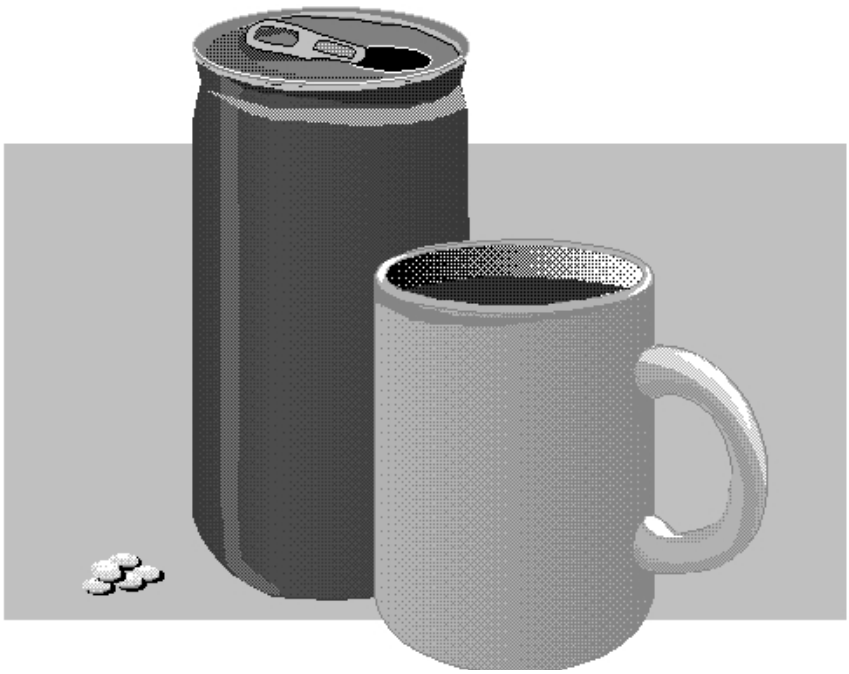
CZE Analysis of Artificial Sweeteners and Preservatives in Drinks

Application Note

Food Analysis

Rainer Schuster,
Angelika Gratzfeld-Hüsgen



Capillary electrophoresis can reduce the complexity associated with the analysis of sweeteners in drinks. Doing away with the need for a multitude of derivatization chemistries and their separations, the method described here has been successfully applied to both beverages and tablet formulations. A single run on an 50-µm id Agilent Extended Path Length capillary at 192 nm, with simultaneous UV-visible absorbance spectral library and peak purity routines, detects and confirms most compounds in the low nanogram range. With buffer replenishment every five injections, repeatability is better than 0.15 % for migration times and approximately 2 % for areas.

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Introduction

Sweetening agents can be classified as belonging to one of two main groups: caloric, or nutritive, and noncaloric or non-nutritive compounds. Nutritive sweeteners are carbohydrates (or their derivatives such as glucose, fructose and maltose) or products hydrolyzed from starch. Non-nutritive sweeteners do not belong to any particular chemical group. Synthetic sweeteners are steadily increasing in importance with increased public awareness of diabetes and its special dietary requirements, and more consumers becoming concerned about obesity and dental caries. The most frequently used synthetic sweeteners are: saccharin, cyclamate, aspartame and acesulfame, see figure 1.

To date, artificial sweeteners (table 1) have been determined by HPLC with reversed phase chromatography using different buffer systems, ion pairing reagents and specific derivatization procedures (aspartame with *o*-phthalaldehyde [OPA]; cyclamate with 4-fluoro-7-nitrobenzofurazone [NBDF]). Derivatization overcomes detection limitations for these compounds in the low UV range.

A number of methods have been published [1,2] for simultaneous determination of aspartame and saccharin. Conditions are shown in table 1.[1]

	Saccharin	Saccharin Aspartame Dulcin	Acesulfame-K	Cyclamate NBDF-Derivative
Column	RP-18	ODS	ODS	RP-18
Mobile phase	0.05 mM H_2PO_4– acetonitrile, 9:1	0.01 mM KH_2PO_4 pH 3.5-acetonitrile, 85:15	0.01 mM TBAHS– H_2O, acetonitrile, 55:45 methanol, 9:1	
Detector	230/260 nm	216 nm	227 nm	490 nm Fluorescence λ_{ex}485nm, λ_{em}530 nm

Table 1
HPLC conditions for determination of sweeteners

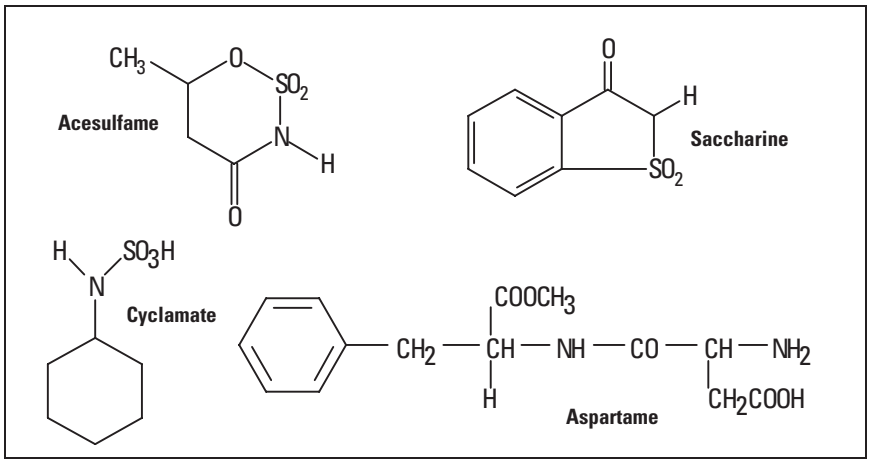


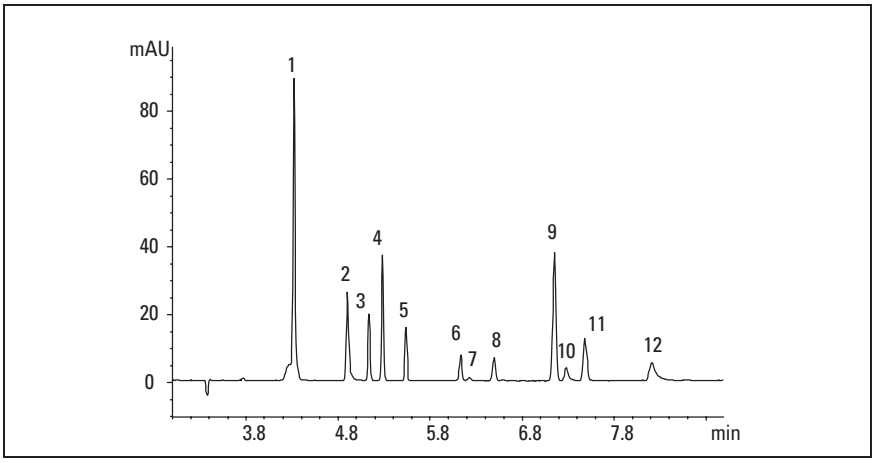
Figure 1
Chemical structure of the common artificial sweeteners

Hermann and coworkers [3] reported on a method for the detection of aspartame, cyclamate, dulcin, and saccharin using an ion-pair HPLC separation with indirect photometric detection.

Toxicological data has led to the use of some artificial sweeteners being controlled, for example cyclamate is banned in the United States, the United Kingdom and Japan. Aspartame is metabolized to aspartic acid, methanol, and *phenylalanine* a substance critical to persons who suffer from phenylketonuria (PKU). Reliable means of obtaining analytical data are required for food samples containing these compounds. However, such varied methods with their differing derivatization protocols make the analysis of artificial sweeteners time consuming and labor intensive. An alternative to HPLC is capillary zone electrophoresis (CZE). All compounds can be separated sufficiently in one run.

Experimental

CZE separations were performed using the Agilent CE system with a built-in diode-array detector and Agilent CE ChemStation (DOS Series) software. Separations were achieved with fused-silica 50-µm id capillaries (64.5 cm total length, 56 cm effective length) with an extended path length or bubble cell at the detector end. All separations were performed at 25°C using a 20-mM sodium tetraborate buffer at pH 9.4. New capillaries were preconditioned by flushing with 1M sodium hydroxide for 3 minutes followed by running buffer for 10 minutes.



Buffer	20mM borate pH 9.4
E	465 V/cm
Effective capillary length	56 cm
Total capillary length	64.5 cm
id 50 µm	
Injection	100 mbars
Temperature	25°C
Detection	
Signal	192/2 nm
Reference	450/100 nm

Key

1	phenylalanine	7	cyclamate
2	aspartame	8	sorbic acid
3	PHB propyl	9	benzoic acid
4	PHB ethyl	10	aspartic acid
5	PHB methyl	11	saccharine
6	Dehydroacetic acid	12	acesulfame

Figure 2
Electropherogram of a standard sample

Meas. Library CalTbl

Time [min]	Time [min]	Time [min]	Sig	Amount [ng/µl]	Purity Factor	#	Library Match		Name	%RSD- (n=5) MT	AREA
4.21	4.10	4.20	1	177.884	1000	1	998		phenylalanine	0.02	9.4
4.78	4.67	4.78	1	87.063	1000	1	999		aspartame	0.006	1.1
5.00	4.90	4.99	1	62.506	1000	1	999		PHB-propyl	0.141	6.0
5.00	5.10	5.12	1	57.134	1000	1	998	?	PHB-ethyl		
5.13	5.10	5.12	1	109.938	1000	1	999		PHB-ethyl	0.03	2.8
5.37	5.30	5.36	1	45.242	1000	1	999		PHB-methyl	0.03	1.4
5.95	5.85	5.94	1	50.572	1000	1	998		dehydroacetic acid	0.02	0.22
6.04	6.00	6.03	1	245.684	-	1	621	x	cyclamate	0.03	7.0
6.29	6.20	6.29	2	69.561	1000	1	998		sorbic acid	0.026	2.3
6.91	6.90	6.89	1	45.588	1000	1	998		benzoic acid	0.03	1.9
6.99	6.86	6.96	1	157.647	1000	1	955		aspartic acid	0.045	0.9
7.22	7.20	7.20	1	53.055	1000	1	1000		saccharine	0.035	1.4
7.88	7.80	7.87	2	136.327	969	1	923	x	acesulfame	0.04	1.7

Table 2
Report of figure 2 for artificial sweeteners and preservatives

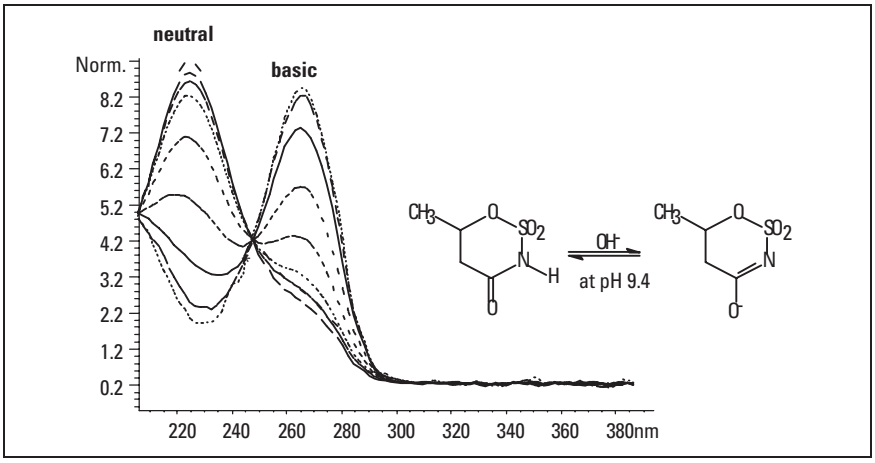
Samples were introduced hydrodynamically in 2 s at 50 mbar and analyzed with an applied voltage of 30 kV and detected at 191 nm (2-nm bandwidth). After each run the column was rinsed with the separation buffer for 2 minutes. Detailed separation conditions are listed alongside figure 2, the separation of common artificial sweeteners: aspartame (and its decomposition products phenylalanine and aspartic acid, cyclamate, saccharine and acesulfame together with the normally occuring preservatives PHB-esters (propyl-, ethyl-, and methyl), sorbic acid and benzoic acid.

Results and discussion

Cyclamate and aspartame lack chromophores and require detection wavelengths in the low UV-range below 200 nm, that part of the spectrum where certain ion pairing reagents also absorb. Monitoring with the CE system's built-in diode-array detector permits detection at 192 nm and simultaneous acquisition of spectra. This spectral information compared to spectra in a library stored on the ChemStation can confirm that the response is indeed from the compound of interest and not from interfering matrix compounds. Peak purity analysis can be achieved by overlaying spectra taken in the peak. The system's software performs all three actions (migration time report, libraray search and peak purity) in one step, producing quantitative reports based on three-dimensional data. Table 2 shows a report based on the analysis of figure 2 with the corresponding library search and peak purity data.

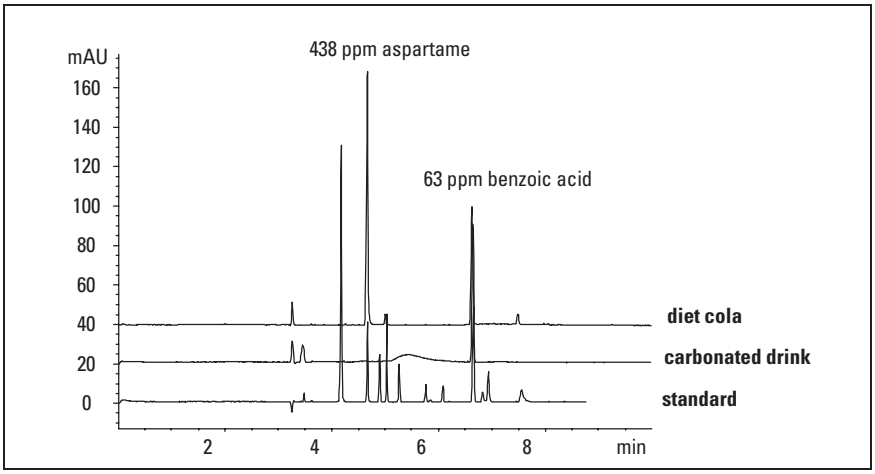
Concentrations injected were in the range 50–200 ppm. The *x* flag in the report shows that acesulfame might contain an impurity: its library match factor of 923 and purity factor of 969 are lower than could be expected for a pure peak. Although flagged, cyclamate concentration is too low, even at 192 nm, to make any conclusive judgements. A spectral overlay of spectra taken over the peak migrating at 7.88 (all spectra in peak) show a rather interesting aspect, see figure 3.

The overlay reveals an isosbestic point (245 nm) —acesulfame exists in a tautomeric equilibrium in that buffer, stable at this pH value.



Buffer	20 mM borate pH 9.4
E	465 V/cm
Effective capillary length	56 cm
Total capillary length	64.5 cm
id	50 µm
Injection	100 mbars
Temperature	25 °C
Detection	
Signal	192/2 nm
Reference	450/100 nm

Figure 3
Overlay of spectra taken from acesulfame



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E	465 V/cm
Effective capillary length	56 cm
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Detection	
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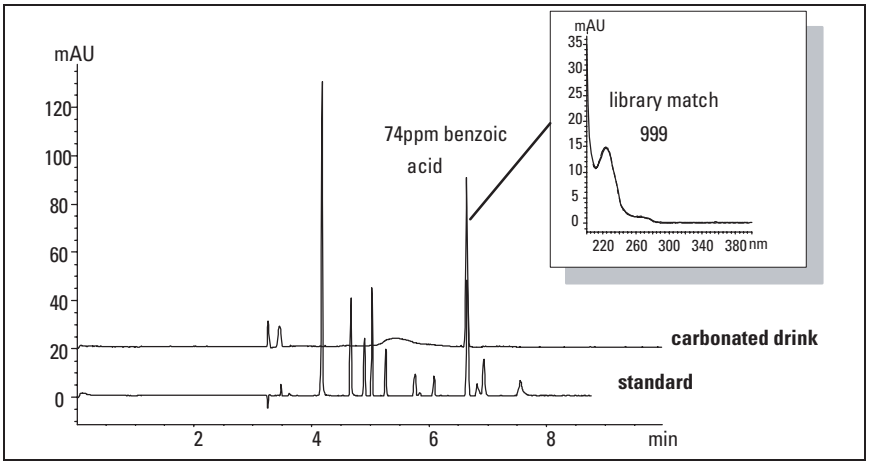
Reference	450/100 nm

Figure 4
Electropherogram of a diet cola containing aspartame and benzoic acid and a carbonated drink containing benzoic acid



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E	465 V/cm
Effective capillary length	56 cm
Total capillary length	64.5 cm
id	50 µm
Injection	100 mbars
Temperature	25 °C
Detection	
Signal	192/2 nm
Reference	450/100 nm

Figure 5
Electropherogram of a carbonated drink with benzoic acid and spectral overlay

Application

The method has been applied to different matrices: beverages, such as diet cola and coffee, and tablets. All compounds have been identified with library search see figure 4.

Reproducibility

The repeatability for all compounds was better than 0.15 % for retention time and between 1 and 7 % (9 % for phenylalanine due to an impurity) for peak area. The calculation was based on five runs with injected amounds of 50 to 250 ng absolute, see table 2 and figure 7. Buffer replenishment after five injections is necessary for highest reproducibility.

CZE is known to have linearity characteristics half that of HPLC, nevertheless the equipment used here is linear up to 600 mAU.[4]

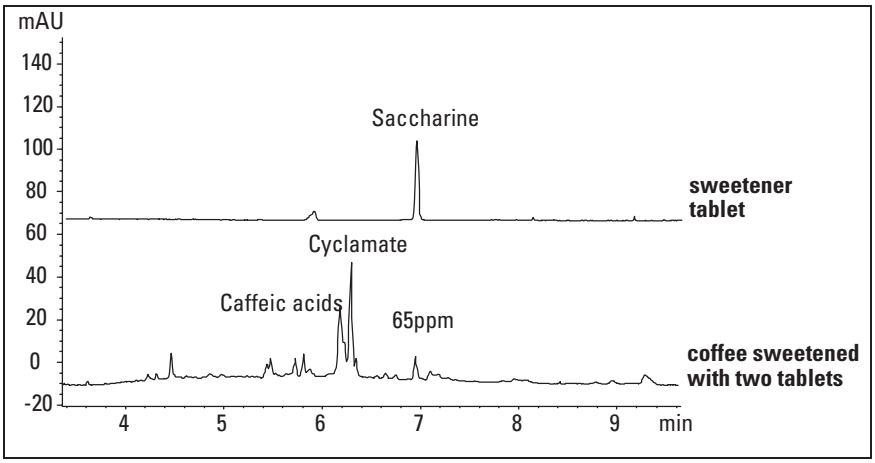

Figure 6
Electropherogram of analysis of a sweetener tablet overlaid with the electropherogram of directly injected coffee sweetened with two such tablets

Buffer	20 mM borate pH 9.4
E	465 V/cm
Effective capillary length	56 cm
Total capillary length	64.5 cm
id	50 µm
Injection	100 mbars
Temperature	25 °C
Detection	
Signal	192/2 nm
Reference	450/100 nm

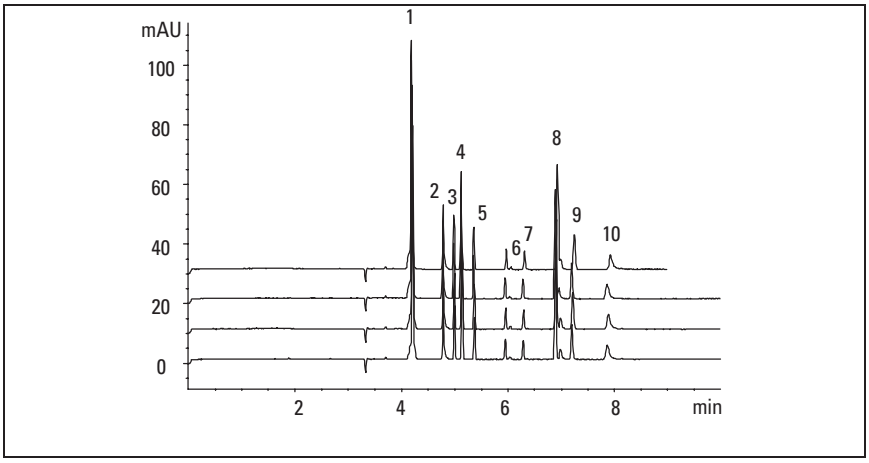

Figure 7
Overlay of five artificial sweetener standards

Buffer	20 mM borate pH 9.4
E	465 V/cm
Effective capillary length	56 cm
Total capillary length	64.5 cm
id	50 µm
Injection	100 mbars
Temperature	25 °C
Detection	
Signal	192/2 nm
Reference	450/100 nm

Key

1	phenylalanine	6	cyclamide
2	aspartame	7	sorbic acid
3	PHB propyl	8	benzoic acid
4	PHB ethyl	9	saccharine
5	PHB methyl	10	acesulfame

Conclusion

We have been able to show that capillary electrophoresis and UV-visible absorbance spectral library search is well suited for controlling food samples for artificial sweeteners and preservatives. Due to the transparency of the borate buffer detection could be done at 192 nm. Detection limit for most of the compounds is in the low nanogram range. Peak purity control for identification and confirmation of the compounds is possible in the same single run.

References

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Rainer Schuster and Angelika Gratzfeld-Hüsgen are HPLC application chemists based at Agilent Technologies Waldbronn Analytical Division, Germany.

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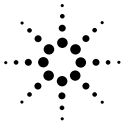

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