

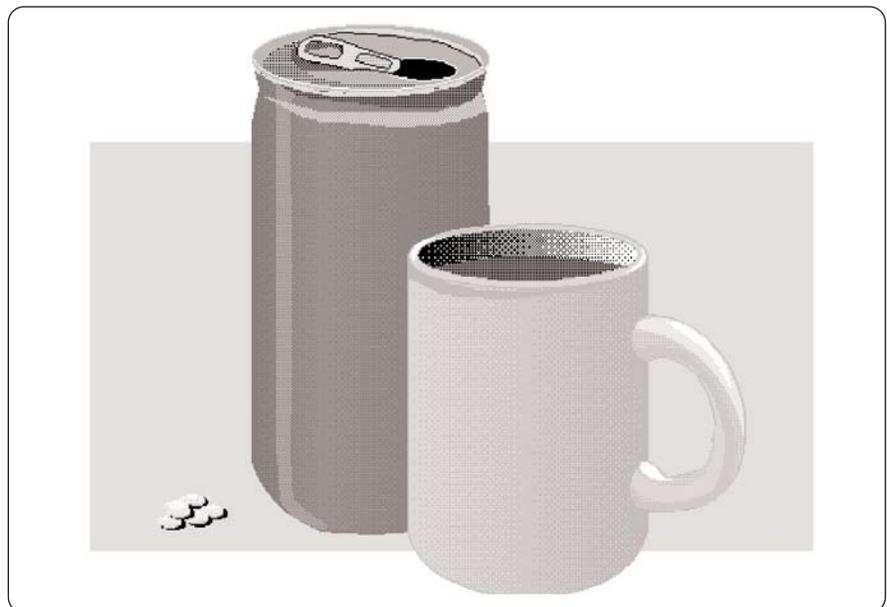
CZE analysis of artificial sweeteners and preservatives in drinks

Application Note

Foods and Flavors

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Abstract

Capillary electrophoresis can reduce the complexity associated with the analysis of sweeteners in drinks. 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 categorized in 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, (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.

Hermann and coworkers³ 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.

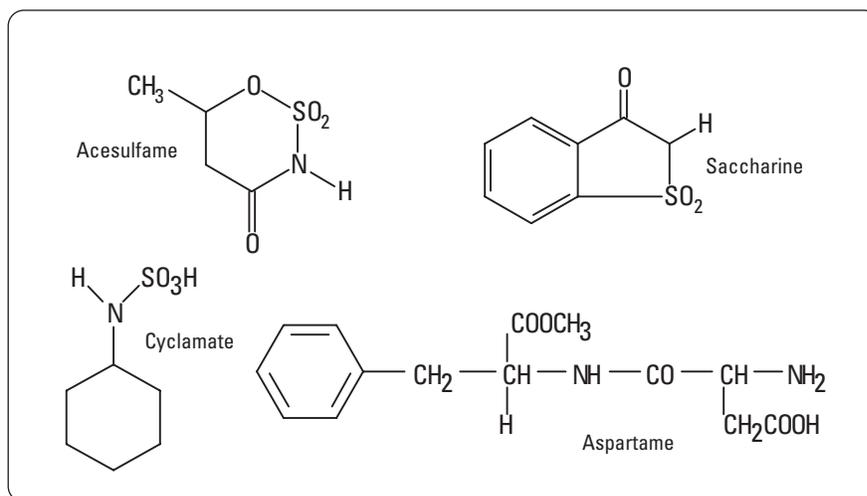


Figure 1
Chemical structure of the common artificial sweeteners.

	Saccharin	Saccharin Aspartame Dulcin	Acesulfame-K	Cyclamate NBDF-Derivative
Column	RP-18	ODS	ODS	RP-18
Mobile phase	0.05 mM H ₂ PO ₄ ⁻ - acetonitrile, 9:1	0.01 mM KH ₂ PO ₄ , pH 3.5-acetonitrile, 85:15	0.01 mM TBAHS- methanol, 9:1	H ₂ O, acetonitrile, 55:45
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.

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 ChemStation 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 1 M sodium hydroxide for 3 minutes followed by running buffer for 10 minutes. Samples were introduced hydrodynamically in 2 seconds 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 occurring preservatives PHB-esters (propyl-, ethyl-, and methyl), sorbic acid and benzoic acid.

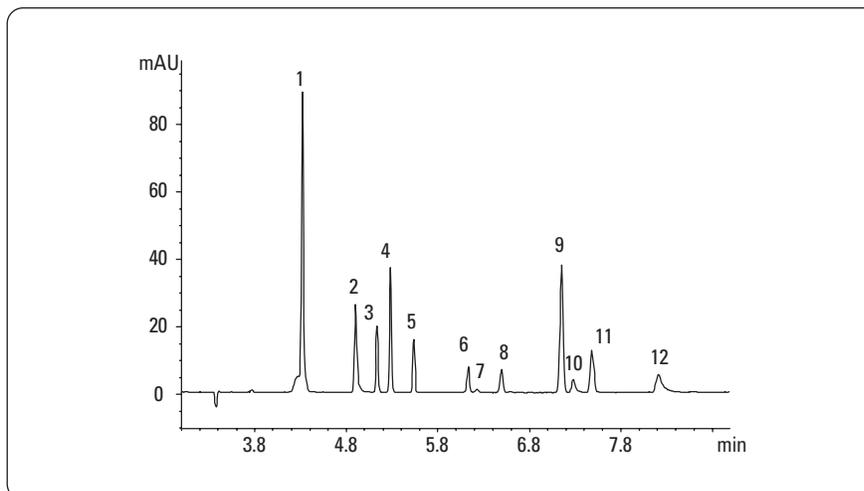


Figure 2
Electropherogram of a standard sample.

Chromatographic conditions

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 mbar x s
 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

Meas. Library CalTbl									
Time [min]	Time [min]	Time [min]	Sig	Amount [ng/ μl]	Purity	Library	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.

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, library 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, (figure 3). The overlay reveals an isobestic point (245 nm) —acesulfame exists in a tautomeric equilibrium in that buffer, stable at this pH value.

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 (figure 4).

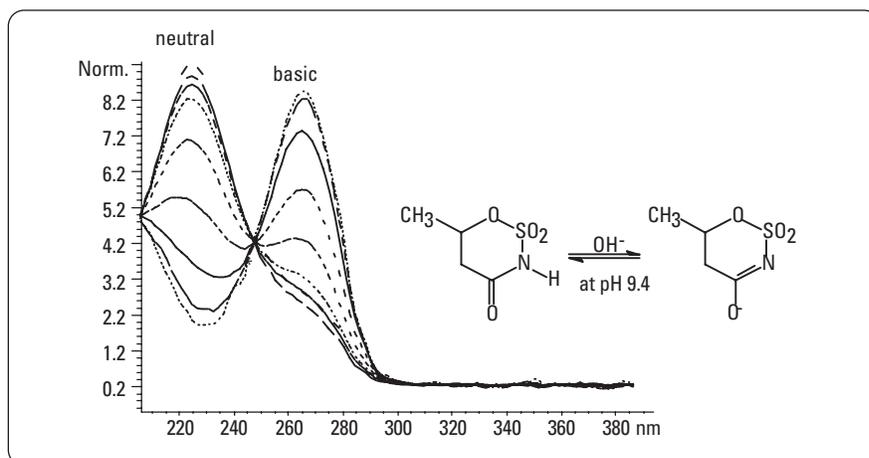


Figure 3
Overlay of spectra taken from acesulfame.

Chromatographic conditions

Buffer: 20 mM borate pH 9.4
E: 465 V/cm
Effective capillary length: 56 cm
Total capillary length: 64.5 cm, id 50 μ m
Injection: 100 mbar x s
Temperature: 25 $^{\circ}$ C

Detection

Signal: 192/2 nm
Reference: 450/100 nm

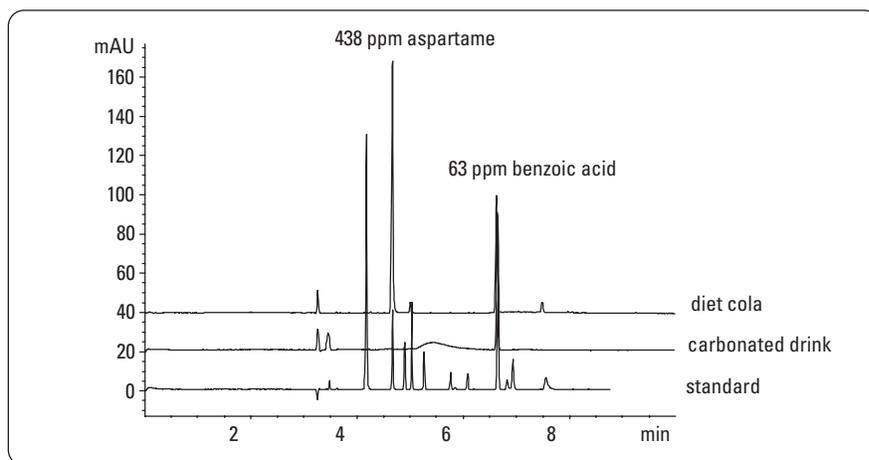


Figure 4
Electropherogram of a diet cola containing aspartame and benzoic acid and a carbonated drink containing benzoic acid.

Chromatographic conditions

Buffer: 20 mM borate pH 9.4
E: 465 V/cm
Effective capillary length: 56 cm
Total capillary length: 64.5 cm, id 50 μ m
Injection: 100 mbar x s
Temperature: 25 $^{\circ}$ C

Detection

Signal: 192/2 nm
Reference: 450/100 nm

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 amounts 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

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.

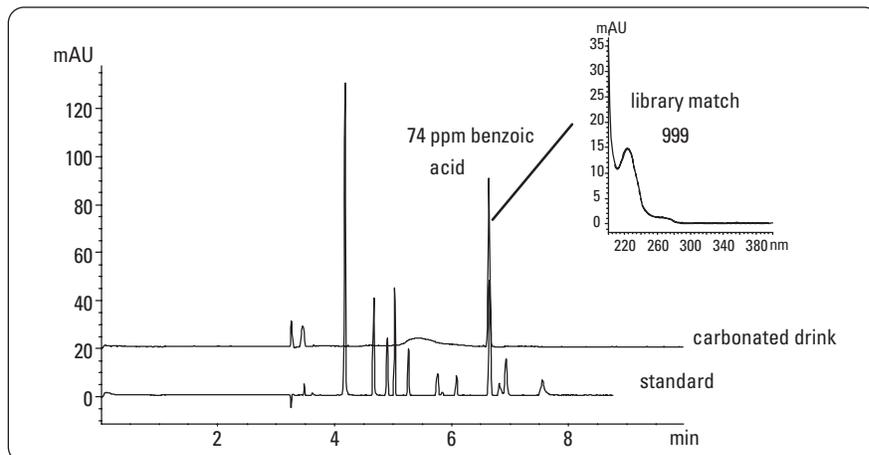


Figure 5
Electropherogram of a carbonated drink with benzoic acid and spectral overlay.

Chromatographic conditions

Buffer: 20 mM borate pH 9.4
E: 465 V/cm
Effective capillary length: 56 cm
Total capillary length: 64.5 cm, id 50 μ m
Injection: 100 mbar x s
Temperature: 25 $^{\circ}$ C

Detection

Signal: 192/2 nm
Reference: 450/100 nm

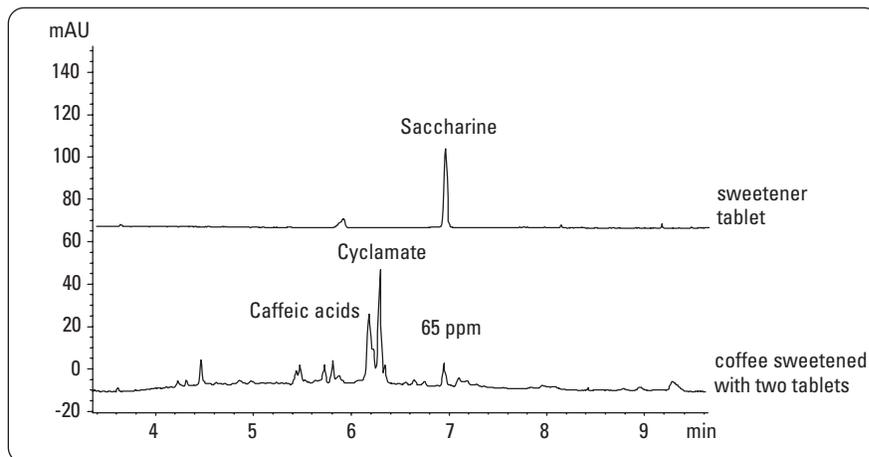


Figure 6
Electropherogram of analysis of a sweetener tablet overlaid with the electropherogram of directly injected coffee sweetened with two such tablets.

Chromatographic conditions

Buffer: 20 mM borate pH 9.4
E: 465 V/cm
Effective capillary length: 56 cm
Total capillary length: 64.5 cm, id 50 μ m
Injection: 100 mbar x s
Temperature: 25 $^{\circ}$ C

Detection

Signal: 192/2 nm
Reference: 450/100 nm

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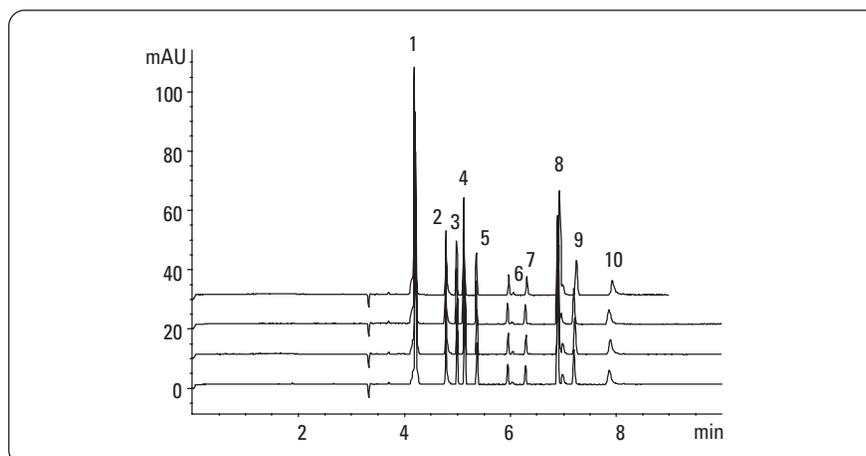


Figure 7
Overlay of five artificial sweetener standards.

Chromatographic conditions

Buffer: 20 mM borate pH 9.4
E: 465 V/cm
Effective capillary length: 56 cm
Total capillary length : 64.5 cm, id 50 μ m
Injection: 100 mbar x s
Temperature: 25 $^{\circ}$ 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

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© Agilent Technologies, Inc., 1994-2009
Published March 1, 2009
Publication Number 5990-3402EN



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