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Journal of Chromatography A, 781 (1997) 481–485

JOURNAL OF CHROMATOGRAPHY A

Short communication

Analysis of beverages by capillary electrophoresis

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Abstract

Rapid analysis of carbonated beverages by capillary electrophoresis (CE) allows the simultaneous determination of aspartame, benzoic acid and caffeine in 2 min using 20 m*M* glycine buffer at pH 9.0 and direct detection at 215 nm. The rapid determination of these compounds with minimal sample preparation offers an excellent method for evaluating stability and shelf-life of commercial products. This method is easily adapted to the analysis of these substances in other aqueous-based consumer products. © 1997 Elsevier Science B.V.

Keywords: Soft drinks; Food analysis; Aspartame; Benzoate; Caffeine

1. Introduction

Soft-drink beverages are consumed throughout the world. The high cost of transportation requires that these products are mixed and bottled regionally. At each of these manufacturing sites, concentrated syrups, aspartame, caffeine and preservatives such as benzoic acid are mixed with water followed by carbonation and bottling. This decentralized, multi-regional mixing generates the need for rapid, reliable and inexpensive testing methods that can be utilized by testing laboratories at mixing facilities.

Three of the more important beverage components which require routine testing are caffeine, benzoate and aspartame. These three substances exhibit strong UV absorptivity and are easily detected by most commercially-available HPLC and CE instruments. CE offers certain advantages over HPLC for beverage analysis, including relative simplicity of operation, lower operational costs, no need for organic solvents in mobile phases and shorter analysis times.

Various CE methods for analyzing beverage constituents have been described, including caffeine [1–3], carboxylic acids [4], benzoic acid [3,5], sorbic acid [6], ascorbic acid [4,7], aspartame [3,8], alkali and alkaline earth metals [9], carbohydrates [10] and sulphite [11]. One of these above methods describes the simultaneous determination of caffeine, benzoic acid and aspartame [3]. We expand this earlier report to include surveys of a larger number of beverages and demonstrate the usefulness of CE in determining the degradation products of aspartame.

2. Experimental

2.1. Apparatus

Separations were performed using a Spectrophoresis 1000 CE system, containing an autosampler, capillary temperature control and a rapid scan UV–Vis detector (ThermoSeparations Products, San Diego, CA, USA). The instrument was interfaced to a Pentium 90 computer running PC1000 ThermoSeparations Products software, version 3.0.

Uncoated, open, 50 μm diameter, silica capillaries

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PII S0021-9673(97)00532-3

were purchases from Polymicro Technologies (Phoenix, AZ, USA) and cut to 44 cm.

2.2. Reagents and samples

Carbonated beverages in metal cans were purchased from refrigerated vending machines. All of the cans were analyzed immediately and all were tested prior to the listed expiration date. Reagents were obtained from the following sources: sodium tetrahydroborate, decahydrate from EM Sciences (Gibbstown, NJ, USA.), glycine (free base) and phenylalanine (free base) from Sigma Chemical (St. Louis, MO, USA), aspartame from The Nutrasweet Company (Deerfield, IL, USA). Water was distilled and deionized before use in preparing buffers and samples.

2.3. Electrophoresis conditions

Silica capillaries were preconditioned by washing with 1 *M* NaOH for 15 min at 60°C, followed by 0.1 *M* NaOH for 15 min at 60°C, and distilled, deionized water for 15 min at 25°C. Prior to each injection, the capillary was washed with two volumes of fresh running buffer, which consisted of 20 m*M* glycine adjusted to pH 9.0 with NaOH. Samples were introduced by hydrodynamic injection for 1 s. Separations were run at 20 kV and 35°C. Rapid scanning was used to collect UV spectra at 200–300 nm. Electropherograms were generated by extracting individual wavelength absorption data during post-run processing.

2.4. Procedure

A portion of each carbonated beverage was withdrawn and degassed under vacuum and sonication to remove carbon dioxide. One volume of this solution was quantitatively mixed with one volume of running buffer and filtered through an 0.45 μm filter into a sample vial. Standard curves for each analyte were prepared in water, diluted 1:1 in running buffer and filtered before analysis.

3. Results

3.1. Electropherograms

As observed in the analysis of Diet Coke (Fig. 1), the peaks for caffeine, aspartame and benzoic acid are well resolved in approximately 2 min. Aspartame is also well-resolved in a mixture of synthetic standards containing phenylalanine and its demethylated dipeptide degradation product, aspartylphenylalanine (Fig. 2).

3.2. Calibration and sensitivity

Sensitivity varies for each of the three analytes, depending on detection wavelength. As is seen from the time-resolved absorption spectra in Fig. 3, longer

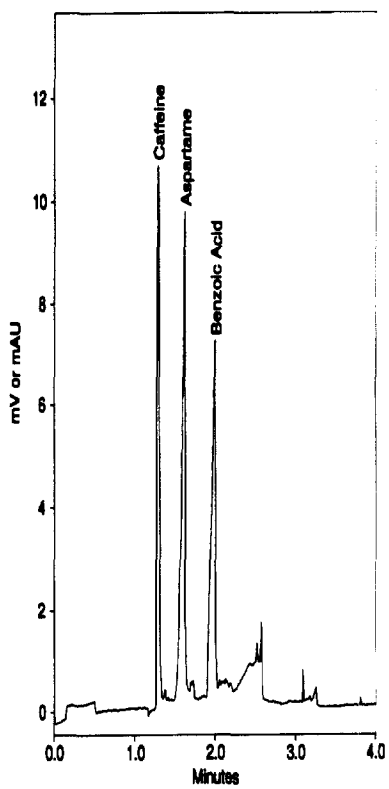

Fig. 1. Electropherogram of Diet Coke. The running buffer was 20 m*M* glycine, pH 9.0; hydrodynamic injection for 1 s; separation at +20 kV, 35°C, UV detection at 215 nm.

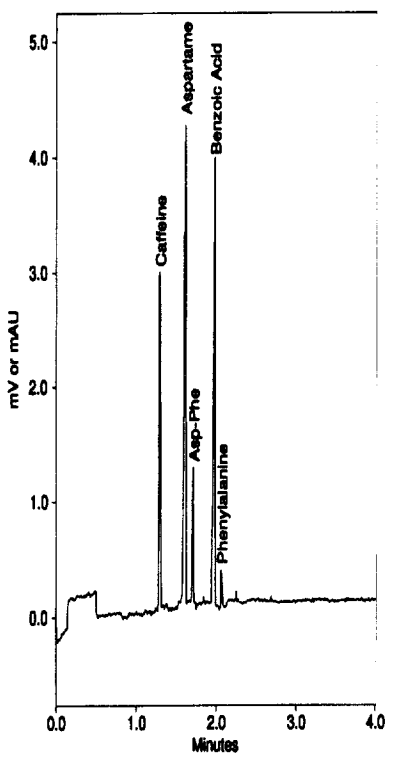

Fig. 2. Electropherogram of calibration standards, under identical conditions to Diet Coke in Fig. 1. The running buffer was 20 m*M* glycine, pH 9.0; hydrodynamic injection for 1 s; separation at +20 kV, 35°C, UV detection at 215 nm.

Table 1
Results of CE calibration and beverage analysis

Statistical data	Caffeine	Aspartame	Benzoic acid
Slope	158.8	44.2	143.6
Intercept	−711	−972	138.6
Std. err. of coeff.	3.0	0.5	4.7
R^2	0.9986	0.9996	0.9958
Avg. migration time (min)	1.28	1.59	1.96
R.S.D. (mig. time)	0.13%	0.32%	0.37%
R.S.D. ($n=18$)	2.0%	3.8%	2.4%
Spike recovery	97.9%	113.9%	100.6%
Min. det. level (μg/ml)	1.6	18	4.0

Analytical results	Amounts per 355 ml can (mg)		
(mg/can)	Caffeine	Aspartame	Benzoic acid
Mountain Dew	48		329
Orange Soda			354
Coke (classic)	35		
Pepsi	33		
Sprite			137
Diet Coke	52	206	56
Diet Dr. Pepper	52	206	70
Diet 7-Up		185	78
Fresca		175	77

Analytical results from CE analysis of beverages; running buffer was 20 m*M* glycine, pH 9.0, separation at +20 kV, 35°C, UV detection 215 nm.

wavelengths at 260–290 nm strongly favor detection of caffeine. Alternatively, detection at 210–230 nm are much more sensitive for benzoic acid. The optimum wavelength for most carbonated beverages was 215 nm, which yielded comparable-sized peaks for all three analytes in samples we tested. Limits of detection reported in Table 1 were determined at 215 nm. These limits vary depending on detection wavelengths. Higher or lower sensitivity levels are possible utilizing wavelengths other than 215 nm.

Standard curves derived from areas of all peaks yielded excellent linear fits, as shown in Table 1. Linearity of the standard curves at this wavelength was excellent up to concentrations of 300 μg/ml for caffeine, 400 μg/ml for benzoic acid and 1500 μg/ml for aspartame. However, standard curves derived from peak heights were not acceptable due to non-linear, hyperbolic deviations at higher concentrations (data not shown). Excellent reproducibility for both peak area and migration times were observed. Relative standard deviations for peak area and migration times were 2.0–3.8% and 0.13–0.37%, respectively ($n=18$). Spike recovery ranged from 98–114%, depending on the analyte (see Table 1).

4. Discussion

Utilization of CE offers an excellent method for the simultaneous, rapid analysis of caffeine, aspartame and benzoic acid in carbonated beverages. The speed of the analysis and simple sample preparation make the method useful in a quality control laboratory. To save time, samples may be analyzed without the extra step of mixing running buffer with the

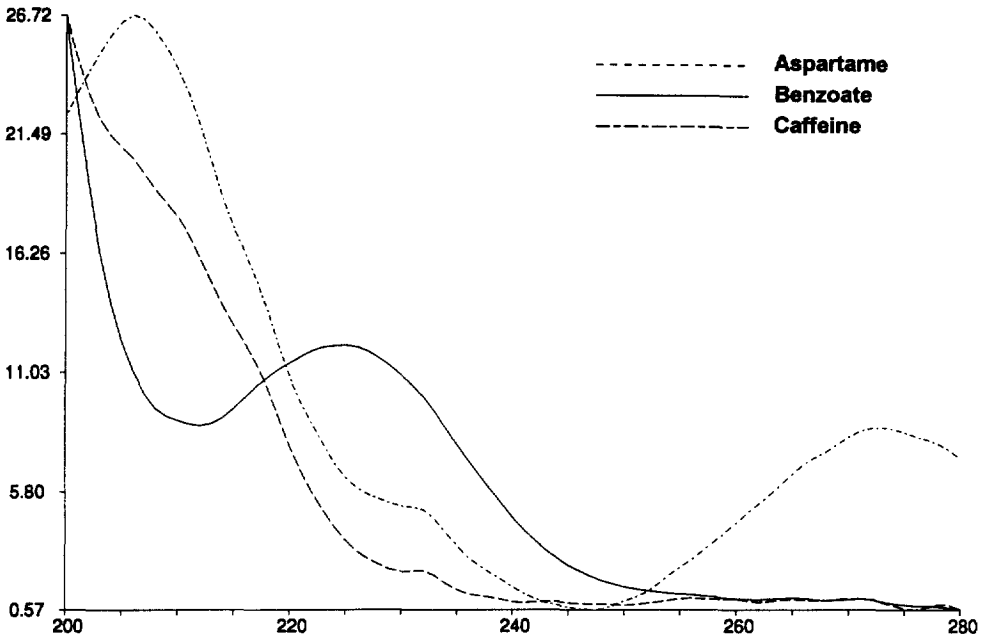

Fig. 3. Absorption spectra for caffeine, aspartame and benzoate obtained during CE separation. Spectra were measured at maximum peak height for each substance as they passed the UV detector window in the capillary.

samples. However, this extra step significantly decreases variance in retention times and peak areas. Obviously, two-minute run times can be decreased further by use of shorter columns or higher voltages, however, we observed less resolution of aspartame degradation products if these techniques were employed. In general, glycine buffers offer more rapid migration times than borate buffers, but also reduce resolution of these minor peaks.

Qualitative identification of the major peaks in the chromatogram can be accomplished easily by their absorption spectra, or by running a series of different beverages whose contents differ and are listed on the product label. Calibration curves are easily accomplished with commercially available standards across ranges often encountered in soft drinks.

Results for caffeine content in common beverages was similar to that obtained by other investigators utilizing CE [1], although our results are slightly higher in some cases. When compared to HPLC data from other works, our results are also very similar [12–14]. During routine testing over a period of two years in our laboratory, we have observed significant batch-to-batch differences in production runs of these beverages, and especially among samples collected from dispensing machines in fast-food restaurants.

We have also observed that when cans of these beverages are stored for extended periods under warm conditions, significant reductions of aspartame can be observed as well as increases in its degradation products, phenylalanine and aspartylphenylalanine. This fact nominates CE as an excellent method for stability testing and expiration dating of these beverages.

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