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Development of a capillary electrophoresis method for the simultaneous analysis of artificial sweeteners, preservatives and colours in soft drinks

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Abstract

A rapid capillary electrophoresis method was developed simultaneously to determine artificial sweeteners, preservatives and colours used as additives in carbonated soft drinks. Resolution between all additives occurring together in soft drinks was successfully achieved within a 15-min run-time by employing the micellar electrokinetic chromatography mode with a 20 m*M* carbonate buffer at pH 9.5 as the aqueous phase and 62 m*M* sodium dodecyl sulfate as the micellar phase. By using a diode-array detector to monitor the UV–visible range (190–600 nm), the identity of sample components, suggested by migration time, could be confirmed by spectral matching relative to standards.

Keywords: Food analysis; Soft drinks; Artificial sweeteners; Preservatives; Dyes

1. Introduction

Most soft drinks contain a combination of food additives designed to enhance their flavour, appearance and/or shelf-life. These food additives fall under strict legislative control as regards their permitted usage, the enforcement of which necessitates the development of rapid and robust analytical methodology. To that end, capillary electrophoresis (CE) represents a powerful and flexible separation technique with the capability to separate sample analytes from a range of compound classes within a single analysis [1,2]. It is this capability that makes CE an ideal technique for the analysis of multi-component mixtures such as soft drinks.

Several recent papers have demonstrated the utility of CE for the analysis of food colours [3–11], artificial sweeteners [12–18] and/or preservatives [14–21]. The separation of food colours has received the most attention, with a number of reports of the application of either micellar electrokinetic chromatography (MEKC) [3,4] or capillary zone electrophoresis (CZE) with buffer additives, such as cyclodextrins [5–7], to achieve resolution of mixtures of colour standards. More straightforward CZE methods have also emerged for the separation of mixtures of colours [8,9]. For example, Liu et al. [8] proposed a simple CZE method to determine six synthetic food colours (amaranth, ponceau 4R, tartrazine, sunset yellow, indigo carmine and brilliant blue), using a 20

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m*M* borate buffer at pH 9.0 without buffer additives. The buffer pH was chosen to ensure dissociation of the carboxylic and sulfonic acid groups in the dye molecules, resulting in multiply charged anions. Recently, Berzas et al. [9] described the development and validation of a CZE method for the simultaneous determination of seven commonly used food colours. Their method employed a 15 m*M* borate buffer at pH 10.5 and achieved detection limits in the range 0.35 to 2.12 mg/l. Interestingly, it was reported that, despite their absorption maxima in the visible range, better detection limits were possible in the UV region at 216 nm, due to higher signal-to-noise ratio. Ionic strength was an important factor, affecting both the migration time and peak shape, increased ionic strength leading to longer run times and peak deformation. Also recently, a CZE method was developed and validated that employed a 50 m*M* carbonate buffer at pH 9.5 for the determination of Class IV caramels in soft drinks [10,11], such caramels being used extensively in the production of cola beverages.

The most commonly used artificial sweeteners in soft drinks are aspartame, acesulfame K and saccharin, each of which may be used individually or blended with sugars or one or more of the others [22]. Pesek and Matyska [12] described a CZE method for quantification of aspartame in foods using a 30 m*M* phosphate–19 m*M* Tris buffer (pH 2.14) with detection at 211 nm. Separation was achieved within a 4-min timescale, and the method was successfully applied to the determination of aspartame in a range of soft drinks. However, using a separation buffer with such an acidic pH had its drawbacks, since aspartame is known to be unstable below pH 3 [22] and there was no detection of other components in the soft drinks.

Other reports have since shown the promise of CZE for the simultaneous separation of aspartame, caffeine and benzoic acid from soft drinks [14–16]. Jimidar et al. [14] used a 25 m*M* sodium phosphate buffer at pH 11 to separate the three components within 10 min, whereas, more recently, the determination of aspartame, benzoic acid and caffeine within a 2-min run-time was achieved using a 20 m*M* glycine buffer at pH 9.0 [15]. A similar method, designed for undergraduate teaching, achieved separation of the three components within a 3-min run-time using a 25 m*M* borate buffer at pH 9.4 and a short (33 cm×50 μm I.D.) capillary [16].

Other artificial sweeteners have also been successfully separated by CE methods [17,18]. Aspartame, saccharin, acesulfame-K, alitame and dulcin were separated and quantified together in a single run together with caffeine, benzoic acid and sorbic acid by MEKC [17]. The method utilised a 10 m*M* phosphate–10 m*M* borate buffer at pH 8.6 with 50 m*M* sodium deoxycholate as the micellar phase and was successfully applied to the analysis of diet soft drinks. A more sophisticated buffer system was developed by Boyce [18] to allow the simultaneous determination of aspartame, saccharin and acesulfame K together with several antioxidants and preservatives. The buffer comprised 20 m*M* borate at pH 9.3 with 35 m*M* sodium deoxycholate–15 m*M* sodium dodecyl sulfate (SDS) as a mixed micellar phase and 10% methanol.

Most of the reports of the CE analysis of artificial sweeteners have also included the separation of preservatives by the same method [14–18]. Further reports concentrate exclusively on the determination of preservatives [19–21]. Ng et al. [19] used a 50 m*M* phosphate–50 m*M* borate buffer at pH 7.0 with 10 m*M* tetrabutylammonium hydrogensulfate added as an ion-pairing reagent to separate mixtures of caprylic acid, sorbic acid, benzoic acid and propionic acid. More recently, Kuo and Hsieh [21] were able simultaneously to determine nine preservatives, including benzoic acid, sorbic acid and a range of alkyl *p*-hydroxybenzoates. A 35 m*M* borate buffer at pH 10.0 was used that was modified with the addition of 2 m*M* α-cyclodextrin. The presence of α-cyclodextrin aided the resolution of the structurally similar *p*-hydroxybenzoates.

While several of the reports described above demonstrated the possibilities for the simultaneous determination of sweeteners and preservatives, CE has not yet been applied to the simultaneous analysis of compounds from all three groups of colours, sweeteners and preservatives. This is despite a certain commonality between the methodologies employed. Therefore, the aim of this paper is to develop a rapid and cost-effective method simultaneously to analyse colours, sweeteners and preservatives in soft drinks, thus filling the gap in current analytical methodology. There is demand for

such a method in quality control and in surveying soft drink ingredients.

2. Experimental

2.1. Chemicals

Acesulfame K, aspartame, benzoic acid, caffeine, saccharin and electrophoresis-grade sodium dodecyl sulfate were purchased from Sigma (Poole, UK). Sodium hydroxide, sodium tetraborate decahydrate, sodium carbonate and sodium hydrogen carbonate were purchased from BDH (Poole, UK). Sorbic acid was purchased from Fluka (Gillingham, UK). Synthetic food colours (black PN, brilliant blue FCF, carmoisine, green S, ponceau 4R, quinoline yellow WS, sunset yellow FCF) were supplied courtesy of Pointing (Prudhoe, Northumberland, UK). Class IV caramel standards were supplied courtesy of British manufacturers. Soft drink samples were purchased from a local supermarket.

2.2. Standards and samples

Standards were prepared by dissolution in high-purity, reverse-osmosis water (Purite, Thame, UK) at stock concentrations of 1 mg/ml. Soft drink samples were degassed by ultrasonication. All standard and sample solutions were filtered through a 0.8/0.2-μm Acrodisc PF filter (Gelman Sciences, Ann Arbor, MI, USA) prior to CE analysis.

2.3. Buffers

Carbonate buffer was prepared by adding *x* m*M* sodium carbonate solution to *x* m*M* sodium hydrogen carbonate solution until the desired pH was achieved. Borate buffers were prepared by preparing a solution of the desired concentration of sodium tetraborate decahydrate and adjusting its pH with 1 *M* sodium hydroxide. Buffers for MEKC were prepared by dissolving the appropriate concentration of SDS into a 20 m*M* carbonate buffer at pH 9.5.

2.4. Apparatus and operating conditions

The CE instrument was a Hewlett-Packard $HP^{3D}CE$ (Hewlett-Packard, Waldbronn, Germany) equipped with diode-array detection and $HP^{3D}CE$ ChemStation software for instrument control, data acquisition and data analysis. Uncoated fused-silica capillaries (Hewlett-Packard) were of 50-μm internal diameter and 48.5 cm (40 cm to detector) or 64.5 cm (56 cm to detector) total length, with a ×3 extended pathlength bubble cell. New capillaries were conditioned by washing with 1 *M* sodium hydroxide (30 min), 0.1 *M* sodium hydroxide (20 min) and water (15 min) prior to first use. The capillaries were then conditioned daily prior to use by washing with 1 *M* sodium hydroxide (10 min), 0.1 *M* sodium hydroxide (5 min) and water (5 min). Samples were injected at the anode by hydrodynamic injection (250 mbar s). Separations were carried out using a voltage of 20 kV and a capillary temperature of 25°C. Before each sample injection, the capillary was rinsed with 0.1 *M* sodium hydroxide (3 min) and buffer (3 min). Buffer was replaced after four sample injections. Spectra were taken at the peak apices.

3. Results and discussion

A survey was made of food additives contained in >50 soft drink brands stocked in a local supermarket. Food additives that fell into the target categories of the method included a range of seven synthetic food colours (quinoline yellow, sunset yellow FCF, carmoisine, ponceau 4R, brilliant blue FCF, green S and black PN), three artificial sweeteners (acesulfame K, aspartame and saccharin) and two preservatives (benzoic acid and sorbic acid). Each of these additives is freely soluble in water, possesses at least one ionisable functional group and, by virtue of its structural characteristics, has sufficient absorbance at 200 nm to allow detection. All of these factors, together with the aim of producing a simple method, pointed to the use of CZE as the mode of separation.

3.1. CZE

The previous literature pertaining to the analysis of food additives by CZE [8,9,14–18,21] and our previous work on the analysis of Class IV caramels in soft drinks [10,11] indicated the use of a buffer system at an alkaline pH. Therefore, CZE was

employed to investigate the performance of borate and carbonate buffer systems at pH 9.5 and 50, 20, 10 and 5 m*M* buffer concentrations for the analysis of the individual standards. Direct comparison of the resulting electropherograms (not shown) clearly showed that the carbonate buffer produced better peak shape and shorter migration times at each buffer concentration. This was particularly evident at 50 m*M* buffer concentration, where use of borate buffer yielded analyte peaks exhibiting substantial peak-broadening and long migration times. The effect of buffer type was less pronounced for sweetener and preservative molecules, but, on the basis of the performance for the food colours, a carbonate buffer system was preferred for further development.

Fig. 1 includes an electropherogram displaying the separation of a 13-component standard mixture at 20 m*M* carbonate buffer concentration. This was the best resolution achieved by CZE within the carbonate concentration range of 5–50 m*M*. Below 20 m*M*, green S and sorbic acid were seen to co-migrate, and the resolution between benzoic acid and saccharin was poor. Above 20 m*M*, the analysis time was unacceptably long, while substantial peak broadening was observed. However, upon analysing some typical mixtures at a carbonate buffer concentration of 20 m*M*, it was found that baseline resolution of benzoic acid and saccharin was not reproducible. This was viewed as an important issue, since these two components often occur together in soft drinks. Variation of the buffer pH in the range pH 9–10.5 (data not shown) offered no improvement to this situation.

One possible approach to improving the resolution between benzoic acid and saccharin was to increase the capillary length. Indeed, using a 64.5-cm (56 cm to detector) capillary with all other conditions the same, was successful for baseline resolution of benzoic acid and saccharin. However, the run time for some other components was unacceptably long, approaching 30 min in the case of black PN. Therefore, it was decided to attempt separation by MEKC.

3.2. MEKC

In the light of the CZE experiments discussed

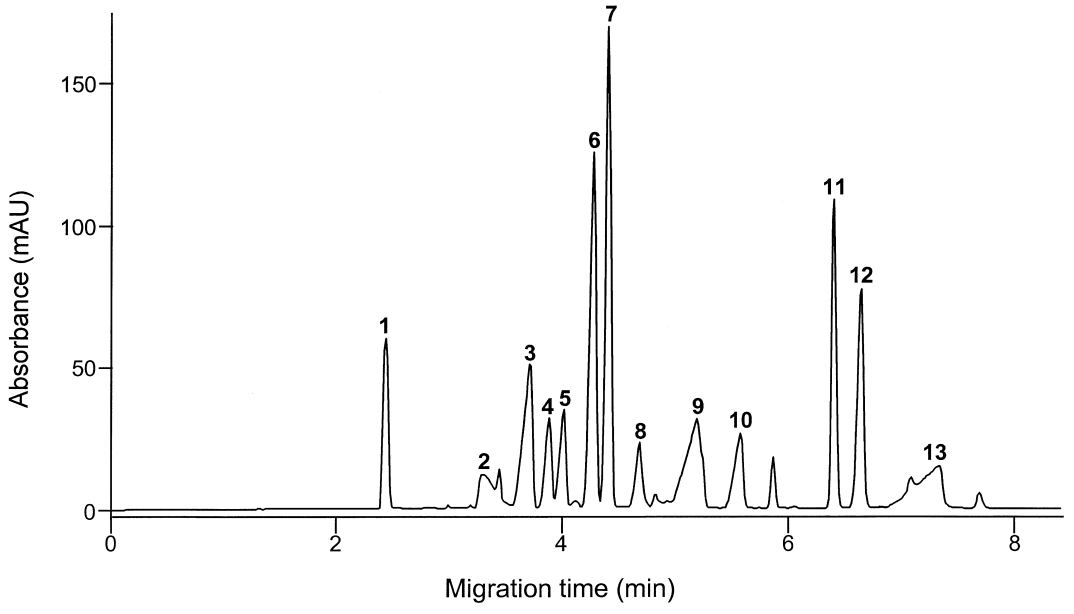

Fig. 1. Electropherogram showing the separation of caffeine (1), aspartame (2), brilliant blue FCF (3), green S (4), sorbic acid (5), benzoic acid (6), saccharin (7), acesulfame K (8), sunset yellow FCF (9), quinoline yellow (10), carmoisine (11), ponceau 4R (12), black PN (13), using 20 m*M* carbonate buffer, pH 9.5. A 48.5-cm×50-μm I.D. fused-silica capillary was used and absorbance was measured at 200 nm.

above, initial MEKC experiments concentrated on achieving resolution between benzoic acid and saccharin. SDS concentrations of 25, 50 and 75 m*M* were added in turn to 20 m*M* carbonate buffers at pH 9.5 to investigate whether the presence of a micellar phase could influence resolution. From these experiments, it was found that the addition of SDS did improve resolution between benzoic acid and saccharin, and that a minimum SDS concentration of 50 m*M* was necessary for reproducible baseline resolution.

Subsequent runs with a more extensive range of analytes showed that the presence of SDS in the buffer significantly affected the migration order and peak shape of certain analytes relative to CZE. Fig. 2 shows electropherograms for the separation of the 13-component standard mixture by CZE (Fig. 2A) and MEKC with 50 m*M* SDS (Fig. 2B) and 75 m*M* SDS (Fig. 2C). From inspection of these electropherograms, it is apparent that the presence of SDS caused a significant shift in the migration times for caffeine, brilliant blue FCF and green S. Indeed, brilliant blue FCF was resolved as two peaks in the presence of SDS. Brilliant blue FCF and green S are both of the same structural family of food colours, namely, the triarylmethanes, which comprise three, conjugated aromatic systems and two hydrophobic tertiary amine substituents. Brilliant blue FCF and green S also carry lower net negative charge than the other colours studied; reflected in their rapid migration in CZE. It can be expected that this low negative charge would allow greater interaction with anionic SDS micelles, since charge repulsion would be minimal.

Further examination of Fig. 2 reveals that, at 50 m*M* SDS, green S co-migrated with saccharin and, at 75 m*M* SDS, green S co-migrated with acesulfame K and the resolution between caffeine and aspartame was poor. Therefore, intermediate SDS concentrations were investigated to attempt to isolate a

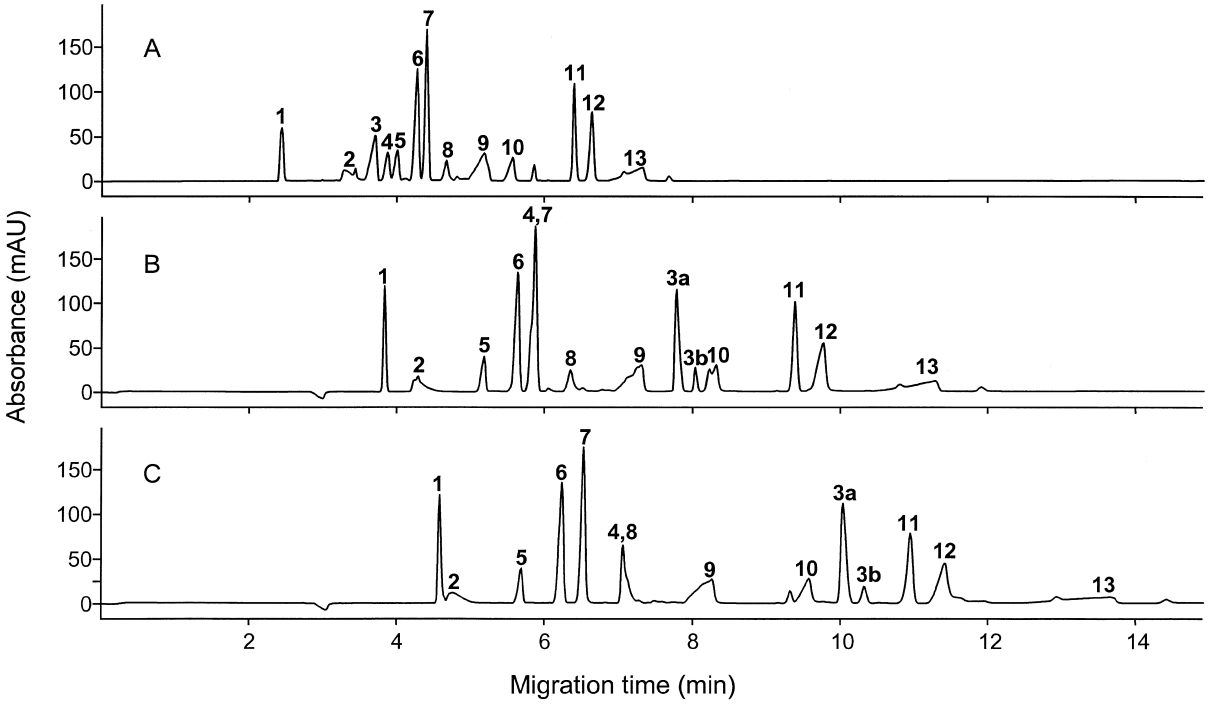

Fig. 2. Electropherograms showing the separation of caffeine (1), aspartame (2), brilliant blue FCF (3), green S (4), sorbic acid (5), benzoic acid (6), saccharin (7), acesulfame K (8), sunset yellow FCF (9), quinoline yellow (10), carmoisine (11), ponceau 4R (12), black PN (13), using 20 m*M* carbonate buffer, pH 9.5, containing (A) no SDS, (B) 50 m*M* SDS, (C) 75 m*M* SDS. A 48.5-cm×50-μm I.D. fused-silica capillary was used and absorbance was measured at 200 nm.

resolved green S peak within the migration time window between saccharin and acesulfame K. Fig. 3 displays the results for these experiments, showing that complete resolution of green S was achieved between 60 and 65 m*M* SDS concentrations. The concentration of SDS was optimal at a value of 62 m*M*, this yielding good peak shapes, particularly for sunset yellow FCF and ponceau 4R. Indeed, over a run of 10 repeat injections of the 13-component standard mixture, peak shapes were maintained and the migration times of each component remained consistent, with relative standard deviations (RSDs) of $<0.6\%$. Brilliant blue FCF and quinoline yellow peaks did co-migrate at 62 m*M* SDS, but this was not perceived to be an issue, since these colours did not appear together in any of the soft drink brands surveyed. In any case, should both brilliant blue FCF and quinoline yellow be present in a sample, then a 65 m*M* SDS could be used.

3.3. Quantification of soft drink components

As a final stage to the development of the method, the quantification of soft drink components by measurement of their peak areas was investigated. For the quantification of brilliant blue FCF, the area of its major peak (labelled '10a' in Fig. 3) was used. Calibration plots were constructed for each of the 13 soft drink components within the range 0.01–1.0 mg/ml. It was found in all cases that a linear relationship exists between concentration and peak area, each with an excellent linear correlation coefficient ($R^2>0.99$). The limit of quantification was determined as 0.01 mg/ml, although several of the

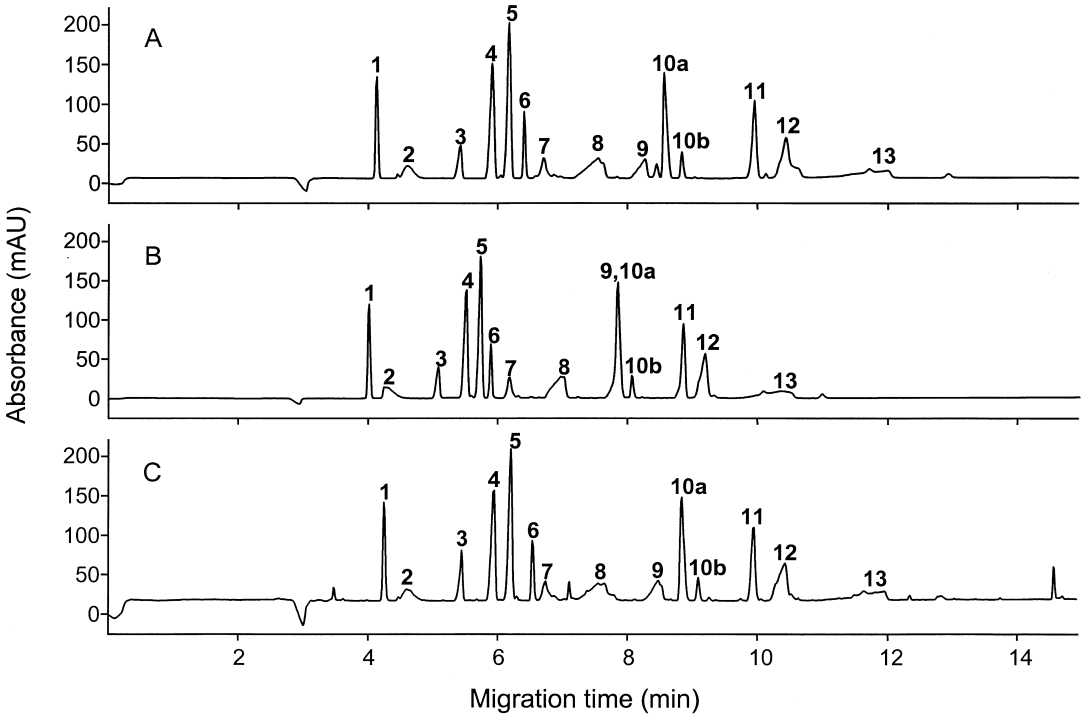

Fig. 3. Electropherograms showing the separation of caffeine (1), aspartame (2), sorbic acid (3), benzoic acid (4), saccharin (5), green S (6), acesulfame K (7), sunset yellow FCF (8), quinoline yellow (9), brilliant blue FCF (10), carmoisine (11), ponceau 4R (12), black PN (13), using 20 m*M* carbonate buffer, pH 9.5, containing (A) 60 m*M* SDS, (B) 62 m*M* SDS, (C) 65 m*M* SDS. A 48.5-cm×50-μm I.D. fused-silica capillary was used and absorbance was measured at 200 nm.

additives could be detected and identified by spectral matching at concentrations of 0.005 mg/ml.

3.4. Application of MEKC method to soft drink samples

Finally, the MEKC method utilising a 20 m*M* carbonate buffer at pH 9.5 with 62 m*M* SDS was tested on a range of soft drink samples, examples of which are shown in Fig. 4. In general, declared ingredients were detected in sufficient amounts to allow their identities to be established by spectral matching relative to standards. Included in the samples for analysis were several soft drinks containing Class IV caramel as the colour additive. An electropherogram for one such sample is shown in Fig. 4C, showing good agreement with previous work on soft drinks containing Class IV caramel [10]. Despite its broad peak shape, quantification is possible of Class IV caramel, since there was a linear relationship between concentration and peak area (R^2>0.99) as with the other soft drink components.

4. Conclusions

A method for the analysis of artificial sweeteners, preservatives and food colours used as food additives in soft drinks has been developed. The separation of mixtures of such additives was successfully accomplished using MEKC under optimised conditions utilising a 20 m*M* carbonate buffer at pH 9.5 with 62

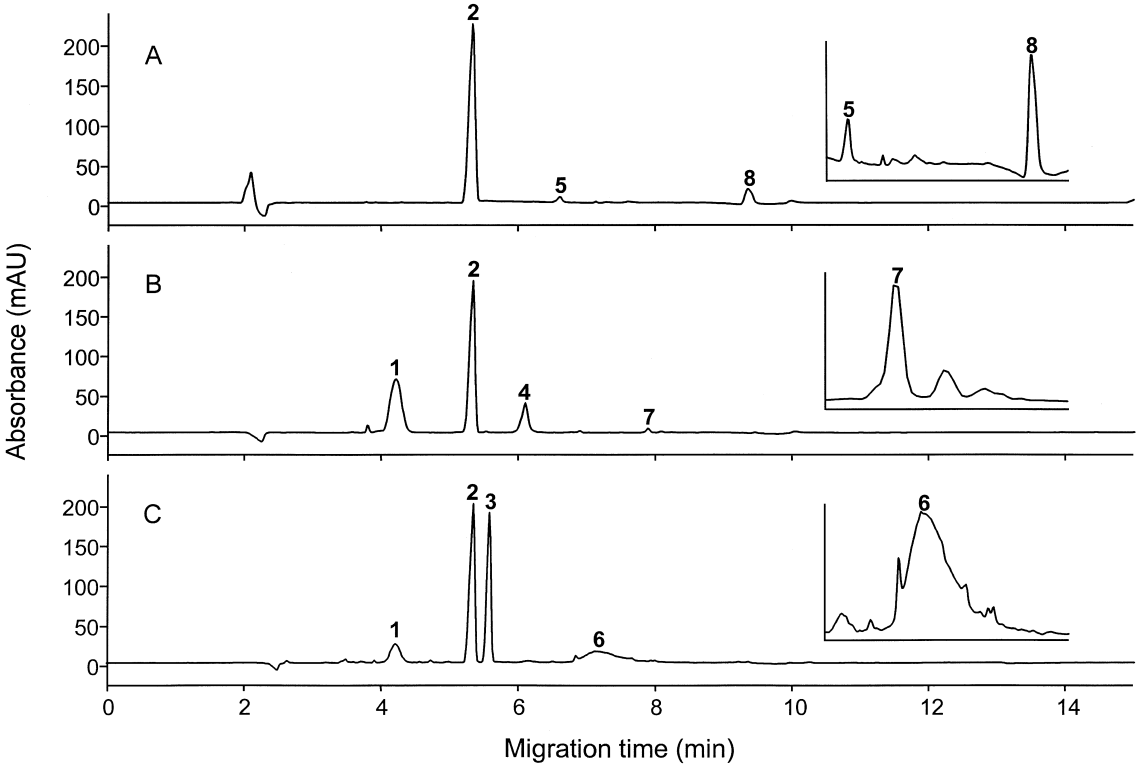

Fig. 4. Electropherograms of (A) mixed-flavours soft drink, (B) sugar-free bubblegum flavour sparkling drink, (C) low-sugar cola, containing combinations of aspartame (1), benzoic acid (2), saccharin (3), acesulfame K (4), sunset yellow FCF (5), Class IV caramel (6), brilliant blue FCF (7), ponceau 4R (8). A 20 m*M* carbonate buffer, pH 9.5, containing 62 m*M* SDS and a 48.5-cm×50-μm I.D. fused-silica capillary were used. Absorbance was measured at 200 nm.

m*M* SDS as the micellar phase. When applied to retail soft drink samples, this method allowed the reliable determination of additives with a limit of quantification of 0.01 mg/ml. However, before this CE method can be used for routine analysis it will be necessary to perform further validation.

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