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Comparison of capillary zone electrophoresis with high-performance liquid chromatography for the determination of additives in foodstuffs

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ABSTRACT

A capillary zone electrophoretic (CZE) method was developed to determine caffeine, aspartame and benzoic acid in diet cola soft drinks and in artificial sweetening powders. The effects of pH, ionic strength, organic solvents and different buffers were investigated to select the optimum conditions. These consisted of a sodium phosphate buffer at pH 11 and ionic strength 0.025. The running voltage was set at 15 kV and the injection was performed hydrostatically for 30 s. The CZE method was then compared with a previously developed high-performance liquid chromatographic (HPLC) method in terms of repeatability, reproducibility, accuracy, linearity, sensitivity and separation efficiency. Both methods gave good repeatability. The relative standard deviations for reproducibility were significantly higher in CZE than in HPLC. The main reason for this is probably the condition of the wall of the capillary, which was difficult to keep constant between the days of analysis. The separation efficiency of CZE was 65–110 times higher than that of HPLC; on the other hand, 10–20 times lower detection limits were obtained in HPLC. Both methods were linear, but the linear ranges were different owing to the lower detection limit of HPLC. In CZE, the effect of the matrix was higher.

INTRODUCTION

Capillary zone electrophoresis (CZE) is an increasingly used technique, the analytical power of which is widely recognized [1–5]. The applications range from biological analysis [1] to food [6] and pharmaceutical analysis [5]. Although CZE can be a powerful qualitative analytical technique, owing to its high separation efficiency, there is still doubt about its quantitative aspects. In comparison with high-performance liquid chromatography (HPLC), parameters such as repeatability, reproducibility and accuracy have received less attention. For this reason, an investigation of these parameters in CZE was carried out.

The aim of this work was first to understand better several parameters of method development in CZE, second to obtain data concerning method validation parameters in CZE and finally to see how

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these differ from HPLC in practical situations. As an HPLC method for the determination of aspartame, benzoic acid and caffeine in diet soft drinks had already been developed at our laboratory [7], a CZE method was developed and compared with the HPLC method.

Aspartame has been determined by HPLC [7], isotachophoresis [8] and enzymatic techniques [9]. To our knowledge, only one method has been published for the determination of aspartame in soft drinks by CZE [6]. By this method caffeine, and benzoic acid could also be determined. For the determination of caffeine methods using CZE [10] and micellar electrokinetic capillary chromatography (MECC) [11] have also been reported.

EXPERIMENTAL

Chemicals

Water purified using a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Sodium monohydrogenphosphate, sodium dihydrogenphosphate, potassium phosphate, sodium hydroxide, sodium carbonate, boric acid, phosphoric acid, sodium tetraborate, sodium phosphate, glycine, benzoic acid, methanol and acetonitrile were purchased from Merck (Darmstadt, Germany), caffeine from Fluka (Buchs, Switzerland) and aspartame from Sigma (St. Louis, MO, USA). Packets of Canderel® and Finn® artificial sweetening powders were obtained at local shops.

Sample pretreatment

Diet cola soft drinks were degassed in an ultrasonic bath and diluted 25- and 100-fold in Milli-Q-purified water for the determinations by CZE and HPLC, respectively. Artificial sweetening powders were dissolved in water in a 100-ml volumetric flask, filtered through a 0.2-μm membrane filter (Schleicher & Schüll, Dassel, Germany) and diluted 10- and 100-fold with water for CZE and HPLC, respectively.

Preparation of solutions

All buffers were prepared with an ionic strength of 0.1 and diluted fourfold prior to use. Following this they were filtered through a 0.2-μm membrane filter (Schleicher & Schüll). The buffers were adjusted to the pH required using an Orion Research digital ionalyzer/501 pH meter. The amounts needed to prepare 1 l of the buffers were as follows: sodium phosphate buffer, $Na_2HPO_4 \cdot 2H_2O$ 5.874 g and $Na_3PO_4 \cdot 12H_2O$ 0.0585 g (pK_a = 12.33), pH = 11.0; borate buffer, $Na_2B_4O_7$ 11.43 g and H_3PO_4 1.21 g (pK_a = 9.2), pH = 10.4; glycine buffer, glycine 8.258 g and NaOH 4.0 g (pK_a = 9.78) pH = 10.8; carbonate buffer, Na_2CO_3 0.827 g and $NaHCO_3$ 0.131 g (pK_a = 10.33), pH = 10.9; potassium phosphate buffer, Na_2HPO_4 7.532 g and Na_3PO_4 0.0086 g (pK_a = 12.33) pH = 10.9; and sodium phosphate buffer for HPLC, $NaH_2PO_4 \cdot H_2O$ 13.799 g and 1 *M* H_3PO_4 0.45 ml (0.85 ml of 85% H_3PO_4 diluted to 100.0 ml) (pK_a = 2.15), pH = 3.9 and μ = 0.1.

Stock solutions of 500 mg l^{-1} of each compound were prepared daily in water. The solubility was increased by using an ultrasonic bath for 5 min. The solutions for the external calibration in CZE were prepared in water in the concentration range 10–100 mg l^{-1}, and for HPLC in the range of 1–10 mg l^{-1}. The solutions for the standard addition calibration were in the same concentration range as in external calibration, but were prepared in 25- and 100-fold diluted and degassed diet cola for CZE and HPLC, respectively.

Instrumentation

CZE was carried out on a Waters Quanta-4000 capillary electrophoresis system equipped with a positive power supply. Fused-silica capillaries (Waters AccuSep®) (60 cm × 75 μm I.D.) were used with a length of 52 cm from the point of sample introduction to the point of detection. The electrophoretic zones were detected with a fixed-wavelength UV detector at 214 nm. Both injection systems (hydrodynamic and electromigration) were used. The electropherograms were recorded and integrated with an Intersmat/Shimadzu IC-R3A data processor and also with a Waters Model 810 data workstation equipped with a W51-watchdog interface.

HPLC was performed on a Perkin-Elmer series 10 liquid chromatograph with a 100-μl loop, coupled with a Perkin-Elmer LC90 UV spectrometric detector. The chromatograms were recorded and integrated on a Merck/Hitachi D-2000 chromato-integrator.

Preparation of the capillary

Each day before starting analysis, the capillary was first purged with 0.5 *M* KOH for 5 min, followed by water for 2 min. Afterwards the capillary had been conditioned for 1 h with the running buffer. Between each run the capillary was flushed for 2 min with the running buffer. On shut-down the capillary was flushed with 0.5 *M* KOH for 2 min, followed by flushing with water for 5 min and purging with air for 2 min.

HPLC conditions

HPLC was carried out as described by Moors *et al.* [7], with some minor modifications. The pH was adjusted to 3.9 instead of the described 4.5 and detection was carried out at 214 nm. The column used was a Waters Nova-Pak C_{18} column (15 cm × 4.6 mm I.D., 5-μm particles). The mobile phase was phosphate buffer (pH 3.9, μ = 0.1)–acetonitrile (85:15, v/v). The flow-rate was set at 1 ml min^{-1}.

RESULTS AND DISCUSSION

HPLC

An example of a chromatogram is shown in Fig. 1. The results are given in Tables I–V.

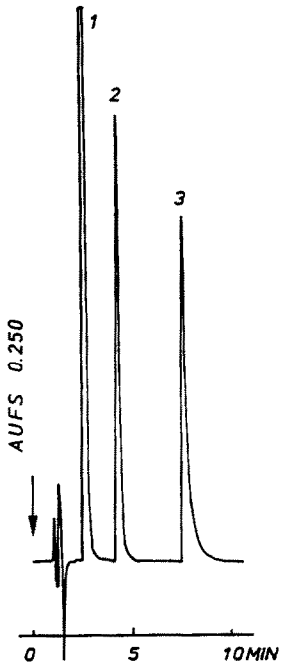

Fig. 1. Chromatogram (10 mg l^{-1} mixture) obtained by HPLC on a C_{18} column with a mobile phase composed of phosphate buffer (pH 3.9, μ = 0.1)–acetonitrile (85:15, v/v). 1 = Caffeine; 2 = aspartame; 3 = benzoic acid.

CZE method development

The parameters that could be important in CZE were investigated in order to obtain the optimum conditions. All the runs were carried out at 15 kV.

Sampling mode. Both sampling modes available on the Quanta-4000 system were tested. By using electromigration as an injection system at the usual 5 kV sampling voltage, benzoic acid was not introduced into the capillary. Compared with hydrodynamic injection, aspartame and caffeine were slightly increased in sensitivity. With the hydrodynamic sample introduction system (which takes a representative part of the sample), all the components could be detected. The hydrodynamic sample introduction system was therefore used for all other injections.

Effect of pH. Two pH effects can occur in CZE: the electroosmotic flow (EOF) increases with increasing pH and weak acids dissociate more [12]. The effect of pH was investigated for caffeine, aspartame and benzoic acid. The pH values employed were 2.5, 7.0, 11.0 and 12.2 in a phosphate buffer with an ionic strength of 0.1. The results are shown in Fig. 2.

At pH 2.5 the EOF is very low; at this pH aspartame alone is positively charged. Caffeine and benzoic acid are not dissociated at this pH [pK_a (caffeine) = ± 14 and pK_a (benzoic acid) = 4.2]. At pH 7 aspartame is slightly negatively charged, so that it migrates more slowly than caffeine, which is still neutral. Benzoic acid is fully negatively charged and is not detected within 20 min. At pH 11 the EOF is sufficient for all three components to migrate to the point of detection within 10 min. This pH was chosen as the optimum. At pH 12.2 it can be seen that there is a large effect on the migration of benzoic acid. Aspartame also migrates faster, but not to the same extent. This is due to the increase in EOF. The effect of this increase is higher with the benzoate ion because of its high electrophoretic mobility. The migration of caffeine is more or less constant. Benzoic acid co-migrates with caffeine at this pH.

It should be noted that at the chosen pH of 11, caffeine is more or less neutral, which means that

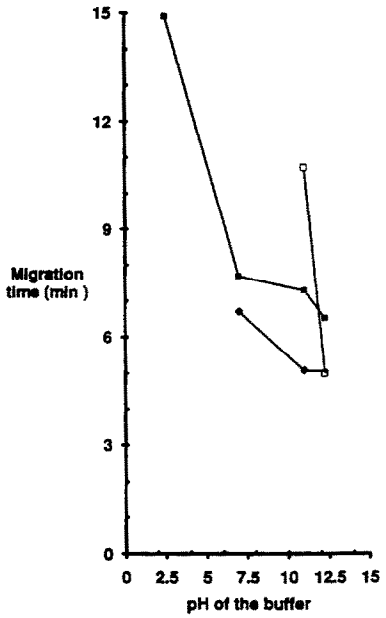

Fig. 2. Effect of pH on migration times. All runs were carried out in phosphate buffer of ionic strength 0.1 with 30-s hydrodynamic injections at 15 kV. ◆ = Caffeine; ■ = aspartame; □ = benzoic acid.

the migration of caffeine is due only to EOF. It also means that all neutral components will migrate with the same velocity as caffeine and that the determination of caffeine can be influenced by a positive error, due to such neutral compounds. If this is found to be a problem, one needs to use MECC instead [11].

Ionic strength. The EOF decreases with increasing ionic strength. Hayes and Ewing [12] reported that the concentration of the buffer was inversely proportional to the endosmotic mobility. If the ionic strength increases, μ_{eof} decreases, giving a decrease in endosmotic velocity (v_{eof}) and an increase in migration times. A decrease in ionic strength also affects the field strength. Owing to the higher resistivity, the field strength increases [13]. A higher field strength means higher velocities, which leads to a decrease in migration times.

Different buffers with the selected pH were prepared with ionic strengths of 0.1, 0.075, 0.05 and 0.025. As expected, there was a nearly linear increase in migration time with increasing ionic strength. The increase in migration time was higher for benzoic acid than for aspartame or caffeine. The buffer with ionic strength 0.025 was chosen, because of the acceptable migration times and good resolution for the components being separated. An example of an electropherogram is shown in Fig. 3.

Organic solvents. The effect of methanol and acetonitrile on migration time and resolution was investigated and the results are shown in Fig. 4. As can be seen, the migration times of all three components increase with increasing proportions of acetonitrile and methanol. This is in agreement with the results of Schwerr and Kenndler [14], where a decrease in EOF with increasing proportions of both acetonitrile and methanol was observed, but in contrast to the results of Fujiwara and Honda [15], where the opposite effect was observed. The electroendosmotic mobility is directly proportional to the permittivity (D_o) and inversely proportional to viscosity (η). With addition of organic solvents, the factor D_o/η decreases and hence the EOF decreases [14]. Apart from the change in EOF, addition of organic solvents also suppresses the dissociation of solutes and increases the field strength due to changes in resistivity. However, as can be seen in Fig. 4, the first two effects are larger than the third.

The increase in the migration time of caffeine is

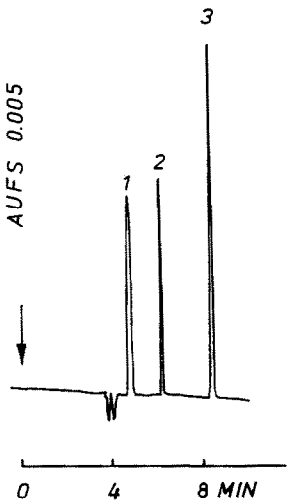

Fig. 3. Example of an electropherogram (10 mg l^{-1}) obtained in CZE at a run voltage of 15 kV and an electrolyte buffer composed of phosphate buffer (pH 11.0, μ = 0.025). The sample was injected using the hydrodynamic injection mode for 30 s. 1 = Caffeine; 2 = aspartame; 3 = benzoic acid.

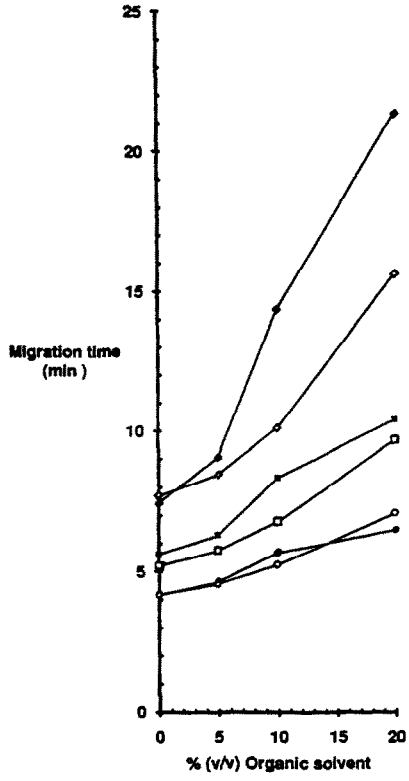

Fig. 4. Effect of organic solvents on the migration of caffeine, aspartame and benzoic acid. Acetonitrile and methanol were mixed with phosphate buffer (pH 11, μ = 0.025). Experimental conditions as in Fig. 3. ● = Caffeine–acetonitrile; ■ = aspartame–acetonitrile; ◆ = benzoic acid–acetonitrile; ○ = caffeine–methanol; □ = aspartame–methanol; ◇ = benzoic acid–methanol

more or less equal for both organic solvents. This indicates that both organic solvents decrease the EOF to the same extent. As discussed earlier, a decrease in EOF has a greater effect on anions with higher electrophoretic mobilities. This is why the effect on benzoic acid is larger than that on the others. However, the effect of acetonitrile was larger than for methanol, especially on benzoic acid. This cannot be explained by a decrease in EOF alone. One should consider also the changes in electrophoretic mobility [15]. Changes in solvent lead to changes in solvation and therefore to changes in electrophoretic mobility. An increase in μ_i (electrophoretic mobility of ion i) can also occur in a medium with acetonitrile because, as observed by Schwerr and Kenndler [14], acetonitrile decreases the viscosity of binary systems with water. Methanol and most other organic solvents show a maximum in viscosity at 60% (v/v) [14] As $\mu_i = q/6\pi\eta r$ (r being the radius), a decrease in η leads to an increase in electrophoretic mobility.

Buffer effect. Several buffer electrolytes were tested, namely sodium phosphate, potassium phosphate, borate, glycinate and carbonate buffer electrolytes. Only small differences in migration times were observed between the buffers, even between sodium and potassium phosphate buffers.

Comparison of CZE with HPLC

The CZE method was compared with the HPLC method with respect to linearity, repeatability, reproducibility, accuracy, sensitivity and separation efficiency.

Repeatability and reproducibility. To determine the repeatability, a 10 mg l^{-1} mixture of the three components was injected sequentially thirteen times for CZE and twelve times for HPLC. The relative standard deviations (R.S.D.s) for HPLC and CZE are given in Table I. For HPLC the R.S.D.s were between 0.48% and 1.24% and for CZE between 1.17% and 2.55%, indicating good repeatabilities for both techniques. The R.S.D.s for the pooled variance of six days of analysis for the two techniques were compared with each other by an F-test (α = 0.05). For aspartame and caffeine a significant difference was observed between the two methods, indicating better repeatabilities for HPLC. Benzoic acid, however, showed no significant difference at this level. As can be seen in Table I, the repeatability calculated using areas or corrected areas (areas normalized by migration time) (CA) gave similar results.

The reproducibility was determined by injecting 10 mg l^{-1} mixtures of the three components during six days of analysis. The R.S.D.s were significantly higher in CZE (6.0–8.7%) than in HPLC (1.4–1.7%) (Table I). The main reason for these high R.S.D.s for in CZE probably is the condition of the wall of the capillary, the characteristics of which are difficult to keep constant for several days. The changes in the wall conditions during the different days affects the EOF, leading to changes in migration times. One would expect the reproducibility calculated using corrected areas to be better, but as

TABLE I

REPEATABILITY AND REPRODUCIBILITY RESULTS FOR CZE AND HPLC

Compound	Repeatability (R.S.D., %)			Reproducibility (R.S.D., %)[d]		
	HPLC[a]	CZE (area)[b]	CZE (CA)[b,c]	HPLC	CZE (area)	CZE (CA)[c]
Caffeine	0.66	1.17	1.23	1.38	8.73	14.81
Aspartame	1.24	1.38	1.22	1.67	5.98	14.00
Benzoic acid	0.48	2.55	2.29	1.49	7.01	14.37

[a] $n = 12$.
[b] $n = 13$.
[c] CA = Corrected area (normalized areas).
[d] $n = 6$.

can be seen in Table I, in this instance they are not. The reason for this was that the areas did not change in accordance with the increasing migration time. Instead of the expected increase in area, they were more or less constant with increasing migration time. Probably the zones had equal velocity as they passed through the detector, but in the previous section of the capillary differences in migration velocity could have occurred.

Separation efficiency. To carry out the comparison with HPLC, the number of theoretical plates (N) was calculated by the expression $N = 16(t_m/W)^2$ where t_m is the migration time and W the peak width [16]. The results are given in Table II. In HPLC the calculated value of N was between 31 and 153 whereas in CZE it was significantly higher, being between 2046 and 14 033.

Limit of detection (LOD). The LOD was calculated as the amount of compound that would still give a signal three times greater than the noise on the baseline. The results are given in Table II. In HPLC the LODs were between 0.1 and 0.5 mg l^{-1} whereas in CZE they were between 2 and 5 mg l^{-1}.

Linearity. Both methods gave fair linearity, but the ranges were different owing to the lower detection limit of HPLC. The calculated correlation coefficients are given in Table III.

Accuracy. By comparing an external calibration line with a standard addition calibration line (Table III), the influence of the matrix can be detected. The slopes of the external and standard addition calibration lines were compared with a t-test. At the 5% confidence level, no significant difference could be detected for the three components in HPLC. The p-values obtained were caffeine $p > 0.9$, aspartame $0.5 < p < 0.9$ and benzoic acid $0.5 < p < 0.9$. In

TABLE II

SEPARATION EFFICIENCY AND DETECTION LIMITS

Compound	Separation efficiency (N)[a]		LOD (mg l^{-1})[b]	
	HPLC	CZE	HPLC	CZE
Caffeine	31.4	2045.8	±0.1	±2
Aspartame	129.1	14 032.8	±0.5	±5
Benzoic acid	152.8	11 615.1	±0.5	±5

[a] Separation efficiency calculated by $N = 16(t_m/W)^2$.
[b] The limit of detection (LOD) is an estimation of 3 × noise.

TABLE III

RESULTS FOR EXTERNAL AND STANDARD ADDITION CALIBRATION LINES IN CZE AND HPLC

Compound	Method	Calibration line	Slope	Ratio (external/addition)	r
Caffeine	HPLC	External	258 821.40	0.999	0.9995
		Addition	259 194.30		0.9998
	CZE	External	1287.48	0.934	0.9996
		Addition	1378.11		0.9996
Aspartame	HPLC	External	70 223.50	1.006	0.9997
		Addition	69 815.63		0.9997
	CZE	External	449.96	0.947	0.9996
		Addition	475.14		0.9996
Benzoic acid	HPLC	External	101 834.30	0.999	0.9980
		Addition	101 948.10		0.9999
	CZE	External	1117.49	0.968	0.9999
		Addition	1154.00		0.9993

CZE, on the other hand, a significant difference was detected for caffeine and aspartame at a confidence level of 5%. At this confidence level, benzoic acid showed no significant difference between the slopes. The p-values obtained were caffeine $0.001 < p < 0.01$, aspartame $0.01 < p < 0.02$ and benzoic acid $0.1 < p < 0.2$.

There was a small but significant difference in migration times between the external and standard addition calibration lines (between 3.1% and 3.4% for all the three components), with higher values for the external calibration lines. This cannot be caused by differences in viscosity between samples and standards, because normally one would expect the sam-

TABLE IV

RESULTS FOR DETERMINATION OF CAFFEINE, ASPARTAME AND BENZOIC ACID IN A SOFT DRINK SAMPLE ($n = 6$)

Compound	Method	Calibration	Concentration found (mg l^{-1})[a]	R.S.D. (%)	Recovery (%)[b]
Caffeine	HPLC	External	120.9	0.22	–
		Addition	132.5		
	CZE	External	142.5	1.07	–
		Addition	157.5		
Aspartame	HPLC	External	487.3	0.15	93.72
		Addition	493.4		94.89
	CZE	External	529.4	1.60	101.80
		Addition	507.7		97.64
Benzoic acid	HPLC	External	150.7	5.60	–
		Addition	114.9		
	CZE	External	113.1	1.60	–
		Addition	121.7		

[a] The cola sample was a Colca-Cola light soft drink, with a claimed content of 520 mg l^{-1} of aspartame. The concentrations of caffeine and benzoic acid are unknown.

[b] Percentage recovery of the claimed amount.

TABLE V

DETERMINATION OF ASPARTAME IN ARTIFICIAL SWEETENING POWDERS

Canderel 36 mg and Finn 38 mg labelled amount per packet.

Method	Canderel			Finn		
	Aspartame found (mg l^{-1})[a]	R.S.D. (%)	Recovery (%)[b]	Aspartame found (mg l^{-1})[a]	R.S.D. (%)	Recovery (%)[b]
HPLC	35.18	0.66	97.71	37.75	0.01	99.34
CZE	34.22	0.74	95.06	36.47	0.36	95.97

[a] Mean (n = 6).
[b] Recovery of aspartame compared with the label claim.

ples to have higher viscosity. The viscosity also plays an important role during sample injection. As this was performed hydrodynamically, lower volumes will be injected for solutions with higher viscosities. This implies that under these conditions smaller areas should be observed. However, the opposite was observed. Hence, the cause of the difference in slopes between the external and standard addition calibration lines is of a different kind. As reported by Swartz [17], matrix effects are more critical in CZE than in HPLC. The ratios of the slopes of external and standard addition calibration lines in HPLC were almost 1.00, whereas in CZE they were between 0.93 and 0.97 for the three components (Table III). The determinations of all components except benzoic acid by CZE gave higher results than by HPLC (Table IV). The difference for benzoic acid could not be explained. However, the amount determined from the standard addition calibration line in HPLC was more or less in agreement with the amount found by CZE.

Matrix effects can be higher in CZE than in HPLC, probably because the amount of matrix used in CZE is normally relatively higher owing to the higher detection limits. The diet cola soft drink was diluted 25-fold in CZE, whereas in HPLC there was a 100-fold dilution. This means that four times more matrix is introduced into the CZE column.

Aspartame was also determined in samples of Canderel and Finn (Brazilian) artificial sweetening powders. The results are given in Table V. HPLC gave recoveries between 97% and 99% and CZE between 95% and 96%. Both results are acceptable.

The validation parameters for CZE in these applications, are less satisfactory than those for HPLC. However, they are still acceptable. Combined with the higher separation efficiency of CZE, the technique can be preferable in some applications. The reproducibility and accuracy in CZE should be investigated further. The choice of the technique should be determined by the specific application involved.

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