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Application of microemulsion electrokinetic chromatography for the detection of preservatives in foods

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Abstract

In this study, a microemulsion electrokinetic chromatographic (MEEKC) method was used to separate seven preservatives which are commonly used as additives in various food products. The RSDs were in the range of 0.64–0.95% for migration time and 0.18–1.21% for reproducibility of sample injection, thus indicating the separation performance was very good even though sample preparations contained high levels of organic solvent (methanol) (up to 20 (v/v)%). Although the separation of soy sauce and wine samples required a C8 solid phase extraction as a pretreatment step in order to reduce matrix interference, this MEEKC method proved to be successful in determining preservatives found in various food products, such as soft drinks, soy sauces and wines.

Keywords: Preservatives; Food additives; MEEKC

1. Introduction

Free-solution capillary electrophoresis (CE) is a very attractive separation technique for charged solutes due to its high efficiency and low cost of analysis, but it is limited in its ability to detect neutral or hydrophobic solutes. Micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC), which are based on CE, are useful techniques in the separation of neutral and charged solutes. In both techniques, charged pseudostationary phase can be used to influence the separation behavior of neutral or charged analytes. In MEKC, normal micelles, which are composed of surfactants in the running buffer, have hydrophobic interiors and hydrophilic exteriors, and are regarded as the pseudostationary phase (Kuo & Hsieh, 1997; Terabe, Otsuka, Ichikawa, Tsuchiya, & Ando, 1984; Watanabe & Terabe, 2000). Like the micelles in MEKC, microemulsion droplets, which are composed of water-immiscible organic solvent (oil), water, surfactant and co-surfactant (medium chain alkyl alcohol), are regarded as the charged pseudostationary phase in MEEKC, and can separate neutral analytes in a similar fashion as MEKC (Gabel-Jensen, Hansen, & Pedersen-Bjergaard, 2001; Hilder, Klampfl, Buchberger, & Haddad, 2001; Pedersen-Bjergaard, Gabel-Jensen, & Hansen, 2000; Watarai, 1991). Most papers which compared separations obtained by MEEKC and MEKC had concluded that MEEKC has a greater separation capability for highly hydrophobic compounds than MEKC, because highly hydrophobic compounds tend to be strongly retained by the micelles. However, separation by MEEKC is more strongly affected by the dissolving solvent of sample (Altria, Clark, & Mahuzier, 2000; Altria, Mahuzier, & Clark, 2003; Klampfl, 2003; Miola, Snowden, & Altria, 1998). To date, MEKC has been more widely studied, and has been demonstrated as a reliable analytical tool in many applications. The application range of MEEKC, however, needs to be further

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investigated (Altria, 2000; Cahours, Cherkaoui, Rozing, & Veuthey, 1998; Huang, Lai, Chiu, & Yeh, 2003; Pedersen-Bjergaard, Naess, Moestue, & Rasmussen, 2000).

The addition of preservatives to various foods is essential for avoiding alteration and degradation by microorganisms during storage. In most literatures on preservative analyses of food products have mainly utilized high performance liquid chromatography, free solution CE methods, or MEKC method (Boyce, 1999, 2001; Kaniansky, Masar, Madajova, & Marak, 1994; Kuo & Hsieh, 1997; Pant & Trenerry, 1995; Pylypiw & Grether, 2000; Rossi & Desiderio, 2002; Waldron & Li, 1996). Reports of preservative analyses in foods by CE or MEKC methods have mostly been for only one or two preservatives with the exception of the nine preservatives that were separated by Kuo and Hsieh (1997). While MEEKC methods have already been successfully applied for analyses of four parabens in pharmaceuticals (Altria, 1999; Mahuzier, Altria, & Clark, 2001), to our knowledge the analysis of preservatives in food products by MEEKC has never been documented. This paper, therefore, examined the potential of MEEKC as a technique for the routine analyses of food additives. In addition, the results of a C8 solid phase extraction, which was used as sample pretreatment for reducing matrix interference when the MEEKC method was applied to analyze preservatives in food samples, are also reported.

2. Experimental

2.1. Preservatives standards

Four parabens (methyl, ethyl, propyl and butyl), sorbic acid, benzoic acid, and dehydroacetic acid, which are seven commonly used preservatives in commercial food products, were chosen as analytes in this study. Triclosan was used as the internal standard for preservative separations. Methyl paraben (Methyl *p*-hydrobenzoate) and butyl paraben (butyl *p*-hydrobenzoate) were purchased from Sigma (Steinheim, Germany). Sorbic acid and benzoic acid were obtained from TCI (Tokyo, Japan). Ethyl *p*-hydrobenzoate, propyl *p*-hydrobenzoate and triclosan were obtained from Aldrich (WI, USA). Dehydroacetic acid was purchased from ACROS (New Jersey, USA). These standards were individually dissolved in ethanol at a stock concentration of 2 mg/ml. The internal standard, triclosan, was added to each standard or sample solution at a concentration of 50 μg/ml before injection.

2.2. Chemicals and real samples

Disodium tetraborate, ethanol (absolute), sodium hydrogen phosphate, and phosphoric acid were bought from Merck (Darmstadt, Germany). Sodium dodecyl sulfate and aspartame were obtained from TCI (Tokyo, Japan). Sodium hydroxide, hydrochloric acid and 1-butanol were obtained from J.T. Baker (NJ, USA). Methanol was bought from Pharmco (CT, USA). Octane was obtained from Fluka (Buchs, switzerland). C8 cartridges (LC-8, 3 ml, 500 mg) used as the extraction column of solid phase extraction was purchased from Supelco (PA, USA). Food samples, such as soy sauces, soft drinks and wines, which are made by manufacturers in various countries, were obtained from supermarkets in Taiwan.

2.3. Preparation of microemulsion running buffer

A microemulsion running buffer was prepared by adding 3.3 g SDS, 0.8 g octane, and 6.6 g 1-butanol to 89.3 ml buffer of pH 9.5. The mixture was then sonicated for 30 min until homogeneous. A running buffer of pH 9.5 was prepared by adding 0.1 M NaOH to 7.5 mM disodium tetraborate solution until the desired pH was achieved.

2.4. Real sample pretreatment

In order to be analyzed by CE, soft drinks were each diluted with the running buffer to a volume ratio of 1:4. For soy sauces and wines, 1.0 ml of each sample was applied to the C8 column of solid phase extraction at the rate of approximately 0.5 ml per minute. The C8 column was conditioned prior to use by washing with methanol (3 ml) followed with deionized water (3 ml). After adding the sample, the extraction column was washed with deionized water (6 ml). The adsorbed preservatives were then eluted with 1 ml of ethanol.

2.5. Apparatus and operating conditions for CE

All experiments were performed with a Beckman Coulter MDQ capillary electrophoresis system equipped with a photo diode-array detector (CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. Separations were performed in a 31.2-cm (21-cm to detector) of 50-μm i.d. uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, USA). The capillaries were conditioned prior to separation by washing with 0.1 M sodium hydroxide (5 min), and then with running buffer (5 min) for MEEKC. Samples and standards solutions were diluted with MEEKC running buffer in the volume ratio of 1:4, and then were pressure-injected into the capillary column at 0.5 psi for 3 s. Separations were carried out using an electrical voltage of 11 kV, and the temperature of the capillary was maintained at 30 °C, while 200 nm was selected as the detection wavelength.

3. Result and discussion

3.1. Effect of temperature for preservatives separation

Previous studies have demonstrated that temperature of microemulsion buffer could effectively alter an analyte's selectivity in MEEKC system (Altria et al., 2000; Huang et al., 2003). Therefore, buffer temperature is an important factor that can be used to optimize a MEEKC separation method, similar to MEKC system (Kuo & Hsieh, 1997). The effect of temperature on separation performance of MEEKC system, however, has not been well characterized. Thus, the separation ability of MEEKC for these preservatives at different temperatures should first be compared. The reproducibility of migration time was examined based on 3 replicated injections of 3 s (0.5 psi) for 100 μg/ml standards, and the relative standard deviations (RSDs) of migration time for the analytes were in the range of 0.57–1.81% for 38 °C, 0.73–1.20% for 34 °C, and 0.64–0.95% for 30 °C. An increase in temperature did not greatly change the RSDs of migration times, thus indicated that the microemulsion solution was relatively stable and the reproducibility of migration times was very good in the range of temperatures that were tested. However, when a higher temperature (over 34 °C) was used in MEEKC system, ethyl paraben and sorbic acid did not separate well (Fig. 1(a)). In contrast, all preservatives had relatively good resolutions (>1.5) when the temperature was under 30 °C (Fig. 1(b)). Consequently, a 30 °C temperature was chosen for the following experiment for preservative separations.

3.2. Effect of buffer pH for preservatives separation

Since the electroosmotic flow (EOF) mobility and electrophoretic mobility of analytes are both highly depended upon the buffer pH in most CE systems, the pH value of running buffer is commonly used to optimize CE separations. Therefore, the effect of buffer pH for the separation of these preservatives in a MEEKC system was examined. Fig. 2 depicts the separation of preservatives by microemulsions of different buffer pH, in which −11 kV was applied for pH 2–6 while 11 kV was employed for pH 8–10 in order to detect all preservatives. The EOF was not present and all analytes were neutral when a buffer of pH 2.0 was used. All analytes, however, could be detected in pH 2.0 which implied each analyte had strongly interacted with oil droplets that coated the SDS anions. Moreover, the degree of interaction between analyte and oil droplet determined the electrophoretic mobility for each analyte. The same migration order for these preservatives was observed at pH 4.0, but the migration time of the preservatives had slightly increased due to small EOF which migrated toward the negative end. In a microemulsion of pH 6.0, all analytes were only detected when a negative voltage was applied, thus indicated the EOF at pH 6.0 was relatively small compared to the electrophoretic mobilities of the analytes. A further increase in buffer pH (in the

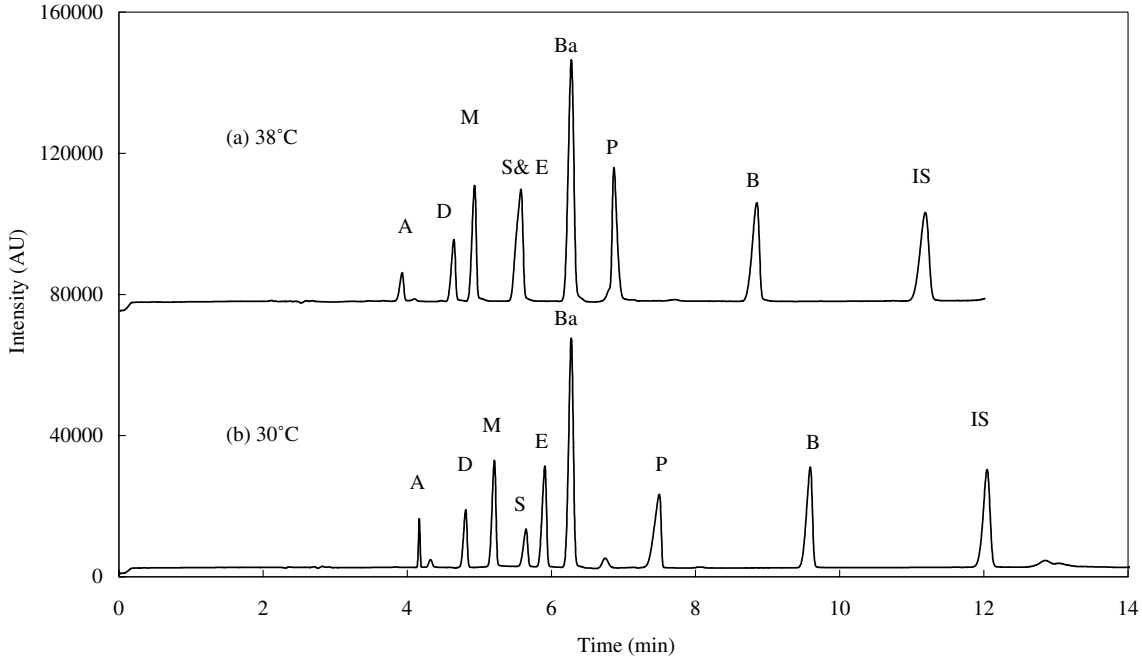

Fig. 1. The electropherogram of preservative standards at different temperatures. Temperature was maintained at 38 (a) or 30 °C (b) and separations were carried out using an electrical voltage of 11 kV. M (methyl paraben), E (ethyl paraben), P (propyl paraben), B (butyl paraben), S (sorbic acid), D (dehydroacetic acid), Ba (benzoic acid), A (aspartame), and IS (triclosan).

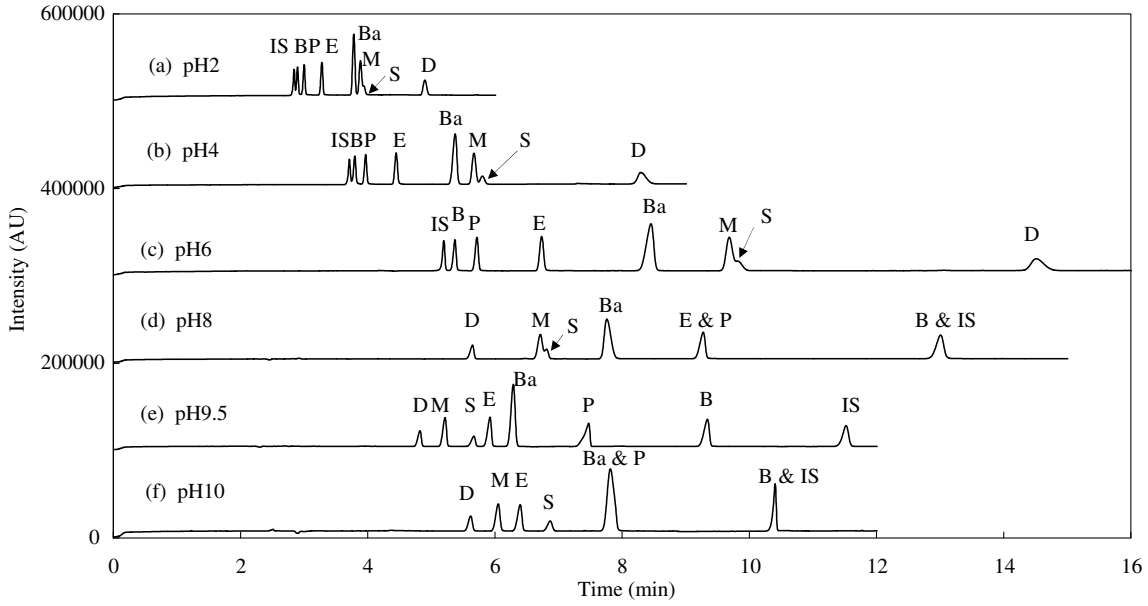

Fig. 2. The electropherogram of preservative standards at different buffer pH. Buffer pH was maintained at 2.0 (a), 4.0 (b), 6.0 (c), 8.0 (d), 9.5 (e) or 10.0 (f), and electrical voltage was kept at −11 kV for (a)–(c), or 11 kV for (d)–(f). Temperature was fixed at 30 °C during separation, and other conditions were the same as in Fig. 1.

range of pH 8–10) resulted in a stronger EOF, and the magnitude of EOF mobility was larger than that of analytes' electrophoretic mobility. As a result, a positive voltage had to be applied in order to migrate all analytes toward the detector (outlet end). In addition, the migration order of the analytes was almost reversed in this higher pH range after positive voltage was used. Even though a lower pH was able to noticeably shorten the separation time of preservatives, but the resolution for methyl paraben and sorbic acid was poor when the pH was below 8.0. Consequently, a microemulsion of pH 9.5, in which a baseline separation could be obtained within 12 min, was chosen for the optimal preservative separations.

3.3. Effect of internal standard for quantitative analysis

An internal standard is usually used in most MEEKC system for improving the reproducibility of sample injection. Thus, the effect of internal standard on the reproducibility of sample injection in the MEEKC system was examined. The reproducibility of migration time was examined based on 3 replicated injections of 3 s (0.5 psi) for 100 μg/ml standards. The RSD of each peak area for three intra-day replicated injections represented reproducibility of sample injection. The RSDs of peak areas for all analytes were in the range of 0.80–1.61% without the addition of internal standard. The RSDs of peak areas, however, were reduced to the range of 0.18–1.21% after triclosan was used as internal standard in this system (Table 1). The result seemed to imply that the effect of internal standard in improving reproducibility of sample injection was not very obvious for preservatives in a simple matrix (a mixture of microemulsion buffer and methanol). However, as the sample matrix became more complicated, the use of internal standard greatly enhanced the quantitative ability for the MEEKC system, especially when real food samples were analyzed.

The correlation coefficients (r) of the calibration curves were greater than 0.999 for each analyte after internal standard calibration (Table 1). In addition, the detection limits for the preservatives were in the range of 0.13–0.79 μg/ml based on S/N ratio of 3. Compare to the results of other CE methods that have been reported previously (0.59–0.99% for the RSDs of migration time, and 0.999 for the correlation coefficients (r) of the calibration curves, 0.4–2.2 μg/ml for detection limits based on S/N ratio of 3 (Boyce, 2001; Kuo & Hsieh, 1997)), the MEEKC method indeed provided a relatively good performance for the analysis of preservatives.

3.4. Effect of sample preparation

As described in previous reports, poor separation was obtained if sample was not dissolved in the microemulsion that was used as the separation buffer (Altria et al., 2003). Hence, a sample was usually prepared in a medium with a high volume ratio of microemulsion. In order to determine the influence of sample medium on preservative separation, standards dissolved in methanol was diluted with the microemulsion buffer in the volume ratio of 1:9, 1:6, 1:4, and 1:3. Separation behavior was almost the same when sample preparation was maintained at ratio of 1:9, 1:6, or 1:4, and the RSDs of migration

Table 1
Average migration times, reproducibilities of sample injection, correlation coefficients of calibration curves, and SPE extraction recoveries of preservatives standards[a]

Preservatives	Migration time (min)[b]	Reproducibility of sample injection[c]		Calibration curves[d]	r	Recovery[b]
		No IS used (%)	IS used (%)			
Dehydroacetic acid	4.80 (0.64%)	1.34	0.78	$Y=0.0035X+0.0002$	0.999	115.3 (1.12%)
Methyl paraben	5.19 (0.79%)	0.95	0.72	$Y=0.0059X+0.021$	0.999	104.0 (3.62%)
Sorbic acid	5.63 (0.74%)	1.54	0.92	$Y=0.0028X-0.0056$	0.999	108.0 (0.87%)
Ethyl paraben	5.89 (0.76%)	1.49	1.03	$Y=0.0055X+0.0509$	0.999	102.3 (0.57%)
Benzoic acid	6.27 (0.82%)	1.61	1.19	$Y=0.015X+0.1063$	0.999	82.3 (1.41%)
Propyl paraben	7.47 (0.95%)	0.80	0.18	$Y=0.0079X+0.0167$	0.999	85.2 (1.22%)
Butyl paraben	9.54 (0.82%)	1.43	1.21	$Y=0.0085X+0.039$	0.999	98.2 (1.46%)

[a] Separation conditions: A microemulsion solution of a pH 9.5 was used as running buffer, temperature was fixed at 30 °C, and 11 kV voltage was applied to a capillary tube with 21 cm of effective length.
[b] Values are means of three intra-day replicates. The value in parenthesis indicates the RSD of migration time in percentage.
[c] The RSD of each peak area for three intra-day replicated injections represented reproducibility of sample injection. Triclosan was used as internal standard (IS) in the method.
[d] The calibration curves were constructed from triplicate measurements at each concentration in the range of 4–500 μg/ml. Triclosan was used as internal standard (IS) in the method.

times and peak areas were similar under these conditions for these standards. However, separation was easily interrupted by current leakage when standards were mixed with microemulsion buffer in the volume ratio of 1:3. The phenomenon was probably due to the disruption of microemulsion environment inside the capillary after high concentration of organic solvent was injected (Altria et al., 2003). Furthermore, several food samples after SPE treatment was diluted with microemulsion buffer in the same ratio as described above, and good separation results were obtained once again. Therefore, in order to keep preservatives in real samples at a high concentration, the ratio of sample to microemulsion buffer was maintained at 1:4 in the following experiments.

3.5. Separation of real food samples

The electropherogram of three soft drinks derived by the MEEKC method when subjected to temperatures under 30 °C is shown in Fig. 3, and it indicated that benzoic acid was determined as preservative present in each

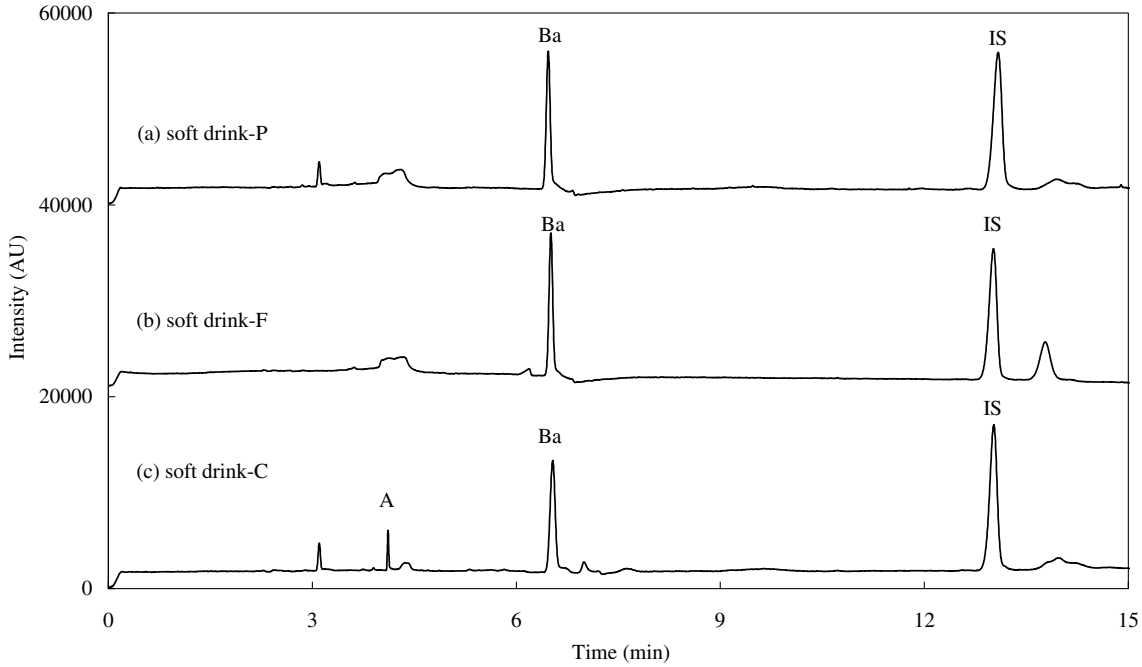

Fig. 3. The electropherograms of commerically available soft drinks determined by MEEKC method. Temperature was fixed at 30 °C during separation, and other conditions were the same as in Fig. 1. Benzoic acid and aspartame were found in the products.

of the soft drinks without any interference. However, preservative peaks were interfered by other unknown peaks for wine and soy sauce samples, hence it was difficult for preservative determination. After a sample of soy sauce was diluted by methanol, some interfering peaks still overlapped with the analytes (Fig. 4), thus indicated that more pretreatment was needed for reducing sample matrix interference. As a result, a C8 solid phase extraction (C8-SPE) was used to pretreat samples of wines and soy sauces. Sorbic acid and methyl paraben were determined as preservatives in three brands of wines without any interference after C8-SPE pretreat-

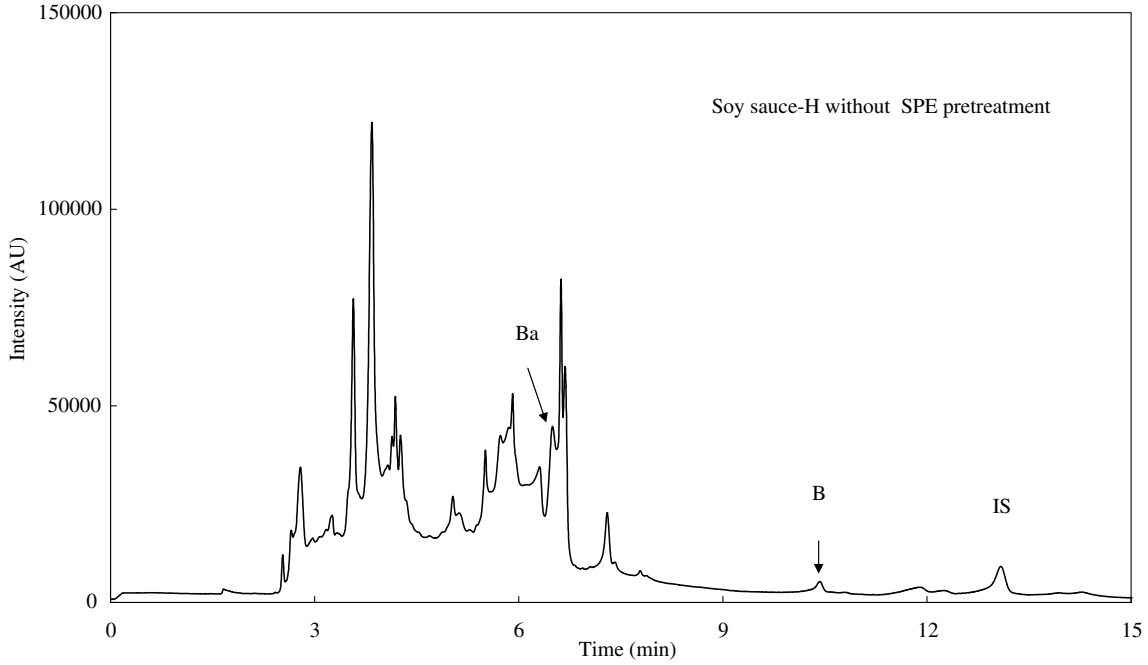

Fig. 4. The electropherogram of a commercially available soy sauce separated by MEEKC method. The sample was directly separated without a C8-SPE pretreatment. All other separating conditions were the same as in Fig. 3. Benzoic acid overlapped with unknown spikes in these products.

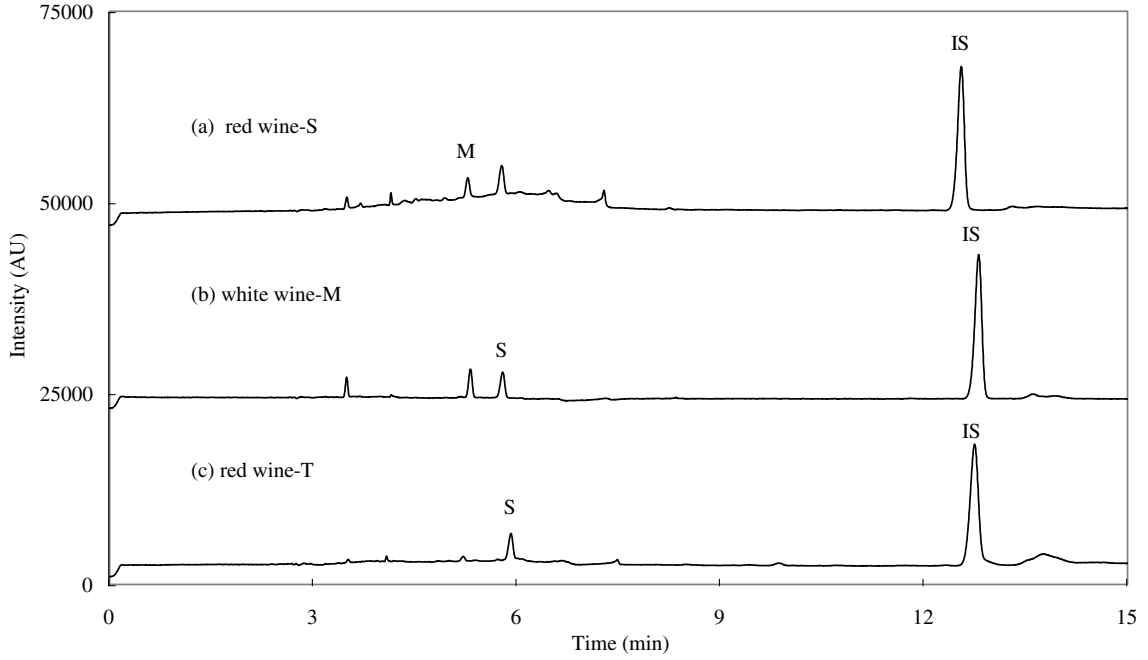

Fig. 5. The electropherograms of commercially available wines separated by MEEKC method. The samples were first treated with C8-SPE, and then separated by MEEKC. All other separating conditions were the same as in Fig. 3. Methyl paraben and sorbic acid were found in these products.

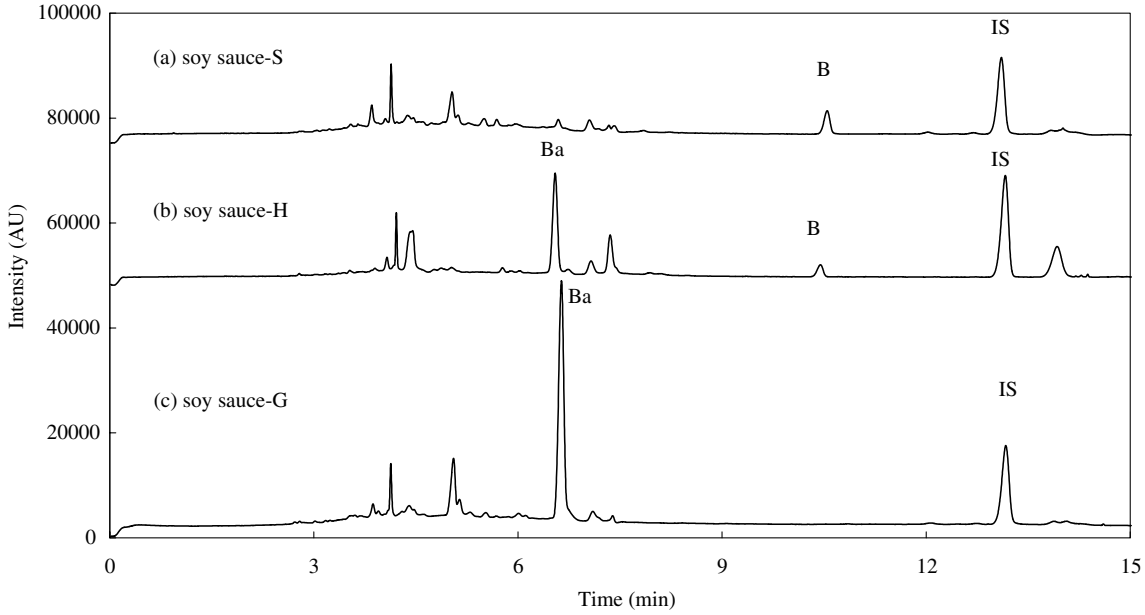

Fig. 6. The electropherograms of commercially available soy sauces determined by MEEKC method. The samples were first treated with C8-SPE, and then separated by MEEKC. All other separating conditions were the same as in Fig. 3. Benzoic acid and butyl paraben were found in these products.

ment (Fig. 5). Similarly, butyl paraben and benzoic acid were clearly detected in soy sauce samples after C8-SPE pretreatment (Fig. 6).

3.6. C8 solid phase extraction

As noted in the previous section, C8-SPE was highly efficient in reducing matrix interference in wines and soy sauces. Hence, the extracting recoveries of the seven preservatives by C8-SPE steps described in the experimental section were further evaluated. The seven preservative standards were used to spike soy sauce samples at a concentration of 200 μg/ml for each preservative standard before C8-SPE. The recoveries, which were determined by triplicate measurements, were in the range of 82.3–115.3% (Table 1). From the results, we concluded that the C8-SPE condition was suitable for extracting preservatives in these food samples.

3.7. Determination of preservatives in food

So far, the optimum separation condition, where all preservative standards were completely separated and the resolutions were more than 1.5, was achieved with a microemulsion solution containing 3.3 g SDS, 0.8 g octane, 6.6 g 1-butanol, and 89.3 ml borate buffer with a pH 9.5 and a temperature of 30 °C. In addition, the electropherograms of these food samples including soft drinks, wines and soy sauces, also demonstrated that the MEEKC method possessed enough separation ability to analyze the preservatives in three different types of food samples with or without C8-SPE (Figs. 3, 5 and 6). Table 2 summarized the content of preservatives in several food samples discussed above. The RSDs of the preservatives contained in these foods were in the range of 0.28–5.94% with triplicate measurements, thus indicating that this method provided good quantitative reproducibility.

Table 2
Contents of preservatives determined in commercial food samples

Food samples	Preservatives	Concentration[a]	RSD (%)
Soy sauce-S	Butyl paraben	126.2 μg/ml	1.94
Soy sauce-H	Butyl paraben	508.6 μg/ml	1.35
	Benzoic acid	101.7 μg/ml	1.11
Soy sauce-G	Benzoic acid	860.6 μg/ml	1.43
Red wine-S	Methyl paraben	19.3 μg/ml	5.94
Red wine-T	Sorbic acid	209.1 mg/g	2.43
White wine-M	Sorbic acid	201.8 μg/g	0.28
Soft drink-C	Benzoic acid	175.1 μg/ml	1.03
Soft drink-P	Benzoic acid	157.6 μg/ml	0.83
Soft drink-F	Benzoic acid	176.8 μg/ml	1.05

[a] Values are means of triplicate determination.

4. Conclusion

In this paper, a method based on microemulsion electrokinetic chromatography (MEEKC) was developed for analyzing seven preservatives commonly used in food samples. The microemulsion solution with a lower temperature produced a better separation for these analytes than that with a higher temperature. Simultaneously, the separation performance of the MEEKC method was very good even though sample

preparations contained high levels of methanol. In addition, the reproducibility of sample injection was enhanced by internal standard calibration. Similar to the results reported from other CE methods, the optimum condition developed for MEEKC has a very good separating ability for preservatives in several food samples.

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