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Separation and Determination of Bromophenols in *Trachypenaeus curvirostris* and *Lepidotrigla microptera* by Capillary Zone Electrophoresis

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ABSTRACT

Bromophenols have been identified as key off-flavor compounds found in seafood. In this report, a new method of capillary zone electrophoresis (CZE) was established for simultaneous assay of five bromophenols, 4-bromophenol (4-BP), 2,4,6-tribromophenol (2,4,6-TBP), 2,4-dibromophenol (2,4-DBP), 2-bromophenol (2-BP) and 2,6-dibromophenol (2,6-DBP), in seafood. The optimum buffer system was 20 mM borate-NaOH buffer (pH 10.00). Voltage was +30 kV and the ultraviolet absorbance detection at 280 nm. The column was an uncoated 50 μm ID fused-silica capillary. Regression equations revealed linear relationships (correlation coefficients: 0.9990, 0.9998, 0.9997, 0.9999 and 0.9989) between the peak area of each compound and its concentration. Calibration curves were constructed in the range of 18.8 - 1200, 15 - 960, 9.4 - 600, 10.6 - 680 and 3.0 - 370 mg/mL for 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP, respectively. The relative standard deviations of migration times and peak areas were < 2.1% and 4.9% within one day, respectively. The recoveries of bromophenols were 91.5 - 103.4%. The detection limits (*S/N* = 3) of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP were 1.6, 1.9, 1.2, 0.9 and 1.4 mg/mL, respectively. The effects of several CE parameters on the resolution were studied systematically. Compared with liquid chromatography, the method described here is relatively rapid and can give symmetrical peaks. The contents of 5 bromophenols in seafood (*Trachypenaeus curvirostris* and *Lepidotrigla microptera*) were determined with satisfactory repeatability and recovery. The contents of 4-BP, 2,4,6-TBP, 2,4-DBP and 2,6-DBP in *T. curvirostris* were 3.9, 2.5, 7.3 and 0.6 ng/g, respectively. The feasibility of this method for the determination of bromophenols in freshwater fish and crustaceans (*Macrobrachium nipponense* and *Carassius auratus var. Pengzesis*) also tested. The result indicated that these flavor compounds in freshwater fish and crustaceans were lower than the detection limits mentioned above.

Key words: bromophenols, capillary zone electrophoresis, flavor compounds, seafoods

INTRODUCTION

The bromophenols have been identified as the off-flavor compounds in a wide variety of seafood species like fish, mollusks, crustaceans and algae[1-6]. Moreover, these off-flavor compounds are widespread in seafood but were virtually absent in freshwater fish and crustaceans[7]. Of these compounds, when present in low concentration levels, the off-flavor of 2,6-DBP and 2,4,6-TBP is described as iodoform-like and the off-flavor of 2-BP as phenolic/iodine-like[8,9]. Two other bromophenols of 4-BP and 2,4-DBP possess weak phenolic-like off-flavor. Alone or in combination, these five bromophenols could enhance the off-flavor of seafoods when added to the diet of artificially cultivated seafoods[10]. The algae-containing feed was used to forfify the bromophenol content in aquacultured fish and the effect of such feeds on the flavor quality of the fish need to be evaluated. Therefore, identification and determination of bromophenol compounds play an important role in the quality control of seafoods and the characteristics of the marine environment.

Several methods such as liquid chromatography (LC), liquid chromatography-mass spectrometry (LC-MS)[11,12] and gas chromatography-mass spectrometry (GC-MS)[13-15] have been established to determine contained in seafoods or water the bromophenols. In those methods, various techniques have been applied to the extraction of bromophenols from samples, including simultaneous steam distillation

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extraction (SDE), direct liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The bromophenols are either analysed directly or derivatized prior to analysis. However, none of these methods of LC and GC-MS is entirely adequate since large amount of organic reagents and many operation steps are often required. Recently, owing to its high resolving power, low solvent consumption and simple pretreatment, capillary electrophoresis (CE) has been used as an attractive method for separating and monitoring food contaminants and additives[16-20]. Ohashi *et al.*[21] have determined 4 flavor components (vanillin, ethylvanillin, 2-methoxyphenol, and 2-ethoxyphenol) in cocoa drink by CE. A non-derivative method was developed to analyze flavor components of methiin and alliin in vegetables by CE[22]. Craston *et al.* reported a CE method for the determination of gellan gum in food products[23]. Although the analysis of phenolic compounds by CE in water was reported[24], only two bromophenols were separated. Moreover, due to the use of the negative applied voltage, the migration time was over 15 min. To the best of our knowledge, the determination of 5 bromophenols in seafood by CE has not been reported. Therefore, the aim of this work was to develop a fast, simple and economic CE method for the identification and determination of the above-mentioned 5 bromophenols in the extract of seafood and freshwater organisms.

MATERIALS AND METHODS

I. *Apparatus and Conditions*

Experiments were carried out on an Agilent HP^{3D} capillary electrophoresis system (Agilent, Palo Alto, CA, USA) with a photo diode array detector. The applied voltage was held constant at 30 kV. The column was an uncoated 50 μm ID fused-silica capillary with a total length of 60 cm and an effective length of 51.5 cm (Yongnian, Hebei Province, China). The temperature of the capillary cartridge during electrophoresis was maintained at 25°C and UV detection was done set at 280 nm. Before each use, the capillary was rinsed with 1 M NaOH for 10 min, and then with water for 10 min; it was then conditioned with running electrolyte for 10 min. Between runs, the capillary was rinsed with water and electrolyte for 5 min each. Samples were loaded by pressure injection at 50 mbar for 5 s.

II. *Materials and Reagents*

T. curvirostris, *L. microptera*, *M. nipponense* and *C. auratus var. Pengzesis* were purchased from the seafood market of Qingdao. The 5 bromophenols of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP (molecular structures shown in Figure 1) were from Aldrich (Milwaukee, WI, USA) with purities ranging from 97 to 99%. All chemicals were of analytical-reagent grade from Beijing Chemical Factory (Beijing, P.R. China). Deionized water was used throughout the experiments. All solutions and samples were filtered through a 0.45 μm syringe filter of cellulose acetate.

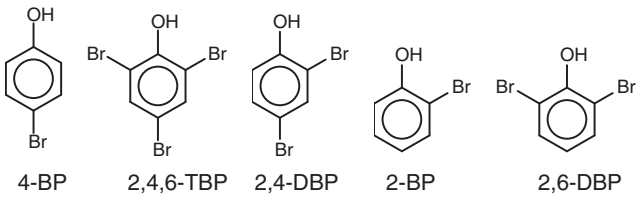

Figure 1. Molecular structures of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP.

The buffer solutions containing borate buffer (10 - 50 mM) adjusted to the desired pH with 0.1 M HCl or 0.1 M NaOH, were all filtered through a 0.45 μm membrane filter and degassed prior to use. A standard solution of 2 mg/mL of each bromophenol was prepared in acetonitrile, filtered, and degassed by the same procedure as for buffer solutions. They were stored at 4°C in the brown glass flasks. Various concentrations of the sample solutions were prepared by appropriate dilution of the stock solution with water when needed.

III. *Sample Preparation for Capillary Electrophoresis*

The samples were prepared as described in the literature[15] with some modifications. Five hundred gram flesh sample of *T. curvirostris*, *L. microptera*, *M. nipponense* and *C. auratus var. Pengzesis* were separately homogenized in 1 L deionized water and the homogenates were acidified to pH 1 with 10 M sulfuric acid and were left to stand at ambient temperature (25°C) about 24 h. The homogenate was transferred to a volatile oil extractor, in which 20 mL diethyl ether was added to the collector. After reflux distillation for 5 h, the diethyl ether containing the volatile components was concentrated at 20°C, with a gentle stream of ultra pure nitrogen. The concentrated extract was dissolved in 0.1 mL acetonitrile and stored in dark-glass vials at 4°C until analysis.

RESULTS AND DISCUSSIONS

I. *Effect of the Buffer pH*

To invesitage the effect of buffer pH on the migration behavior, experiments were performed using the running buffers 20 mM borate-NaOH with various pH (9.00-11.00). The effect of buffer pH on the migration time of 5 bromophenols was shown in Figure 2. The results indicated that the migration time increased with the increase of buffer pH from 9.00 to 10.50 and hardly changed from 10.50 to 11.00. Besides, as can be seen from Figure 2 that the peak orders of the 4-BP, 2,4-DBP and 2-BP were inverted with the increase of buffer pH. All of the compounds under investigation have weakly acidic hydroxyl groups so CZE at high or moderate pH might be suitable for their seperation. The p*K*a values in water of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP

in water are 9.36, 6.10, 7.80, 8.29, 6.60, respectively[25-28]. Therefore, 2,4,6-TBP and 2,6-DBP are fully deprotonated in the pH range considered. The results indicated that the migration time of 2,4,6-TBP and 2,6-DBP increased at basic pH. The reason could be that the complex formation between borate and hydroxyl groups of 2,4,6-TBP and 2,6-DBP was a strongly pH-dependent equilibrium[29]. With the increase of buffer pH, the negative charges of 4-BP, 2,4-DBP and 2-BP increased, which resulted in the invertion of the peak orders and the increase of the migration time. Consequently, Taking account of the resolution and analytical time, pH of 10.00 was chosen for further experiments.

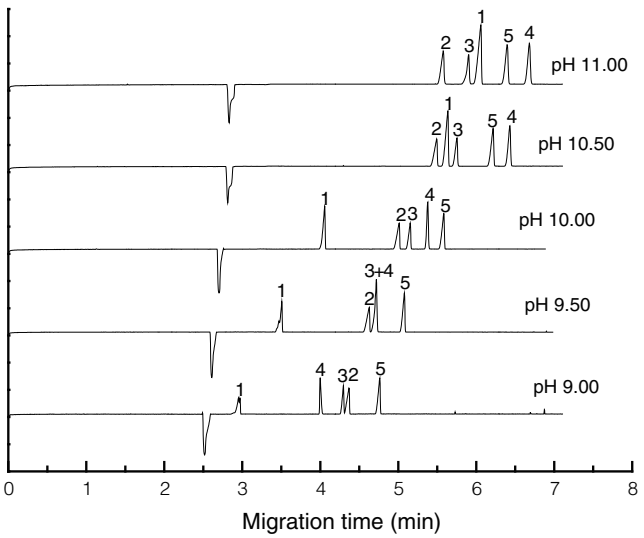

Figure 2. Effect of pH on the migration time. Analytical conditions: 20 mM borate buffer. voltage, 30 kV; temperature, 25°C; UV monitoring at 280 nm. 1: 4-BP; 2: 2,4,6-TBP; 3: 2,4-DBP; 4: 2-BP; 5: 2,6-DBP.

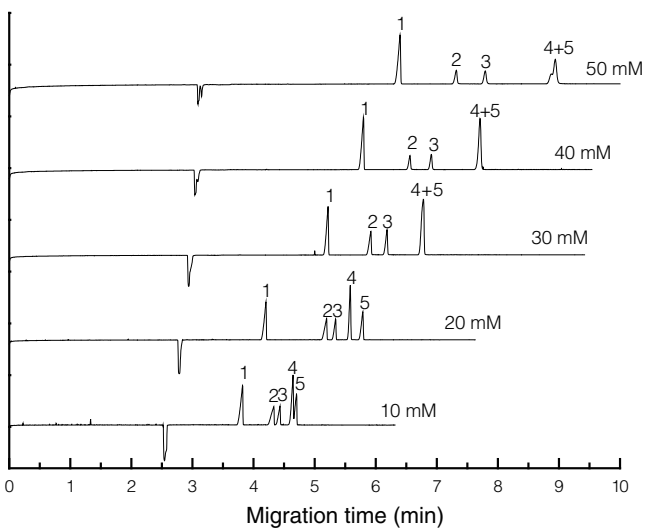

Figure 3. Effect of the buffer concentration on the migration time. Analytical conditions: Borate buffer at pH 10.00. Other conditions were similar to Figure 2. 1: 4-BP; 2: 2,4,6-TBP; 3: 2,4-DBP; 4: 2-BP; 5: 2,6-DBP.

II. *Effect of Buffer Concentration*

To understand the effect of buffer concentration on migration behavior, the running buffer consisting of sodium tetraborate at various concentrations (10, 20, 30, 40 and 50 M) at pH 10.00 were tested. The result was shown in Figure 3. The migration time and resolution of five analytes increased with an increase of the concentration of running buffer. This was resulted of the decreased electroosmotic flow since this effect is directly related to the decrease of the zeta potential at the capillary wall-solution interface[30]. Additionally, the higher the running buffer concentration, the stronger the complex formation between borate and the naturally occurring compounds in the extract. Moreover, the increase of the migration time for each analyte was almost the same so that the migration order did not alter. However, the separation became worsen as the buffer concentration increased to 30 mM and 2-BP and 2,6-DBP co-migrate. The best resolution was achieved at 20 mM borate buffer, in which the five analytes were completely separated under the optimal conditions described above.

III. *Effect of Applied Voltage*

The high voltage was necessary for rapid CE analysis. It was found that the resolutions of five bromophenols were not improved with the applied voltage ranging from 10 kV to 30 kV. However, the migration time increased when a lower voltage was applied. 30 kV was thus used as the running voltage.

From the above results, the best condition was obtained, an electrolyte containing 20 mM borate at pH 10.00. The applied voltage was 30 kV. Figure 4 showed the CE electropherogram of a mixture of five bromophenols (4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP).

IV. *Linearity, Reproducibility, Detection Limit and Recovery*

Calibration curves (peak area, y, vs. concentration, x (μg/mL)) were constructed in the range of 18.8 - 1200 μg/mL for 4-BP, 15 - 960 μg/mL for 2,4,6-TBP, 9.4 - 600 μg/mL for 2,4-DBP, 10.6 - 680 μg/mL for 2-BP and 3.0 - 370 μg/mL for 2,6-DBP. The regression equation of these curves and their correlation coefficients were calculated as follow: 4-BP: $y = 0.0713 x - 0.5503$ ($r = 0.9990$); 2,4,6-TBP: $y = 0.059 x + 0.4793$ ($r = 0.9998$); 2,4-DBP: $y = 0.0942 x + 0.2725$ ($r = 0.9997$); 2-BP: $y = 0.1418 x + 0.2285$ ($r = 0.9999$; 2,6-DBP: $y = 0.1183 x + 0.8094$ ($r = 0.9989$). The detection limits ($S/N = 3$) of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP were 1.6, 1.9, 1.2, 0.9 and 1.4 μg/mL, respectively. The quantitative limits ($S/N = 10$) of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP were 5.6, 7.0, 3.9, 3.5 and 3.0 mg/μL, respectively.

The method was validated for reproducibility of the migration time and the peak area of the analytes. The relative standard deviations (RSD) of the migration time and

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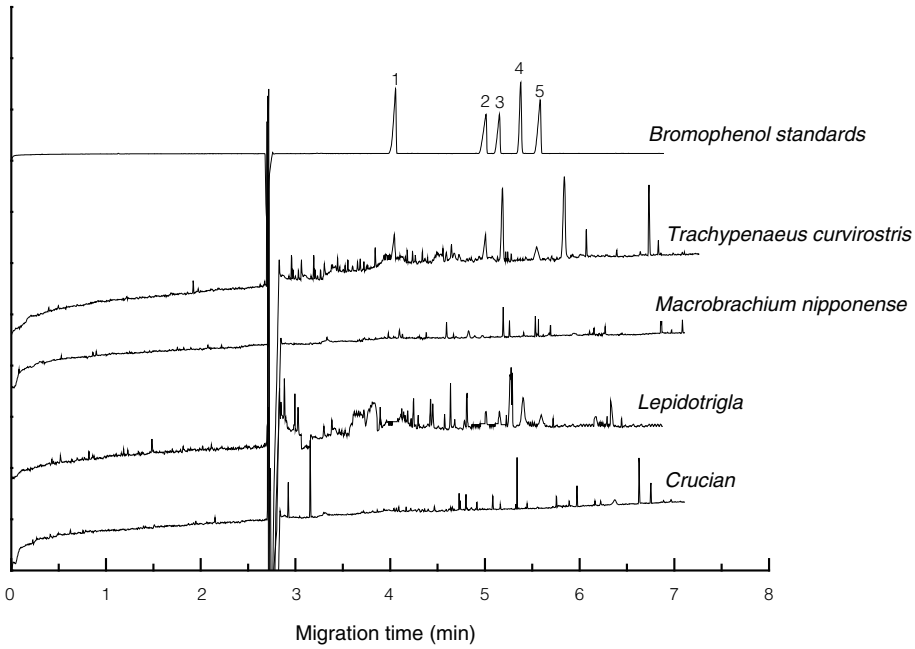

Figure 4. Chromatograms of a mixture of bromophenols, seafood and freshwater foods. Analytical conditions: 20 mM borate buffer at pH 10.00. Other conditions were similar to Figure 2. 1: 4-BP; 2: 2,4,6-TBP; 3: 2,4-DBP; 4: 2-BP; 5: 2,6-DBP.

Table 1. Contents of five bromophenols in *T. curvirostris*, *L. microptera*, *M. nipponense* and *Crucian* (n=3).

Samples	Contents (ng/g)				
	4-BP	2,4,6-TBP	2,4-DBP	2-BP	2,6-DBP
T. curvirostris	3.9	2.5	7.3	ND	0.6
M. nipponense	ND	ND	ND	ND	ND
L. microptera	ND	1.4	1.6	3.9	0.6
C. auratus var. Pengzesis	ND	ND	ND	ND	ND

the peak area of each peak were determined for six replicate injections. The intra-day reproducibility (n = 6) of the migration time and the peak area of the analytes were lower than 2.1 and 4.9% of RSD, respectively, while the inter-day reproducibility (n = 6) of the migration time and the peak area of the analytes lower than 3.9 and 5.1% of RSD, respectively. Accuracy of the method was determined by spiking three different amounts of the analytes (20, 100 and 500 μg/mL) to seafood extracts. The recoveries of 4-BP, 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP were 94 - 97%, 92 - 103%, 95 - 103%, 98 - 100% and 92 - 99%, respectively.

V. *Determination of the Bromophenols in the Extract of Seafood and Freshwater Fish and Crustaceans*

The acetonitrile solutions of the extracts of *T. curvirostris*, *L. microptera*, *M. nipponense* and *C. auratus var. Pengzesis* were injected directly and separated under the optimum conditions, mentioned above. Each sample was injected in triplcate. Concentration of each bromophenol in the samples was calculated based on the peak area in the electropherograms and the standard equation. The analytical results were summarized in Table 1. No bromophenol was detected in *M. nipponense* and *C. auratus var. Pengzesis*. The concentrations of 4-BP, 2,4,6-TBP, 2,4-DBP and 2,6-DBP in *T. curvirostris* were 3.9, 2.5, 7.3 and 0.6 ng/g, respectively. The contents of 2,4,6-TBP, 2,4-DBP, 2-BP and 2,6-DBP in *L. microptera* were 1.4, 1.6, 3.9 and 0.6 ng/g, respectively.

CONCLUSIONS

The results demonstrated that the proposed CZE method is very suitable for fast determination of bromophenols in the extract of seafood. In addition, the method promises to be applicable to the quality control of seafood and, since analysis time and cost are much lower than those of LC and GC-MS[12,13], CZE is a good alternaive for food analysis.

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REFERENCES

1. Kotterman, M., van der Veen, I., van Hesselingen, J., Leonards, P., Osinga, R. and de Boer, J. 2003. Preliminary study on the occurrence of brominated organic compounds in dutch marine organisms. Biomol. Eng. 20: 425-427.
2. Chung, H. Y., Ma, W. C. J., Ang, P. O., Kim, J. S. and Chen, F. 2003. Seasonal variations of bromophenols in brown algae (*Padina arborescens*, *Sargassum siliquastrum* and *Lobophora variegata*) collected in Hong Kong. J. Agric. Food Chem. 51: 2619-2624.
3. Chung, H. Y., Ma, W. C. J. and Kim, J. 2003. Seasonal distribution of bromophenols in selected Hong Kong seafoods. J. Agric. Food Chem. 51: 6752-6760.
4. Bickmeyer, U., Drechsler, C., Köck, M. and Assmann, M. 2004. Brominated pyrrole alkaloids from marine *Agelas* sponges reduce depolarization-induced cellular calcium elevation. Toxicon. 44: 45-51.
5. Whitfield, F. B., Helidoniotis, F., Shaw, K. J. and Svoronos, D. 1999. Distribution of bromophenols in species of marine algae from eastern Australia. J. Agric. Food Chem. 47: 2367-2373.
6. Thomas, H., Sabine, P., Jyotsna, P., Jessica, L., Michael, A. and Ulf, B. 2006. Bromophenols, both present in marine organisms and in industrial flame retardants, disturb cellular Ca^{2+} signaling in neuroendocrine cells (PC12). Aquatic Toxicology. 76: 37-45.
7. Whitfield, F. B., Helidoniotis, F., Shaw, K. J. and Svoronos, D. 1997. Distribution of bromophenols in Australian wild-harvested and cultivated prawns (Shrimp). J. Agric. Food Chem. 45: 4398-4405.
8. Boyle, J. L., Lindsay, R. C. and Stuiber, D. A. 1993. Contributions of bromophenols to marine-associated flavors of fish and seafood. J. Aquat. Food Prod. Technol. 1: 43-63.
9. Whitfield, F. B., Last, J. H., Shaw, K. J. and Tindale, C. R. 1988. 2,6-dibromophenol: the cause of an iodoform-like off-flavour in some Australian crustacea. J. Sci. Food Agric. 46: 29-42.
10. Ma, W. C. J., Chung, H. Y., Ang Jr., P. O. and Kim, J.-S., 2005. Enhancement of bromophenol levels in aquacultured silver seabream (*Sparus sarba*). J. Agric. Food Chem. 53: 2133-2139.
11. Sílvia, M., Albert, C., Sílvia, L., Anna, J. and Romà, T. Comprehensive description of the photodegradation of bromophenols using chromatographic monitoring and chemometric tools. 2011. Talanta. 83: 1134-1146.
12. da Silva, V. M, da Cunha Veloso, M. C., de Oliveira, A. S., Santos, G. V., de P. Pereira, P. A. and de Andrade, J. B. 2005. Determination of simple bromophenols in marine fishes by reverse-phase high performance liquid chromatography (RP-HPLC). Talanta 68: 323-328.
13. Blythe, J. W., Heitz, A., Joll, C. A. and Kagi, R. I. 2006. Determination of trace concentrations of bromophenols in water using purge-and-trap after in situ acetylation . J. Chromatogr. A 1102: 73-83.
14. Sim, W.-J., Lee, S.-H., Lee, I.-S., Choi, S.-D. and Oha, J.-E. 2009. Distribution and formation of chlorophenols and bromophenols in marine and riverine environments. Chemosphere. 77: 552-558.
15. Xu, J. L., Yan, X. J., Xu, N. J., Shen, J. and Shen, L. J. 2004. Analysis of bromophenol-like marine flavors in marine aquatic products by gas chromatography-mass spectrometry. J. Fish. China 28: 100-106.
16. Bermudo, E., Núñez, O., Puignou, L. and Galceran, M. T. 2006. Analysis of acrylamide in food products by in-line preconcentration capillary zone electrophoresis. J. Chromatogr. A 1129: 129-134.
17. Cataldi, T. R. I., Nardiello, D., Carrara, V., Ciriello, R. and De Benedetto, G. E. 2003. Assessment of riboflavin and flavin content in common food samples by capillary electrophoresis with laser-induced fluorescence detection. Food Chem. 82: 309-314.
18. Han, F., He, Y. Z., Li, L., Fu, G. N., Xie, H. Y. and Gan, W. E. 2008. Determination of benzoic acid and sorbic acid in food products using electrokinetic flow analysision pair solid phase extraction-capillary zone electrophoresis. Anal. Chim. Acta 618: 79-85.
19. Horie, M., Ishikawa, F., Oishi, M., Shindo, T., Yasui, A. and Ito, K. 2007. Rapid determination of cyclamate in foods by solid-phase extraction and capillary electrophoresis. J. Chromatogr. A 1154: 423-428.
20. Tang, Y. J and Wu, M. J. 2007. The simultaneous separation and determination of five organic acids in food by capillary electrophoresis. Food Chem. 103: 243-248.
21. Ohashi, M., Omae, H., Hashida, M., Sowa, Y. and Imai, S. 2007. Determination of vanillin and related flavor compounds in cocoa drink by capillary electrophoresis. J. Chromatogr. A 1138: 262-267.
22. Horie, H. and Yamashita, K.-I. 2006. Non-derivatized analysis of methiin and alliin in vegetables by capillary electrophoresis. J. Chromatogr. A 1132: 337-339.
23. Craston, D. H., Farnell, P., Francis, J. M., Gabriac, S., Matthews, W., Saeed, M. and Sutherland, I. W. 2001. Determination of gellan gum by capillary electrophoresis and CE-MS**.** Food Chem. 73: 103-110.
24. Blanco, E., Casais, M. C., Mejuto, M. C. and Cela, R. 2005. Comparative study of aqueous and non-aqueous capillary electrophoresis in the separation of halogenated phenolic and bisphenolic compounds in water samples. J. Chromatogr. A 1068: 189-199.
25. Broderius, S. J., Kalh, M. D. and Hoglund, M. D. 1995. Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. Environ. Toxicol. Chem. 14: 1591-1605.
26. Lepri, L., Desideri, P. G. and Heinler, D. 1980. Reversed-phase and soap thin-layer chromatography of phenols. J.

Chromatogr. A 195: 339-348.
27. Rived, F., Roses, M. and Bosch, E. 1998. Dissociation constants of neutral and charged acids in methyl alcohol. The acid strength resolution. Anal. Chim. Acta 374: 309-324.
28. Vandenbelt, J. M., Heinrich, C. and Vanden Berg, S. G. 1954. Comparison of $pK_{á}$ values determined by electrometric titration and ultraviolet absorption methods. Anal. Chem. Acta 26: 726-727.
29. Morin, P., Villard, F. and Dreux, M. 1993. Borate complexation of flavonoid-O-glycosides in capillary electrophoresis: I. Separation of flavonoid-7-O-glycosides differing in their flavonoid aglycone. J. Chromatogr. A 628: 153-160.
30. Jorgenson, J. W. 1984. Zone electrophoresis in open-tubular capillaries. TRAC-Trend. Anal. Chem. 3: 51-54.