

Analytical, Nutritional and Clinical Methods

The simultaneous separation and determination of five organic acids in food by capillary electrophoresis

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Abstract

The simultaneous separation of quinic acid, anisic acid, salicylic acid, benzoic acid and sorbic acid was performed by capillary electrophoresis with 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) as the background electrolyte. The choices of the background electrolytes and the applied voltage were optimized. The effects of tetrabutylammonium bromide and sodium dodecylsulfate on the separation were investigated in detail. Under the optimum condition, the linearity, reproducibility and detection limit of five organic acids were shown, respectively. As an application of the method proposed, 10 samples of soy sauce and vinegar were analysed to determine benzoic acid and sorbic acid. The procedure described provides the advantages of good selectivity, rapid speed and simplicity for the separation and determination of organic acids.

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1. Introduction

Organic acids were widely distributed in fruits, cheese, wine, and vegetables (Yang & Choong, 2001). Quinic acid, anisic acid, salicylic acid and benzoic acid belong to phenolic acid. Benzoic acid and sorbic acid are widely used as antifungal preservatives against molds and yeasts to ensure long-term microbial stability (Mikami, Goto, Ohno, Matsumoto, & Nishida, 2002; Pant & Trenerry, 1995). Benzoic acid is permitted for use in non-dairy dips where the pH is 4.5 or less. As a preservative, sorbic acid inhibits the growth of yeasts, fungi and bacteria over a much wider pH range than benzoic acid and is effective up to a pH of 6.5. The appropriate amount of benzoic acid or sorbic acid appears to be safe, which has been tested by many laboratories throughout the world. Meanwhile, individuals may

be sensitive to different preservatives, so the kinds and the amount of the preservatives must be controlled.

Organic acids have been separated and determined by chromatographic techniques such as high-performance liquid chromatography (HPLC) (Escobal, Gonzalez, & Iriando, 1997; Falque-Lopez & Fernández-Gómez, 1996; Shui & Leong, 2002; Vonach, Lendl, & Kellner, 1998; Zhao, Guo, Chang, & Wang, 2001), gas chromatography (GC) (Deng, 1997), thin-layer chromatography (Thompson & Hedin, 1966), gas-liquid chromatography (Sarkar & Malhotra, 1979), and ion chromatography (IC) (Ding, Suzuki, & Koizumi, 1995; Morales, Gonzalez, & Troncoso, 1998). Compared with the methods above, capillary electrophoresis (CE) offers high resolution efficiency, rapid separation speed, simple sample pretreatment, small consuming sample, and short analysis time, while having no derivatization often needed in GC and sample extraction encountered in HPLC or IC.

In this paper, a simple capillary zone electrophoresis (CZE) method for the simultaneous separation and

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determination of quinic acid, anisic acid, salicylic acid, benzoic acid and sorbic acid was performed with 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) as the background electrolyte (BGE). The effects of tetrabutylammonium bromide (TBAB) and sodium dodecylsulfate (SDS) on the separation were investigated in detail. As an application of this method, 10 samples of soy sauce and vinegar were analysed to determine benzoic acid and sorbic acid, which were often added as preservative.

2. Materials and methods

2.1. Chemicals

Quinic acid was purchased from Takeda Chemical Industries Ltd. (Japan), sorbic acid from the Pharmacy Faculty of Second Military Medical University (China), anisic acid from International Dispensary Co., Ltd. (China), benzoic acid from Shanghai Laize Fine Chemicals Factory (China), salicylic acid from Xinzhong Chemical Factory Co., Ltd. (China), cinnamic acid from Beijing Chemical Reagent Research Institute (China), TBAB from E.MERCK (West Germany), SDS from Zibo Jujin Chemical Co., Ltd. (China). Sodium hydroxide was from Beijing Chemical Plant (China), (hydroxymethyl) aminomethane (Tris) from Changshu Fushan Biology and Chemistry Reagents Factory (China), boric acid from Beijing Chemical Reagent Factory (China), borax from Beijing Xinguang Chemical Reagent Factory (China), disodium hydrogen phosphate from Hongxing Chemical Reagent Factory (Beijing, China) and carbon tetrachloride from Beijing Chemical Reagent Factory (China). All reagents were of analytical purity and used without further purification.

2.2. Apparatus and method

All experiments were performed on a laboratory-built CE unit, which comprised a ± 3 kV high voltage supply (Shanghai Institute of Nuclear Research), a CV⁴ variable-wavelength detector (ISCO, Lincoln, NE, USA), and a 75- μ m-I.D., 360- μ m-O.D. fused-silica capillary tube (Yongnian Optical Conductive Fiber Plant, Hebei, China). The total length of the capillary was 55 cm, and the detection was performed at 40 cm downstream. The samples were introduced into the anode end of the capillary by gravity injection at 6.5 cm height for 8 s. The pH meter was from Shanghai Analytical Instrument Factory of China.

Boric acid, borax, disodium hydrogen phosphate, TBAB, SDS and Tris were dissolved in double-distilled water, respectively, to prepare the concentration of 0.1 mol L⁻¹ as the stock solutions. Stock standard solutions of organic acids were prepared at a concentration of 1×10^{-3} mol L⁻¹ using double-distilled water, respectively. In all cases, the pH value was measured in a PHS-3CT pH meter (Shanghai Analytical Instrument Factory, China) with a combined electrode. The required pH value

was adjusted with H₃BO₃ or NaOH. The samples and BGEs were filtered through a 0.45 μ m cellulose acetate filter membrane (Jiangsu Qilin Medical Instrument Factory, China).

The capillary was conditioned for 30 min with 0.1 mol L⁻¹ NaOH and 10 min with water. Additionally, the capillary was washed for 2 min with 0.1 mol L⁻¹ NaOH, 2 min with water and 5 min with the running buffer before each run. Between runs, the capillary was washed with running buffer. The experiment was done under constant humidity (40%).

2.3. Samples and sample preparation

The real samples were Taoda soy sauce (Shanghai Taoda Food Co. Ltd.), Hubao seasoning sauce (Tianjin Hubao Flavour-Making Co. Ltd.), Donghu mature vinegar (Shanxi Meiheju Mature Vinegar Co. Ltd.), Zilin mature vinegar (Shanxi Qingxu Laifu Mature Vinegar Co. Ltd.), Haitian light soy sauce (Foshan Haitian Flavour-Making Co. Ltd.), Beikang dark soy sauce (Jilin Beikang Fermentation Food Co. Ltd.), Haitian seafood soy sauce (Foshan Haitian Flavour-Making Co. Ltd.), Beikang vinegar (Jilin Beikang Fermentation Food Co. Ltd.), Beikang rice vinegar (Jilin Beikang Fermentation Food Co. Ltd.) and Beikang flavor vinegar (Jilin Beikang Fermentation Food Co. Ltd.). All the samples were purchased from a supermarket in Changchun. Soy sauce and vinegar with the labeling of food preservatives were shown in Table 1. Vinegar was directly diluted with double-distilled water to 1:10 (v/v) and filtered through a 0.45 μ m filter membrane. Two millilitres of soy sauce and 1 ml CCl₄ were mixed and the solution was vortexed for 10 min. Then, the lipids extracted into the organic phase were discarded. The aqueous phase solution obtained was transferred into a dry vial. The aqueous phase solution was diluted with double-distilled water to 1:10 (v/v) and filtered through a 0.45 μ m filter membrane.

2.4. Linearity, reproducibility and detection limit

Using the optimized instrumental and operational parameters for the standard solution, the linearity of the method was calculated and expressed as the correlation coefficient, and the reproducibility of the method was mea-

Table 1
Description of samples analysed

Sample name	Labeling of food preservatives	
Taoda soy sauce	Benzoic acid	Sorbic acid
Beikang dark soy sauce	Benzoic acid	
Haitian light soy sauce	Benzoic acid	Sorbic acid
Hubao seasoning sauce		Sorbic acid
Haitian seafood soy sauce		Sorbic acid
Beikang vinegar	Benzoic acid	
Beikang rice vinegar	Benzoic acid	
Donghu mature vinegar	Benzoic acid	Sorbic acid
Zilin mature vinegar	Benzoic acid	
Beikang flavor vinegar	Benzoic acid	

Table 2
Summary of calibration and precision data for the method

Acids	The regression equation	<i>r</i>	RSD (%)	Linearity (mg L ⁻¹)	LOD (mg L ⁻¹)
Quinic acid	$y = 0.11x + 0.02^a$	0.9978	2.59	5.41–288.67	1.80
Anisic acid	$y = 0.12x + 0.03$	0.9969	2.70	3.19–106.54	1.21
Sorbic acid	$y = 0.24x + 0.03$	0.9979	2.58	1.12–112.13	0.44
Benzoic acid	$y = 1.31x + 0.05$	0.9965	2.80	2.93–73.27	2.19
Salicylic acid	$y = 1.23x + 0.05$	0.9966	2.78	3.17–96.68	2.07

Mean values of seven determinations ($n = 7$).

BGE: 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8); applied voltage: 20 kV; injection: 6.5 cm × 8 s.

^a *y*, the peak area ratio (*y*) of the respective organic acid to cinnamic acid; *x*, concentration (mg L⁻¹).

sured by replicate injections of the standard solution. Cinnamic acid was used as the internal standard. The regression equations of five organic acids, the correlation coefficient, the relative standard deviation (RSD) ($n = 7$), the linearity range and the limits of detection (LOD) (signal-to-noise ratio of three) were shown in Table 2.

2.5. Validation of the proposed methods

Typical electropherograms of the separation of five organic acids by UV detection were shown in Figs. 4(a) and 5(a). The reproducibility of peak area and migration time in this experiment was determined by injecting a solution of the mixture of five organic acids (5×10^{-5} mol L⁻¹ in each) into the system for seven times, with 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) as BGE under the optimum conditions. RSDs of peak area and migration time were 2.0% and 0.9%, respectively, for quinic acid, 2.1% and 1.1%, respectively, for anisic acid, 2.1% and 1.2%, respectively, for sorbic acid, 2.3% and 1.3%, respectively, for benzoic acid and 2.6% and 1.4%, respectively, for salicylic acid. The high reproducibility indicates that this method is suitable for the analysis of real samples.

3. Results and discussion

3.1. Optimization of the background electrolyte

BGE affects the migration time and the separation between compounds, directly. It is necessary to provide high electroosmotic flow (EOF) in basic conditions for high-speed and high-resolution separations of organic acids.

Faster separations are obtained in CE when the electrophoretic vector and the electroosmotic flow are in the same direction. The separations of five organic acids in four different BGEs including 20 mmol L⁻¹ borax–20 mmol L⁻¹ H₃BO₃, 20 mmol L⁻¹ Tris–20 mmol L⁻¹ H₃BO₃, 20 mmol L⁻¹ Na₂HPO₄–20 mmol L⁻¹ H₃BO₃ and 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃, were tried and the migration times were shown in Table 3. Benzoic acid and salicylic acid had the same migration time in 20 mmol L⁻¹ borax–20 mmol L⁻¹ H₃BO₃ (pH 8.8). Anisic acid and sorbic acid could not be separated completely and synchronously in 20 mmol L⁻¹ Tris–20 mmol L⁻¹ H₃BO₃ (pH 8.8). In 20 mmol L⁻¹ Na₂HPO₄–20 mmol L⁻¹ H₃BO₃ (pH 8.8), five organic acids were separated, but benzoic acid and salicylic acid had leading peaks. In 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) five organic acids were separated and detected absolutely with sharp and symmetrical peaks, compared with the other BGEs.

Dilute electrolyte solutions are desirable to shorten migration time. However, it is important that electrolyte solutions have sufficient buffer capacity to maintain a fixed pH and guarantee the strong mutual action in the presence of the analytes. Inadequate buffer capacity may result in the poor resolution. So, we alternated the BGE from 5 mmol L⁻¹ NaOH–5 mmol L⁻¹ H₃BO₃ (pH 8.8) to 25 mmol L⁻¹ NaOH–25 mmol L⁻¹ H₃BO₃ (pH 8.8). The effects of different electrolyte concentrations on the separation of five organic acids showed that 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) gave complete separation while the higher electrolyte concentrations brought about longer migration time and broader peak. The electropherograms of the above BGEs revealed that 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) was suitable.

3.2. The effect of TBAB concentration

Cationic surfactants can affect the EOF in micellar electrokinetic chromatography (MEKC) owing to the electrostatic interactions between the positively charged surfactant monomers and the negatively charged fused-silica wall. The feasibility of making use of the interactions between charged analytes and TBAB in the capillary was studied. The resolution variation of five organic acids as the function of TBAB concentration was shown in Fig. 1.

Table 3
Migration times of organic acids obtained in different BGEs

BGE	Migration time					
	Quinic acid	Cinnamic acid	Anisic acid	Sorbic acid	Benzoic acid	Salicylic acid
20 mmol L ⁻¹ borax–20 mmol L ⁻¹ H ₃ BO ₃	2.44	4.88	4.97	5.08	5.61	5.61
20 mmol L ⁻¹ Tris–20 mmol L ⁻¹ H ₃ BO ₃	2.23	4.04	4.11	4.16	4.45	4.60
20 mmol L ⁻¹ Na ₂ HPO ₄ –20 mmol L ⁻¹ H ₃ BO ₃	2.36	4.10	4.16	4.29	4.72	5.05
20 mmol L ⁻¹ NaOH–20 mmol L ⁻¹ H ₃ BO ₃	2.19	3.93	4.01	4.08	4.43	4.65

Mean values of five determinations ($n = 5$).

Applied voltage: 20 kV; injection: 6.5 cm × 8 s.

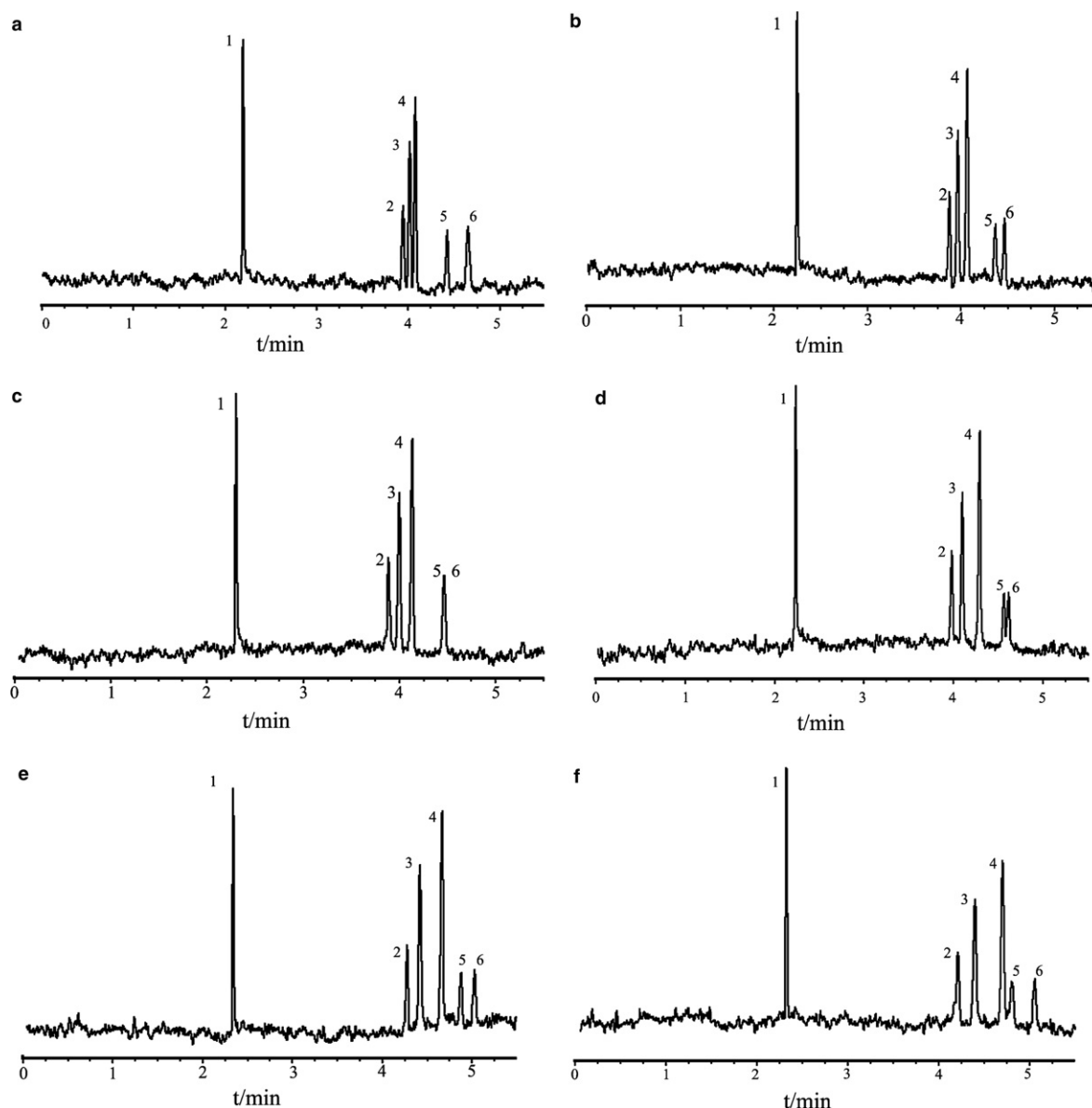


Fig. 1. The effect of TBAB concentration on the separation of organic acids. (1) quinic acid; (2) cinnamic acid; (3) anisic acid; (4) sorbic acid; (5) benzoic acid; (6) salicylic acid. (a) 0 mmol L⁻¹ TBAB; (b) 5 mmol L⁻¹ TBAB; (c) 10 mmol L⁻¹ TBAB; (d) 15 mmol L⁻¹ TBAB; (e) 20 mmol L⁻¹ TBAB; (f) 25 mmol L⁻¹ TBAB. BGE: 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8); applied voltage: 20 kV; injection: 6.5 cm × 8 s; UV wavelength: 240 nm.

The migration order of the organic acids was quinic acid, followed by cinnamic acid, anisic acid, sorbic acid, benzoic acid and salicylic acid. When 5 mmol L⁻¹ TBAB was added to the electrolyte, the separation window between benzoic acid and salicylic acid was diminished. Benzoic acid and salicylic acid had the same migration time when TBAB concentration increased to 10 mmol L⁻¹. The separation between benzoic acid and salicylic acid became better gradually along with the TBAB concentration changed from 15 to 20 mmol L⁻¹. However, when the concentration of TBAB reached 25 mmol L⁻¹, benzoic acid overlapped with sorbic acid, partly. During all the processes, the separation window between anisic acid and sorbic acid got enlarged.

3.3. The effect of SDS concentration

SDS is the most regularly used anionic surfactant in MEKC separations. SDS of 10, 20, 30, 40 and 50 mmol L⁻¹ were added sequentially to the electrolyte solution of 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8) to investigate whether the presence of SDS could influence the resolution. From these experiments, it was found that the resolution between cinnamic acid and anisic acid reduced from 1.97 to 1.20 after the addition of SDS, and that the migration times of all the organic acids increased. Fig. 2 showed the electropherograms of the separation of five organic acids by MEKC with SDS as the anionic surfactant.

3.4. The choice of applied voltage

The efficiency of the separation improves and the migration time shortens when the applied voltage is increased. But the higher applied voltage will result in the efficiency of the separation falling because of the Joule heating effect. On the other hand, the reproducibility becomes worse and the migration time prolongs if the applied voltage gets lower. In this experiment, the five organic acids were separated as the applied voltage increased from 12.5 to 22.5 kV (Fig. 3). The current increased slightly and was always below 10 μ A. Under the applied voltage above, five organic acids were well resolved except for 22.5 kV. A 20 kV applied voltage was found to be an acceptable compromise between adequate resolution and short migration time.

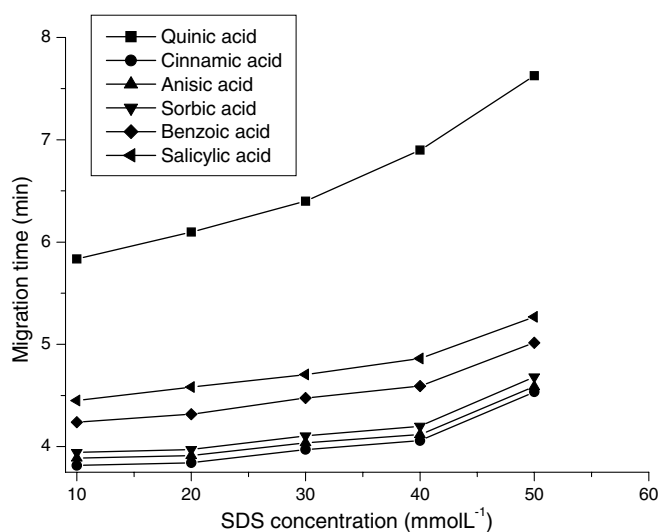


Fig. 2. The effect of SDS concentration on the separation of five organic acids (the values expressed as average of five injections). Other conditions were the same as in Fig. 1.

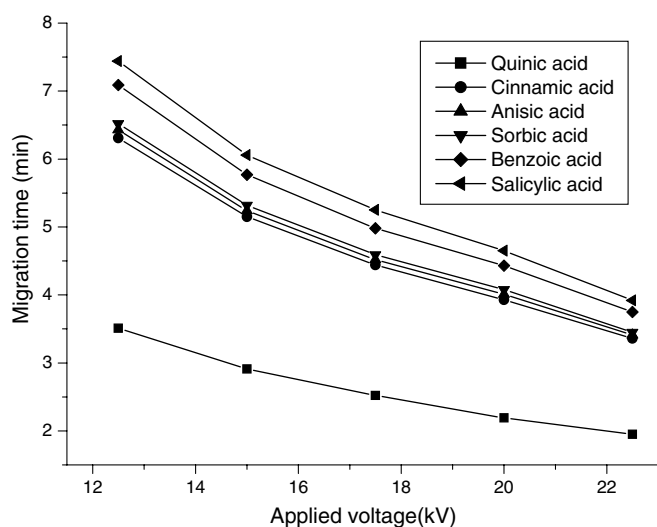


Fig. 3. Migration times of organic acids under different applied voltage (the values expressed as average of five injections). Other conditions were the same as in Fig. 1.

3.5. Application

As an application, soy sauce and vinegar were prepared using the procedure described in Section 2.3. Cinnamic acid was used as the internal standard and the electropherograms were depicted (Figs. 4 and 5). By standard addition method and comparing migration times of analytes with those of the standard mixture solution, benzoic acid and sorbic acid in soy sauce and vinegar were determined. The recovery and reproducibility experiments under the optimum conditions were conducted to evaluate the precision and accuracy of the method. Recovery was determined by standard addition method and the determination results of the real samples were listed in Tables 4 and 5. Nowadays, HPLC (García, Ortiz, Sarabia, Vilches, & Gredilla, 2003; Saad, Bari, Saleh, Ahmad, & Talib, 2005) is the most common analytical procedure for the determination of benzoic acid and sorbic acid. Compared with HPLC, the method proposed in this paper simplifies considerably the analysis, reducing its cost and shortening migration time. The procedure described provides the advantages of good

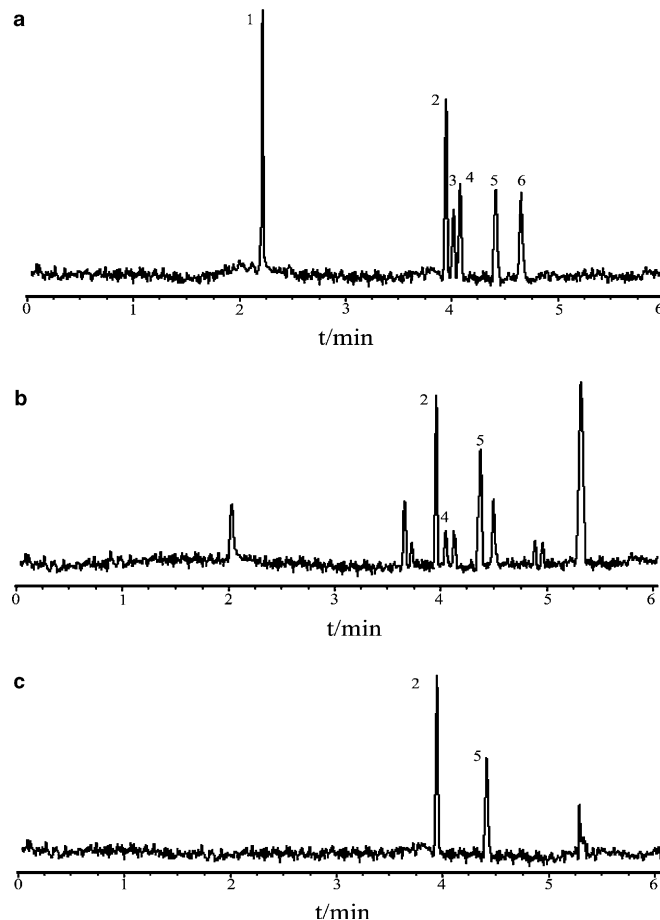


Fig. 4. The electropherograms of determining benzoic acid in the samples. (a) The standard solution; (b) Taoda soy sauce + 5×10^{-5} mol L⁻¹ cinnamic acid; (c) Beikang vinegar + 5×10^{-5} mol L⁻¹ cinnamic acid. BGE: 20 mmol L⁻¹ NaOH–20 mmol L⁻¹ H₃BO₃ (pH 8.8); applied voltage: 20 kV; injection: 6.5 cm \times 8 s; UV wavelength: 225 nm.

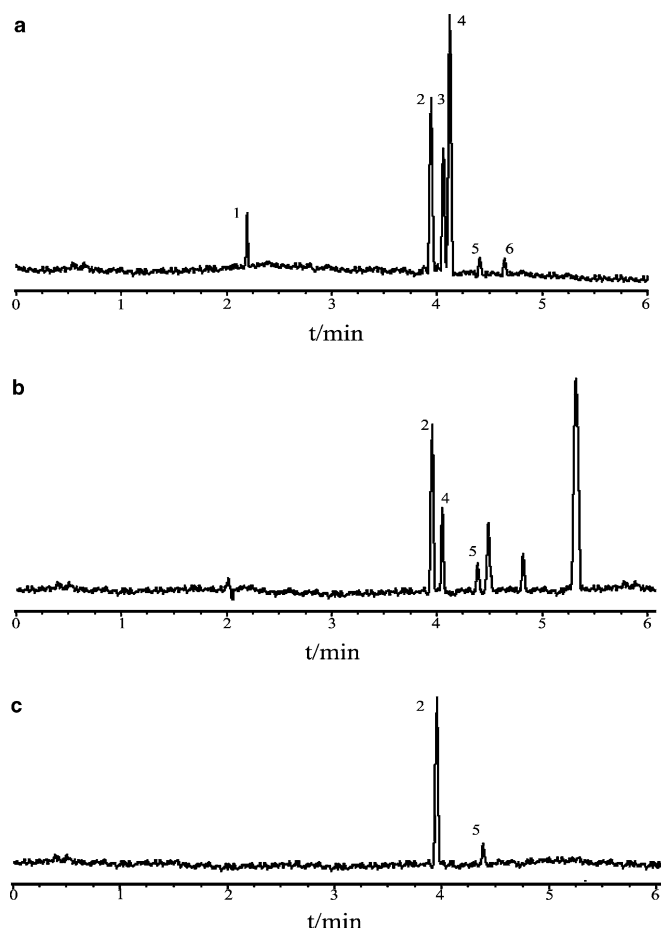


Fig. 5. The electropherograms of determining sorbic acid in the samples. UV wavelength: 255 nm. Other conditions were the same as in Fig. 4.

Table 4
The results for CE analysis of benzoic acid in the samples

Sample	The average value found (mg L ⁻¹)	RSD (%)	Recovery (%)
Taoda soy sauce	78.15	2.90	98.30
Beikang dark soy sauce	60.98	2.95	98.25
Haitian light soy sauce	60.27	2.97	98.21
Beikang vinegar	63.50	2.85	98.90
Beikang rice vinegar	65.24	2.86	98.85
Donghu mature vinegar	112.31	2.87	98.83
Zilin mature vinegar	89.22	2.86	98.85
Beikang flavor vinegar	90.04	2.85	99.01

Mean values of five determinations ($n = 5$).

Other conditions as in Fig. 4.

Table 5
The results for CE analysis of sorbic acid in the samples

Sample	The average value found (mg L ⁻¹)	RSD (%)	Recovery (%)
Taoda soy sauce	17.94	2.67	99.00
Haitian light soy sauce	15.21	2.70	98.20
Hubao seasoning sauce	23.46	2.65	99.18
Haitian seafood soy sauce	15.34	2.65	99.03
Donghu mature vinegar	24.01	2.60	99.24

Mean values of five determinations ($n = 5$).

Other conditions as in Fig. 5.

selectivity, rapid speed and simplicity for the separation and determination of organic acids and these results indicates that the proposed method can be useful for research and routine analysis of organic acids in many application areas.

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