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Determination of benzoic acid and sorbic acid in food products using electrokinetic flow analysis–ion pair solid phase extraction–capillary zone electrophoresis

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ABSTRACT

An electrokinetic flow analysis system (EFA), consisting of one electroosmotic pump, five solenoid valves and one on-line homemade solid phase extraction (SPE) unit, combined with capillary zone electrophoresis (CZE) was proposed to determine benzoic acid and sorbic acid in food products. Tetrabutylammonium bromide (TBAB) was adopted as an ion pair reagent to improve the retention of the preservatives on C_8-bonded silica sorbent, which was also used to remove sample matrices. By using the SPE unit, the EFA–SPE–CZE system was able to perform the SPE operation and CZE separation simultaneously. With a modified interface of EFA and CZE, the buffer consumption was reduced to 130 μL for each running. The preservatives were separated and determined under optimized conditions with *p*-hydroxybenzoic acid as an internal standard. The relative standard deviation (R.S.D.) of peak area for each analyte was less than 3.1% ($n = 5$) and the limits of detection (LODs) ranged from 10 to 20 ng mL^{-1} ($K = 3$, $n = 11$).

1. Introduction

Benzoic acid, sorbic acid and their salts are commonly used as chemical preservatives [1] in food products to prevent alteration and degradation by microorganisms during storage. However, excessive addition of these preservatives may be harmful to consumers, because of the tendency to induce allergic contact dermatitis, convulsion and hives etc. [2,3]. Therefore, the development of convenient and inexpensive analysis methods of these preservatives is of great importance for food safety.

High-performance liquid chromatography (HPLC) is the main method [4–6] in benzoic acid and sorbic acid analysis. Nowadays, capillary electrophoresis (CE) has become an attractive analytical technique for preservatives owing to its high separation efficiency, low sample consumption and fast analysis velocity [7–11]. In food analysis, sample pretreatment [12] prior to determination includes protein precipitation, liquid–liquid extraction and ion exchange clean-up etc. However, liquid–liquid extraction has the shortcomings of low enrichment factor and high solvent consumption. Solid phase extraction (SPE) [13,14] can eliminate these shortcomings.

Sequential injection analysis (SIA) introduced by Ruzicka and Marshall [15] has become a conventional flow analysis technique with versatility and simplicity. Multicommutation developed by Reis et al. [16] provided flow analysis technique with flexibility and controllability. As a fluid delivery device, electroosmotic pump has proved to be suitable for flow analysis with its large flow range (10 μL min^{-1} to 5.0 mL min^{-1}), stable flow rate (<4.0%, 4 h), pulseless driving, simplified apparatus, convenient control operation and proper back pressure (≤1.1 MPa) [17–19] etc. However, electrolyte solutions cannot

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be introduced into the porous core of the pump because of the influence on the surface charge density of porous core and the electroosmotic flow (EOF). The problem can be solved by aspirating sample and reagent solutions into a holding coil, as performed by SIA. Based on the flow analysis techniques of SIA, multicommutation and electroosmotic pump, electrokinetic flow analysis (EFA) was proposed [20–22].

Flow injection analysis (FIA) coupling with SPE [23,24] or combining with CE separation [25–32] were reported to set up automated analysis systems in recent years. Our previous work [33] reported a FIA–SPE–MEKC system using a modified split–flow interface to avoid running buffer contamination and reduce buffer consumption, especially, for the buffer solutions containing expensive reagents. In this paper, a simple and controllable EFA system with stable flow rate and pulseless driving was employed to deliver reagent and sample solution; an on-line SPE unit was used to clean up matrices and preconcentrate analytes, furthermore, the SPE operation and CZE separation were able to be performed simultaneously to save total analytical time; and an ion-pairing reagent of tetrabutylammonium bromide (TBAB) [5] was added into sample solutions to enhance the breakthrough content of the preservatives on the SPE column. In addition, the interface of low buffer consumption reported in the previous work [33] was also employed in this system. To verify the proposed method, the preservatives in three kinds of food products were determined by the EFA–SPE–CZE system.

2. Experimental

2.1. Reagents and solutions

Benzoic acid, sorbic acid and *p*-hydroxybenzoic acid, hexamethylene tetramine, cetyltrimethylammonium bromide (CTAB), TBAB, sodium tetraborate, sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol, methanol and acetonitrile were of analytical grade and purchased from the Chemical

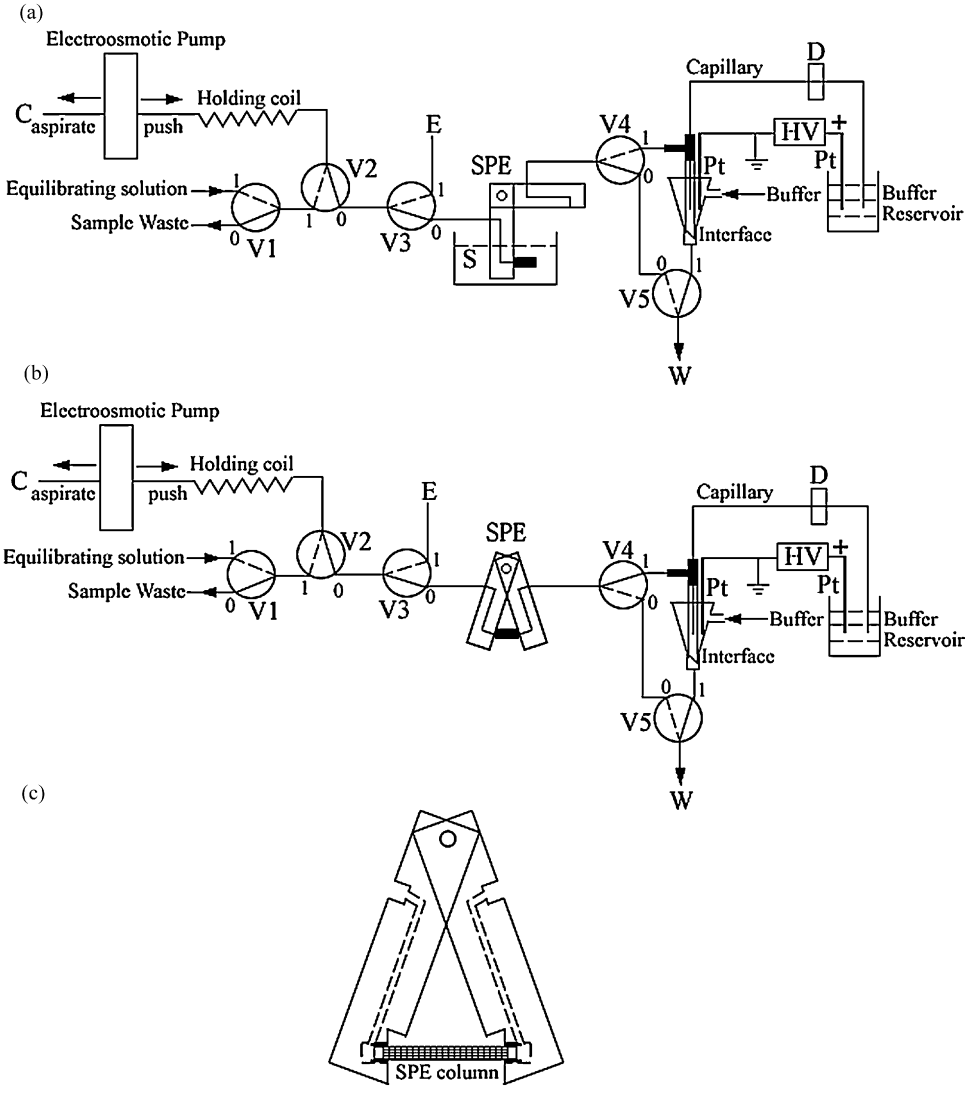

Fig. 1 – Schematic diagram of EFA–SPE–CZE system in sample loading (a) and eluting (b), and on-line SPE unit (c). V_1–V_5: solenoid valves; SPE: homemade SPE unit; HV: high voltage power supply; Pt: platinum electrodes; D: detector; C: pump carrier; CL: C_8-bonded silica column; S: sample; E: eluent; W: waste.

Reagent Ltd. (Shanghai, China). Tetradecyltrimethylammonium bromide was obtained from Sigma (St. Louis, MO, USA). All aqueous solutions were prepared with deionized water from Lanlan Water Ltd. (Hefei, China).

Benzoic acid, sorbic acid and *p*-hydroxybenzoic acid were individually dissolved in ethanol at a stock concentration of 0.20 mg mL^{-1}. The mixed standard solutions were prepared by diluting the stock solutions with 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 10% ethanol (v/v). *p*-Hydroxybenzoic acid and TBAB were used as the internal standard and ion pair reagent, and added into each mixed standard and sample solution with the concentration of 2.0 μg mL^{-1} and 1.0 mmol L^{-1}, respectively.

The separation buffer was prepared by adjusting 20 mmol L^{-1} sodium tetraborate solution containing 0.04 mmol L^{-1} CTAB to pH 11.5 with 1.0 mol L^{-1} NaOH. The SPE eluent was 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 70% methanol (v/v). All the solutions were degassed for 15 min by an S–2200 ultrasonic bath (120 W, 35 kHz, J & L Ltd., Shanghai) before use.

2.2. EFA–SPE–CZE system

A 1229-HPCE Analyzer (Institute New Tech. Appl., Beijing, China) detecting at 214 nm and an N-2000 double-channel chromatography station (Institute Info. Engineering, Zhejiang Univ., Zhejiang, China) were employed in the CZE separation, which was performed in a 50 μm i.d. fused-silica capillary with 70 cm total length and 40 cm effective length (Yongnian Chromatogr. Components Ltd, Hebei, China) at 15 kV. The operation safety was ensured with ground potential at the interface and positive high potential at the downstream end of the capillary. An XW-80A vortex mixer (Jingke, Shanghai) was used to blend sample solutions. A UV-9100 spectrophotometer (Ruili Anal. Instr. Ltd., Beijing) detecting at 214 nm was employed to measure the breakthrough volume of the SPE column.

The schematic diagram of the EFA–SPE–CZE system is shown in Fig. 1a and b. The EFA system consisted of one homemade electroosmotic pump, five three-way solenoid valves (161T031, Nreseach Inc., USA) and one on-line homemade SPE unit. The pump and solenoid valves were controlled by a personal computer with a homemade interface card and a Visual C program written by our group. The flow rate and flow direction of the pump were regulated by the working voltage and voltage polarity, which were provided by an electrophoretic power supply (DYY-III-4, Liuyi Instr. Factory, Beijing). The R.S.D. of the pump flow rate was 3.2%, measured each 20 min in 4 h, with the pump carrier of 0.5 mmol L^{-1} hexamethylene tetramine. The injection of 10.8 mL sample solution and 180 μL eluent were regulated by the aspirating flow rate of the pump and the switch time of correlative valves, as referred to Table 1. The carrier solution inside the pump chambers and electrode cells should be replaced after daily performance of the pump.

The homemade SPE unit was simplified from a bidirectional electrostacking [34–37] unit reported in [34] and could be used for on-line sample pretreatment of SPE or bidirectional electrostacking. This unit consisted of a nylon pincers and a quartz SPE microcolumn (2 cm × 2.0 mm i.d.) packed tightly with 30 mg C_8-bonded silica (50 μm, Daisogel, Japan) inside the column and quartz wool at both ends. As shown in Fig. 1c, two

Table 1 – Operation process of on-line EFA–SPE–CZE system

Step	Flow rate (mL min^{-1}) (pump voltage, V)	Duration (s)	Valve position					Operation	Appendix
			V1	V2	V3	V4	V5		
1	2.4 (−800)	25	1	1	0	0	0	Aspirating equilibrium solution	Equilibrating and washing
2	2.0 (+800)	30	0	0	0	1	1	Pushing equilibrium solution	SPE unit closed
3	1.8 (−600)	60	0	0	0	0	0	Aspirating sample solution	Analyte loading, recycling six times
4	2.0 (+600)	54	0	1	0	0	0	Pushing sample solution	SPE unit opened
5	2.2 (−600)	5	0	0	1	0	0	Aspirating eluent	Analyte eluting
6	0.9 (+350)	12	0	0	0	1	1	Pushing eluent	SPE unit closed
7	0(0)	20	0	0	0	0	0	Hydrodynamic injection	8 cm height difference, SPE unit closed
8	1.0 (−350)	8	0	0	0	1	0	Aspirating running buffer	SPE unit closed
9	2.4/2.0 (−800/+800)	270	1/0	1/0	0	0	0	CZE separation	Data processing
	1.8/2.0 (−600/+600)		0	0/1	0	0	0	Column equilibrium and sample loading	Functions as Step 1–4, SPE unit opened

holes of 8 mm × 5 mm i.d. on the inflexed surface of two nylon arms were used to join the SPE column sealed with silicone sheath. The flow conduits inside each arm were 16 mm × 1 mm i.d. and 5.5 mm × 1 mm i.d. that were connected to the EFA manifold by M6 fittings. By opening the SPE pincers and isolating the SPE unit from the CZE system, it was found that the column equilibrating-sample loading and CZE separation could be carried out simultaneously.

2.3. Sample pretreatment

Food products of milk beverages, soy sauces and fruit jams were obtained from local supermarkets. For liquid and solid sample analysis, 0.200 g sample was mixed with 10-mL 50 mmol L^{-1} sodium tetraborate, 4.5 mL ethanol, 0.5-mL 0.20 mg mL^{-1} *p*-hydroxybenzoic acid and 1.0-mL 50 mmol L^{-1} TBAB by the vortex mixer for 3 min, filtered through 0.45 μm nylon membrane and washed with 30 mL deionized water. The pretreated sample solutions were adjusted to pH 10.0 with 1.0 mol L^{-1} NaOH, diluted to 50 mL with deionized water and degassed for 15 min by the ultrasonic bath before the EFA–SPE–CZE analysis. The analytical recovery was examined by spiking the standard solutions of the analytes into real samples.

To compare with the samples pretreated by SPE, 1.00 g soy sauce and 1.00 g fruit jam was mixed with 5.0-mL 50 mmol L^{-1} sodium tetraborate and 2.5-mL 0.20 mg mL^{-1} *p*-hydroxybenzoic acid by the vortex mixer for 3 min, filtered through 0.45 μm nylon membrane and washed with 5.0 mL deionized water. All the collected solutions were diluted to 25 mL with deionized water. Then the untreated sample solution was directly analyzed by CZE with the same separation conditions as the EFA–SPE–CZE method.

2.4. Analytical procedure

Daily start-up procedure was the separation capillary and SPE column washing. The former was successively rinsed with 1.0 mol L^{-1} HCl for 5 min, deionized water for 1 min, 1.0 mol L^{-1} NaOH for 10 min, deionized water for 1 min and separation buffer for 10 min via the capillary outlet by pressure. And the latter was flushed with 3.0-mL 80% acetonitrile (v/v) by the pump.

The analytical steps of the EFA–SPE–CZE method are listed in Table 1. In Steps 1 and 2, the SPE column was equilibrated and the EFA manifold was washed with the solution of 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 10% (v/v) ethanol in the first running, as shown in Fig. 1b. From the second running as shown in Fig. 1a, the column equilibrating solution was expelled through the opened SPE column directly after removing the sample reservoir. During the sample loading steps of 3 and 4, the sample solution was aspirated and loaded onto the SPE column at a flow rate of 1.8 mL min^{-1} for 60 s by the pump, and then expelled through the sample waste outlet of V_1. The sample loading was implemented between Step 3 and 4 for six times to achieve the sample volume of 10.8 mL. During the eluting steps of 5 and 6, the nylon pincers and the SPE column were closed. After aspirating 180 μL eluent at 2.2 mL min^{-1} for 5 s, the retained analytes were eluted by pushing the eluent through the SPE column, and transported into the EFA–CZE interface, as shown in Fig. 1b. With the pump stopped in Step 7, a sample zone was hydrodynamically injected into the capillary at 8 cm height difference for 20 s. Then V_4 switched from 0 to 1 and V_5 kept at 0 in Step 8, 130 μL separation buffer was aspirated from the pipette reservoir into the PTFE tube containing the capillary inlet with a flow rate of 1.0 mL min^{-1} for 8 s. In Step 9, the CZE separation was carried out at +15 kV for 4.5 min. At the same time, the SPE column equilibrating and sample loading was carried out in turn with the nylon pincers opened. The next analysis procedure started from Step 5. Data processing was carried out during the whole analytical period except the first running from Step 9. The operations of the system were implemented automatically except for opening and closing the SPE unit and moving the sample reservoir.

3. Results and discussion

For on-line analysis of benzoic acid, sorbic acid and their salts by the EFA–SPE–CZE system, the conditions of the CZE separation and SPE pretreatment were investigated, respectively.

3.1. Conditions of CZE separation

Benzoic acid and sorbic acid can be separated using 20 mmol L^{-1} sodium tetraborate buffer (pH 9.3) within 12 min by CZE and the running of EFA–SPE–CZE procedure requires 25 min. In this situation, the analyte anions migrate towards anode in the opposite direction of EOF, which can reduce the separation velocity. Cationic surfactants can neutralize the negative surface charges of fused-silica capillary, and even more reverse the surface charges and EOF direction of the capillary. Two cationic surfactants of CTAB and tetradecyltrimethylammonium bromide were investigated in this work. For CTAB has stronger modification effect, lower working current and baseline noise than the other, CTAB was adopted as the EOF modifier in this work.

According to our experiment result [38], the CTAB concentration higher than 0.005 mmol L^{-1} can reverse the EOF direction in fused-silica capillary and the reversed EOF was enhanced by increasing the CTAB concentration. The influence of CTAB concentration ranging from 0.01 to 0.15 mmol L^{-1} on separating resolution and migration time was tested. The resolution of the analytes became worse with CTAB concentration higher than 0.04 mmol L^{-1}. Although they could be separated on baseline with CTAB concentration lower than 0.04 mmol L^{-1}, the migration time was increased rapidly. 0.04 mmol L^{-1} CTAB was employed in the separation buffer and the separation time was reduced to 4.5 min.

Since both the EOF and analyte mobility are highly dependent on the pH value of separation buffer in CZE separation, the effect of buffer pH ranging from 8.0 to 12.5 was examined. Three anions could not be separated on baseline with pH lower than 11.0, whereas their migration time and electric current increased with pH higher than 11.5. By compromising the resolution and migration time, the pH value was established at 11.5. Therefore, 20 mmol L^{-1} sodium tetraborate buffer (pH 11.5) containing 0.04 mmol L^{-1} CTAB was used as the separation buffer in this method.

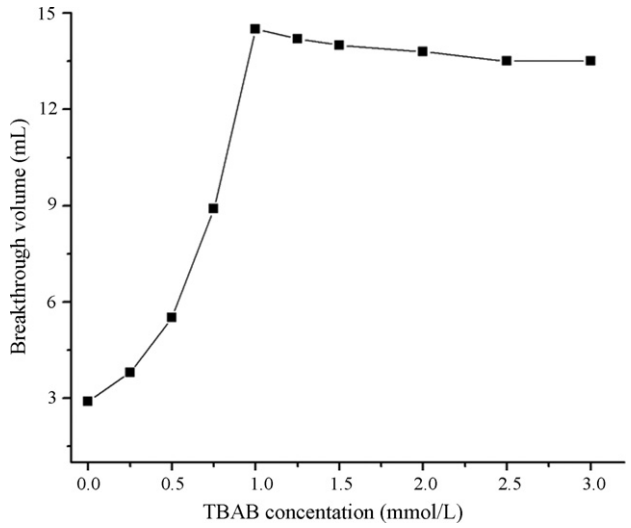

Fig. 2 – Effect of TBAB concentration on breakthrough volume of C_8-bonded silica column. Analyte concentrations: 20 μg mL^{-1} benzoic acid, 20 μg mL^{-1} sorbic acid and 2 μg mL^{-1} *p*-hydroxybenzoic acid.

3.2. *Conditions of SPE pretreatment*

C_8- and C_{18}-bonded silica was investigated as the SPE sorbent without the ion-pairing reagent, respectively. For C_{18}-bonded silica could cause a lot of bubbles during eluting process, which affected the CZE sampling and spectrophotometric detection seriously, C_8-bonded silica was employed as the sorbent packed in the SPE microcolumn.

3.2.1. *Breakthrough volume of SPE column*

To improve the retention of the preservatives on C_8-bonded silica column and enhance their enrichment factor, the ion pair reagent of TBAB was added into the sample solutions. The effect of TBAB concentration ranging from 0 to 3.0 mmol L^{-1} on breakthrough volume was examined with the concentration of 20 μg mL^{-1} of each analyte and 2.0 μg mL^{-1} *p*-hydroxybenzoic acid by the spectrophotometer. As shown in Fig. 2, the breakthrough volume of the SPE column enhanced rapidly from 2.9 to 14.5 mL when the TBAB concentration was increased from 0 to 1.0 mmol L^{-1}. However, a slightly decrease was observed with the TBAB concentration higher than 1.0 mmol L^{-1}. Thus, 1.0 mmol L^{-1} TBAB was adopted in the sample solutions.

3.2.2. *Selection of SPE conditions*

The analytical samples should be pretreated to remove sample matrices and eliminate their interferences before CZE analysis. The analytes were retained on the SPE column, whereas sample matrices could not be done during sample loading.

To obtain an effective elution, three eluents of 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 50% (v/v) methanol, 50% (v/v) acetonitrile or 50% (v/v) ethanol were investigated, respectively. The peak area responses were tested with 20 μg mL^{-1} of each analyte and 2.0 μg mL^{-1} *p*-hydroxybenzoic acid and the eluent volume ranging from 50 to 500 μL at the flow rate of 0.8 mL min^{-1}. It was found that the peak area responses were enhanced in the order of acetonitrile, ethanol and methanol. The eluting volume lower than 180 μL could reduce the peak area due to the incomplete elution, whereas that higher than 180 μL resulted in the analyte dispersion. The influence of methanol concentration on the peak area was also investigated. When the methanol concentration increased from 10% to 70% (v/v), the peak area was enhanced rapidly. However, the peak area kept almost constant with the concentration higher than 70%. As a consequence, 180-μL, 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 70% (v/v) methanol was used as the SPE eluent in this work.

In addition, the effect of the pH value of sample solutions was also studied. The pH value was adjusted to 5, 6, 8, 10 and 12 with 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH. The analyte recoveries were enhanced by increasing the pH value. For example, 92.7%, 97.4% and 98.1% of benzoic acid recovery were obtained with the pH value of 8, 10 and 12, respectively. To avoid the damage of the silica sorbent by high pH value, pH 10 was finally chosen for the sample solution.

The sample loading volume ranging from 5 to 13 mL was investigated with the mixed standard solution of 20 μg mL^{-1} of each analyte and 2 μg mL^{-1} *p*-hydroxybenzoic acid. The peak area of the preservatives was enhanced by increasing the sample volume from 5 to 10.8 mL, whereas the response kept almost constant with the volume larger than 10.8 mL. It implied that the sampling volume of 10.8 mL was close to the breakthrough one. 10.8 mL sample volume was established in this work.

With 180 μL eluent and 10.8 mL sample solution, the influence of sample loading and eluting flow rate on the peak area was also investigated. It was found that the peak area decreased with the loading flow rate higher than 1.8 mL min^{-1} due to the incomplete retention on the SPE column. On the other hand, the loading flow rate lower than 1.8 mL min^{-1} could reduce the sample throughput. In addition, the peak area increased with the eluting flow rate from 0.3 to 0.9 mL min^{-1} and decreased rapidly with the flow rate higher than 0.9 mL min^{-1}. It manifested that the eluting flow rate could affect the intercepted part of the eluted sample zone injected into the CZE system, and the flow rate higher than 0.9 mL min^{-1} might lead to an incomplete elution. In accordance with the results obtained above, 1.8 and 0.9 mL min^{-1} was selected as the sample loading and eluting flow rate in this work, respectively.

3.3. *Performance of EFA–SPE–CZE system*

The corresponding linear regressive equations, limits of detection (LODs) and relative standard deviation (R.S.D.) of the proposed method were listed in Table 2. The analyte peaks were identified by their relative retention time compared with *p*-hydroxybenzoic acid. The linear regressive equations were obtained by injecting the mixed standard solutions of five concentration levels, of which each point corresponded to the mean value for three times of independent measurement. The LODs were calculated from three-times relative standard deviation of baseline noise ($K = 3$, $n = 11$) and the R.S.D. of peak area was less than 3.1% ($n = 5$). The peak area ratio vs. concentration ratio between

Table 2 – Analytical characteristics of EFA–SPE–CZE

Preservative	BA	SA
Regressive equations[a]	$Y = 0.924X + 0.368$	$Y = 2.057X - 0.428$
Correlative coefficient	0.9989	0.9994
Linear range ($\mu g\,mL^{-1}$)	0.06–20	0.03–20
Peak area R.S.D. (%, $n = 5$)	3.1	2.5
LOD ($\mu g\,mL^{-1}$, S/N = 3, $n = 11$)	0.02	0.01

[a] Y, peak area ratio of analyte to IS; X, concentration ratio of analyte to IS.

the analyte and *p*-hydroxybenzoic acid was employed for the quantitative analysis in this work. The electropherogram of a mixed standard solution containing 2.0 $\mu g\,mL^{-1}$ benzoic acid, 2.0 $\mu g\,mL^{-1}$ sorbic acid and 2.0 $\mu g\,mL^{-1}$ *p*-hydroxybenzoic acid with three consecutive injections is presented in Fig. 3.

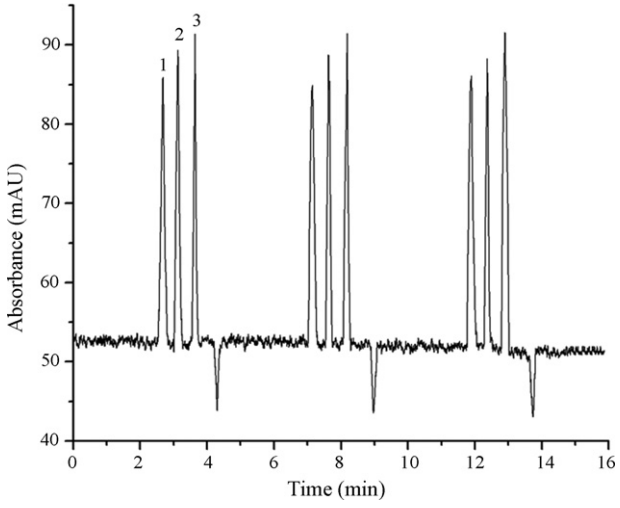

Fig. 3 – Electropherogram of three consecutive injections with a mixed standard solution by EFA–SPE–CZE system. Peak identification: IS, 2 $\mu g\,mL^{-1}$ *p*-hydroxybenzoic acid; 1, 2.0 $\mu g\,mL^{-1}$ benzoic acid; 2, 2.0 $\mu g\,mL^{-1}$ sorbic acid in 10 mmol L^{-1} sodium tetraborate buffer (pH 10.0) containing 10% ethanol (v/v); separation buffer, 20 mmol L^{-1} sodium tetraborate buffer (pH 11.5) containing 0.04 mmol L^{-1} CTAB; sample loading, 10.8 mL sample solution at 1.8 mL min^{-1}; sample eluting, 0.9 mL min^{-1} for 20 s; separation voltage, 15 kV; hydrodynamic injection, 8 cm height difference for 20 s; detection wavelength, 214 nm.

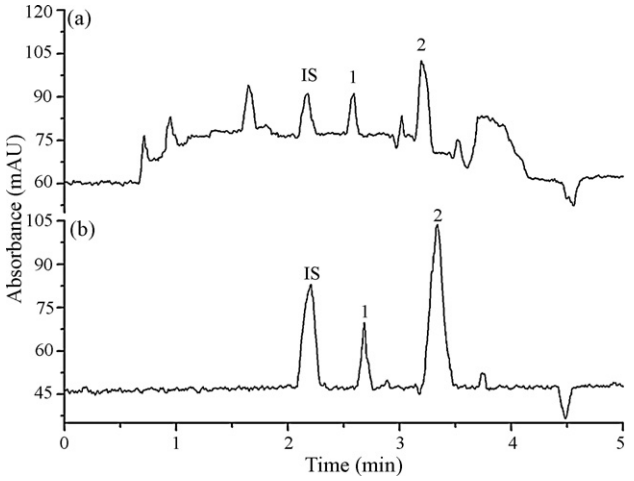

Fig. 4 – Electropherograms of soy sauce by CZE (a) and EFA–SPE–CZE (b). Peak identification: IS, *p*-hydroxybenzoic acid; 1, benzoic acid; 2, sorbic acid. (a) 20 $\mu g\,mL^{-1}$ *p*-hydroxybenzoic acid and (b) other conditions are the same as Fig. 3.

3.4. *Determination of benzoic acid and sorbic acid in real food samples*

Benzoic acid and sorbic acid in milk beverages, soy sauces and fruit jams were analyzed to evaluate the proposed EFA–SPE–CZE method. After the pretreatment described in Section 2.3, the sample solution was introduced into the EFA–SPE–CZE system. The analyte recoveries were obtained from 92.5% to 102% by spiking the standard solutions into the real samples. The amounts of the preservatives found in nine food products are listed in Table 3. Without the SPE pretreatment, the samples of soy sauce and fruit jam containing 20 $\mu g\,mL^{-1}$ *p*-hydroxybenzoic acid were analyzed by CZE directly. The electropherograms of the samples without (Figs. 4a and 5a) and with (Figs. 4b and 5b) the SPE pretreatment are compared. It was found that the matrix interference was eliminated and the sensitivity was improved by the ion pair SPE pretreatment. The determination results of benzoic

Table 3 – Contents of BA and SA determined in commercial food samples

Sample	Preservative	Detected concentration ($\mu g\,mL^{-1}$)	Sample concentration ($mg\,kg^{-1}$)	Recovery (%)
Milk beverage a	BA	1.0	250	93.4
Milk beverage b	SA	1.6	400	96.8
Mile beverage c	BA	2.8	700	97.9
Fruit jam a	BA	1.8	450	95.1
Fruit jam b	BA	0.8	200	102
	SA	1.1	275	99.4
Fruit jam c	SA	2.3	575	92.5
Soy sauce a	BA	1.1	300	97.6
	SA	1.7	175	92.8
Soy sauce b	BA	3.3	825	93.7
Soy sauce c	BA	3.2	800	94.0

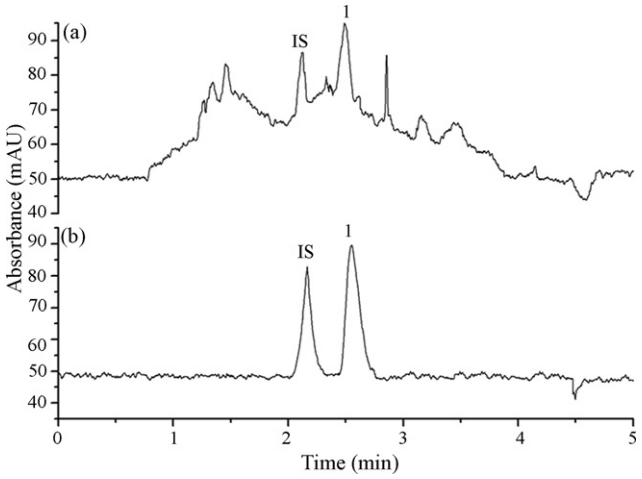

Fig. 5 – Electropherograms of fruit jam by CZE (a) and EFA–SPE–CZE (b). Peak identification: IS, *p*-hydroxybenzoic acid; 1, benzoic acid; 2, sorbic acid. (a) 20 μg mL^{-1} *p*-hydroxybenzoic acid and (b) other conditions are the same as in Fig. 3.

acid and sorbic acid in real food products were lower than the maximum addition levels established by China [39].

4. Conclusions

The on-line pretreatment and determination method was proposed to analyze benzoic acid and sorbic acid in food products by using the EFA–SPE–CZE system. With the home-made SPE unit, the SPE operation and CZE separation could be performed simultaneously. The modified split–flow interface could reduce the consumption of separation buffer, especially, for that containing expensive reagent. To improve the retention of the preservatives on C_8-bonded silica column, the ion pair reagent of TBAB was adopted in this work. From the analysis of benzoic acid and sorbic acid in three kinds of food products, it verified that the EFA–SPE–CZE system could improve sample throughput, analytical sensitivity and selectivity. The EFA system is developing to be a convenient and inexpensive total analysis system including high-performance separation [19,40]. More automatic operations of the method and more applications of the EFA system will be our due course.

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