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Determination of preservatives by integrative coupling method of headspace liquid-phase microextraction and capillary zone electrophoresis

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ABSTRACT

An integrative coupling method of headspace liquid-phase microextraction (HS-LPME) and capillary zone electrophoresis (CZE) was proposed in this paper. In the method, a separation capillary was used to create a microextraction droplet of the running buffer solution of CZE, hold the droplet at the capillary inlet, extract analytes of sample solutions in the headspace of a sample vial, inject concentrated analytes into the capillary and separate the analytes by CZE. The proposed method was applied to determine the preservatives of benzoic acid and sorbic acid in soy sauce and soft drink samples, in which the running buffer solution of 50 mmol/L tetraborate (pH 9.2) was directly used to form the acceptor droplet at the capillary inlet by pressure, and the preservatives in a 6-mL sample solution containing 0.25 g/mL NaCl were extracted at 90 °C for 30 min in the headspace of a 14-mL sample vial. Then the concentrated preservatives were injected into the capillary at 10 cm height difference for 20 s and separated by CZE. The enrichment factors of benzoic acid and sorbic acid achieved 266 and 404, and the limits of detection (LODs) were 0.03 and 0.01 μg/mL (S/N = 3), respectively. The recoveries were in the range of 88.7–105%. The integrative coupling method of HS-LPME and CZE was simple, convenient, reliable and suitable for concentrating volatile and semi-volatile organic acids and eliminating matrix interferences of real samples.

1. Introduction

Preservatives are often used as food additives to prevent food products from alteration and degradation by microorganisms. Benzoic acid, sorbic acid and their salts are most commonly used to inhibit the activity of a wide range of fungi, yeasts, molds and bacteria [1,2]. However, excessive addition of the preservatives may be harmful to consumers and cause some symptoms of urticaria, asthma, intolerance [3], etc. So the maximum permitted concentrations of preservatives in food products have been regulated in many countries [4,5].

The analytical methods of benzoic acid, sorbic acid and their salts in food products have been reported, including spectrophotometry [6], high performance liquid chromatography (HPLC) [3,7–12], thin layer chromatography (TLC) [13], gas chromatography (GC) with methylation [14]. Nowadays, capillary electrophoresis (CE) has become an attractive analysis method for the preservatives [15–18] because of its high separation efficiency, low sample consumption and fast analysis velocity.

However, laborious sample preparation should be carried out prior to the determination of preservatives in food products with complex matrices, including filtration, extraction and evaporation. Development of effective, convenient, miniaturized and environmentally friendly sample preparation methods is the trend in analytical chemistry. Liquid-phase microextraction (LPME) has been developed quickly since 1996 [19,20] and becomes an effective sample pretreatment technique in analytical chemistry. LPME has been applied to chromatography and CE, including single drop microextraction (SDME) [21,22], hollow fiber protected liquid-phase microextraction (HF-LPME) [23–25] and headspace liquid-phase microextraction (HS-LPME) [26,27]. As acceptor phases do not contact with samples, HS-LPME can concentrate volatile and semi-volatile analytes, and clean up sample matrices effectively. In addition, solvent bar microextraction (SBME) [28,29], dispersive liquid-liquid microextraction (DLLME) [30] and directly suspended droplet microextraction (DSDME) [31] have been proposed recently. Moreover, ionic liquids (ILs) are also adopted as acceptor phases of LPME [32–34].

In LPME, organic solvents are frequently used as acceptor phases. However, hydrophobic solvents are not compatible with aqueous buffer solutions of CE. To improve the compatibility, liquid-liquid-liquid microextraction (LLLME) and HF-LPME were adopted in the combination [23–25]. In addition, water-based acceptor phases, such as acidic and alkaline solutions, were also used as acceptor phases in the extraction [35]. Especially, the analyte solubility and the electric conductivity of acceptor phases were

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extremely important for in-line coupling methods of LPME and CE. Several in-line coupling methods were reported recently, such as in-line LLLME-CE [36] and SDME-CE [37,38]. To eliminate the peak broadening by injecting large acceptor volume during in-line LLLME-CE, a dual preconcentration method of on column LLLME-base stacking-CE was proposed [39]. To improve the reliability for in-line SDME-CE with a nanilitre solvent layer, an in-line HS-LPME-CE means was reported [40].

The aim of this paper is to develop a simple, convenient and reliable integrative coupling method of HS-LPME and capillary zone electrophoresis (CZE). In the method, a separation capillary was used to create a microextraction droplet of the running buffer solution of CZE, hold the droplet at the capillary inlet, extract analytes of sample solutions in the headspace of a sample vial, inject the concentrated analytes into the capillary and separate the analytes by CZE, in which the running buffer solution was directly used as the acceptor phase. The microextraction conditions were investigated in detail. The proposed method has been applied to determine benzoic and sorbic acids in soy sauce and soft drink samples.

2. Experimental

2.1. Reagents and solutions

Benzoic acid, sorbic acid, sodium tetraborate, sodium hydroxide, sodium chloride and ethanol were of analytical grade and purchased from Chemical Reagent Ltd. (Shanghai, China). All aqueous solutions were prepared with deionized water obtained from Lanlan Water Ltd. (Hefei, China).

The stock solutions of benzoic acid and sorbic acid, with the concentration of 2.0 mg/mL, were prepared by dissolving each acid in 5 mL ethanol and diluting to 25 mL with deionized water. The calibration solutions of five concentration levels were prepared by diluting the stock solutions with deionized water. The buffer solution, viz. acceptor phase, was 50 mmol/L $Na_2B_4O_7$, being adjusted to pH 9.2 with 1.0 mol/L NaOH.

2.2. Instrumentation

A 1229-HPCE Analyzer (Institute of New Technology Application, Beijing, China) detecting at 214 nm was employed throughout the experiment. An N-2000 double-channel chromatography processor (Institute of Information Engineering of Zhejiang University, Zhejiang, China) was used for data acquisition and processing. A 78-1 magnetic stirrer with hotplate (Jintan Experimental Instrument, Jiangsu, China) and a temperature controller (Shanghai Medical Instrument, Shanghai, China) were used to regulate the extraction temperature of sample solutions by a water bath. A 50-μm I.D. fused-silica capillary (Handan Xinnuo Fiber Chromatography, Hebei, China) with its total length of 65 cm, effective length of 50 cm and inner volume about 1 μL was adopted in the method. The capillary was flushed daily from its outlet by pressure, in the order of 1 mol/L HCl for 3 min, 1 mol/L NaOH for 5 min, and the running buffer for 5 min. The capillary was washed with the running buffer solution for 2 min between the electrophoretic runs. The separation voltage was 22 kV.

2.3. Headspace microextraction procedure

A laboratory-made cover unit of a 14-mL sample vial consisted of a vial cover and a cover plug, made of polytetrafluoroethylene (PTFE) and silicone rubber, respectively [40]. Each of them had a slot from its edge to centre. The inlet part of the separation capillary was placed in the centre of the cover unit with the two slots at the same direction, and then the silicone plug was turned 180° to seal the capillary in the cover unit. The schematic diagram of the coupling setup is presented in Fig. 1.

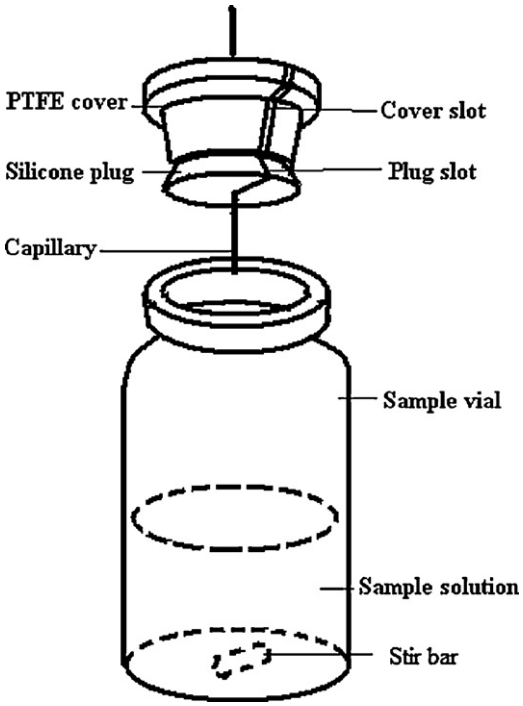

Fig. 1. Schematic of integrative coupling setup for HS-LPME and CZE.

After washing steps, the separation capillary was filled with the running buffer solution. A 6.0-mL sample solution was transferred into the sample vial and 1.5 g sodium chloride was added into the sample solution. The sample vial was closed with the cover unit and the capillary tip was fixed at 1 cm height above the surface of the sample solution. The buffer solution from the capillary outlet was propelled to create a droplet hanging at the capillary tip at a pressure of 55 kPa for 30 s. Then the extraction was carried out at 90 °C for 30 min in the water bath on the magnetic stirrer. After the extraction, the acceptor solution was hydrodynamically injected into the capillary at 10 cm height difference for 20 s and the excessive acceptor phase was removed by touching the capillary tip on the vial wall. At last, the capillary inlet was placed in a high potential buffer solution to carry out CZE.

3. Results and discussion

3.1. Selection of acceptor phase

Benzoic and sorbic acids are weak acids with the ionization constant (pK_a) of 4.20 and 4.77, respectively. Hydroxide solutions or basic buffer solutions can be adopted as the acceptor phase of the organic acids in HS-LPME. In this experiment, 0.1 mol/L NaOH solution and 50 mmol/L $Na_2B_4O_7$ buffer solution (pH 9.2) were examined in the microextraction. For the extraction efficiency, the former was better than the latter. Since the latter was the running buffer solution of CZE in the preservative analysis, it was more convenient to be used as the acceptor phase.

The acceptor droplet was created by propelling the buffer solution from the capillary outlet at a pressure of 55 kPa. In the HS-LPME, the final volume of the aqueous acceptor droplet was decided by both the propelling time and the evaporation in the sample vial. In the experiment, the propelling time was tested from 20 s to 40 s, and the optimal time was 30 s, being close to 1 μL. With the time shorter than 30 s, the repeatability could become unsatisfac-

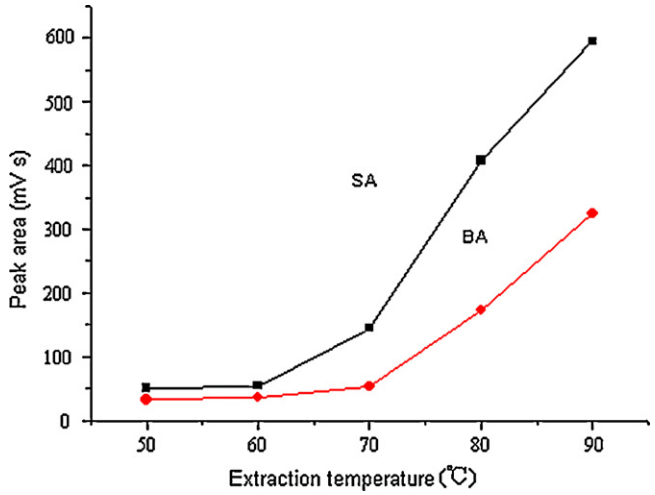

Fig. 2. Effect of extraction temperature on peak area of benzoic acid (BA) and sorbic acid (SA). Extraction time, 20 min; stirring rate, 800 rpm; sample concentration, 0.67 μg/mL each preservative; sample volume, 8.0 mL; acidity of sample solution, pH 4.

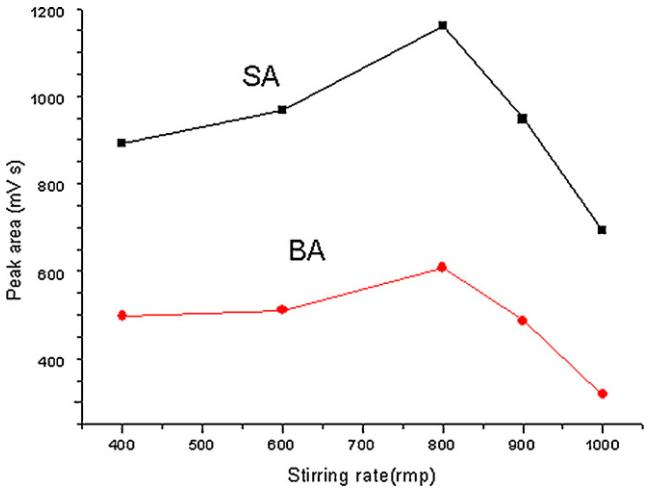

Fig. 3. Effect of stirring rate on peak area of BA and SA. Extraction time, 30 min. Other conditions are the same as in Fig. 2.

tory due to the evaporation of the aqueous droplet, but a longer time could reduce the enrichment factor and make the droplet unstable in the extraction.

3.2. Effect of extraction temperature and time

Benzoic acid and sorbic acid are semi-volatile compounds and have relative high melting and boiling points in the range of 122–134 °C and 228–249 °C, respectively. Extraction temperature is an important parameter in HS-LPME. The effect of extraction temperature was examined from 50 °C to 90 °C in the water bath. It was found that the peak area of the preservatives was evidently enhanced with the extraction temperature higher than 70 °C, as shown in Fig. 2. With the temperature higher than 90 °C, the acceptor phase could be lost more than 30%. The evaporated volume of the acceptor droplet could be measured by propelling a 1-μL buffer droplet at the capillary tip by a 1-μL microsyringe connected with the capillary, examining at different temperatures for 30 min under the extraction conditions, and then withdrawing the residual droplet into the microsyringe. To reduce the evaporation of the aqueous acceptor drop, the extraction temperature was selected at 90 °C.

HS-LPME is dependent on the equilibrium processes of the preservatives transferring from a donor solution to headspace and acceptor phase. The evaporated analytes should take a period of time to achieve the equilibrium concentration in the acceptor phase. The influence of extraction time on peak area of the preservatives was investigated from 10 min to 40 min. The peak area was increased by increasing the extraction time from 10 min to 30 min, but kept nearly constant with the time longer than 30 min. After the extraction equilibrium was obtained in the acceptor phase, the increase of extraction time was not able to improve the extraction efficiency further and the aqueous acceptor phase could be lost more.

3.3. Effect of stirring rate and sample volume

To speed up the mass transfer and keep a uniform temperature in sample solutions, an agitating step should be adopted in the extraction. The stirring rate was investigated from 400 rpm to 1000 rpm, as shown in Fig. 3. The analytical responses were enhanced by increasing the stirring rate from 400 rpm to 800 rpm, but decreased by raising the stirring rate further. It meant that excessive stirring rate might result in the uniformity of sample solutions and temperature.

Based on the theoretical prediction [40], the optimal analysis responses of HS-LPME were obtained when the volume of sample solution was half of the sample vial. The volume of sample solutions was examined from 4 mL to 12 mL, as shown in Fig. 4, the optimal responses were observed with the sample solution of 6 mL, which was close to the prediction.

3.4. Effect of pH value and salt concentration in sample solution

To investigate the effect of sample acidity on peak area, the pH value of sample solutions was tested from 1 to 5. It was found that the highest responses were observed at pH 2, because both benzoic and sorbic acids were easily evaporated in their neutral forms.

Salt-out effect is widely used in LPME to increase the extraction efficiency. The concentration was varied from 0.05 g/mL to 0.30 g/mL NaCl. It was found that the maximal peak area of the preservatives was observed with the concentration of 0.25 g/mL NaCl.

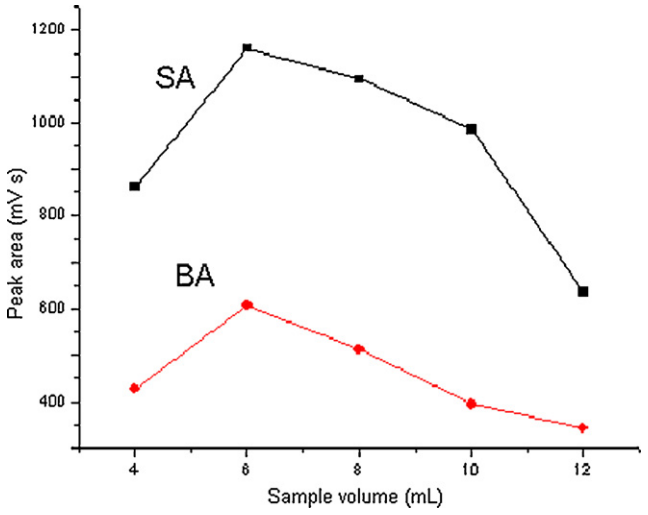

Fig. 4. Effect of sample volume on peak area of BA and SA. Extraction time, 30 min. Other conditions are the same as in Fig. 2.

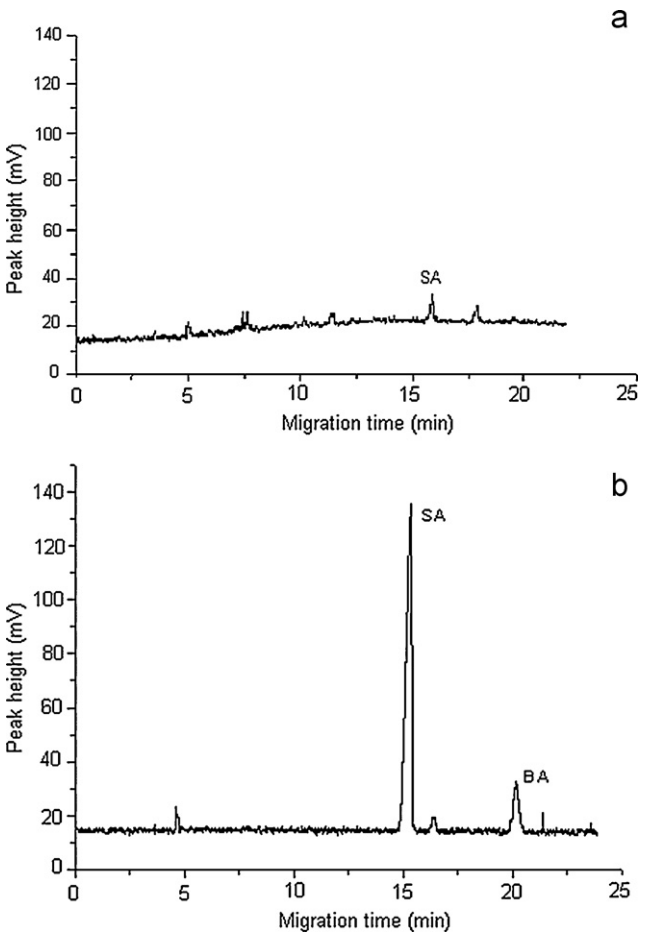

Fig. 5. Electropherograms of BA and SA in a real sample. The sample solution is diluted to 1/5 and analyzed by CZE (a), and diluted to 1/100 and analyzed by HS-LPME–CZE (b). Extraction time, 30 min; sample volume, 6.0 mL and acidity of sample solution, pH 2. Other conditions are the same as in Fig. 2.

3.5. Determination of benzoic and sorbic acids in real samples

Benzoic acid and sorbic acid in soy sauce and soft drink samples were analyzed to evaluate the proposed method. These food products were obtained from local supermarkets. After being filtered through 0.45 μm nylon membrane, a 10-mL sample solution was mixed with 30 mL deionized water, adjusted to pH 2 with 0.1 mol/L HCl and diluted to 50 mL with deionized water. The analytical sample was prepared by diluting the above sample solution to twentieth with 0.01 mol/L HCl. The preservative concentrations of the samples were lower than the maximum permitted concentrations [4]. The relative standard deviations (RSDs) of peak area were less than 4.4% ($n = 3$). The recoveries were obtained in the range from 88.7 to 105%, examined by spiking the standard solutions of 0.7 and 1.4 μg/mL each preservative into the diluted sample solutions and calculated by the ratio of the found spiked concentration divided by enrichment factor to the actual spiked one. The electropherograms of the samples without and with the HS-LPME pretreatment are presented in Fig. 5(a) and (b), respectively. The peak of benzoic acid could not be identified even though the sample concentration in Fig. 5(a) was 20 times as that in Fig. 5(b), and the matrix interference of the baseline could be observed in Fig. 5(a).

The enrichment factors of benzoic acid and sorbic acid were 135 and 280 for the analytical samples of diluted soy sauce, and 266 and 404 for those of diluted soft drink, respectively, which were defined as the ratio of the preservative concentrations (spiked in standard addition method) after the extraction to before one. The limits of detection (LODs) of benzoic acid and sorbic acid were 0.03 and 0.01 μg/mL (S/N = 3), respectively. The quantitative concentration ranges were 0.10–10 μg/mL for benzoic acid and 0.03–3.0 μg/mL for sorbic acid, respectively. The concentration range of calibration solutions was 3.0–1000 μg/mL each preservative, which were determined by CZE directly. The linear regressive equations were Y (mV min) = 4.49 + 10.1X (μg/mL SA, $r = 0.9973$) and $Y = 2.96 + 4.11X$ (μg/mL BA, $r = 0.9994$), in which the analyte concentration was the calibration concentration divided by the enrichment factor. The analytical results of soy sauce and soft drink samples are listed in Table 1.

Table 1
Analytical results with HS-LPME–CZE.

Sample	Paraben	Found (mg/mL)	RSD (%, $n = 3$)	Recovery (%)
Soy sauce 1	SA	0.22	3.2	94.7
	BA	0.077	2.5	105
Soy sauce 2	SA	0.033	3.8	88.7
	BA	0.26	4.4	95.8
Soft drink 1	BA	0.10	3.0	99.6
Soft drink 2	BA	0.19	1.9	93.4

4. Conclusion

The integrative coupling method of HS-LPME and CE was proposed using a capillary in both the extraction and separation, and the buffer solution as both the acceptor phase and running buffer. The proposed method was successfully applied to analyze the preservatives of benzoic and sorbic acids in soy sauce and soft drink samples, and proved to be a simple, convenient and reliable method, being suitable for the analysis of volatile and semi-volatile organic acids in real samples with complex matrices.

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