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Study on the Performance of the Headspace Liquid-Phase Microextraction, Gas Chromatography−Mass Spectrometry in the Determination of Sorbic and Benzoic Acids in Soft Drinks and Environmental Water Samples

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A simple, efficient and virtually solventless headspace liquid-phase microextraction (HS-LPME) technique, combined with gas chromatography−mass spectrometry (GC−MS), was developed for the analysis of sorbic acid (SA) and benzoic acid (BA) in soft drinks and environmental water samples. A microdrop of organic solvent was suspended from the tip of a microsyringe needle over the headspace of the stirred sample solution, containing the analytes for a desired time. The microdrop was then retracted into the microsyringe and directly injected into the GC−MS, without any further pretreatment. Initially, microextraction efficiency factors were optimized, and the optimum experimental conditions found were as follows: 2.5 μL toluene microdrop exposed for 20 min over the headspace of a 6.5 mL aqueous sample (45 °C), containing 3 M of NaCl with pH of 1.5 and stirred at 1000 rpm. Under the optimized extraction conditions, preconcentration factors of 154 and 198, limits of detection of 0.3 and 0.1 μg L^{-1} (S/N = 3) with dynamic linear ranges of 1−500 and 0.5−500 μg L^{-1}, were obtained for SA and BA respectively. A good repeatability (RSD < 10.3%, $n = 8$) and satisfactory linearity ($r^2 \geq 0.99$) of results were achieved. The accuracy of the method was tested by the relative recovery experiments on spiked samples, with results ranging from 90 to 113%. The method proved to be rapid and cost-effective and is a green procedure for screening purposes.

KEYWORDS: Headspace liquid-phase microextraction; gas chromatography−mass spectrometry; sorbic acid; benzoic acid; soft drink; environmental water sample

INTRODUCTION

Sorbic acid (SA) and benzoic acid (BA) are generally used as preservatives in a great variety of foods and beverages. These compounds exhibit inhibitory activity against a wide range of fungi, yeasts, molds and bacteria (*1*−*4*). Since the maximum allowed concentrations of preservatives are controlled by legislation, their determination is a mandatory step in routine food analysis (*5*). Moreover, with the growing use of the additives, the preservative residues can be considered as environmental contaminants (*6*−*9*).

Analytical techniques used in the determination of SA and BA are mainly thin layer chromatography (TLC) (*10, 11*), high-performance liquid chromatography (HPLC) (*12, 13*), capillary electrophoresis (CE) (*14, 15*), and also micellar electrokinetic chromatography (MEKC) (*16, 17*). Additionally, gas chromatography (GC) is a common tool for the analysis of the analytes, usually after derivatization (*18, 19*). Other methods have been reported including second-order derivative spectrophotometry (*20, 21*), chemometrics enhanced spectrophotometry (*22, 23*), polarography (*24*) and enzymatic determination (*25*). The preservatives in complex matrixes were determined by these techniques after laborious manipulation of the sample, including filtration, extraction and evaporation before the instrumental analysis.

Recent research activities are oriented toward the development of efficient, economical and miniaturized sample preparation methods. The invention of solid-phase microextraction (SPME) by Pawliszyn and co-workers (*26*) basically initiated the interest

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for microextraction techniques in analytical chemistry. SPME satisfies most of the requirements of a good sample preparation technique, including simplicity of use, automation, and low consumption of materials (*27*). Therefore, it has been used for many applications consisting of environmental, biological, and pharmaceutical monitoring (*28*), as well as preservatives analyses (*29, 30*).

An alternative solvent-minimized sample preparation approach to complement SPME appeared in the middle-to-late 1990s (*31*−*33*); liquid-phase microextraction (LPME) utilizes only a small amount of solvent (low microliter range) for concentrating analytes from aqueous samples. It is simply a miniaturized format of liquid−liquid extraction (LLE). It overcomes many of the disadvantages of LLE as well as some of those of SPME (e.g., nondependence on a commercial supplier and sample carryover). LPME is simple to implement and use, is generally fast, and is characterized by its affordability and reliance on widely available apparatus or materials (*34*).

Recently, some reports on headspace (HS) LPME (or headspace solvent microextraction) have been reported (*35, 36*). A microdrop of organic solvent suspended at the tip of a microsyringe is placed in the headspace of the sample solution to extract volatile analytes. HS-LPME has similar capabilities in terms of precision and speed of analysis compared to HS-SPME (*37*). Furthermore, as in HS-SPME, nonvolatile matrix interferences are reduced, if not eliminated (*34*). Today, HS-LPME has been successfully applied for the extraction and preconcentration of organic compounds from a variety of matrixes (*38*−*40*), owing to its advantages.

The aim of the present study is to investigate the applicability of the HS-LPME method for environmental water monitoring of the preservative residues and also determination of them in soft drinks. The factors affecting microextraction efficiency were studied in detail, and the optimal conditions were established. The resulting method was validated for quantitative purposes, and applied to real sample analysis in combination with gas chromatography−mass spectrometry (GC−MS).

EXPERIMENTAL PROCEDURES

Reagents. SA and BA were purchased from Merck (Darmstadt, Germany). Proper amounts of SA and BA were dissolved in methanol to obtain stock solutions of each analyte with a concentration of 2000.0 mg L^{-1}. Working standard solutions (in the range of 0.1−1000 μg L^{-1}) were freshly prepared by diluting the mixed standard solution of the analytes with water to the required concentration. The stock and working standard solutions were stored at 4 °C in a refrigerator. The used water was purified with a Milli-Q water purification system (Millipore, Bedford, MA). *p*-Methylbenzoic acid was supplied by Fluka (Busch, Switzerland). All of organic solvents, sodium chloride and hydrochloride acid were of analytical grade and obtained from Merck (Darmstadt, Germany).

GC−MS Analysis. The analysis was performed on a Hewlett-Packard (Agilent Technologies, Palo Alto, CA) HP 6890 series GC, equipped with a split/splitless injector and an HP 5973 mass selective detector system. Helium (99.999%) was employed as carrier gas at the flow rate of 0.8 mL min^{-1}. The analytes were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-1MS gas chromatographic column (J&W Scientific, Folsom, CA) with the following oven temperature program: initial temperature 75 °C (held for 2 min), ramped to 285 at 15 °C min^{-1} and held for 4 min. The injection port was operated at 280 °C and was used at the split mode with a split ratio of 1:10. The MS was operated at the electron impact (EI) mode (70 eV). The EI ion source, quadrupole mass analyzer and interface temperature were maintained at 230 °C, 150 °C and 280 °C, respectively. Acquisition was performed on the full scan mode (*m*/*z* 40−300) for identification purposes, and at the selected ion monitoring (SIM) mode for quantification. For the SIM mode, six ions were monitored, based on the selection of some mass peaks of the highest intensity for each compound: *m*/*z* 97, 112 for SA, *m*/*z* 105, 122 for BA and *m*/*z* 91, 136 for *p*-methylbenzoic acid internal standard.

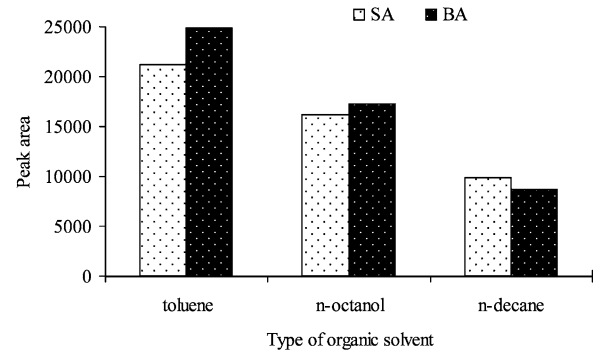

Figure 1. Extraction efficiencies obtained for different organic solvents. Conditions: sample volume, 7.0 mL; extraction solvent volume, 2.5 μL; stirring rate, 700 rpm; extraction time, 25 min, without salt addition and adjust the pH.

Analytical Procedure. A 6.5 mL aliquot of the sample solution (containing 250.0 μg L^{-1} of each analyte) was placed in the 10.0 mL vial with a PTFE-silicon septum and a 7 mm × 3 mm magnetic stir bar. A fixed concentration (5.0 mg L^{-1}) of *p*-methylbenzoic acid as an internal standard was prepared in organic solvent as the extracting solvent. A 5.0 μL microsyringe (bevel tip needle, SGE, Australia) was thoroughly washed with methanol, and then with acetone. It was rinsed and primed at least six times with the solvent/internal standard solution. A specified volume of organic solvent is drawn into the microsyringe. The needle of the syringe pierced the vial septum, and it was fixed so that the tip of the needle was located in a constant position in the headspace. After extraction for a prescribed time, the syringe plunger was withdrawn and the microdrop was retracted into the microsyringe. It was then injected into the GC inlet without any further pretreatment for analysis.

Sample Pretreatment. Soft drinks (cola, malt beverage and orange juice) were bought at a local market and diluted 250 times with reagent water. The carbonated drinks, including cola and a malt beverage, were degassed by ultrasonication for 5 min to decrease CO_2 interference in the matrix. Furthermore, to avoid the losses of the analytes, degasification was performed after the addition of the proper amount of internal standard (IS) in ice bath. Caspian seawater (Anzali, Gilan Province, Iran), well water (University of Tehran) and surface water (Tehran Water and Sewerage Company) were selected as environmental water samples. All of the samples were stored at 4 °C until their analysis time.

RESULTS AND DISCUSSION

A univariate approach was employed to optimize the influential factors in this study. Quantifications were based on the relative peak area of the analyte to the internal standard from the average of 3 replicate measurements. A fixed concentration, 250.0 μg L^{-1} of each analyte, was used in optimization. Also, the concentration of the internal standard was 5.0 mg L^{-1} in all of experiments.

Effect of the Extracting Solvent and Drop Volume. The selection of extraction solvent is of major importance in HS-LPME, in order to obtain efficient extraction. The extraction solvent has to meet the following requirements: (a) relatively high boiling point and low vapor pressure so that it can stand under higher extraction temperature without apparent loss, (b) high affinity for the interested analytes and (c) good chromatographic behavior (*37, 39, 41*). According to these considerations, three extracting solvents, including *n*-decane, *n*-octanol and toluene, were examined. Among the tested extracting solvents, toluene presented the highest extraction efficiency (**Figure 1**) and was chosen as the extracting solvent. In order to improve

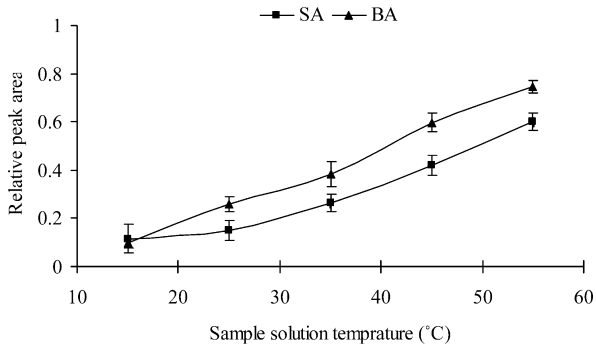

Figure 2. Effect of the sample solution temperature on the extraction efficiency. Conditions: as with **Figure 1**; extraction solvent, toluene.

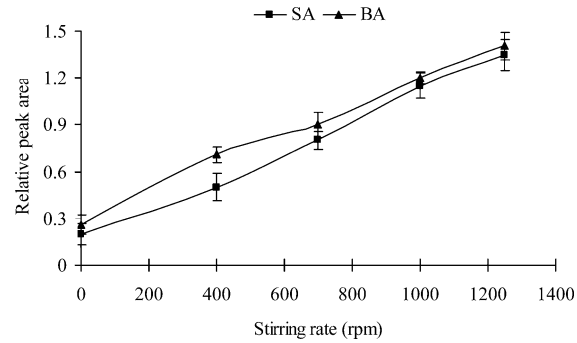

Figure 3. Influence of the stirring rate on the relative peak area. Conditions: as with **Figure 2**; extraction temperature, 45 °C.

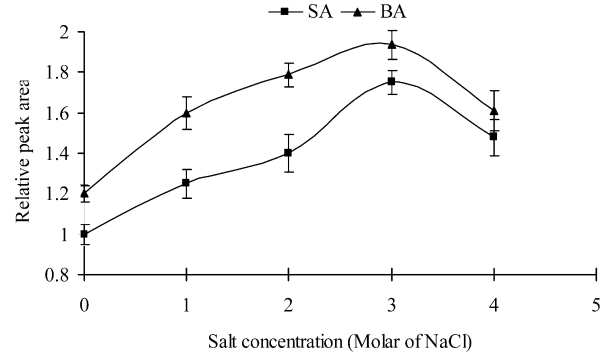

Figure 4. Effect of the salt addition on the extraction efficiency. Extraction conditions as with **Figure 3**; stirring rate, 1000 rpm.

the precision and accuracy of the method in all of experiments, *p*-methylbenzoic acid with the concentration of 5.0 mg L^{-1} was used as the internal standard and was added into the extracting solvent.

The influence of the microdrop volume on the extraction efficiency of the system was studied in the range of 1.5−3.0 μL. As it was expected, an increase in the volume of the microdrop (up to 2.5 μL) resulted in a sharp increase in the extraction efficiency of the analytes. However, a further increase of the microdrop from 2.5 to 3.0 μL decreased extraction, and the resulting analytical signal was approximately the same as for the 2 μL. This observation is in agreement with the previous published reports dealing with the HS-LPME (*42, 43*). Furthermore, when the drop volume was above 2.5 μL, such microdrops were difficult to manipulate and less stable to be kept in the needle tip. Accordingly, the following investigations were carried out with the drop volume of 2.5 μL.

Effect of the Sample Solution Temperature and Stirring Rate. In headspace mode sampling, the analytes need to be transported through the barrier of air before reaching the drop. Temperature affects the kinetics of sorption in the microdrop by changing the vapor pressure of analytes and diffusion coefficient values in all three phases. Thus, the time required to reach equilibrium decreased (*43, 44*). The effect of the sample temperature on the extraction efficiency was studied in the range of 15−55 °C. **Figure 2** shows that the analytical signals improve significantly by increasing the temperature. Nevertheless, high temperatures can alter the microdrop size dramatically and cause overpressurization in the sample vial, making the extraction system unstable. Thus, the sample temperature was held at 45 °C for further analysis.

Agitation of the aqueous donor solution has been used universally to improve microextraction efficiency (*45*). The stirring can regenerate a new sample solution surface, thus accelerating the mass transfer from the donor phase to the headspace to increase the convection and evaporation rate (*46, 47*). For investigating the influence of this parameter on the HS-LPME performance, samples were agitated at different stirring rates (0, 400, 700, 1000 and 1250 rpm). As shown in **Figure 3**, the results confirmed that agitation of the sample greatly enhances extraction reaching a maximum at 1250 rpm, but at this speed splattering of solution occurred and the stability of the drop was affected. Therefore, 1000 rpm was selected as optimum on the basis of these observations.

Effect of the Ionic Strength and pH. Control of salt concentration and sample pH can be used to enhance extraction efficiency. To study the salt effect on the analytical signal, water samples containing different concentrations of sodium chloride (0−4 M) were analyzed. The results (**Figure 4**) revealed that the extraction efficiency gradually increased with the increase of the NaCl concentration. The maximum signal was achieved at the NaCl concentration of 3 M and decreased afterward. This can be explained by the engagement of more water molecules in the hydration spheres around the ionic salt. It reduces the amount of water available to the dissolved analytes. Consequently, solubility of the analytes in the aqueous sample is reduced, and the distribution constant of compounds between aqueous phase and headspace is enhanced, due to the salting-out phenomena (*48, 49*). Moreover, by increasing viscosity of the sample solution at higher concentration of NaCl (>3 M), diffusion of analytes toward the headspace and organic solvent becomes difficult (*50*) and, consequently, a small decrease in extraction efficiency was obtained (**Figure 4**). So, a fixed concentration of 3 M NaCl was used as the optimum quantity.

The pH of the sample solution is known to play a key role in the headspace extraction of ionizable analytes (*51, 52*). Ionizable analytes should be changed to their neutral form in order to reduce their solubility within the donor phase and reach the maximum extraction efficiency. SA and BA with pK_a of 4.8 and 4.2, exists in neutral form (un-ionized) at low pH, while they are completely ionized at a pH of higher than 5. In order to evaluate this parameter, the pH of sample solutions was changed in the range of 0.5−5.0 with the addition of the concentrated HCl. To prevent the concentration changing of the analytes, a micropipette was used for addition of appropriate amount of the HCl. **Figure 5** shows that the best results were obtained in pH 1.5. At pH values lower than 1.5, protonation of the carboxylic group can occur, thus the analytes change to ionic form and decrease the extraction efficiencies. Therefore, the pH was adjusted to 1.5 for further analysis.

Effect of the Ratio Sample/Vial Volume and Extraction Time. During the headspace extraction process, sample volume can influence the magnitude of the headspace, and thus might influence the extraction efficiency. The optimal ratio of the aqueous volume to the headspace volume, for headspace analysis

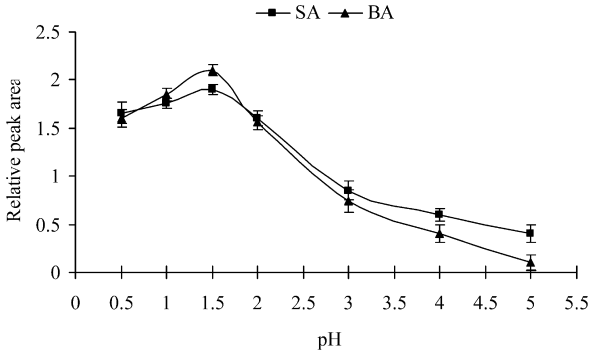

Figure 5. Influence of the sample pH on the relative peak area. Extraction conditions as with **Figure 4** and 3 M NaCl.

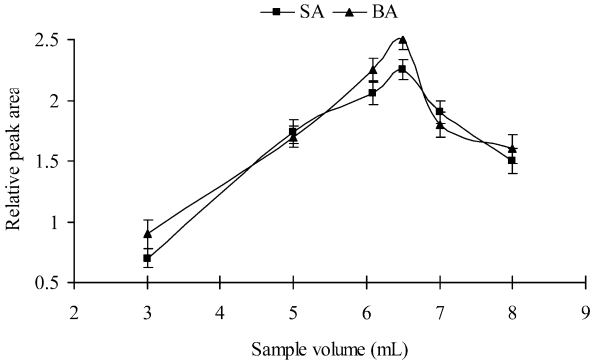

Figure 6. Effect of the sample volume on the extraction efficiency. Extraction conditions as with **Figure 5**; pH = 1.5.

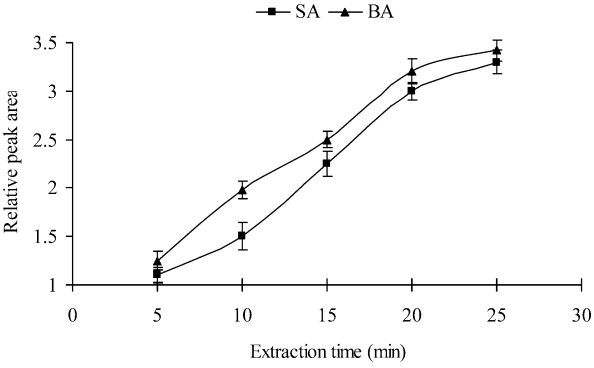

Figure 7. HS-LPME time profiles obtained for the studied preservatives. Extraction conditions as with **Figure 6**; sample volume, 6.5 mL.

in 10 mL vials, was determined by varying the sample volume (3.0, 5.0, 6.0, 6.5, 7.0 and 8.0 mL, containing a fixed amount of the analytes and the IS). The results are illustrated in **Figure 6**. The extracted amounts of SA and BA increase continuously with increasing sample volumes, reaching a maximum at an aqueous volume of 6.5 mL, and decreasing afterward. At the beginning, by increasing the sample volume, the headspace volume decreases, which accelerates the diffusion of the analytes into the drop until saturation. Upon stirring the solution at a fixed rate with a large volume, the convection is not as good in the aqueous phase, resulting in less extraction (*53, 54*). On the basis of the above considerations, 3.5 mL of headspace volume (aqueous volume of 6.5 mL) was selected since this quantity provided best results.

HS-LPME is a process dependent on equilibrium rather than exhaustive extraction. For increasing repeatability of the extraction, it is necessary to choose a suitable extraction time during which the equilibrium between the microdrop, the headspace and sample solution is reached (*39, 55*). The extraction time profiles were examined by monitoring the variation of analytical signal of the analytes as a function of exposure time, in the range of 5−25 min. The results (**Figure 7**) revealed that when the extraction time was set at 20 min, satisfactory extraction efficiency was achieved, while equilibrium in the extraction of the analytes was not reached even at 25 min. However, for the microdrop-based extraction methods, it is not necessary for the analytes to have reached equilibrium, only to allow sufficient mass transfer into the organic drop and the exact reproducible extraction time (*56*). Due to the considerable loss of extraction solvent volume at longer times (>20 min), we have chosen an extraction time of 20 min as the optimum.

Table 1. Quantitative Data Obtained after the HS-LPME and GC−MS Determination of the Preservatives

compound	LOD[a] (μg L^{-1})	r^2	LR[b] (μg L^{-1})	PF[c]	RSD %[d] (n = 8)
SA	0.3	0.994	1−500	154	8.6
BA	0.1	0.992	0.5−500	198	7.2

[a] Limit of detection for S/N = 3. [b] Linear range. [c] Preconcentration factor. [d] Relative standard deviation at the concentration of 50.0 μg L^{-1} of each analyte.

Table 2. The Results Obtained from the Analysis of Real Samples

	SA	BA
Environmental Water Samples		
Surface Water (5.0 μg L^{-1} Added)		
concentration (μg L^{-1})	ND[a]	4.21
found (μg L^{-1})	5.39	9.84
relative recovery (%)	108	113
RSD % (n = 8)	8.5	8.8
Well Water (25.0 μg L^{-1} Added)		
concentration (μg L^{-1})	ND	ND
found (μg L^{-1})	23.48	25.76
relative recovery (%)	94	103
RSD % (n = 8)	9.0	10.3
Seawater (50.0 μg L^{-1} Added)		
concentration (μg L^{-1})	ND	ND
found (μg L^{-1})	44.82	47.42
relative recovery (%)	90	95
RSD % (n = 8)	7.8	8.8
Soft Drink Samples		
Orange Juice (10.0 mg L^{-1} Added)		
concentration (mg L^{-1})	ND	39.42
found (mg L^{-1})	11.13	29.12
relative recovery (%)	110	103
RSD % (n = 8)	8.6	10.1
Malt Beverage (15.0 mg L^{-1} Added)		
concentration (mg L^{-1})	61.08	ND
found (mg L^{-1})	76.41	16.38
relative recovery (%)	102	109
RSD % (n = 8)	8.7	9.5
Cola (20.0 mg L^{-1} Added)		
concentration (mg L^{-1})	ND	87.17
found (mg L^{-1})	18.23	106.34
relative recovery (%)	91	96
RSD % (n = 8)	9.4	9.9

[a] Not detected.

Evaluation of the Method Performance. To evaluate the practical applicability of the proposed method, calibration curves were plotted using 10 spiked levels including 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 μg L^{-1}. Each standard sample was extracted by the proposed method at the optimum conditions. For each level, 3 replicate extractions were conducted. The limits of detection (LODs), based on the signal-to-noise ratio (S/N) of 3 under the SIM mode, the correlation coefficients (r^2), the linear ranges (LRs), the relative standard deviations (RSDs) and

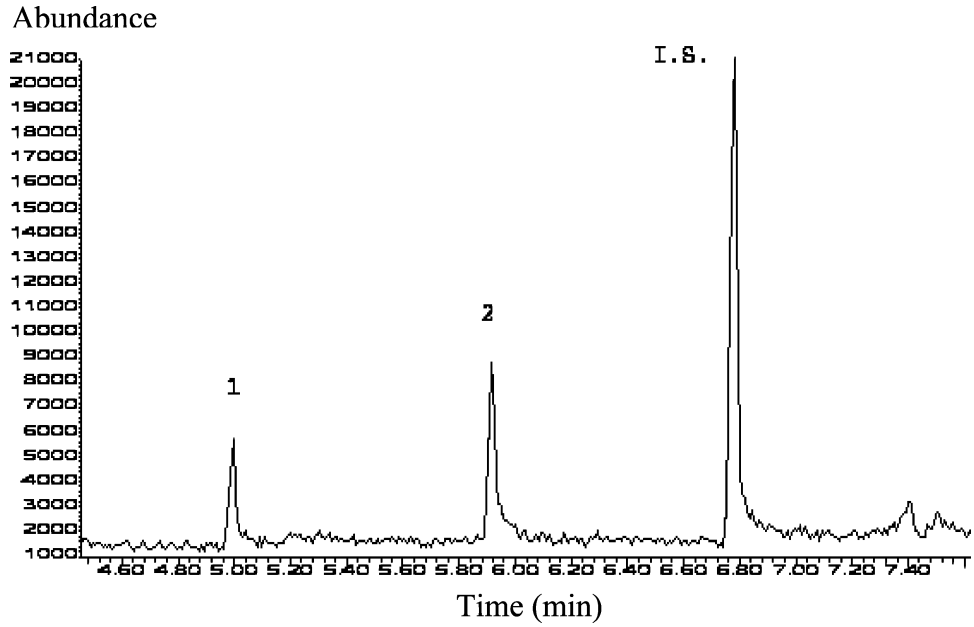

Figure 8. Chromatogram obtained by GC−MS of the seawater after performing HS-LPME, spiked with the preservatives at the concentration level of 50.0 μg L^{-1} of each analyte. Peak numbers correspond to (1) sorbic acid; (2) benzoic acid; (I.S.) internal standard, *p*-methylbenzoic acid.

the preconcentration factors (PFs) were calculated and are summarized in **Table 1**.

In order to examine the PF of each analyte, a series of standard solutions (at concentration of 0.1, 0.5, 1.0, 2.5, 5.0, 10, 25, 50, 75 and 100 mg L^{-1}) in the extracting solvent were prepared and 2 μL samples of them were injected into the GC. Then the plots of relative peak area against the concentration of each analyte were drawn. The PF was calculated as the slope ratio of the LPME calibration curve to that of the nonextraction curve.

Real Sample Analysis. Under the selected optimum experimental conditions, the performance of this method was tested by analyzing the preservatives in six different real samples. Each sample extracted using the HS-LPME after the addition of the proper amount of salt and IS and the pH adjustment.

The results listed in **Table 2** indicate that, in the surface water sample, BA was detected with the concentration level of 4.21 μg L^{-1}, while other environmental samples were free of the contaminants. Thus, the preservative's entrance in the aquatic environment can be considered as a challenging issue. Since the consequences of the presence of these compounds in the aquatic environment are still largely unknown, substantial scientific efforts should be devoted to evaluate their disruptive effects.

All of the real samples were spiked with the analyte standards at different concentration levels to assess the matrix effects. HS-LPME is a nonexhaustive extraction procedure, and the relative recovery (determined as the ratio of the concentrations found in real sample and reagent water sample, spiked with the same amount of analytes), instead of the absolute recovery (used in exhaustive extraction procedures), was employed. The relative recoveries of the analytes are given in **Table 2** and varied between 90 and 113%, which indicated that the real matrixes in our present context had little effect on HS-LPME. In comparison with the AOAC official methods (*10, 18*) and those previously published, the protocol demonstrated a satisfactory reliability, accuracy and repeatability for the determination of BA and SA in soft drinks with complex matrixes. The chromatogram obtained by GC−MS of seawater sample spiked with the target compounds at the concentration level of 50.0 μg L^{-1} of each analyte after the developed method is shown in **Figure 8**.

In conclusion, this paper has demonstrated the successful development and application of the HS-LPME technique, combined with the capillary GC−MS for the qualitative and quantitative analysis of SA and BA in soft drinks and environmental water samples. The simplicity, ease of operation, no possibility carry-over, good precision and high preconcentration factor with sufficient sensitivity, are clear advantages of the proposed analytical procedure. Moreover, there was no need for evaporation of solvent and derivatization of the analytes prior to injection into the GC. Most importantly, sample cleanup that is an essential step to reduce interferences from the complex matrix, was omitted in our recommended technique. Also, it is nearly a solvent-free sample preparation method, therefore being an environmentally friendly approach. The analytical performance of this method, particularly short analysis time and low cost, verifies its potential applicability for routine analysis of the preservatives in quality control of soft drinks and also their monitoring in aquatic environment.

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LITERATURE CITED

(1) Kubo, I.; Lee, S. H. Potentiation of antifungal activity of sorbic acid. *J. Agric. Food. Chem.* **1998**, *46*, 4052–4055.
(2) Brul, S.; Coote, P. Mode of action and microbial resistance mechanisms. *Int. J. Food Microbiol.* **1999**, *50*, 1–17.
(3) Techakriengkrai, I.; Surakarnkul, R. Analysis of benzoic acid and sorbic acid in Thai rice wines and distillates by solid-phase sorbent extraction and high-performance liquid chromatography. *J. Food Compos. Anal.* **2007**, *20*, 220–225.
(4) Marsili, N. R.; Lista, A.; Fernandez Band, B. S.; Goicoechea, H. C.; Olivieri, A. C. New method for the determination of benzoic and sorbic acids in commercial orange juices based on second-order spectrophotometric data generated by a pH gradient flow injection technique. *J. Agric. Food. Chem.* **2004**, *52*, 2479–2484.

(5) European Parliament and Council Directive No. 95/2/EC, February 1995.
(6) Schou, L.; Krane, J. E. Organic micropollutants in a Norwegian water-course. *Sci. Total Environ.* **1981**, *20*, 277–286.
(7) Stuermer, D. H.; Ng, D. J.; Morris, C. J. Organic contaminants in groundwater near underground coal gasification site in northeastern Wyoming. *Environ. Sci. Technol.* **1982**, *16*, 582–587.
(8) Goerlitz, D. F.; Troutman, D. E.; Godsy, E. M.; Franks, B. J. Migration of wood-preserving chemicals in contaminated groundwater in a sand aquifer at Pensacola, Florida. *Environ. Sci. Technol.* **1985**, *19* (10), 955–961.
(9) Barker, J. F.; Barbash, J. E.; Labonte, M. Groundwater contamination at a landfill sited on fractured carbonate and shale. *J. Contam. Hydrol.* **1988**, *3*, 1–25.
(10) Mandrou, B.; Bressolle, F. Thin layer chromatography reflectometric determination of benzoic and sorbic acids in fruit beverages. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 675–678.
(11) Khan, S. H.; Murawski, M. P.; Sheama, J. Quantitative high-performance thin-layer chromatographic determination of organic acid preservatives in beverages. *J. Liq. Chromatogr. Relat Technol.* **1994**, *17*, 855–865.
(12) Tfouni, S. A. V.; Toledo, M. C. F. Determination of benzoic and sorbic acids in Brazilian food. *Food Control* **2002**, *13*, 117–123.
(13) Ferreira, I. M. P.; L, V. O.; Mendes, E.; Brito, P.; Ferreira, M. A. Simoultanouse determination of benzoic and sorbic acids in quince jam by HPLC. *Food Res. Int.* **2000**, *33*, 113–117.
(14) Mato, I.; Huidobro, J. F.; Simal-Lozano, J.; Sancho, T. Simultaneous determination of organic acids in beverages by capillary zone electrophoresis. *Anal. Chim. Acta* **2006**, *565*, 190–197.
(15) Costa, A. C. O.; Perfeito, L.; d, S.; Tavares, M. F. M.; Micke, G. A. Determination of sorbate and benzoate in beverage samples by capillary electrophoresis-Optimization of the method with inspection of ionic mobilities. *J. Chromatogr. A* **2008**, *1204*, 123–127.
(16) Boyce, M. C. Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography. *J. Chromatogr. A* **1999**, *847*, 369–375.
(17) Huang, H.; Lai, Y.; Chiu, C.; Yeh, J. Comparing micellar electrokinetic chromatography and microemulsion electrokinetic chromatography for the analysis of preservatives in pharmaceutical and cosmetic products. *J. Chromatogr. A* **2003**, *993*, 153–164.
(18) Method 983.16. *Benzoic acid and sorbic acid in food. Gas chromatographic method*; Association of Official Analytical Chemists (AOAC): Gaithersburg, MD, 1995.
(19) Pan, Z.; Wang, L.; Mo, W.; Wang, C.; Hu, W.; Zhang, J. Determination of benzoic acid in soft drinks by gas chromatography with on-line pyrolytic methylation technique. *Anal. Chim. Acta* **2005**, *545*, 218–223.
(20) García Castro, J. C.; Rodríguez Delgado, M. A.; Sánchez, M. J.; García Montelongo, F. Simultaneous 2nd order derivative spectrophotometry determination of sorbic and benzoic acids in soft drinks. *Anal. Lett.* **1992**, *25*, 2367–2376.
(21) Lozano, V. A.; Ibañez, G. A.; Olivieri, A. C. Three-way partial least-squares/residual bilinearization study of second-order lanthanide-sensitized luminescence excitation-time decay data analysis of benzoic acid in beverage samples. *Anal. Chim. Acta* **2008**, *610*, 186–195.
(22) Marsili, N. R.; Sobrero, M. S.; Goicoechea, H. C. Spectrophotometric determination of sorbic and benzoic acids in fruit juices by a net analyte signal-based method with selection of the wavelength range to avoid non-modelled interferences. *Anal. Bioanal. Chem.* **2003**, *376*, 126–133.
(23) Lozano, V. A.; Caminã, J. M.; Boeris, M. S.; Marchevsky, E. J. Simultaneous determination of sorbic and benzoic acids in commercial juices using the PLS-2 multivariate calibration method and validation by high performance liquid chromatography. *Talanta* **2007**, *73*, 282–286.
(24) Fung, Y.; Luk, S. Polarographic-determination of sorbic acid in fruit juices and soft drinks. *Analyst* **1990**, *115*, 1219–1221.
(25) Hamano, T.; Mitsuhashi, Y.; Aoki, N.; Semma, M.; Ito, Y. Enzymic method for the spectrophotometric determination of benzoic acid in soy sauce and pickles. *Analyst* **1997**, *122*, 259–262.
(26) Arthur, C. L.; Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145–2148.
(27) Pawliszyn, J. *Solid phase microextraction: theory and practice*; Wiley-VCH Inc.: New York, 1997.
(28) Pawliszyn, J., Ed. *Applications of solid phase microextraction*; Royal Society of Chemistry: Cornwall, U.K., 1999.
(29) Dong, C.; Meib, Y.; Chena, L. Simultaneous determination of sorbic and benzoic acids in food dressing by headspace solid-phase microextraction and gas chromatography. *J. Chromatogr. A* **2006**, *1117*, 109–114.
(30) Dong, C.; Wang, W. Headspace solid-phase microextraction applied to the simultaneous determination of sorbic and benzoic acids in beverages. *Anal. Chim. Acta* **2006**, *562*, 23–29.
(31) Liu, H.; Dasgupta, P. K. Analytical chemistry in a drop. Solvent extraction in a microdrop. *Anal. Chem.* **1996**, *68*, 1817–1821.
(32) Cantwell, F. F.; Jeannot, M. A. Solvent microextraction into a single drop. *Anal. Chem.* **1996**, *68*, 2236–2240.
(33) He, Y.; Lee, H. K. Liquid-phase microextraction in a single drop of organic solvent by using a conventional microsyringe. *Anal. Chem.* **1997**, *69*, 4634–4640.
(34) Xu, L.; Basheer, C.; Lee, H. K. Developments in single-drop microextraction. *J. Chromatogr. A* **2007**, *1152*, 184–192.
(35) Shen, G.; Lee, H. K. Headspace liquid-phase microextraction of chlorobenzenes in soil with gas chromatography-electron capture detection. *Anal. Chem.* **2003**, *75*, 98–103.
(36) Theis, A. L.; Waldack, A. J.; Hansen, S. M.; Jeannot, M. A. Headspace solvent microextraction. *Anal. Chem.* **2001**, *73*, 5651–5654.
(37) Kokosa, J. M.; Przyjazny, A. Headspace microdrop analysis an alternative test method for gasoline diluent and benzene, toluene, ethylbenzene and xylenes in used engine oils. *J. Chromatogr. A* **2003**, *983*, 205–214.
(38) Fragueiro, S.; Lavilla, I.; Bendicho, C. Hydride generation-headspace single-drop microextraction-electrothermal atomic absorption spectrometry method for determination of selenium in waters after photoassisted preproduction. *Talanta* **2006**, *68*, 1096–1101.
(39) Zhao, R. S.; Lao, W. J.; Xu, X. B. Headspace liquid-phase microextraction of trihalomethanes in drinking water and their gas chromatographic determination. *Talanta* **2004**, *62*, 751–756.
(40) Bagheri, H.; Salemi, A. Headspace solvent microextraction as a simple and highly sensitive sample pretreatment technique for ultra trace determination of geosmin in aquatic media. *J. Sep. Sci.* **2006**, *29*, 57–65.
(41) Adam, M.; Dobiáš, P.; Eisner, A.; Ventura, K. Headspace single-drop microextraction of herbal essential oils. *J. Sep. Sci* **2008**, *31*, 356–363.
(42) Przyjazny, A.; Kokosa, J. M. Analytical characteristics of the determination of benzene, toluene, ethylbenzene and xylenes in water by headspace solvent microextraction. *J. Chromatogr. A* **2002**, *977*, 143–153.
(43) Psillakis, E.; Kalogerakis, N. Fast extraction, clean-up and detection methods for the rapid analysis and screening of seven indicator PCBs in food matrices. *TrAC Trends Anal. Chem.* **2002**, *21*, 53–63.
(44) Bagheri, H.; Saber, A.; Mousavi, S. R. Immersed solvent microextraction of phenol and chlorophenols from water samples followed by gas chromatography-mass spectrometry. *J. Chromatogr. A* **2004**, *1046*, 27–33.
(45) Zhang, Z.; Pawliszyn, J. Headspace solid-phase microextraction. *Anal. Chem.* **1993**, *65*, 1843–1852.
(46) Zhao, L.; Lee, H. K. Application of static liquid-phase microextraction to the analysis of organochlorine pesticides in water. *J. Chromatogr. A* **2001**, *919*, 381–388.
(47) Psillakis, E.; Kalogerakis, N. Application of solvent microextraction to the analysis of nitroaromatic explosives in water samples. *J. Chromatogr. A* **2001**, *907*, 211–219.

(48) Marcus, Y. *Ion Solvation*; Wiley: New York, 1985.
(49) Boyd-Boland, A. A.; Pawliszyn, J. Solid-phase microextraction of nitrogen-containing herbicides. *J. Chromatogr. A* **1995**, *704*, 163–172.
(50) Shamsipur, M.; Hassan, J.; Salar-Amoli, J.; Yamini, Y. Headspace solvent microextraction-gas chromatographic thermionic specific detector determination of amitraz in honey after hydrolysis to 2,4-dimethylaniline. *J Food Compos. Anal.* **2008**, *21*, 264–270.
(51) De Jager, L. S.; Andrews, A. R. J. Development of a screening method for cocaine and cocaine metabolites in urine using solvent microextraction in conjunction with gas chromatography. *J. Chromatogr. A* **2001**, *911*, 97–105.
(52) Hwang, B. H.; Lee, M.-R. Solid-phase microextraction for organochlorine pesticide residues analysis in Chinese herbal formulations. *J. Chromatogr. A* **2000**, *898*, 245–256.
(53) Shariati-Feizabadi, S.; Yamini, Y.; Bahramifar, N. Headspace solvent microextraction and gas chromatographic determination of some polycyclic aromatic hydrocarbons in water samples. *Anal. Chim. Acta* **2003**, *489*, 21–31.
(54) Peng, J.; Liu, J.; Jiang, G.; Tai, C.; Huang, M. Ionic liquid for high temperature headspace liquid-phase microextraction of chlorinated anilines in environmental water samples. *J. Chromatogr. A* **2004**, *1072*, 3–6.
(55) Vidal, L.; Canals, A.; Kalogerakis, N.; Psillakis, E. Headspace single-drop microextraction for the analysis of chlorobenzenes in water samples. *J. Chromatogr. A* **2005**, *1089*, 25–30.
(56) Pereira, F. J. P.; Bendicho, C.; Kalogerakis, N.; Psillakis, E. Headspace single drop microextraction of methylcyclopentadienyl-manganese tricarbonyl from water samples followed by gas chromatography-mass spectrometry. *Talanta* **2007**, *74*, 47–51.

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