Anal Bioanal Chem (2013) 405:2077–2081
DOI 10.1007/s00216-012-6646-7

TECHNICAL NOTE

Sensitive determination of organic acid preservatives in juices and soft drinks treated by monolith-based stir cake sorptive extraction and liquid chromatography analysis

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Received: 24 October 2012 / Revised: 4 December 2012 / Accepted: 7 December 2012 / Published online: 11 January 2013

Abstract A simple, efficient, and sensitive method for simultaneous determination of sorbic acid (SA), benzoic acid (BA), and cinnamic acid (CA) in juices and soft drinks was developed by stir cake sorptive extraction (SCSE) coupling to high-performance liquid chromatography with diode array detection. The SCSE based on polymeric ionic liquid-based monolith (PILM) as extractive medium was used to concentrate these three organic acid preservatives. Because hydrophobic and ion-exchange interactions co-contributed to the extraction, the PILM-SCSE exhibited a high extractive capability towards analytes. To obtain optimum extraction performance, several SCSE parameters were investigated and discussed, including desorption solvent, pH value, ionic strength in the sample matrix, and the extraction and desorption time. Under the optimized extraction conditions, limits of detection of 0.16, 1.08, and 0.18 μg/L (S/N=3) and quantification limits of 0.52, 3.42, and 0.61 (S/N=10) were obtained for SA, BA, and CA, respectively. The method also showed good linearity and reproducibility, as well as advantages such as simplicity, low cost, and high feasibility. Finally, the proposed method was successfully applied to the determination of SA, BA, and CA in real juices and soft drinks, and the recoveries ranged from 63.0 to 107 %.

Keywords Stir cake sorptive extraction · Polymeric ionic liquid-based monolith · Preservative · Juices · Soft drinks · HPLC

Introduction

Sorbic acid (SA), benzoic acid (BA), and cinnamic acid (CA) have played an increasingly important role in the food industry because they exhibit inhibitory activity against a wide range of fungi, yeasts, molds, and bacteria [1]. However, the excessive use of these preservatives may be harmful to consumers and cause some adverse effects such as urticaria, intolerance, and hyperpnea in humans [2]. Therefore, their maximum permitted concentrations in different kinds of food have been regulated in many countries [3, 4]. Hereby, in order to ensure food safety, it is necessary to develop an effective and reliable analytical method to monitor the preservative levels in food.

A variety of analytical methods for determining preservatives have been reported to date. Because of the effective power in qualification and quantification, chromatographic methods such as GC [5], HPLC [6], and capillary electrophoresis [7] are the most popular techniques to determine SA, BA, and CA. The Ministry of Agriculture of China has regulated GC [8] as the national standard method for detecting SA, BA, and CA, and HPLC [9] method for detecting SA and BA in food, respectively. When using a chromatographic method, suitable sample preparation is necessary in order to eliminate the inference of matrices and concentrate the target analytes. Liquid–liquid extraction (LLE) [5, 10] and solid-phase extraction (SPE) [11] have been used as popular techniques in the extraction of SA, BA, and CA from food such as juices and soft drinks. However, the LLE technique requires large volumes of poisonous solvents. SPE requires multi-step procedures that are

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Electronic supplementary material The online version of this article (doi:10.1007/s00216-012-6646-7) contains supplementary material, which is available to authorized users.

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complex, laborious, and time consuming. Recently, miniaturized sample pretreatment techniques such as solid-phase microextraction (SPME) [6] and stir-bar sorptive extraction (SBSE) [12] have also been successfully applied to the analysis of organic acid preservatives in several types of food. SPME possesses some advantages such as simplicity and solventless nature of the extraction, but the low extraction capacity limits its application. Ochiai N's group has used commercial SBSE to extract preservatives from several types of food [12]. However, the coating of commercial SBSE is polydimethylsiloxane, which is mainly applied to extract nonpolar and weakly polar compounds [13]. SA, BA, and CA possess carboxyl groups in their molecules and belong to strongly polar compounds. Therefore, in their work, the extractive performance for SA and BA was low. When using GC-MS to separate and determine the analytes, the limit of detection (LOD) for SA and BA was as high as 220 and 3,300 μg/L. Hereby, there is still an urgent necessity to develop convenient, high extraction capacity, cost-effective, and environmentally friendly sample preparation methods to analyze preservatives in food.

Stir cake sorptive extraction (SCSE) using monoliths as extractive medium is a new extractive format which developed in our group [14]. There are many advantages for SCSE, such as simple operation, high extractive capacity, good flexibility, cost-effectiveness, and being environmentally friendly. More important, the SCSE possesses excellent longevity because the sorbent does not come in contact with the vessel wall during stirring and there is no friction loss of the extractive medium. In our previous study [15], a new SCSE sorbent based on polymeric ionic liquid-based monolith (PILM) was prepared. Because there are abundant anion exchange groups in the PILM (the structure of PILM can be seen in electronic supplementary material Fig. S1), the SCSE-PILM shows high anion exchange capacity. SA, BA, and CA belong to organic acids; the carboxyl groups can dissociate and become organic anions under an appropriate pH value. Furthermore, the alkyl groups in the monolith can come in contact with analysts through hydrophobic interaction. Therefore, the PILM-SCSE is expected to show high extraction capability towards SA, BA, and CA compounds through anionic exchange and hydrophobic interaction. The aim of this research was to develop and validate a SCSE-PILM–HPLC/diode array detector (DAD) method for the simultaneous determination of SA, BA, and CA in juices and soft drinks. The results showed that the proposed method was convenient, sensitive, and environmentally friendly.

Experimental

Chemicals and materials

1-ally-3-Methylimidazolium chloride (AMIC) (99 %) and ethylene dimethacrylate (EDMA) (97 %) were supplied by Alfa Aesar (Tianjin, China); azobisisobutyronitrile (97 %, recrystallized before use), 1-propanol (97 %), and *N*,*N*-dimethylformamide (97 %) were purchased from Shanghai Chemical Co. (China); HPLC-grade acetonitrile (ACN) and methanol were purchased from Tedia Company (Fairfield, USA); water used throughout the study was purified using a Milli-Q water purification system (Millipore, USA). Benzoic acid (99.5 %), sorbic acid (99 %), and cinnamic acid (98 %) were bought from the Shanghai Chemical Reagent Corporation. They were prepared as 100-μg/mL mixtures in methanol and stored at 4 °C in the dark. The sample solution was spiked with these standard stock solutions to the desired concentration for each experiment.

The PILM-SCSE based on poly (AMIC–EDMA) was prepared according to reference [14]. The dimensions of monolithic cake of PILM were 12 mm in diameter and 3.0 mm in monolithic thickness. Fig.S1 (electronic supplementary material) shows the chemical structure of poly (AMIC–EDMA) monolith, and it can be seen that there are abundant imidazolium groups which can be activated under a suitable pH value.

Instrumentation and chromatographic conditions

HPLC analyses were carried out on an LC chromatographic system (Shimadzu, Japan) equipped with a binary pump (LC-20AB) and a DAD (SPD-M20A). Sample injection was carried out using a RE3725i manual sample injector with a 20-μL loop (Rheodyne, Cotati, CA, USA), all experiments were performed at room temperature.

The separation of SA, BA, and CA was performed on a Supelco C18 column (5 lm particle size, 250 mm×4.6 mm i.d.). The mobile phase was ACN/0.3 % acetic acid aqueous solution (40:60, *V*/*V*), and the flow rate was maintained at 1.0 mL/min. The detector wavelength was set at 271 nm, and injection volume was 20 μL.

Preparation of juices and soft drinks

All of the samples studied were bought at a local market. The juice drinks were filtered with a 0.45-μm membrane; then, 10 mL of the filtrate was diluted with Milli-Q water to 100 mL. The PILM-SCSE was directly put into the sample and stirred at 400 rpm at room temperature for a certain time. After finishing the extraction procedure, the PILM-SCSE was removed and immersed in 3.0 mL desorption solvent, stirred for a certain time to release the extracted analytes. The stripping solution was used directly for HPLC/DAD analysis. For soft drinks, the pretreatment was similar to that of the juice drink samples but without the sample filtering step. In the present study, the above sample preparation procedure was very simple; no additional cleanup step was needed.

After each sample preparation, the SCSE-PILM can be stirred in 50 % methanol aqueous solution for 1.0 h, then dipped

in methanol for 0.5 h to remove the residue on the monoliths. We also recommend that the SCSE should be stored in methanol. Because the extractive medium does not come in contact with the vessel wall during stirring, the SCSE-PILM shows excellent longevity, and it can be reused more than 250 times.

Results and discussion

Optimization of SCSE-PILM extraction conditions

In order to obtain the optimum extractive performance, several extractive conditions, including desorption solvent, pH value and ionic strength in the sample matrix, extraction, and desorption time, were investigated in detail.

The effect of desorption solvent

During the preliminary experiment, we found that using methanol or ACN as desorption solvent, the target analyst adsorbed on the monolith could not be desorbed completely, and even the desorption time was prolonged to 12 h. However, when low content of acetic acid was added in the desorption solvent, SA, BA, and CA could be desorbed from the PILM completely. The reason may be that acetate ions can weaken the ionic exchange of analytes and PILM. Figure S2 (electronic supplementary material) shows the effect of the content of acetic acid in desorption solvent on extraction performance. The results indicate that the extraction efficiencies reach maximum for all studied preservatives when acetic acid content is 8 %. Therefore, the mixture of ACN/acetic acid (*V*/*V*=92/8) was chosen as the desorption solvent.

The effect of pH values

The effect of sample pH on the extraction efficiency was investigated in the range of 3.0–11.0. As shown in Fig. S3 (electronic supplementary material), when the other conditions were constant, the pH value affected the extraction efficiency of SCSE-PILM for SA, BA, and CA strongly. The results showed that the extraction efficiencies improved significantly with the increase of pH value from 2.0 to 5.0 and decreased sharply with the pH value increased continuously. This interesting trend may be explained as follows: at low pH value, the imidazolium groups in PILM were not activated; at the same time, there was no ionization for the three analytes. Therefore, SA, BA, and CA were extracted by hydrophobic interaction. The imidazolium groups were activated, and the three organic acids produced ionization with the increase of pH value, so the anion exchange and hydrophobic interactions between PILM and analytes contributed to the extraction, and the extractive performance reached maximum when pH value was 5.0, in which all the three organic acids possessed negative charges (the p*K*a values for SA, BA, and CA were 4.76, 4.20, and 4.37, respectively). When the pH value increased continuously, in the one hand, overmuch OH^- groups would compete with the sorptive sites on the PILM with analytes; thus, the anion exchange interaction was weakened. On the other hand, more and more analytes produced ionization which reduced the hydrophobic interaction with PILM. The above two factors resulted in the obvious decrease of extractive performance of SCSE-PILM at high pH values. According to the results, setting the pH values of the matrix at 5.0 was used in the following research.

The effect of ionic strength

In the present study, the ionic strength of the matrix was adjusted by the addition of NaCl from 0 to 25 % (*w*/*v*) (Fig.S4, electronic supplementary material). The results show that the extraction efficiencies of SCSE-PILM decrease rapidly when NaCl concentration increases from 0 to 5 %; then, there is no obvious change in extraction efficiencies from 5 to 15 %; the extraction efficiencies improve obviously when NaCl concentration increased continuously. But the extraction efficiencies at 25 % ionic strength were still lower than the corresponding values achieved at 0 % ionic strength. Therefore, no addition of any salt was adopted in the following studies.

The effect of extraction and desorption time

The extraction time was varied from 0.5 to 3.5 h. Results indicated that the extraction efficiencies increased quickly when the extraction time increased from 0.5 to 3.0 h and reached equilibrium afterwards (Fig.S5, electronic supplementary material). The sharp slopes of the profiles between 0.5 and 3.0 h well demonstrates that the monolithic materials show remarkable extraction capacity towards the three organic acid preservatives. The effect of liquid desorption time on the result was also studied. It was found that 1.0 h was enough for desorption of target compounds from SCSE-PILM when the extraction time was 3.0 h. Consequently, 3.0 and 1.0 h were adopted for the extraction and desorption procedure, respectively, in the following research.

From the above experimental result, the optimized parameters for the extraction of SA, BA, and CA with SCSE-PILM are the followings: using ACN/acetic acid binary solvent (*V*/*V*=92/8) as desorption solvent; the pH value of the matrix was 5.0; no salt was added in the matrix; extraction and desorption time were 3.0 and 0.5 h, respectively.

Proposed method performance

Figure 1 shows typical chromatograms for SA, BA, and CA obtained with SCSE-PILM–HPLC/DAD and those obtained

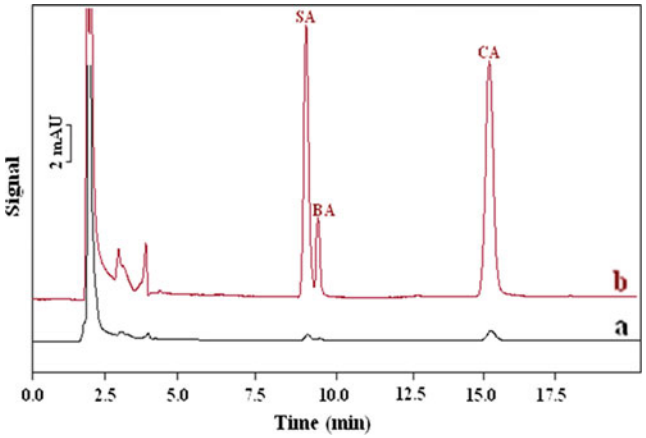

Fig. 1 HPLC chromatograms of SA, BA, and CA preservatives. **a** Direct injection of spiked water sample with each analyte at 100.0 μg/L; **b** spiked water sample with each analyte at 100.0 μg/L and treated with SCSE-PILM. Conditions: extraction and desorption time were 3.0 and 1.0 h, respectively; no salt was added in the sample; pH value was adjusted to 5.0; using ACN/acetic acid (*V*/*V*=92/8) as desorption solvent. The sample pH values were adjusted by 0.1 mol/L HCl

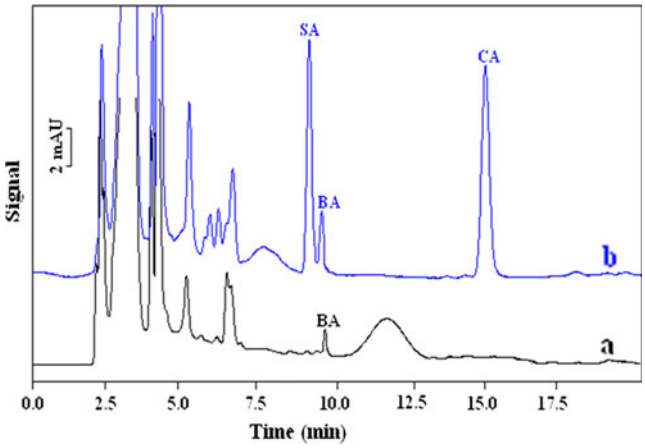

Fig. 2 HPLC chromatograms of real juice sample. **a** No spiked analytes juice sample and treated with SCSE-PILM; **b** spiked juice sample with each target analyte at 100 μg/L and treated with SCSE-PILM. Conditions: extraction and desorption time were 3.0 and 1.0 h, respectively; no salt was added in the sample; pH value was adjusted to 5.0; using ACN/acetic acid (*V*/*V*=92/8) as desorption solvent. The sample pH values were adjusted by 0.1 mol/L HCl

after direct injection of a 100-μg/L standard sample. It can be seen from the figure that the peak heights for the target compounds obviously increase after enrichment. The enrichment factors for SA, BA, and CA were 29, 23, and 25, and extractive efficiencies were 87, 69, and 75 %, respectively. The results well indicate that the PILM-SCSE possesses expected extraction capacity for the three organic acids.

The data of linear dynamic range, correlation coefficients, limits of detection (LOD), limits of quantification (LOQ), and reproducibility for the analytes under the optimized experimental conditions are listed in Table S1 (electronic supplementary material). It can be seen from the data that the SCSE-PILM–HPLC/DAD methodology presents a good performance. The linear dynamic ranges of a 100-mL sample are 1.0–2,000 μg/L for SA and CA, and 3.0–2,000 μg/L for BA. The correlation coefficients (R^2) for all analytes are above 0.997. The LOD and LOQ were determined at a concentration at which signal-to-noise ratios were equal to 3 and 10, and those were in the range of 0.16–1.03 and 0.52–3.42 μg/L, respectively. The LOD and LOQ are low enough to analyze trace SA, BA, and CA in food. The precision of the proposed method was evaluated using inter-assay repeatability calculated as RSD on four replicates, and the RSDs were found below 6.0 %.

A comparative study of our developed method with previous reported analytical methods was performed, and the results were presented in Table S2 (electronic supplementary material). Comparing with other methods, lower LOD can be obtained in the present method than other methods

Table 1 Results of determination and recoveries of juices and soft drinks spiked with three target analytes

Water sample	Spiked (μg/L)	Detected (μg/L)			Recovery (%RSD, *n*=3)		
		SA	BA	CA	SA	BA	CA
Juice sample 1	0.0	0.0	0.0	52.0			
	100	67.0	75.0	141	67.3 (6.7)	75.2 (4.7)	89.0 (3.5)
	1,000	630	736	704	63.0 (4.2)	73.6 (4.5)	65.2 (7.7)
Juice sample 2	0.0	0.0	35	0.0			
	100	64.0	127	68.1	64.1 (4.6)	92.0 (7.0)	68.1 (4.5)
	1,000	729	824	861	72.9 (2.7)	78.9 (4.7)	86.1 (3.8)
Soft drink 1	0.0	48.0	0.0	0.0			
	10.0	126	86.9	86.0	78.1 (3.8)	86.9 (4.2)	86.0 (6.5)
	1,000	911	766	1,020	86.3 (5.6)	76.6 (2.3)	102 (5.8)
Soft drink 2	0.0	86.0	56.0	0.0			
	100	174	131	89.0	88.1 (4.6)	75.1 (6.8)	89.0 (7.2)
	1,000	1054	962	1,070	96.6 (3.8)	90.6 (5.3)	107 (6.4)

with the same kind of detector. Typically, using MS as a detector can achieve higher sensitivity than a UV detector. However, for the determination of SA, the proposed method exhibits a greater sensitivity than HS-LPME–GC/MS and SBSE–GC/MS. Furthermore, no additional cleanup step is required in the present method, the whole analytical procedure is very convenient.

The experimental and comparative results well indicate that the SCSE-PILM–HPLC/DAD method can be used to effectively monitor organic acid preservers in food.

Application in the analysis of juices and soft drinks

To further demonstrate the applicability of the developed methodology, different juices and soft drinks were analyzed. Low contents of SA, BA, and CA were detected in the samples, but all the concentrations were below MRL values regulated in China (Table 1) [3]. To validate the feasibility of the proposed method, extraction recoveries were assessed by spiking juice and soft drink samples with trace mixed standard solutions (100 and 1,000 μg/L, respectively) (Fig. 2). It can be seen from the data in the table that acceptable recoveries and good reproducibility are obtained; the recoveries vary from 63.0 to 107 %, and the RSDs for reproducibility are less than 8.0 % for the target analytes in all the samples, demonstrating good method feasibility.

Conclusions

In this work, a SCSE based on polymeric ionic liquid-based monolith as extractive medium was used to extract sorbic acid, benzoic acid, and cinnamic acid preservatives. Because hydrophobic and anion exchange interactions co-contribute to the extraction, the SCSE-PILM showed expected extraction performance for the three organic acid preservatives. The combination of SCSE-PILM with HPLC/DAD provided a convenient and robust method for the simultaneous determination of SA, BA, and CA in juices and soft drinks. In comparison with the existing extraction methods for organic acid preservative determination, the proposed method was simple, sensitive, cost-effective, stable, and environmentally friendly. Therefore, we hope that the proposed method could become a useful and practical approach in the routine monitoring of SA, BA, and CA in juices and soft drinks or other food matrices.

Acknowledgments The work described in this article was supported by the National Natural Science Foundation of China (grant 21077085); Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry; New Century Excellent Talents in Fujian Province University and Fundamental Research Funds for the Central Universities (no. CXB2011037).

References

1. Kubo I, Lee SH (1998) J Agric Food Chem 46:4052–4055
2. Tfouni SAV, Toledo MCF (2002) Food Control 13:117–123
3. Ministry of Health (2011). Hygienic standards for uses of food additives, Ministry of Health, P.R. China, GB 2760, 2011
4. The European Parliament and the Council of the European Union (1995). European Parliament and Council Directive No. 95/2/EC, February 1995
5. Kokya TA, Farhadi K, Kalhori AA (2012) Food Anal Methods 5:351–358
6. Wen Y, Wang Y, Feng YQ (2007) Anal Bioanal Chem 388:1779–1787
7. Ling DS, Xie HY, He YZ, Gan WE, Gao Y (2010) J Chromatogr A 1217:7807–7811
8. National Standard of the People's Republic of China GB/T 5009.26 (1996) Standards Press of China, Beijing
9. National Standard of the People's Republic of China GB/T 5009.29 (1996) Standards Press of China, Beijing
10. González M, Gallego M, Valcárcel M (1999) J Chromatogr A 848:529–536
11. Han F, He YZ, Li L, Fu GN, Xie HY, Gan WE (2008) Anal Chim Acta 618:79–85
12. Ochiai N, Sasamoto K, Takino M, Yamashita S, Daishima S, Heiden AC, Hoffmann A (2002) Anal Bioanal Chem 373:56–63
13. Silva ARM, Portugal FCM, Nogueira JMF (2008) J Chromatogr A 1209:10–16
14. Huang XJ, Chen LL, Lin FH, Yuan DX (2011) J Sep Sci 34:2145–2151
15. Huang XJ, Chen LL, Yuan DX, Bi SS (2012) J Chromatogr A 1248:63–73