Hollow fiber supported ionic liquid membrane microextraction for determination of sulfonamides in environmental water samples by high-performance liquid chromatography

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ABSTRACT

By using ionic liquid as membrane liquid and tri-*n*-octylphosphine oxide (TOPO) as additive, hollow fiber supported liquid phase microextraction (HF-LPME) was developed for the determination of five sulfonamides in environmental water samples by high-performance liquid chromatography with ultraviolet detection. The extraction solvent and the parameters affecting the extraction enrichment factor such as the type and amount of carrier, pH and volume ratio of donor phase and acceptor phase, extraction time, salt-out effect and matrix effect were optimized. Under the optimal extraction conditions (organic liquid membrane phase: [C₈MIM][PF₆] with 14% TOPO (w/v); donor phase: 4 mL, pH 4.5 KH₂PO₄ with 2 M Na₂SO₄; acceptor phase: 25 μ L, pH 13 NaOH; extraction time: 8 h), low detection limits (0.1–0.4 μ g/L, RSD $\leq$ 5%) and good linear range (1–2000 ng/mL, $R^2 \geq 0.999$) were obtained for all the analytes. The presence of humic acid (0–25 mg/L dissolved organic carbon) and bovine serum albumin (0–100 μ g/mL) had no significant effect on the extraction efficiency. Good spike recoveries over the range of 82.2–103.2% were obtained when applying the proposed method on five real environmental water samples. These results indicated that this present method was very sensitive and reliable with good repeatabilities and excellent clean-up in water samples. The proposed method confirmed hollow fiber supported ionic liquid membrane based LPME to be robust to monitoring trace levels of sulfadiazine, sulfamerazine, sulfamethazine, sulfadimethoxine and sulfamethoxazole in aqueous samples.

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1. Introduction

Sulfonamides (SAs) belong to an important group of bacteriostatic agents used to prevent infections, treat diseases and to promote growth [1]. Due to their wide-ranging activity and low cost, SAs have been widely used in human and veterinary medicine. After normal applications, SAs and/or their metabolites can be excreted from human body or animal organisms through urine and feces [2]. Ultimately these residues can find their way into the environment and consequently, trace of SAs has been frequently detected in surface water worldwide [3,4]. Although SAs are present in the environment at low concentration levels, they can promote the

development of antibiotic-resistant bacteria, cause allergic reactions in human, and even possess carcinogenic potency [5,6]. In the research of Halling-Sørensen et al., SAs have a long life-time in the environment and can resist biodegradation and accumulate in all kinds of organisms through the food-chain. Further, photo-enhanced toxicity of SAs are reported by Jung et al. [7]. Huang et al. suggested that SAs are the most common water pollutants among various antibiotics, based on their environmental fate and predicted concentrations [8]. Stringent guidelines have been established in the US and the EU to conduct environmental assessment of pharmaceutical compounds [9,10]. Therefore, it is imperative to develop reliable, highly sensitive and easily operated methods to monitor their concentrations in the environment.

In the past decades, various methods have been employed to determine sulfonamide drugs such as gas chromatography–mass spectrometry (GC–MS) [11,12], GC-atomic emission detection (GC-AED) [13], high-performance liquid chromatography ultraviolet detection (HPLC–UV) [14,15] or fluorescence detection [16], liquid chromatography–mass spectrometry (LC–MS) [17], capillary

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electrophoresis-ultraviolet detection (CE-UV) [18], etc. Prior to GC analysis, derivatization of non-volatile and thermally labile sulfonamides is required. Although HPLC-fluorescence detection has been successfully applied to determine SAs with high sensitivity, derivatization is also required to enhance the fluorescence signal [16]. LC-MS [17] or HPLC-UV [14,15] seems to be the most promising methods since derivatization is not needed.

Sample preparation and clean-up in order to eliminate the interference of matrices for environmental samples is of great importance. Due to the very low concentrations of sulfonamide residues and the complexity of the matrices, an effective pretreatment approach is of vital importance for analysis of SAs prior to those quantitative chromatography-based techniques. Various sample preparation procedures have been applied for SAs, such as liquid-liquid extraction (LLE) [19,20] and on/off-line solid-phase extraction (SPE) [16,21,22]. These procedures are usually tedious and time-consuming, in addition to the large volume of sample and large consumption of toxic organic solvents required. In recent years, microextraction techniques were introduced as an alternative to the conventional SPE and LLE due to the simplicity of operation, solvent-saving/free and being time-efficient.

Since 1999, hollow fiber based LPME introduced by Pedersen-Bjergaard and Rasmussen [23], have been widely applied as an alternative to conventional techniques mentioned above. Besides the advantages of preconcentration, sample clean-up, and solvent-saving, this technique requires the equipment to be simple and inexpensive. And in the hollow fiber based LPME device, the extraction solvent is contained within the lumen of a porous hollow fiber, so the solvent is not in direct contact with the sample solution. Thus, the hollow fiber based LPME is a reliable and robust technique which can provide high enrichment and low limits of detection. To date, many works published have successfully applied the hollow fiber based LPME for extraction of several different classes of drugs from various matrices [24–27]. Final analysis of extraction solutions has been reported with CE [23], HPLC [28], GC-MS [29], and LC-MS [30].

For the hollow fiber based LPME, appropriate selection of the organic solvent is of great importance to obtain efficient extraction. Toluene, undecane, 1-octanol and dihexyl ether are the most popular solvents used in HF-LPME. Nevertheless, based on the “like dissolves like” principle in LPME and the amphoteric properties of SAs (Table 1), these traditional solvent might be not suitable for SAs. Room temperature ionic liquids (RTILs) are novel solvents that have good solubility for inorganic and organic compounds [31]. RTILs

have displayed good extractability for various organic compounds and metal ions [32]. Moreover, with the properties of non-volatility and innocuity to chromatographic column, ionic liquids can be compatible with liquid phase chromatography and directly injected for analysis [33,34]. To our knowledge, the applicability of ionic liquids for extracting SAs has yet to be investigated. Previous studies [35,36] demonstrated that the ionic liquids could be firmly immobilized in the microspores of a supported membrane, thus it is attractive to explore the use of ionic liquid based liquid membrane extraction for SAs.

In this present work, three-phase hollow fiber supported ionic liquid membrane extraction was developed for preconcentration of trace levels of five sulfonamides in environmental water samples. Parameters including the organic membrane phase, the content of TOPO added in organic liquid, pH of donor phase and acceptor phase, volume ratio of donor to acceptor phase, extraction time, shaking speed and salinity of sample solution were optimized and the method performance was evaluated and was applied for extracting SAs in environmental water samples.

2. Experimental

2.1. Reagents and materials

Sulfadiazine (SD), sulfamerazine (SM₁), sulfamethazine (SM₂), sulfadimethoxine (SDM) and sulfamethoxazole (SMZ) with purity of 99.0% were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 1-Octyl-3-methylimidazolium hexafluorophosphate [C₈MIM][PF₆] (99.0%) was purchased from Meisibei Co., Ltd. (Shanghai, China). Dihexyl ether (DHE) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Kita-Ku, Tokyo, Japan). Tri-*n*-octylphosphine oxide (TOPO), undecane and terrestrial origin humic acid sodium salt with 35.1% of dissolved organic carbon (DOC) were purchased from Sigma-Aldrich (Steinheim, Germany) and used as obtained. Bovine serum albumin (BSA) (total protein 99%) was obtained from Xijingke Biotechnology Co., Ltd. (Beijing, China). HPLC-grade methanol was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade or above and ultrapure water, prepared by an Easypure LF purification system (Barnstead International, Dubuque, IA, USA), was used throughout.

Individual standard stock solutions of SAs were prepared by dissolving 50 mg standard in 100 mL of HPLC-grade methanol. Working solutions were prepared by appropriate dilution of the stock solutions with water. The standard stock solutions and work-

Table 1
Target sulfonamide antibiotics structures and physicochemical properties.

	Sulfadiazine (SD)	Sulfamerazine (SM ₁)	Sulfamethazine (SM ₂)	Sulfadimethoxine (SDM)	Sulfamethoxazole (SMZ)
CAS No.	68-35-9	127-79-7	57-68-1	122-11-2	723-46-6
Formula
Molecular weight	250.3	264.3	278.3	310.3	253.3
log <i>K</i> _{ow} ^a	−0.09	0.14	0.89	1.63	0.89
p <i>K</i> _{a1} ^b	1.6 ± 0.1	1.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	1.4 ± 0.1
p <i>K</i> _{a2} ^c	6.8 ± 0.5	6.9 ± 0.5	7.0 ± 0.5	6.5 ± 0.5	7.7 ± 0.5

^a log *K*_{ow} and p*K*_a are calculated using the ACD/p*K*_a and ACD/log *P* DB soft programs (Advanced Chemistry Development, Toronto, Canada).

^b p*K*_{a1} is the dissociation constant of the amino group which is the basic dissociation constant.

^c p*K*_{a2} is the dissociation constant of sulfanilamido group which is the acidic dissociation constant.

ing solution were stored at 4 °C in refrigerator, but thawed to room temperature before use.

Q3/2 Accurel® PP polypropylene microporous hollow fiber membrane (200 µm wall thickness, 600 µm inner diameter, 0.2 µm pore size, 75% porosity) was obtained from Membrana GmbH (Wuppertal, Germany). Aseptic Syringe (with a needle of 0.6 mm outer diameter and 25 mm length, 1 mL) for single use, obtained from Shoukang Medical Instrument Co., Ltd (Shanghai, China) was used to fill the acceptor solution into the lumen of the hollow fiber for extraction and to flush out the acceptor phase.

2.2. Instrument

Chromatographic analysis was performed in Agilent 1100 series HPLC system (Wilmington, DE, USA), consisting of G1312A binary pump and G1314 VW detector. All separations were achieved on an analytical reversed-phase column (Eclipse XDB-C₁₈ column, 150 mm × 4.6 mm, particle size 5 µm) under isocratic conditions at column temperature of 25 °C. The mobile phase was a mixture of acetonitrile and pH 5.5 KH₂PO₄–K₂HPO₄ buffer (20 mmol/L) at a flow rate of 1 mL/min. The ultraviolet detector was operated at 265 nm. Gradient elution program used was the following: initial concentration of acetonitrile at 95%, linearly decreasing to 75% in 0–3 min, linearly decreasing to 60% in 3–7 min and then linearly decreasing to 35% in 7–9 min and maintaining at 35% for 1 min. The five SAs were baseline separated and eluted within 10 min.

2.3. Water samples

Five environmental water samples including wastewater from pig, fish and duck farm, and paddy water and river water were collected for validating the proposed method. River water sample was collected from Tiaoxi river in north of Zhejiang province. The other water samples were collected from suburbs of Hangzhou city. All samples were collected in September 2008. The collected waters were maintained in brown glass bottles and stored at 4 °C. Before extraction, the water samples were adjusted to pH 4.5 with the addition of 0.02 M KH₂PO₄ at room temperature.

2.4. Extraction procedure

The HF-LPME unit set-up used was similar to the one described in previous work by Lezamiz and Jönsson [37]. In brief, the hollow fiber (13 cm) was cleaned with acetone by ultrasonication and air dried before use. The lumen of the fiber used was filled with water, and then the fiber was dipped into the membrane liquid ([C₈MIM][PF₆] with 14% (w/v) TOPO) for 5 min to form the organic liquid membrane in the fiber pores. Before extraction, the surface and lumen of the fiber were flushed with water to remove excess membrane liquid. The acceptor phase (pH 13 NaOH) was slowly injected into the fiber lumen by a disposable syringe until the lumen was full. Then two ends of the fiber were folded and sealed with aluminum foil. The prepared extraction set-up had an effective length of 12 cm and the corresponding volume of acceptor was 25 µL. After that, the set-up was placed and submerged into the donor phase (0.02 M KH₂PO₄, pH 4.5). The whole extraction was performed in a 5-mL brown capped vial with 8 h shaking at 300 rpm on multipurpose desk homothermal shaker (DDHZ-300, Taicang Experimental Instrument Factory, Jiangsu, China). At the end of the extraction time, the fiber was carefully removed from the sample solution and both sealed ends were cut. One end was connected to the needle of Aseptic Syringe full of air and the acceptor phase containing the target analytes was flushed out from the fiber lumen into a clean glass liner tube (100 µL, Alltech, Deerfield, IL, USA). Finally, the whole acceptor phase (~25 µL) was manually injected into HPLC system for analysis. In order that the extraction was free of memory effects,

and the membrane life was not a concern, a new fiber was used for each extraction.

Optimization of the extraction parameters were performed with sample solutions spiked with 5 µg/L of five sulfonamide compounds. All experiments were replicated three times and the mean values were obtained.

2.5. Calibration and data processing

The operation of the extraction was evaluated by the concentration enrichment factor (EF):

$$EF = \frac{C_{AP,final}}{C_{DP,initial}}$$

where $C_{DP,initial}$ and $C_{AP,final}$ are the initial analyte concentration in the donor phase (DP) and the final analyte concentration in the acceptor phase (AP), respectively. $C_{AP,final}$ was calculated from the calibration curves by directly injecting 20 µL of different concentrations of standards into the HPLC system. The concentrations of analytes in environmental samples were determined by the proposed procedure and calculated from the standard curve. All results were expressed as mean values of at least three replicates.

3. Results and discussion

3.1. Selection of liquid membrane

Generally, there are several selection criteria for organic solvents as the liquid membrane: (1) immobility in the pores of hollow fiber; (2) immiscibility with water; (3) reasonably higher solubility of analytes in the organic phase than in the aqueous phase. Based on those main requirements, three organic solvents including *n*-undecane, DHE and [C₈MIM]PF₆ were tested at fixed conditions as follows: 4 mL, pH 4.5 KH₂PO₄ buffer solution as DP, 10 µL, 0.1 M NaOH as AP, shaking speed 100 rpm and 1 h of extraction time. As shown in Table 2, when *n*-undecane, a non-polar organic solvent, was used, none of the five sulfonamides compounds was extracted into AP; When DHE with weakly polar solvent was used, the enrichment of all the five compounds was improved, but the EF (3.3–9.1) was unacceptable. Comparatively, [C₈MIM]PF₆ with higher polarity could provide relative high EF (4.5–12.5). The phenomena were in good agreement with the principle of “like dissolving like”. As SAs belong to the polar compounds, [C₈MIM]PF₆ with higher polarity than DHE would be more applicable as the extraction solvent. On the other hand, as sulfonamides are hydrophilic drugs (log *K*_{ow} < 1) with high solubility in aqueous media, their direct extraction by passive diffusion from the DP through the organic membrane immobilized in the fiber pores into AP would be very difficult. To obtain higher EF, the commonly-used additives, TOPO [38–40] and Aliquat 336 [41,42], were dissolved into the organic solvents in membrane liquid, which would change the behavior of the membrane liquid. The results (Table 2) showed more efficient extraction in the presence of TOPO dissolved in [C₈MIM]PF₆, i.e. the EFs doubled compared to that in pure [C₈MIM]PF₆. Fortunato et al. demonstrated that no leakage of ionic liquid from membrane pores was found and the ionic liquid membrane showed high operational stability [43]. Thus, the mixture of [C₈MIM]PF₆/TOPO was selected as membrane liquid in subsequent experiments.

Following that, the influence of TOPO concentration in membrane liquid on EF was further studied. Fig. 1 shows EFs of all sulfonamide compounds increased with increase of TOPO content and reached maximum at 14% TOPO in [C₈MIM]PF₆. In this case, more addition of TOPO into [C₈MIM]PF₆ would become insoluble and result in the non-homogeneity of the membrane liquid.

Table 2
Extraction enrichment factor (EF) of different organic solvents as membrane liquid.

Organic membranes	SD	SM ₁	SM ₂	SMZ	SDM
DHE	3.3	3.9	4.8	9.0	9.1
<i>n</i> -Undecane	–	–	–	–	–
[C ₈ MIM][PF ₆]	4.5	5.6	5.5	12.5	11.2
DHE/4% TOPO	4.2	4.3	5.1	12.4	12.2
<i>n</i> -Undecane/4% TOPO	1.6	2.1	1.8	2.5	3.7
[C ₈ MIM][PF ₆]/4% TOPO	7.7	8.5	9.1	17.0	20.8
DHE/4% Aliquat 336	3.1	3.5	4.7	8.6	10.4
<i>n</i> -Undecane/4% Aliquat 336	–	–	–	–	–
[C ₈ MIM][PF ₆]/4% Aliquat 336	3.9	5.0	4.1	7.2	8.3

DP: 4 mL, pH 4.5 KH₂PO₄ solution spiked with 5 µg/L target compounds; AP: 10 µL, 0.1 M NaOH; stirring speed: 100 rpm; extraction time: 1 h.

Accordingly, the extraction flux would be reduced from DP into AP. Therefore, 14% TOPO was adopted for further studies.

3.2. Effect of donor and acceptor pH

SAs are ampholytes, and in order to efficiently extract them into the organic membrane liquid, the properties of DP should be adjusted for SAs to be in their neutral forms. In our experiment, pH of DP in the range of 1–7 was studied using two systems with the constant volume of 4 mL: (1) the buffer solution of KH₂PO₄; (2) the solution of HCl. The pH value of DP was adjusted to the test value by 5 mol/L HCl and NaOH. The results showed that the same trends of changes were obtained by two kinds of DP solutions and the maximum extraction efficiency was obtained at pH 4.5 of KH₂PO₄, as shown in Fig. 2a. The probable reasons might be concerned with the ampholyte property of SAs. In view of properties of the five target analytes (Table 1), the presence of the amino group and the sulfanilamido group would cause a two-step protolysis and increase their hydrophilicity, impeding their direct extraction into hydrophobic media ($\log K_{ow} < 1$). Theoretically it is feasible to control the ionizing and non-ionizing forms of SAs by adjusting the pH. In addition, the hydrophobicity of the neutral species is logically greater than that of their corresponding ions [44]. When pH is adjusted to the average of pK_{a1} and pK_{a2} of SAs, the neutral molecule form is the dominant species [14]. Therefore, the hydrophilic–hydrophobic character of sulfonamides can be changed by adjusting the pH. Like other hydrophilic pharmaceutical residues, such as salbutamol and terbitaline [41], SAs might also be extracted by three-phase HF-LPME. As mentioned above, pH of DP might be adjusted to the average of pK_{a1} and pK_{a2} of the target SAs to convert them into their

neutral species. pK_{a1} and pK_{a2} of analytes are in the range of 0.4 ± 0.1 to 1.6 ± 0.1 and 6.5 ± 0.5 to 7.7 ± 0.5 , respectively (Table 1). Accordingly, KH₂PO₄ at pH 4.5 was used as DP in subsequent experiments.

Based on the extraction principles of HF-LPME, the pH of AP should ionize the trapped analytes to prevent them from being back-extracted into the liquid membrane. When KH₂PO₄ of pH 4.5 was adopted as DP, pH over 10 should be adopted for AP. The acceptor concentration of NaOH was further optimized in the range of 0.001–2 M. As the results in Fig. 2b indicated, maximum EFs were obtained at 0.25 M NaOH as AP. Subsequently, EFs decreased with the increase of the concentration of NaOH over 0.25 M. The probable reason was that excess concentration of NaOH leaked into DP and weakened the DP capability to convert SAs into neutral species. In the following experiments, 0.25 M NaOH was used as acceptor solution.

3.3. Effect of volume ratio of donor to acceptor

In three-phase HF-LPME, the analytes are extracted by passive diffusion from the aqueous donor phase, through the organic solvent immobilized in the pores of the hollow fiber (organic phase), and further into the acceptor phase present inside the lumen of the hollow fiber. The analyte enrichment factor in this process can be calculated by the equation as follows: $EF = (V_d R) / (V_a \times 100)$, where V_a is the volume of acceptor phase and V_d is the volume of donor phase. It is clear that EFs were positively related to the ratio of V_d/V_a from the equation. In the present work, keeping a constant donor phase volume of 4 mL with analyte concentration of 1 µg/L, the effect of acceptor volume on EFs was studied in the range of 10–90 µL. The results, shown in Fig. 3, illustrated EFs of five target analytes reaching maximum at 25 µL of AP. The probable reason was that the small volume of acceptor phase would result in relatively high extraction efficiency (R) and thus high enrichment factors. However, the enrichment factor could never become larger than the volume ratio of V_d/V_a . Therefore, 25 µL of AP and 4 mL of DP were adopted in the following studies.

3.4. Effect of stirring and shaking

In order to accelerate the mass transfer velocity from donor through organic membrane into acceptor in the extraction, magnetic stirring and shaking are commonly used speedup means. Three speed rates (50, 150, and 300 rpm) representing low, medium and high-speed respectively were investigated in experiments. The graphs (see Appendix B Fig. S1 in Supplementary Material) indicated EFs markedly increased with the increasing of speed rates by both stirring and shaking. Nevertheless, stirring at the rate of 300 rpm made the fiber spin intensively in the donor solution and as consequence, continuously produced air bubbles that attached to the fiber surface, resulting in slightly inferior repeatability (RSD%:

Fig. 1. Effect of TOPO content on the extraction enrichment factor. DP: 4 mL, pH 4.5 KH₂PO₄ buffer solution; AP: 10 µL, 0.1 M NaOH; liquid membrane: [C₈MIM][PF₆] with TOPO; shaking speed: 100 rpm; extraction time: 1 h. (□) SD; (○) SM₁; (△) SM₂; (▽) SMZ; (<) SDM.

Fig. 2. Effect of pH of donor and acceptor on the extraction enrichment factor. DP: 4 mL KH₂PO₄ buffer solution; AP: 10 μ L NaOH; liquid membrane: [C₈MIM][PF₆] with 14% TOPO; shaking speed: 100 rpm; extraction time: 1 h. (□) SD; (○) SM₁; (Δ) SM₂; (▽) SMZ; (◁) SDM.

0.3–11.5%) and lower EFs (18–35) than were obtained by shaking (RSD%: 0.2–7.1%; EFs: 22–41). Therefore, subsequent experiments were performed by shaking at 300 rpm.

3.5. Effect of extraction time

HF-LPME can be performed in the kinetic range or the equilibrium range. The EF linearly increases with the increase of the extraction time in the first stage, whereas it becomes independent of the extraction time in the equilibrium range [35]. In three-phase HF-LPME, the amount of extracts should be expected to increase with the prolonged extraction time and reach maximum in the equilibrium stage. Fig. 4 displays the extraction time dependency for the equilibration of all analytes. As seen, the extraction of all analytes reached equilibrium after 8 h. In most cases, HF-LPME is performed with extraction times approaching equilibrium to maximize recovery (typical extraction times of 30–90 min). The probable reason for the longer equilibrium time in the present work was that the relatively high viscosity of [C₈MIM]PF₆ reduced mass transfer speed between donor and acceptor. On the other hand, the micropore structures of the hollow fiber would affect the molecules of SAs to transfer into the DP. Although the extraction time seemed relatively long, the repeatability of extraction was much better than

that with shorter extraction time. Meanwhile higher EFs (45–70) were obtained. Therefore, the extraction time of 8 h was chosen in further experiments.

3.6. Effect of salt addition in donor phase

In practicing conventional liquid–liquid extraction, the salting-out effect is often used to increase the partition coefficient of the polar analytes to the organic liquid. The effect in liquid–liquid extraction shows it is possible to promote dissolution of the analytes from the aqueous donor to the organic membrane liquid. According to the literatures, the effect of increasing ionic strength on the enrichment factor by addition of a salt is not beneficial for short extraction times, but for longer extractions [45]. Therefore, in this case, the content of salt added in the donor solution was varied to investigate its effect on the maximum EFs. The findings (Fig. 5) showed that the EFs for each compound increased with the increase of salt content. For SMZ and SDM, EFs did not increase significantly after the addition of 0.2 M Na₂SO₄. However, EFs of SD, SM₁ and SM₂ increased remarkably with the content of Na₂SO₄ increasing and reached 128, 135, 100 at 2 M Na₂SO₄ in donor phase, the concentration in which Na₂SO₄ is almost saturated at room temperature. This phenomenon could be explained by different

Fig. 3. Effect of volume ratio of donor to acceptor on the extraction enrichment factor. DP: 4 mL, pH 4.5 KH₂PO₄ buffer solution; AP: 0.25 M NaOH; liquid membrane: [C₈MIM][PF₆] with 14% TOPO; shaking speed: 100 rpm; extraction time: 1 h. (□) SD; (○) SM₁; (Δ) SM₂; (▽) SMZ; (◁) SDM.

Fig. 4. Effect of extraction time on the extraction enrichment factor. DP: 4 mL, pH 4.5 KH₂PO₄ buffer solution; AP: 25 μ L, 0.25 M NaOH; liquid membrane: [C₈MIM][PF₆] with 14% TOPO; shaking speed: 300 rpm. (□) SD; (○) SM₁; (Δ) SM₂; (▽) SMZ; (◁) SDM.

Table 3
Analytical performance of the proposed extraction procedure.

Analytes	Linear range ($\mu\text{g/L}$)	Correlation equation	Correlation coefficient, r	Enrichment factor	Detection limit ($\mu\text{g/L}$)
SD	1–2000	$y = 6.5247x + 47.562$	0.9995	128	0.4
SM ₁	1–2000	$y = 6.7724x + 67.747$	0.9997	135	0.1
SM ₂	1–2000	$y = 6.232x + 67.338$	0.9997	100	0.3
SMZ	1–2000	$y = 5.6663x + 45.895$	0.9998	73	0.1
SDM	1–2000	$y = 2.8348x + 21.029$	0.9998	58	0.1

DP: standard solutions prepared in 4 mL, pH 4.5 KH_2PO_4 with 2 M Na_2SO_4 ; AP: 25 μL , 0.25 M NaOH; membrane liquid: $[\text{C}_8\text{MIM}][\text{PF}_6]$ with 14% TOPO; extraction time: 8 h with shaking speed 300 rpm.

Fig. 5. Effect of sample salinity on the extraction enrichment factor. DP: 4 mL, pH 4.5 KH_2PO_4 buffer solution; AP: 25 μL , 0.25 M NaOH; liquid membrane $[\text{C}_8\text{MIM}][\text{PF}_6]$ with 14% TOPO; shaking speed: 300 rpm; extraction time: 8 h. ($\square$) SD; ($\circ$) SM₁; ($\triangle$) SM₂; (∇) SMZ; ($\leftarrow$) SDM.

pKa and hydrophobic character of different compounds in donor solution. Because high salt content resulted in high EFs and was beneficial for longer extraction, 2 M Na_2SO_4 was added into the sample solution.

3.7. Matrix effect

Compounds with high molecular mass (such as humic acids that are commonly found in environmental matrices) are known to affect the ionization of lower mass molecules [46], and polar analytes such as sulfonamide antibiotics are the most susceptible to these effects [47]. Humic acid (0–25 $\mu\text{g/mL}$ DOC) and bovine serum albumin (0–100 $\mu\text{g/mL}$) as common high molecular compounds in environment and organisms were individually selected to study the matrix effect. The relative extraction efficiency was calculated by the peak area normalization method. The relative extraction efficiencies of all compounds were in the range of 97–104% and 98–113% over the studied concentration range of humic acid and BSA, respectively (see AppendixB Fig. S2 in Supplementary Material). The results indicated there was no significant matrix effect on the relative extraction efficiencies of all the analytes.

Table 4
Analyte concentration (C_0 , C_s $\mu\text{g/L}$) and spike recovery ($R = \text{mean} \pm \text{SD}$, $\%$, $n = 3$) in samples by the proposed method.

Samples	SD			SM ₁			SM ₂			SMZ			SDM		
	C_0	C_s	R	C_0	C_s	R	C_0	C_s	R	C_0	C_s	R	C_0	C_s	R
Wastewater ^a	N.D.	4.94	98.7 ± 5.8	0.12	5.24	102.4 ± 6.4	N.D.	4.67	93.3 ± 8.6	N.D.	4.46	89.1 ± 6.7	0.22	5.06	96.8 ± 6.4
Wastewater ^b	N.D.	5.04	82.5 ± 7.2	0.18	5.48	99.6 ± 5.9	N.D.	5.31	86.9 ± 7.5	N.D.	4.35	82.2 ± 5.5	0.15	4.72	91.4 ± 8.7
Duckery water	N.D.	5.16	103.2 ± 5.5	N.D.	4.16	83.2 ± 7.3	N.D.	4.78	95.5 ± 6.7	N.D.	4.89	97.7 ± 8.4	N.D.	4.91	98.2 ± 6.1
Fishery water	N.D.	4.75	95.0 ± 6.3	0.26	4.75	95.0 ± 5.6	N.D.	4.88	97.5 ± 7.6	N.D.	4.84	96.8 ± 7.6	0.2	4.92	94.3 ± 6.9
Paddy water	N.D.	4.64	92.7 ± 7.8	N.D.	5.05	101.5 ± 8.3	N.D.	4.60	90.2 ± 8.4	N.D.	4.59	91.8 ± 8.1	N.D.	4.60	91.9 ± 7.8

C_0 is the initial concentration of analytes without spike. C_s is the concentration of analytes determined by spiking 5 $\mu\text{g/L}$. N.D.: not detected.

^a Collected from a sewage reservoir in the pig farm.

^b Collected from a pool in the pig farm.

3.8. Method performance and real sample analysis

Calibration curves of the method were plotted by 15 spiking levels in the range of 1–4000 $\mu\text{g/L}$ for all compounds. Under the optimized conditions, wide linearity range (1–2000 $\mu\text{g/L}$), good correlative coefficients ($r = 0.995$ – 0.9998) and satisfactory enrichment factors (58–135) were obtained for the analytes. The limits of detection (LODs) were calculated at a signal-to-noise ratio of 3. The LODs ($\mu\text{g/L}$, with corresponding compounds in the parentheses) were 0.4 (SD), 0.1 (SM₁), 0.3 (SM₂), 0.1 (SMZ), and 0.1 (SDM). Details about the parameters for the analytical performance of the proposed method can be found in Table 3. According to the research by Balakrishnan et al. [48], SPME and SPE were successfully employed for the determination of SAs. The LODs were respectively 9.04–55.3 $\mu\text{g/L}$ of SPME and 2.88–9 ng/L of SPE. The SPME responses of sulfonamide antibiotics adhered to non-linear equations and all of studied SAs followed an excellent linear relationship with coefficients of determination ($r > 0.97$) by SPE. By a comparison of the performances of the present method with SPME and SPE, the sensitivities of HF-LPME were better than SPME and poorer than SPE; the LODs of HF-LPME were higher than SPME and lower than SPE. Whereas, the total sample volume required was 4 mL in the present work less than in SPE (500 mL) and the organic solvents was used in HF-LPME dozens of times less than in SPE. Therefore, it could be concluded that the present work was practicable for the determination of SAs in environmental water samples.

Five environmental water samples were analyzed to test the applicability of the proposed extraction procedure. One group of samples was extracted by the proposed procedure; the parallel group of samples was analyzed after spiking with 5 $\mu\text{g/L}$ of target compounds. As shown in Table 4, only SM₁ (0.12–0.26 $\mu\text{g/L}$) and SDM (0.15–0.22 $\mu\text{g/L}$) were detected in wastewater from the pig and fish farm, whereas other compounds were not detected in any sample. Good spike recovery (82–103%) was obtained. Moreover, the method displayed good reproducibility with RSD values in the range of 0.5–4.8% ($n = 3$). Fig. 6 shows the HPLC chromatograms obtained by the proposed procedure: (a) 20 $\mu\text{g/L}$ standard solution; (b) water sample spiked with 5 $\mu\text{g/L}$ of each analyte; and (c) blank water sample. It was further demonstrated that the method showed effectively the interference free performance.

Fig. 6. Chromatograms obtained by UV detection at 265 nm for (a) standard solution of 20 µg/L, (b) blank water sample spiked with 5 µg/L and (c) blank water sample. 1, SD; 2, SM₁; 3, SM₂; 4, SMZ; 5, SDM.

4. Conclusions

A hollow fiber supported ionic liquid based three-phase LPME was developed to detect trace level of amphoteric sulfonamides in environmental water samples. With the precise control of donor and acceptor pH and the addition of TOPO, as suitable additive into the ionic liquid membrane, the method provided high *EFs* and combined extraction, enrichment and clean-up using small volume of samples in one step. In addition, ionic liquid as membrane phase would not only provide good extractability for SAs but also form an efficient barrier to big molecule compounds in complicated matrix. With few organic solvents being used in the total procedure, the proposed extraction procedure can be considered as an environmentally friendly, safe and simple technique for analyzing sulfonamides residues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.06.025.

References

- [1] K. Dost, D.C. Jones, G. Davidson, *Analyst* 125 (2000) 1243.
- [2] W. Baran, J. Sochacka, W. Wardas, *Ecotoxicol. Environ. Safe.* 63 (2006) 113.
- [3] K. Kümmerer, *Chemosphere* 45 (2001) 957.
- [4] M. Carballa, F. Omil, J.M. Lema, M. Llombart, C. García-Jares, I. Rodríguez, M. Gómez, T. Ternes, *Water Res.* 38 (2004) 2918.
- [5] N.A. Littlefield, W.G. Sheldon, R. Allen, D.W. Gaylor, *Food Chem. Toxicol.* 28 (1990) 157.
- [6] M.S. Díaz-Cruz, M.J. López de Alda, D. Barceló, *Trends Anal. Chem.* 22 (2003) 340.
- [7] J. Jung, Y. Kim, J. Kim, D.H. Jeong, K. Choi, *Ecotoxicology* 17 (2008) 37.
- [8] C.H. Huang, J.E. Renew, K.L. Smeby, K. Pinkston, D.L. Sedlak, The 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, Minneapolis, MN, USA, 2001.
- [9] US FDA, Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications. CMC 6 Revision 1. Food and Drug Administration, Rockville, MD, 1998. Available at <http://www.fda.gov/cder/guidance/1730fnl.pdf> (last accessed 10/19/2004).
- [10] EMEA, Draft CPMP discussion paper on environmental risk assessment of non-genetically modified organism (non-GMO) containing medicinal products for human use. CPMP/SWP.4447/00. European Agency for the Evaluation of Medicinal Products, London, 25 January 2001.
- [11] N. Assassi, A. Tazerouti, J.P. Canselier, *J. Chromatogr. A* 1071 (2005) 71.
- [12] A. Cannavan, S.A. Hewitt, W.J. Blanchflower, D.G. Kennedy, *Analyst* 121 (1996) 1457.
- [13] B. Chiavarino, M.E. Crestoni, A. Di Marzio, S. Fornarini, *J. Chromatogr. B* 706 (1998) 269.
- [14] C.Y. Lin, S.D. Huang, *Anal. Chim. Acta* 612 (2008) 37.
- [15] G.Z. Fang, J.X. He, S. Wang, *J. Chromatogr. A* 1127 (2006) 12.
- [16] J. Raich-Montiu, J. Folch, R. Compañó, M. Granados, M.D. Prat, *J. Chromatogr. A* 1172 (2007) 186.
- [17] K.H. Lu, C.Y. Chen, M.R. Lee, *Talanta* 72 (2007) 1082.
- [18] T. Li, Z.G. Shi, M.M. Zheng, Y.Q. Feng, *J. Chromatogr. A* 1205 (2008) 163.
- [19] L. Verzeznassi, M.C. Savoy-Perroud, R.H. Stadler, *J. Chromatogr. A* 977 (2002) 77.
- [20] M.Y. Haller, S.R. Müller, C.S. Mc Ardell, A.C. Alder Marc, J.F. Suter, *J. Chromatogr. A* 952 (2002) 111.

- [21] G. Font, A. Juan-García, Y. Picó, J. Chromatogr. A 1159 (2007) 233.
- [22] J. Raich-Montiu, J. Folch, R. Compañó, M. Granados, M.D. Prat, J. Chromatogr. A 1172 (2007) 186.
- [23] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
- [24] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
- [25] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
- [26] S. Pedersen-Bjergaard, T.S. Ho, K.E. Rasmussen, J. Sep. Sci. 25 (2002) 141.
- [27] S. Andersen, T.G. Halvorsen, S. Pedersen-Bjergaard, J. Chromatogr. A 963 (2002) 303.
- [28] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Uglund, T. GrPnhaug, J. Chromatogr. A 873 (2000) 3.
- [29] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 3.
- [30] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. B 760 (2001) 219.
- [31] J.F. Liu, J.Á. Jönsson, G.B. Jiang, Trends Anal. Chem. 24 (2005) 20.
- [32] R. Liu, J.F. Liu, Y.G. Yin, X.L. Hu, G.B. Jiang, Anal. Bioanal. Chem. 393 (2009) 871.
- [33] J.F. Liu, G.B. Jiang, Y.G. Chi, Y.Q. Cai, Q.X. Zhou, J.T. Hu, Anal. Chem. 75 (2003) 5870.
- [34] J.F. Liu, Y.G. Chi, G.B. Jiang, C. Tai, J.F. Peng, J.T. Hu, J. Chromatogr. A 1026 (2004) 143.
- [35] R. Fortunato, C.A.M. Afonso, M.A.M. Reis, J.G. Crespo, J. Membr. Sci. 242 (2004) 197.
- [36] J.F. Peng, J.F. Liu, X.L. Hu, G.B. Jiang, J. Chromatogr. A 1139 (2007) 165.
- [37] J. Lezamiz, J.Á. Jönsson, J. Chromatogr. A 1152 (2007) 226.
- [38] Y. Shen, V. Obuseng, L. Grönberg, J.Á. Jönsson, J. Chromatogr. A 725 (1996) 189.
- [39] D.W. Kou, X.Y. Wang, S. Mitra, J. Chromatogr. A 1055 (2004) 63.
- [40] G.H. Zhao, J.F. Liu, M. Nyman, J.Á. Jönsson, J. Chromatogr. B 846 (2007) 202.
- [41] Y. Yamini, C.T. Reimann, A. Vatanara, J.Á. Jönsson, J. Chromatogr. A 1124 (2006) 57.
- [42] A. Drapala, J.Á. Jönsson, P. Wiczorek, Anal. Chim. Acta 553 (2005) 9.
- [43] R. Fortunato, C.A.M. Afonso, J. Benavente, E. Rodriguez-Castellón, J.G. Crespo, J. Membr. Sci. 256 (2005) 216.
- [44] A. Pagliara, P.A. Carrupt, G. Caron, P. Gaillard, B. Testa, Chem. Rev. 97 (1997) 3385.
- [45] L. Chimuka, N. Megersa, J. Norberg, L. Mathiasson, J.Á. Jönsson, Anal. Chem. 70 (1998) 3906.
- [46] J.L. Sterner, M.V. Johnston, G.R. Nicol, D.P. Ridge, J. Mass Spectrom. 35 (2000) 385.
- [47] R. Bonfiglio, R.C. King, T.V. Olah, K. Merle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
- [48] V.K. Balakrishnan, K.A. Terry, J. Toito, J. Chromatogr. A 1131 (2006) 1.