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A sensitive emulsification liquid phase microextraction coupled with on-line phase separation followed by HPLC for trace determination of sulfonamides in water samples

Behnam Ebrahimpour · Yadollah Yamini · Maryam Rezazadeh

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Abstract For the first time, ion-pair based emulsification liquid phase microextraction coupled with a novel approach for phase separation followed by high performace liquid chromatgraphy (HPLC) was utilized for trace determination of sulfonamides in water samples. After the formation of ion-pair complex with a cationic surfactant, sulfonamides were extracted into the drops of dispersed organic extracting solvent. Then, the cloudy solution was passed through an in-line filter located in a suitable holder and was separated based on emulsion filtration. By changing the HPLC valve position, the filter was laid in the mobile phase path, and the extraction phase was eluted by the mobile phase and introduced into the separation column for analysis. The effects of important parameters, such as type of extraction solvent, type of ion-pair agent and its concentration, pH of sample solution, ionic strength, and volume of extraction phase, on the extraction efficiency, were investigated and optimized. Under optimal conditions, the linear range, limits of detection, and precision (relative standard deviations) were 0.3–100, 0.1–0.3 μg L^{-1}, and 4.7–5.8 %, respectively. Preconcentration factors (PFs) for the compounds studied were obtained in the range of 268–664. These PFs correspond to extraction recoveries in the range of 41–97 %. The sample throughput of the method was 3 samples per hour, regarding 20 min analysis time for a single procedure. Finally, the method was successfully applied to determine the selected sulfonamides in some water samples.

Keywords Ion-pair based emulsification microextraction · On-line phase separation · Emulsion filtration · Aliquat-336 · High-performance liquid chromatography · Sulfonamides

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Introduction

Agriculture and animal husbandry mostly utilize concentrated feeding operations, and therefore, various antibiotics are used to improve the productivity (Lin and Huang 2008). Sulfonamides (SAs), also known as "sulfa drugs," derived from sulfanilamide (p-aminobenzenesulfonamide) are widely used in both veterinary and human medicine (Evanthia et al. 2010). The presence of SAs in the aquatic environment has two risks. These compounds are (a) potentially toxic to aquatic organisms and to humans (through drinking water), and (b) furthermore, they induce drug resistance in disease-causing bacteria (Yang and Carlson 2003). After normal applications, SAs and/or their metabolites can be excreted from human body or animal organisms through urine and feces (Baran et al. 2006). Ultimately, these residues can find their way into the environment, and consequently, trace of SAs has been frequently detected in surface waters worldwide (Kümmerer 2001). They can be present in the environment (soils, ground, and surface waters) for a long period of time, leading to the appearance of antimicrobial resistance

(Dı'az-Cruz et al. 2003). Thus, there is a great need to monitor the trace levels of these compounds in the discharges of SAs from agriculture, animal husbandry, and environmental samples.

So far, various detection methods including liquid chromatography (HPLC; Costi et al. 2010; Soto-Chinchilla et al. 2007), capillary electrophoresis (Lin et al. 2010), gas chromatography–mass spectrometry (Stanway et al. 2007), and electrochemical methods (Msagati and Ngila 2002; Campestrini et al. 2010) have been widely used for analysis of SAs. Also, numerous classic extraction methods such as dissolution (Caballero et al. 2001; Vinas et al. 1995), solid-phase extraction (SPE; Posyniak et al. 2002), and liquid–liquid extraction (LLE) followed by SPE (Maudens et al. 2004) have been developed for sample treatment of SAs.

One of the most important objectives of modern analytical chemistry is miniaturization, simplification, and automation of the whole analytical procedure, especially to speed up the sample treatment which is currently the bottleneck of analysis. As a result, since the implementation of SPE, conventional LLE has been overshadowed, though it is currently undergoing further development as demonstrated by novel miniaturized preconcentration techniques using only small volumes of organic solvents (Kocúrová et al. 2010).

Microextraction techniques are environmentally friendly, less expensive, and simple to operate. Liquid phase microextraction (LPME) techniques using small volumes of solvent as extraction phase have emerged as new attractive alternative for traditional sample preparation methods since 1996 (Jeannot and Cantwell 1996). Recently, some LPME methods were published for sample treatment in the analysis of SAs (Lin and Huang 2008; Costi et al. 2010). One of the LPME methods is dispersive liquid–liquid microextraction (DLLME) that was firstly proposed in 2006 by Rezaee et al. (2006) and received increasing attentions due to its advantages such as simplicity of operation, rapidity, low cost, high-recovery, high enrichment factor, and environmental benignity, with wide application prospects in trace analysis (Zang et al. 2009).

A new version of DLLME, namely surfactant-assisted dispersive liquid–liquid microextraction (SA-DLLME), based on surfactant as disperser agent, was reported in 2010 (Moradi et al. 2010). In comparison with traditional DLLME, SA-DLLME uses small amounts of environmentally friendly emulsifier agents (Ebrahimpour et al. 2012a, b). The main drawback of DLLME or SA-DLLME is their inability to extract hydrophilic compounds into the extraction solvent. To overcome this limitation, a new method for extraction of ionic and polar compounds has been reported, namely ion-pair based surfactant assisted microextraction (Saleh et al. 2009). In this new methodology, analytes are extracted as their ion-pair (IP) complexes with an ionic surfactant that also acts as emulsifier agent. IP extraction is a method for partitioning the ionic compounds with the aid of lipophilic counter ions. IP liquid phase microextraction is also a possible approach to extract polar compounds from an aqueous phase directly to an organic phase.

Centrifugation, which is considered to be the most time-consuming step in these methods, has been applied in the majority of DLLME and emulsification liquid phase microextraction (ELPME) procedures up to now (Zhang et al. 2011). Also, it is assumed as a bottleneck in the automation of these techniques. In the recent published work of our research group (Ebrahimpour et al. 2012a, b), a new method based on emulsion filtration was introduced for phase separation instead of centrifugation, making it possible to use DLLME and ELPME methods as on-line approaches coupled to HPLC. In the present study, a sensitive and effective microextraction method combined on-line with HPLC was introduced to extract and analyze trace levels of SAs in different water samples.

Materials and methods

Chemicals and reagents

Standards of SAs (sulfocetamide (SC), sulfothiazole (ST), sulfomethazine (SM), and sulfometoxazole (SX)) were obtained from Sigma (St. Louis, MO, USA). Stock standard solutions of SAs were prepared in methanol. A mixed standard solution was prepared by adding an appropriate amount of each stock standard solution into a 50-mL volumetric flask and diluting it to the mark by methanol. The concentrations of SAs were 10, 20, and 200 mg L^{-1} in the mixed standard solutions. Working standards were prepared by spiking the aqueous solution with appropriate amounts of the mixed standard solution. All standard solutions were stored at 4 °C in a fridge. HPLC-grade methanol and acetonitrile were purchased from Caledon (Georgetown, ON, Canada). Toluene, 1-octanol, carbon tetrachloride, and chloroform

were purchased from Merck (Darmstadt, Germany) and were used as extraction solvents. Tetradecyl trimethyl ammonium bromide, TTAB ($C_{17}H_{38}BrN$), and cetyl trimethyl ammonium bromide, CTAB ($(C_{16}H_{33})N(CH3)_3Br$), were purchased from Sigma. Trioctylmethylammonium chloride, Aliquat-336 ($C_{25}H_{54}ClN$), was obtained from Fluka (Buchs, Switzerland). All other chemicals used were of reagent grade or of the highest purity available. Ultra-pure water was prepared by an Aqua Max-Ultra Youngling ultra-pure water purification system (Dongan-gu, South Korea). Plastics and glassware used for the experiments were previously washed with acetone and rinsed carefully with ultra-pure water.

Apparatus and HPLC conditions

Separation and detection of target analytes were performed by a Varian HPLC comprising a 9012 HPLC pump (California, USA), a six-port Cheminert HPLC valve from Valco Instruments (Houston, TX, USA), and a Varian 9050 UV–vis detector. Chromatographic data were recorded and analyzed using Chromana software version 3.6.4 from Marjaane Khatam (Tehran, Iran). The separations were carried out on an ODS-3 column (250 mm×4.6 mm, with 5 μm particle size) from MZ-Analysentechnik (Mainz, Germany). The elution was carried out under gradient conditions consisting of phase A (20 mmol L^{-1} of ammonium acetate buffered at pH= 5.0) and phase B (acetonitrile) running at flow rate of 1 mL min^{-1} in the following way: After sample loading (or standard solution injection), the linear gradient started from 90 % A: 10 % B and increased to 45 % A: 55 % B within 15 min. Then, within 3 min after the linear gradient of the run, the mobile phase composition was changed to 100 % B, and this condition was remained for 9 min. Finally, within 3 min the mobile phase composition was returned to the starting conditions and held constant for another 3 min before the next run. The detection was performed at the wavelength of 260 nm.

The filter used for separation of the extraction solvent was a traditional HPLC in-line filter (made of polytetrafluoroethylene (PTFE) with dimensions of 10 mm length and 4.3 mm outer diameter) from Agilent (Palo Alto, CA, USA)). It was fixed in a holder made of stainless steel with 30 mm length, 4.3 mm i. d., and 5.0 mm o. d.

IP–ELPME followed by on-line phase separation

Aliquots of 10 mL sample solution containing the analytes were adjusted to pH 10 and placed in a 12-mL glass vial. A 0.5-mL of pure water (pH= 10), containing 30 μL of extraction phase (12 % (*w*/*v*) of Aliquat-336 as emulsifier and ion-pairing agent in carbon tetrachloride), was injected rapidly into the sample solution by using a 5.0-mL gas-tight syringe. As a result, a cloudy solution was formed in the test tube. In this step, the extraction solvent was dispersed in the aqueous sample as very fine droplets. This process greatly enlarged the contact area between the extraction solvent and the aqueous phase. Afterwards, the ion pair-complex of SAs with Aliquat-336 was extracted into the fine droplets of extraction solvent. Then, phase separation was performed at two following stages: (1) Loading step: the emulsion (nanometer dispersed droplets of carbon tetrachloride in aqueous phase) was passed through the in-line filter (approximately with flow rate of 3 mL min^{-1}) and separation of the organic phase was performed based on emulsion filtration. (2) Injection step: by changing the position of the HPLC valve, the in-line filter was located in the path of the mobile phase. The organic phase was eluted and introduced into the HPLC column. After separation and detection of the analytes, the filter was washed with acetonitrile (mobile phase of HPLC) at the end of each HPLC run and prepared for the next separation. The schematic design of the procedure is shown in Fig. 1.

Calculation of preconcentration factor, extraction recovery, and relative recovery

Preconcentration factor (PF) was defined as the ratio of the final analyte concentration in the extraction phase ($C_{f,a}$) to the initial concentration of analyte ($C_{i,s}$) in the sample solution.

$$PF = \frac{C_{f,a}}{C_{i,s}} \quad (1)$$

where $C_{f,a}$ was calculated from a calibration graph obtained from direct injection of SAs standard solutions (5–100 mg L^{-1}) into methanol. Extraction recovery

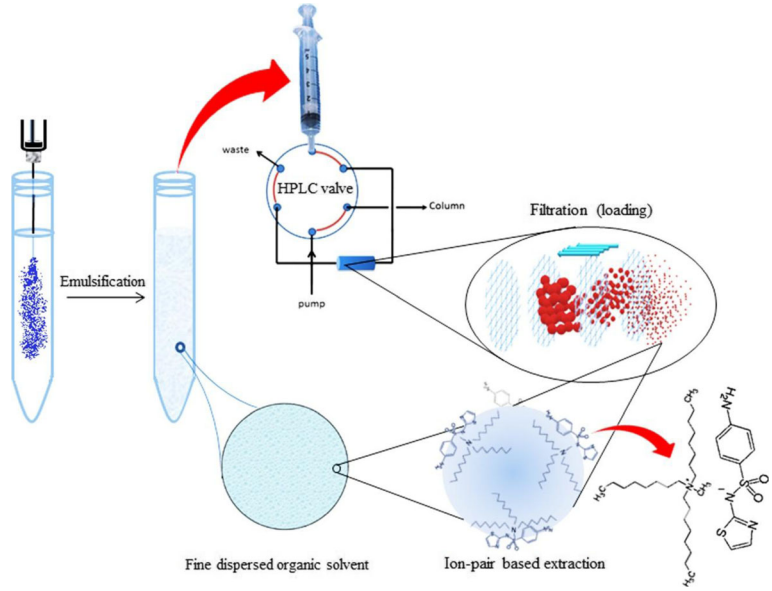


Fig. 1 Schematic design of the microextraction procedure coupled on-line to HPLC and separation of phases using in-line filter. In-line PTFE filter was inserted in its holder and was located in the loop position of the HPLC valve. In loading step, the extraction phase was trapped in the filter; then, by changing the valve to the injection position, the trapped phase was eluted from inverse direction by mobile phase and transferred to HPLC column

(ER) was calculated for each analyte according to the following equation (Eq. (2)):

$$ER(\%) = \frac{n_{f,a}}{n_{i,s}} \times 100 \quad (2)$$

where $n_{i,s}$ and $n_{f,a}$ refer to the number of moles of analyte initially present in the sample solution and the number of moles of analyte present in the extraction phase, respectively.

Relative recovery (RR) was acquired from the following equation (Eq. (3)):

$$RR(\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

Where C_{found}, C_{real}, and C_{added} are the concentration of analyte after addition of a known amount of standard into the real sample, the concentration of analyte in the real sample, and the concentration of a known amount of standard spiked into the real sample, respectively.

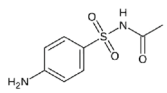
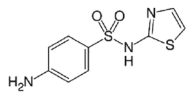
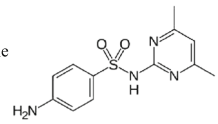
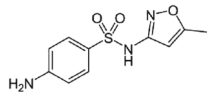
Results and discussion

In view of properties of the four target analytes (Table 1), presence of the amino and sulfanilamido groups would cause a two-step protolysis and increase their hydrophilicity, impeding their direct extraction into hydrophobic media (log P<1). Theoretically, it is feasible to control the ionized and non-ionized forms of SAs by adjusting the pH. In addition, the hydrophobicity of the neutral species is logically greater than that of their corresponding ions (Pagliara et al. 1997). When pH is adjusted to the average of pK_{a1} and pK_{a2} of SAs, the neutral molecule form is the dominant species (Lin and Huang 2008). Therefore, the hydrophilic–hydrophobic character of SAs can be changed by adjusting the pH.

In preliminary experiments, the pH of the sample solution was adjusted to the average of pK_{a1} and pK_{a2} (in the ranges of 4.0–6.0) of the target SAs to convert them into their neutral forms, and then the extraction was performed. Due to high polarity, none of the target analytes were extracted into the extraction solvents (1-octanol, dihexyl ether, carbon tetrachloride, toluene, and chloroform) or the extraction efficiencies were very low. Therefore, by adjusting the pH of sample solution, the target analytes were converted to their anionic forms in order to produce IP complexes with a cationic surfactant that can be extracted into the organic solvent (IP based LPME).

In order to optimize the ion-pair based emulsification liquid phase microextraction (IP-ELPME) of SAs from aqueous solutions, several parameters controlling the optimum performance of extraction were studied. There are different factors that affect the IP-ELPME and the partition coefficient of the analyte-surfactant IP complexes between the organic solvent and the aqueous phase. Some of these factors include type of the extraction solvent, type of ion-pairing reagent and its concentration, ionic strength, pH of sample solution, and volume of extraction phase. These parameters were

Table 1 Chemical structures and some physicochemical properties of the studied sulfonamides

Analyte	Chemical structure	Log*P*	MW[a]	pK_a
Sulfacetamide		-0.26	236.22	1.8, 5.4
Sulfathiazole		0.35	255.32	1.8, 7.24
Sulfamethazine		0.43	278.33	1.8, 7.45
Sulfamethoxazole		0.79	253.28	1.7, 5.81

separately evaluated to develop the optimized extraction conditions. The chromatographic peak area was used as the analytical signal to evaluate the influence of factors on the extraction efficiencies of the target analytes using on-line microextraction procedure.

Optimization of IP-ELPME

Effect of extraction solvent

The selection of an extraction solvent is of great importance in solvent microextraction methods in order to achieve efficient extraction. The selection of a suitable extraction solvent is limited by several characteristics: the solvents must be able to extract the analytes of interest, have low water solubility, and be compatible with the analytical instrumentation to be used.

On the basis of the procedure introduced, phase separation was performed based on filtration, so this method possesses the merits that there is no need to use centrifugation and special vessels. Therefore, any extraction solvent could be used without regarding its density. A number of solvents, including: toluene, 1-octanol, dihexyl ether, chloroform and carbon tetrachloride, were selected as extracting solvents.

A 0.5-mL of ultra-pure water (with adjusted pH equal to the sample solution pH), containing 40 μL of extraction phases (2 % *w/v* of Aliquat-336 in each organic solvent), was injected into the sample solution (10 mL) by a 5.0-mL gas-tight syringe, and the emulsion formed was passed through the in-line filter. As shown in Fig. 2, the extraction efficiencies obtained using chloroform or carbon tetrachloride as extracting solvents are higher than those achieved from other studied solvents. Despite the high extraction efficiencies, chloroform changed the Gaussian shape of the peaks, and thus, it was not used in the next experiments. Carbon tetrachloride was selected as the suitable extracting solvent in the subsequent experiments.

Effect of type of surfactant as ion-pairing and emulsifier reagent

As mentioned in the previous section, SAs have very low solubility in organic solvents in their molecular forms. By increasing the pH to basic conditions, SAs are converted to their anionic forms and can form IP complex with a cationic surfactant. Therefore, they can be extracted into the organic extracting solvent.

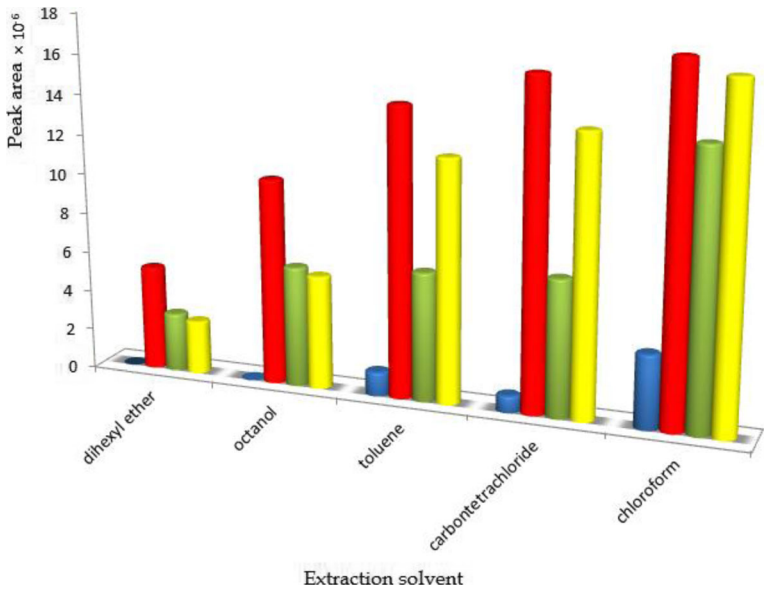


Fig. 2 Effect of extraction solvent on the peak areas of analytes. Concentrations of ST, SM, SX, 50 μg L^{-1} and for SC, 100 μg L^{-1}; sample volume, 10 mL; pH=10; extracting phase: 2 % (*w*/*v*) of Aliquat-336 in each solvent

Surfactants are organic compounds that are amphiphilic so that they contain both hydrophobic and hydrophilic groups. Therefore, they are soluble in both organic solvents and water. Surfactants reduce the interfacial tension between oil and water by adsorbing at the liquid–liquid interface. This phenomenon can contribute to the dispersion of organic solvent in aqueous phase (acting as emulsifier reagent).

Two inherent specifications of ionic surfactants (emulsification and IP formation) in extraction phenomena were used to extract SAs simultaneously. Different surfactants (TTAB, CTAB, and Aliquat-336) were investigated as IP reagents. The obtained results revealed that the Aliquat-336 has the highest efficiency for producing the IP complex, and thus, extracting the SAs among the selected surfactants. Therefore, Aliquat-336 was used as emulsifier and IP reagent in the subsequent experiments.

Effect of pH

As mentioned in the previous section, theoretically, it is feasible to control the ionized and non-ionized forms of SAs by adjusting the pH. At basic conditions, the anionic form is the dominant species, and therefore, hydrophobic IP complexes can be formed and SAs can be extracted into the organic phase.

In order to study the effect of pH of sample solution on the extraction efficiency, the pH was changed in the range of 6.0–12.0. Figure 3 reveals that when pH increases, the molecules are changed to their anionic forms, and thus, the extraction efficiency improves due to an increase in IP formation. As pH was increased to 10.0 in the sample solution, a significant growth in the peak areas was observed. Thus, at pH=10.0, Aliquat-336 emulsifies carbon tetrachloride in the sample solution, and in addition, forms IP complexes with the analyte ions and efficiently extracts them into the organic solvent. Thus, the pH of sample solution was adjusted to 10.0 for subsequent experiments.

Surfactant concentration

The concentration of surfactant (as emulsifier and ion-pairing reagent) in IP-ELPME is a critical factor (Ebrahimpour et al. 2012a, b). As the Aliqut-336 concentration was increased from 1.0 to 12.0 % (*w*/*v* in carbon tetrachloride), the extraction efficiencies increased (Fig. 4) due to the formation of finer droplets, causing an improved mass transfer into the organic phase and well IP formation. Further increase in the concentration of surfactant did not change the extraction efficiencies significantly. Based on the obtained results, the best extraction efficiencies were achieved by using 12.0 % Aliquat-336 in carbon tetrachloride.

Effect of salt addition

The influence of salt addition on the extraction efficiency was investigated by increasing the NaCl

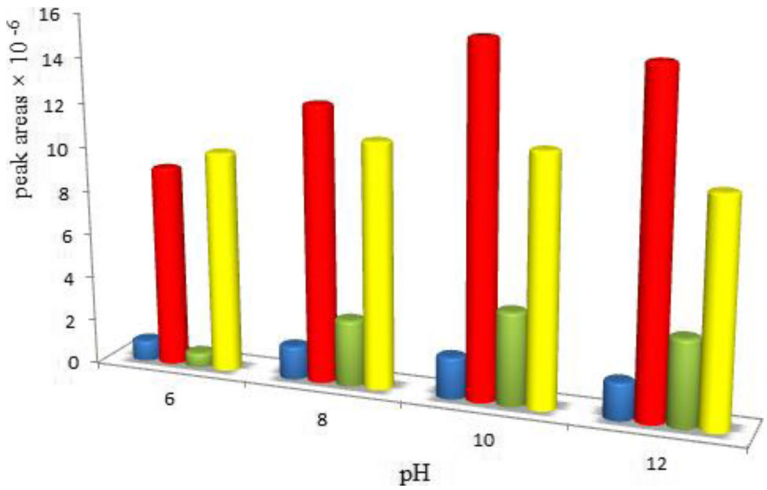


Fig. 3 Effect of sample pH on the peak areas of analytes. Carbon tetrachloride as extraction solvent and pH of the sample solution was varied; other conditions were the same as Fig. 2

concentration in the sample solution in the range of 0–6 (% *w*/*v*). Figure 5 indicates that the presence of salt reduces the extraction efficiencies. Two main competitive effects occur in LPME when an increase in the salt concentration takes place, namely, salting-out effect and change of the physical properties of the Nernst diffusion film. In the case of salting-out effect, salt addition has a favorable impact on the extraction since it decreases water solubility of the analyte, thus, improving mass transfer to the organic phase. On the contrary, it has been reported that the diffusion rate of the analyte into the extracting phase may decrease due to the change in the properties of the Nernst diffusion film. Also, the presence of the salt reduces the efficiency of emulsification so that the extraction efficiency may be decreased. Moreover, the presence of anions can reduce the ion-pairing efficiency. Therefore, the next experiments were performed without salt addition.

Effect of extracting phase volume

To study the effect of extracting phase (14 % (*w*/*v*) Aliquat-336 in carbon tetrachloride) volume, different volumes of the organic phase in the range of 10–45 μL were subjected to IP-ELPME while other parameters were kept constant (Fig. 6).

As can be seen in Fig. 6, the extraction recoveries are increased by increasing the extraction phase volume up to 30 μL, which is well expected. After that, the ERs were decreased by further increase in the extracting

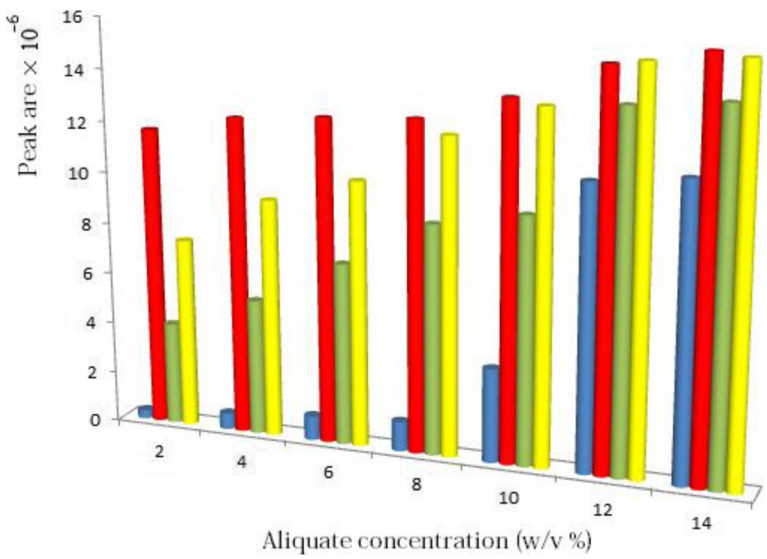


Fig. 4 Effect of ion-pair agent concentration on the peak areas of analytes. Sample solution pH was adjusted to 10.0 and concentration of Aliquat-336 in extracting phase was varied; other conditions were the same as Fig. 3

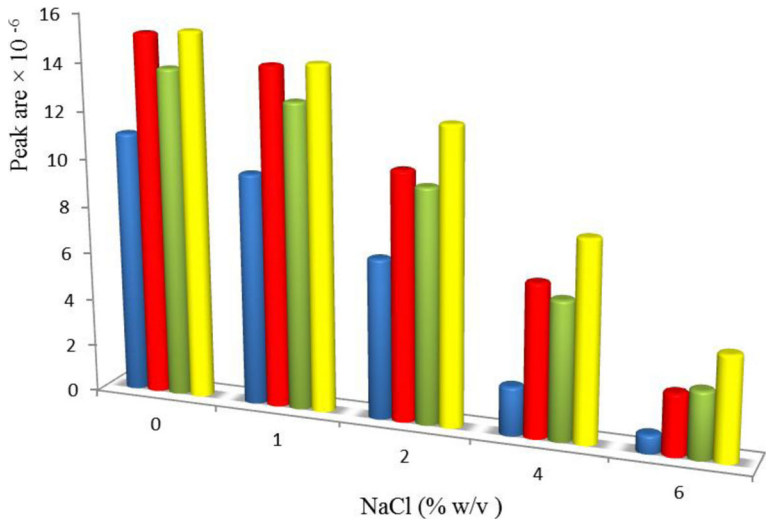


Fig. 5 Effect of ionic strength on the peak areas of analytes. Extracting phase, 12 % (*w*/*v*) Aliquat-336 in carbon tetrachloride, and NaCl was added in order to study the effect of salt addition. Other conditions were the same as Fig. 4

phase volume (from 30 to 45 μL). It could be said that the filtration was not performed well in volumes more than 30 μL and resulted in losing some amount of the extraction phase. According to the obtained results, 30 μL was selected as the optimum volume of the extracting phase for extraction and determination of SAs.

Effect of flow rate of sample loading

When the formed emulsion in the ELPME procedure was passed through the in-line filter, the drops of dispersed extraction phase were combined together and the extraction phase was filtered and separated from water medium. The effect of loading speed of emulsified solution was studied using the flow rates of 1, 2, and 3 mL min^{-1}. The results showed that the loading speed of the sample within the studied range did not have significant effect on the recoveries of the analytes. Therefore, loading speed of 3 mL min^{-1} was used for subsequent experiments.

Method validation

Analytical performance of the method

The figures of merit of the proposed on-line microextraction method, including PFs, ERs, linear ranges, and limits of detection (LODs), for the extraction of target analytes from 10-mL aqueous solutions

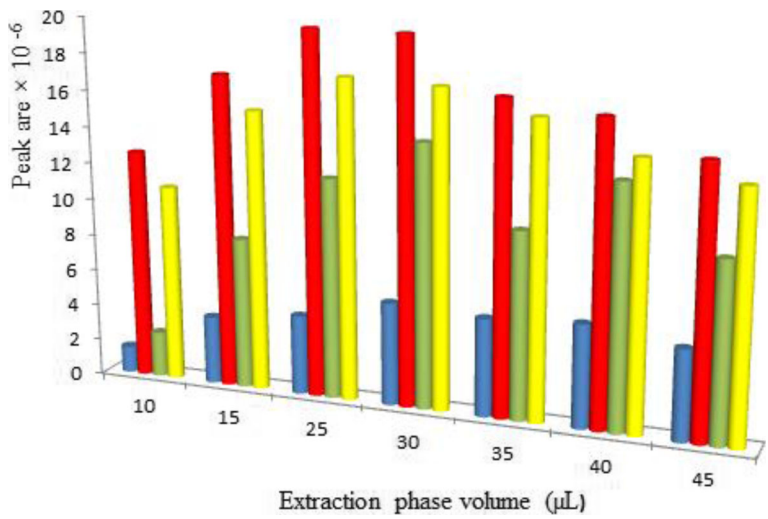


Fig. 6 Effect of volume of extracting phase on the peak areas of analytes. Conditions as Fig. 5, except for the volumes of extracting phase and without NaCl addition

Table 2 Figures of merit for on-line IP-ELPME of target analytes

Analyte	R^2	m[a]	b[b]	LOD[c]	LR[c]	PF	ER[d]	RSD[e]	RSD[f]
SC	0.9988	55,888	6166	0.3	0.5–100	268	41	5.83	5.23
ST	0.9978	202,077	31,102	0.1	0.3–100	664	98	5.03	5.14
SM	0.9992	145,408	5944	0.1	0.3–100	534	82	5.18	4.89
SX	0.9980	175,222	10,323	0.1	0.3–100	648	97	5.62	4.78

[a] Slope of the calibration curve

[b] Intercept of the calibration line

[c] Concentrations are reported in micrograms per liter

[d] Extraction recovery (%)

[e] RSDs (%) were calculated at concentration of 10 $\mu g L^{-1}$ (n=3)

[f] RSDs (%) were calculated at concentration of 25 $\mu g L^{-1}$ (n=3)

were investigated under optimal conditions. The results are summarized in Table 2. Calibration curves were plotted using ten spiking levels of analytes in the concentration range of 0.1–100 μg L^{-1} for SAs. For S/N=3, LODs were in the range of 0.1–0.3 μg L^{-1} for the analytes. The PFs for the 10-mL aqueous sample

Table 3 Determination of sulfonamides in water samples

Sample	analyte	$C^a_{initial}$	C_{added}	C^b_{found}	RSD[c] (%)	RR (%)
Tap water	SC	n.d.[d]	10	9.9	5.2	99.0
			25	25.6	5.1	102.4
	ST	n.d.	10	9.7	5.7	97.0
			25	24.4	5.1	97.6
	SM	n.d.	10	9.7	5.5	97.0
			25	25.1	5.3	100.4
	SX	n.d.	10	9.9	5.1	99.0
			25	24.7	5.1	98.8
Effluent water	SC	n.d.	10	9.3	5.7	93.0
			25	23.6	5.3	94.4
	ST	n.d.	10	9.7	5.9	97.0
			25	24.3	5.6	97.2
	SM	n.d.	10	9.4	5.8	94.0
			25	24.3	5.3	97.2
	SX	n.d.	10	9.6	5.5	96.0
			25	23.8	5.1	95.2
Influent water	SC	n.d.	10	9.0	6.0	90.0
			25	22.3	5.8	89.2
	ST	3.2	10	12.4	5.8	92.0
			25	26.7	5.6	94.0
	SM	n.d.	10	9.2	6.1	92.0
			25	22.6	5.9	90.4
	SX	n.d.	10	9.6	5.7	96.0
			25	23.6	5.3	94.4

[a] Concentration of analytes (micrograms per liter) in the sample

[b] Concentration of analytes (micrograms per liter) in the sample after spiking of target analytes

[c] Relative standard deviation (n=3)

[d] Not detected

were 268, 664, 534, and 648 for SC, ST, SM, and SX, respectively. These PFs correspond to the ERs of 40, 98, 82, and 97 %, respectively. The precision of the proposed IP-ELPME followed by on-line phase separation coupled to HPLC-ultraviolet (HPLC-UV) method was evaluated in terms of repeatability, and relative standard deviations in (RSDs) in percentage <5.9 (n=3) were obtained.

Possibility of carry over effect

Some experiments were designed in order to study the possibility of carry over effect. After a loading and elution procedure, another run was performed without loading the sample (this experiment was repeated three times at three different concentration levels). The obtained results showed that there was no carry over effect between the runs and that one in-line filter could be used several times without any problems.

Real sample analysis

To evaluate the applicability of the proposed method, it was applied for extraction and determination of the analytes in influent and effluent water samples from waste water refinery (Tehran, Iran) and in tap water

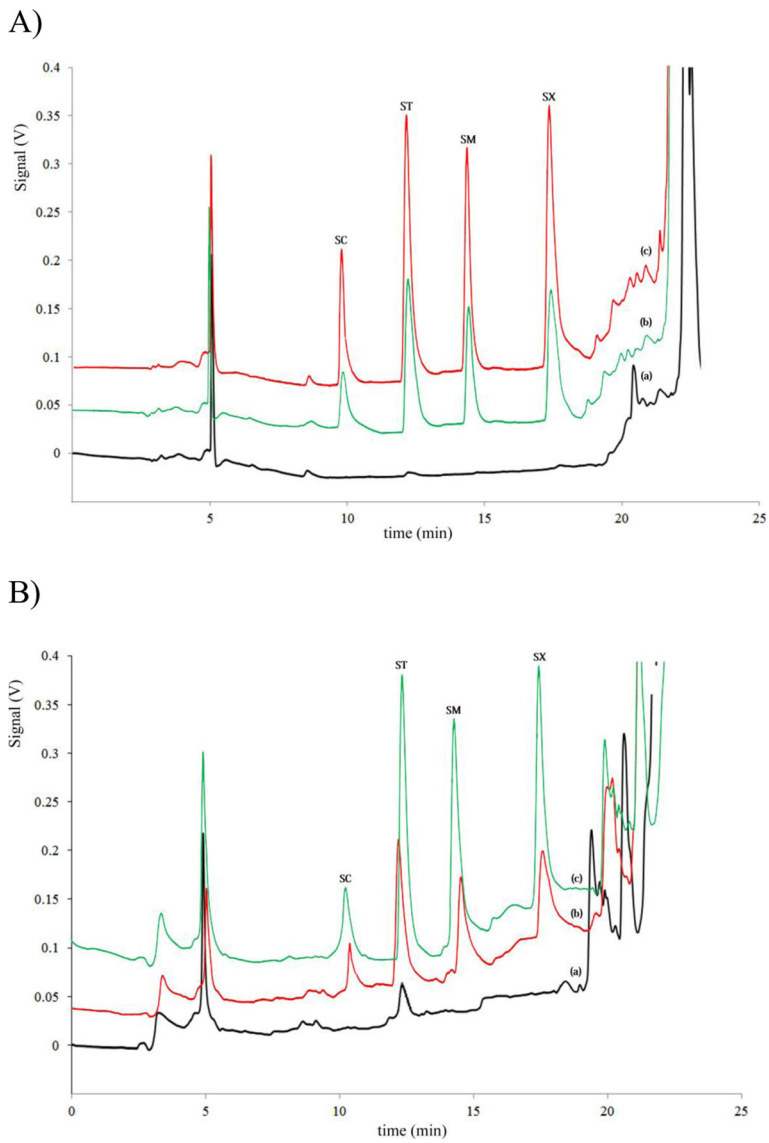


Fig. 7 Chromatograms of *a* non-spiked, *b* 10 μg L^{-1}, and *c* 25 μg L^{-1} spiked for **a** effluent water sample and **b** influent water sample

sample. As shown in Table 3, only ST (3.2 μg L^{-1}) is detected in the influent water sample of the refinery, whereas other compounds are not detected in any of the water samples. To investigate the accuracy of the method, the water samples were spiked with the analytes. The final concentrations of the analytes in the sample solution were 10 and 25 μg L^{-1}; the proposed method was applied for determination of the analytes. The RRs% for all of the analytes were in the range of 89.2–102.4 % over the studied concentration range. The results indicated that there was no significant matrix effect on the extraction efficiencies of all the analytes. Moreover, the method displayed good reproducibility with RSD values in the range of 5.1–6.1 % ($n=3$). Figure 7 shows the HPLC chromatograms obtained by the proposed procedure. In each figure (Fig. 7a, b), the chromatograms of (a) blank water sample, (b) the sample spiked at 10 μg L^{-1}, and (c) the sample spiked at 25 μg L^{-1} of each analyte are depicted.

Comparison of the proposed method with other reported methods

Comparison of the proposed method with different existing methods for extracting and determining the SAs is provided in Table 4. It was shown that along with its simplicity, this technique demonstrated wide linearity range, high sensitivity, acceptable reproducibility with an important emphasis on the extraction time seemed to be short, and no need for centrifugation step, making it possible to perform the emulsification microextraction procedure on-line coupled to HPLC.

Conclusions

In this study, an on-line ion-pair based emulsification liquid phase microextraction coupled to HPLC was utilized for analysis of the sulfonamides in water

Table 4 Comparison of the proposed method with other methods for analyzing the sulfonamides

Analytical method	Analyte	LR (μg L^{-1})	LOD (μg L^{-1})	RSD (%)	Sample volume (mL)	Time (min)	Ref.
HF-LPME[a]-HPLC-UV	SX	1.0–2,000	0.1	5.0	4	~480	Tao et al. (2009)
UA-IL/IL-DLLME[b]-	ST	32.9–432.0	9.26	3.5	4	~30	Gao et al. (2012)
HPLC-DAD	SX	12.1–442.8	3.35	2.0			
HF-LPME-HPLC-DAD	SM	0.05–1,000	0.015	1.2	50	~360	Payan et al. (2011)
	SX	0.003–1,000	0.001	1.2			
SPME[c]-LC-MS/MS	SC	–	47.5	–	25	~80	Balakrishnan et al. (2006)
	ST	–	26.3	–			
	SM	–	16.2	–			
	SX	–	14.0	–			
SUPRAS[d]-HPLC-FD	SX	–	4.0	3.6	–	~90	Costi et al. (2010)
LLLME/AMADPe-	SC	5.0–500	0.57	3.8	12	~30	Lin and Huang (2008)
HPLC-UV	ST	5.0–500	0.77	4.1			
	SX	1.0–500	0.11	3.6			
IP-ELPME-HPLC-UV	SC	0.5–100	0.3	5.8	10	~5	This method
	ST	0.3–100	0.1	5.0			
	SM	0.3–100	0.1	5.2			
	SX	0.3–100	0.1	5.6			

[a] Hollow fiber-based liquid phase microextraction

[b] Ultrasound-assisted ionic liquid/ionic liquid-dispersive liquid–liquid microextraction

[c] Solid phase microextraction

[d] Supramolecular solvent based microextraction

[e] Liquid–liquid–liquid microextraction technique in utilizing automated movement of acceptor and donor phases

samples. The organic extraction solvent was dispersed in the aqueous phase as fine droplets. The ion pair-complex of sulfonamides with Aliquat-336 was extracted into the droplets of extraction solvent. Then, the emulsion of extraction phase in the aqueous solution was passed through the in-line filter and the extraction phase was separated based on emulsion filtration. After that, the extracted analytes were transferred to HPLC column for analysis. Elimination of the centrifugation step and no need to use special vessels for microextraction made this methodology a simple and rapid sample preparation method. Also, 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. The results showed that there was no carry over effect between runs and one in-line filter can be used several times without any problems.

Obtained results in this study have proved that the centrifugation step which is mentioned as a bottleneck in the automation of DLLME and ELPME methods can be replaced by filtration successfully. Finally, the results showed that the method is applicable for extraction and determination of the analytes in very complicated matrices such as influent and effluent water samples of the waste water refinery.

Conflict of interest The authors declare that they have no conflict of interest.

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