Hollow fiber-based liquid phase microextraction (HF-LPME) for a highly sensitive HPLC determination of sulfonamides and their main metabolites

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ABSTRACT

In this paper, three phase-hollow fiber-based liquid phase microextraction (HF-LPME) combined with a HPLC procedure using diode array (DAD) and fluorescence detection (FLD) has been developed for the determination of four widely used sulfonamides: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole and their main metabolites, the corresponding N^4-acetyl derivatives: N^4-acetyl-sulfadiazine, N^4-acetyl-sulfamerazine, N^4-acetyl-sulfamethazine, N^4-acetyl-sulfamethoxazole. A Q3/2 Accurel KM polypropylene hollow fiber supporting 1-octanol was used between a 2 M Na_2SO_4 aqueous solution (pH 4) as a donor phase and aqueous solution (pH 12) as an acceptor phase. The procedure allows very low detection and quantitation limits of 0.3–33 ng L^{-1} and 0.9–100 ng L^{-1}, respectively. The proposed method was applied to the determination of the analytes in environmental water samples (surface, tap and wastewater).

1. Introduction

The analysis of complex samples and the analyte detection or quantitation at very low levels are nowadays two of the main analytical problems. The analytical complexity increases in those cases where both problems are present. The use of clean-up procedures is an old analytical tool that, in the last years has undergone very important developments. Liquid phase microextraction (LPME), also known as supported liquid membranes extraction (SLM), is an attractive alternative to the widely used solid phase extraction (SPE). The use of hollow fibers for LPME (HF-LPME) not only allows in most cases an efficient clean-up procedure, but also produces high degree of pre-concentration. Additionally, the low cost of the polypropylene fibers used and the low organic solvent consumption make HF-LPME an interesting and environmental friendly analytical procedure.

The growing worldwide consumption of pharmaceuticals and their proved occurrence in the environment has become an important issue in recent years, and in the last decade, the focus in environmental research has been extended from more classical environmental pollutants as PCBs, PAHs or pesticides to pharmaceuticals and personal care products. The amount of human pharmaceuticals reaching the environment depends on the consumption amount, and excretion rate via faeces and urine. Effluents of wastewater treatment plants (WWTPs) are considered the principal source of drugs in the aquatic environment. A smaller contribution to the presence of pharmaceuticals in the environment is due to the disposal of outdated medicines down household drains [1] and to the pharmaceutical industry waste [2,3]. The low levels expected and the matrix complexities make it necessary to use adequate preconcentration and clean-up procedures.

Solid phase extraction (SPE), using several sorbent types, has been the preferred sample preparation technique to extract pharmaceuticals from environmental waters [4–6] but in the last years there has been a high interest in developing new clean-up procedures.

Liquid–liquid extraction (LLE) is a classical and common technique used for preconcentration and clean-up prior to chromatographic or electrophoretic analysis that leads to large organic solvent consumption. It is also tedious and the frequently analyte is lost due to the multi-stage operations that cannot be neglected. Liquid-phase microextraction (LPME) based on a droplet of water-immiscible organic solvent hanging at the end of a microsyringe needle (single drop microextraction, SDME) [7,8], is a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. However, SDME is not very robust, and the droplets may be lost from the needle tip of the microsyringe during extraction.

Audunsson [9] introduced an alternative concept for LPME that was developed by Thordarson et al. [10], and Pedersen-Bjergaard and Rasmussen [11], based on the use of single, low-cost, disposable, porous, hollow fibers made of polypropylene. In this

concept, the analytes of interest are extracted from aqueous samples, through a thin layer of organic solvent (several microlitres) immobilised within the pores of a porous hollow fiber, and into an acceptor solution inside the lumen of the hollow fiber; when acceptor phase is an aqueous phase the procedure is known as three-phase HF-LPME. The disposable nature of the hollow fiber totally eliminates the possibility of sample carryover and ensures reproducibility. In addition, the small pore size prevents large molecules and particles present in the donor solution from entering the accepting phase and, at the same time, most macromolecules do not enter the hollow fiber because they are not soluble in the organic phase present in the pores in the extraction conditions, thus yielding very clean extracts [12]. Several reviews on hollow fiber-based LPME have been reported [13–16].

Sulfonamides play an important role as effective chemotherapeutics of bacterial and protozoal diseases and also exhibit growth-promoting properties in veterinary medicine [17,18]. For several authors, sulfonamides have been implicated in the growing prevalence of antibiotic resistance in humans [19–21] so their possible presence in foods (products of animal origin, essentially) and in the environment is a public health concern.

Several methods have been reported for the analysis of sulfonamides, using many kinds of analytical tools that include photometric methods [22], the Bratton–Marshall method [23,24], titrimetric assay methods [25], thin layer chromatography [26], FIA with amperometric detection [27], ELISA [28], high-performance liquid chromatography [29–33], gas chromatography and gas chromatography–mass spectrometry [34–36] and capillary electrophoresis (CE) [37–39]. Generally, for the application of these methods to real complex samples, a high number of extraction, concentration and purification steps are necessary, usually solid phase extraction (SPE) or more laborious approaches.

Two methods that use liquid phase microextraction for the determination of sulfonamides have been previously published. Msagati and Muzi-Nindi [40] use a porous PTFE membrane (FG type Millipore filter) impregnated with 5% (w/v) tri-n-octylphosphine oxide (TOPO) dissolved in hexyl amine and placed between two circular polyvinylidene difluoride (PVDF) blocks in a continuous configuration with two separated channels controlled by two peristaltic pumps. The extracts were analysed by HPLC–MS allowing the simultaneous determination of 16 sulfonamides in spiked water, urine, milk and animal tissues with recoveries between 34 and 93% (34–70% for sulfamerazine, sulfamethazine and sulfamethoxazole) and detection limits between 1.8 and 24.3 $\mu g\,L^{-1}$. Yong et al. [41] use an ionic liquid (1-octyl-3-methylimidazolium hexafluorophosphate [C_8MIMI]) as liquid membrane and 14% (w/v) TOPO as additive in a three phases HF-LPME configuration using a Q3/2 Accurel KM polypropylene hollow fiber. The procedure allows the HPLC-UV determination of five sulfonamides (sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole and sulfadimethoxine) in aqueous samples (farm wastewaters) with recoveries >82%, detection limits between 0.1 and 0.4 $\mu g\,L^{-1}$ and quantitation limits of 1.0 $\mu g\,L^{-1}$.

The aim of this work was to develop a highly sensitive determination of sulfonamides and their metabolites that can be easily applicable to environmental waters (including wastewaters). The organic solvent consumption of several microlitres, in contrast to other clean-up/preconcentration alternatives like SPE is according to the current trends to a "Green Chemistry".

In this work, a HPLC DAD-FLD method combined with preceding HF-LPME was developed for the highly sensitive determination of four sulfonamides: sulfadiazine (SDI), sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMX) and their main metabolites N^4-acetyl-sulfadiazine (NSDI), N^4-acetyl-sulfamerazine (NSMR), N^4-acetyl-sulfamethazine (NSMZ) and N^4-acetyl-sulfamethoxazole (NSMX). (Table 1 shows their

structures, pK_a and IUPAC names.) The method has been successfully applied to their determination in several water types: wastewaters from the different treatment steps of a WWTP, and water samples from river, lake and tap water.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade or better. All solutions and dilutions were prepared with ultrapure water form a Milli-Q Plus water purification system (Millipore, Billerica, MA, USA). SDI, SMR, SMZ, SMX, dihexyl ether and 1-octanol were purchased from Fluka-Sigma-Aldrich (Madrid, Spain) and the rest of products were obtained from Merck (Darmstadt, Germany). N^4-acetyl sulfonamides metabolites (NSDI, NSMR, NSMZ and NSMX) were synthesised according to Pfeifer et al. [42].

Methanolic working solutions of SDI, SMR, SMZ, SMX, NSDI, NSMR, NSMZ and NSMX were daily prepared by adequate dilutions from methanolic 100 mg L^{-1} stock solutions. Q3/2 Accurel KM polypropylene hollow fiber (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany).

2.2. Chromatographic conditions

The chromatographic separation was performed at 10 °C using a LaChrom® VWR-Hitachi (Barcelona, Spain) liquid chromatograph with a quaternary L-2130 pump. The injector was a Rheodyne manual injection valve Model 7725i, fitted with a 20-μL sample loop. Separations were carried out using a Eclipse® XDB-C18 3.5 μm (150 mm × 3.0 mm i.d.) (Agilent., Palo Alto, CA (USA)) column preceded by a guard column Kromasil® 100 Å, C18, 5 μm, (15 mm × 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of 0.1% formic acid (pH 2.6) (component A) and acetonitrile (component B) at a flow rate of 0.4 mL min^{-1}. An initial 90% component A was used in isocratic mode for 10 min and then a linear elution gradient was programmed from 90% to 85% A for another 5 min, finally an elution gradient was programmed from 85% to 60% A for another 15 min. Three minutes were waited between injections which allowed re-equilibration of the column to the initial conditions.

Table 2 shows the monitoring wavelengths for DAD and FLD detections and the retention times for the analysed compounds.

2.3. Synthesis of the metabolites

The main sulfonamides metabolites are their corresponding N^4-acetyl derivatives [43,44] and they were synthesised in our laboratory. The synthesis reaction can be schematised by

$$H_2N-\langle\bigcirc\rangle-SO_2NH-(A) \longrightarrow H_3COC-HN-\langle\bigcirc\rangle-SO_2NH-(A)$$
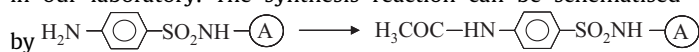
where (A) is the corresponding heterocyclic rest for each sulfonamide.

The synthesis of the sulfonamides metabolites was carried out by slight modifications of the procedure proposed by Pfeifer et al. [42]. Briefly the procedure consisted on: 100 mg of sulfonamide was mixed with 1.5 mL of pyridine and 1.5 mL of acetic anhydride, and the mix was stirred and heated under reflux at 40 °C in a glycerin bath for a variable time depending on the sulfonamide: 6.30 h for SDI, 7 h for SMR, 3 h for SMZ and 6 h for SMX. After reaction the mix was cooled into an ice-bath and extracted with three portions of 20 mL of dichloromethane. Organic extracts were washed with two portions of 200 mL 2 N H_2SO_4 and 20 mL of saturated NaHCO$_3$. The resulting organic extract was dried with MgSO$_4$, filtered and concentrated under vacuum.

Table 1
Structure and IUPAC name of the examined antibiotics and their corresponding N^4-acetyl metabolites.

Drug	Structure	IUPAC name
SDI		Benzenesulfonamide, 4-amino-N-2-pyrimidinyl pK_{a1} = 1.6; pK_{a2} = 6.5
NSDI		Acetamide, N-[4-[(2-pyrimidinylamino)sulfonyl]phenyl]
SMR		Benzenesulfonamide, 4-amino-N-(4-methyl-2-pyrimidinyl) pK_{a1} = 1.58; pK_{a2} = 6.90
NSMR		Acetamide, N-[4-[[(4-methyl-2-pyrimidinyl)amino]sulfonyl]phenyl]
SMZ		Benzenesulfonamide, 4-amino-N-(4,6-dimethyl-2-pyrimidinyl) pK_{a1} = 2.07; pK_{a2} = 7.49
NSMZ		Acetamide, N-[4-[[(4,6-dimethyl-2-pyrimidinyl)amino]sulfonyl]phenyl]
SMX		Benzenesulfonamide, 4-amino-N-(5-methyl-3-isoxazolyl)- pK_{a1} = 1.85; pK_{a2} = 5.60
NSMX		Acetamide, N-[4-[[(5-methyl-3-isoxazolyl)amino]sulfonyl]phenyl]-

The concentrated extract was purified by chromatography using a silica column and dichloromethane:methanol (50:1) as mobile phase and the purity of the collected fractions were controlled by thin layer chromatography (silica as a stationary phase and dichloromethane:methanol (10:1) as a mobile phase). Detection was carried out with ethanolic 1% ninhydrine or UV irradiation (254 nm). Purified sulfonamide metabolite fraction was identified by mass spectrometry.

2.4. Supported liquid membrane preparation and extraction procedure

Hollow fibers were cut into 27 cm pieces, washed with acetone in an ultrasonic bath and dried. The fiber was soaked with 1-octanol during 10 s to impregnate the pores, and rinsed with water on the outside by placing it into the ultrasonic bath for 30 s in order to remove the excess of organic solvent. The lumen of the prepared

Table 2
Monitoring wavelengths and retention times.

	DAD			FLD			
	λ_{max} (nm)	t_R (min)	S.D. (min)	λ_{exc} (nm)	λ_{em} (nm)	t_R (min)	S.D. (min)
SDI	270	8.69	0.008	280	450	9.21	0.003
SMR	270	12.45	0.006	275	445	13.18	0.012
NSDI	262	15.52	0.01	270	356	15.98	0.009
SMZ	270	16.99	0.005	275	445	17.51	0.006
NSMR	262	18.96	0.011	275	351	19.38	0.008
NSMZ	262	21.84	0.007	275	351	22.21	0.011
SMX	270	28.91	0.013	275	382	29.30	0.002
NSMX	262	29.94	0.014	280	351	30.41	0.004

fiber piece was filled with 50 μL of acceptor phase (pH 12 aqueous solution) using a HPLC syringe. Both open ends of the fiber were closed by means of a hot soldering tool and a plastic film (Parafilm®, Pechiney Plastic Packaging Company, Chicago, IL, USA). During extraction the membrane portion that contains the acceptor phase was immersed in the 50 mL sample solution (pH 4, Na_2SO_4 2 M) contained into a 50 mL glass beaker. The sample was stirred for 6 h by means of a magnetic stirrer (ANS-00/1 Science Basic Solutions; Rubí, Barcelona, SPAIN) at 300 rpm. After extraction, the fiber was taken out, one of the ends was cut and the acceptor phase was extracted using a HPLC syringe and injected into the HPLC system.

2.5. Preparation of environmental water samples

Wastewater samples were obtained from "Guadalquivir"-ALJARAFESA Wastewater Treatment Plant which is located in Palomares del Río, Seville, SPAIN. The WWTP essentially receives urban wastewaters. The capacity of this WWTP is 100.000 inhabitants and the discharged flow is 12,433,313 m^3/year (2008 data). Grab samples of the influent (raw water, WWR), after the primary sedimentation tank (WW1), after the aeration tank (WW2) and the effluent (treated water after anaerobic digestion, WWT) were collected in 11 January 2010.

Two samples from Guadalquivir River were analysed. One (RIVER1) from Coria del Río, Seville, 2 km downstream the WWTP previously mentioned and other sample (RIVER2) was taken at the mouth of Guadalquivir River (Sanlúcar de Barrameda, Cádiz) where water has a high seawater proportion. Lake water samples (LAKE) were obtained from "Lagos del Serrano" (Guillena, Seville). Tap water sample (TAP) was obtained directly from the laboratory tap.

All samples, except tap water, were filtered through a GDU1 glass fiber filter bed (10–1 μm) (Whatman, Mainstone, UK) and through Pall Nylaflo™ nylon membrane filter 0.45 μm (Pall Corporation, Ann Arbor, MI, USA) and adjusted to pH 4 with HCl. Filtered samples were stored in the dark at 4 °C prior to HF-LPME extraction.

Water samples, were directly analysed after Na_2SO_4 addition for a 2 M final concentration; HCl was added just to obtain pH 4 prior to be submitted to the HF-LPME procedure.

3. Results and discussion

3.1. Chromatographic conditions

Looking for a fast and high resolution separation an Eclipse® XDV-C18 3.5 μm was selected as working column. This column is a high packing HPLC column that allows high resolution separations using low flow-rates which implies low solvent consumption. The selected column provides good resolution and good peak symmetry.

The mobile phase consisted of 0.1% formic acid and acetonitrile. Different gradient elution conditions were tested searching for the shortest time of analysis without sacrificing peak shape. The gradient elution program described in Section 2.2, was the best option in terms of time of analysis, shape of the peaks and reproducibility.

N^4-acetyl metabolites are not stable at high pH values like the used for the acceptor phase and they are transformed to the corresponding N^4-hydroxy derivatives; we have checked that a complete transformation carry out in 4 h at pH 12, so the measured chromatographic peaks for the N^4-metabolites correspond to their N^4-hydroxy derivatives, and the chromatographic conditions were accordingly optimised for these compounds. It is remarkable that N^4-hydroxy derivatives only can be generated from the corresponding N^4-acetyl metabolites. Fig. 1 shows representative chromatograms from aqueous standards submitted to the HF-LPME procedure.

The efficiency and selectivity chromatographic parameters of the proposed procedure are shown in Table 3, N (number of theoretical plates), T (asymmetry factor), $W_{1/2}$ (peak half-width), k (retention factor), α (selectivity factor) and R_s (peak resolution). As it can be seen, all parameter values are adequate according to their critical values.

3.2. Optimization and evaluation of experimental conditions for HF-LPME extraction

First, several tests with donor phases pHs 3–5 and acceptor phases pHs 10–12 were carried out in order to choose the more adequate liquid supported on the polypropylene membrane; dihexyl ether and 1-octanol were checked and the best results
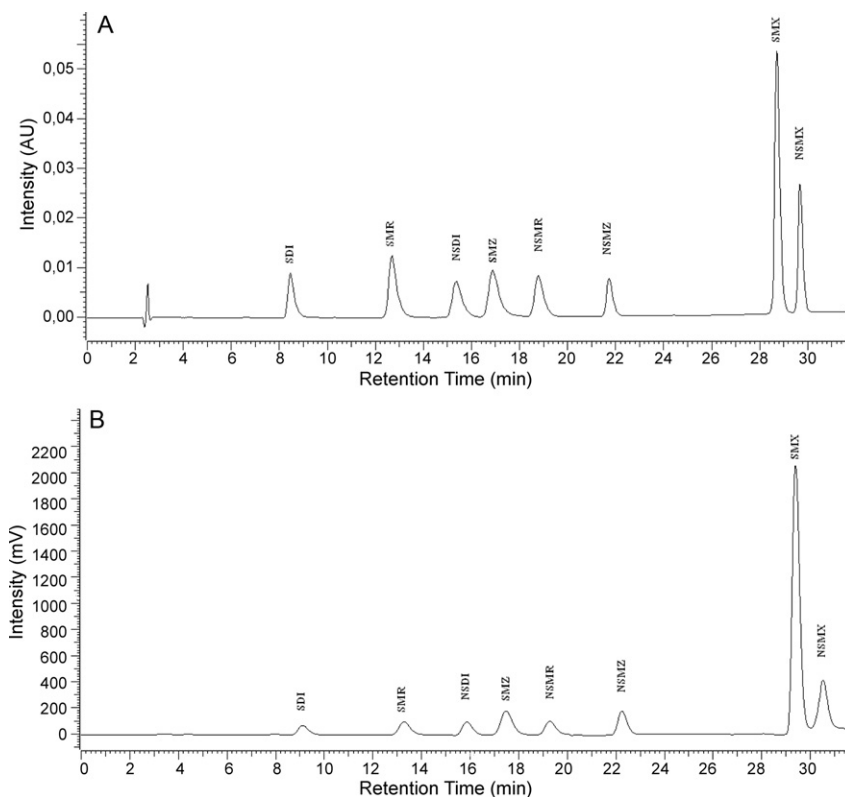
Table 3
Efficiency and selectivity chromatographic parameters for the proposed HPLC procedure (for abbreviation see text).

	$W_{1/2}$ (min)	T	N	k	α	R_s
SDI	0.2577	1.21	6299	3.35	1.56	7.56
SMR	0.3290	1.33	7933	5.23	1.93	5.03
NSDI	0.3919	1.44	8688	6.76	1.11	2.14
SMZ	0.4178	1.41	9161	7.50	1.13	2.84
NSMR	0.4017	1.40	12,342	8.48	1.17	4.45
NSMZ	0.3613	1.36	20,243	9.92	1.36	10.34
SMX	0.4453	1.44	23,351	13.46	1.04	1.66
NSMX	0.2873	1.28	60,164	13.97	1.04	1.66
Critical values		<1.5	>2000	>2	>1	>1.5

Fig. 1. DAD (a) and FLD (b) chromatograms from standard aqueous solutions (10 and 5 ng L^{-1}, respectively).

were obtained with 1-octanol, so this was the liquid supported selected.

Sulfonamides have two dissociation constants. A sulfonamide contains one basic amine group (–NH$_2$) and one acidic group (–NH–SO$_2$–) which correspond to pK_{a1} and pK_{a2}, respectively. The amine group is able to gain a proton, while the amide group is able to release a proton under specific pH conditions. When pH is adjusted to the average of pK_{a1} and pK_{a2} the neutral molecule form is the dominant specie. When pH is above pK_{a2} sulfonamides become neutral and then changed to anionic forms. N^4-acetyl metabolites have pK_a values slightly lower than the corresponding sulfonamide ones. For these reasons donor HCl aqueous solutions within 3.3–5 pH range were tested using 50 mL of aqueous solutions containing 10 μg L^{-1} of each analyte. The extraction was carried out 300 rpm during 30 min and 50 μL of aqueous pH 11 NaOH solution was used as acceptor phase. In general, maximum recoveries were observed when donor pH varies between 3.5 and 4.3, so a pH 4 aqueous solution was selected as donor phase. When pH values between 10 and 12.5 (adjusted with aqueous NaOH solutions) were tested as acceptor phase, maximum extraction efficiencies were observed within 11.5 and 12.2. Thus, pH 12 was selected as optimum for acceptor phase.

Once the donor and acceptor pHs were optimised, a possible influence of salting out effect was tested. Aqueous pH 4 solutions containing NaCl (2–6 M) or Na$_2$SO$_4$ (0.5 M to saturation) were checked as donor phases using a pH 12 acceptor phase and stirring at 300 rpm during 30 min. In general, salting out allows an increase in the extraction efficiency with the salt concentration that is more pronounced with Na$_2$SO$_4$. Aqueous saturated (approximately 2 M) Na$_2$SO$_4$ pH 4 solution was selected as optimum donor phase.

Using the selected optimum donor and acceptor phases, the influence of the stirring time was checked between 3 and 8 h; maximum extraction was obtained for time values around 6 h for all the analytes, so a stirring time of 6 h at 300 rpm was selected as an optimum value.

All the figures corresponding to the optimization and evaluation of experimental conditions for the proposed HF-LPME extraction have been supplied as Supplementary Electronic Material.

3.3. Linearity, sensitivity, precision and robustness for the HF-LPME extraction

Linearity of the response function was studied from external calibration. A 10-point (in triplicate) calibration curve, was constructed using a least-square linear regression analysis of standards mixtures of the analytes at different concentrations. Using the selected HF-LPME conditions, aqueous pH 4 solutions with different analyte concentrations were submitted to the liquid microextraction procedure and analysed according to the described HPLC procedure. Peak areas were proportional to concentrations in the donor phase. A linear relationship was obtained with correlation coefficients $r \geq 0.999$ and the calibration curves obtained showed no changes over the course of one month. Detection and quantitation limits were calculated as the minimum concentration of an analyte giving peaks whose signal-to-noise ratio is 3 and 10, respectively. Enrichment factor was calculated as the increase in the concentration between the aqueous external donor phase and the internal (acceptor) phase. Table 4 shows the corresponding data and, as can be seen, the high enrichment values obtained for all the analytes allow the detection and determination of low concentration levels.

To evaluate the repeatability and the intermediate precision, aqueous samples at three concentrations levels 10^2 (25 for SMX and NSMX), 10^3 and 10^5 ng L^{-1} (in triplicate) were subjected to the entire analytical procedure and measured in one single day and one day per week during two months, respectively. Intermediate precision was performed using the prediction of actual concentrations from the validation standards selected for the analytical assay in the $m \times p \times n$ design (m = analytical levels, p = days and n = replications). From the corresponding ANOVA, the intermediate precision was

Table 4
Detection limits, linear ranges, % linearity and enrichment factors for the HPLC method (DAD and FLD detection) combined with prior HF-LPME.

	DAD			FLD			Enrichment
	LOD (ng L^{-1})	Linear range (ng L^{-1})	% linearity	LOD (ng L^{-1})	Linear range (ng L^{-1})	% linearity	
SDI	15	50–10^6	99.81	15	50–10^5	99.91	200
SMR	5	15–10^6	99.67	15	50–10^5	99.63	1000
NSDI	15	50–10^6	99.59	8	25–10^5	99.96	400
SMZ	15	50–10^6	99.86	15	50–10^5	99.83	250
NSMR	15	50–10^6	99.69	8	25–10^5	99.97	500
NSMZ	15	50–10^6	99.92	33	100–10^5	99.88	175
SMX	1	3–10^6	99.77	0.3	0.9–10^5	99.95	1000
NSMX	3.5	10–10^6	99.84	8	25–10^5	99.90	600

computed [45]. The repeatability, expressed as relative standard deviation, was in the range 0.8–1.2%. Intermediate precision also expressed as relative standard deviation, was in the range 1.0–1.8%.

The robustness study is based on the procedure suggested by Youden [46]. A design matrix with two factors in eight experiments was used when the +1 and −1 levels correspond to high and low pH values (4.5 and 3.5 for donor phase and 12.5 and 11.5 for the acceptor phase). Stirring time is not considered as a variable for robustness study due to its high optimum value (6 h) and the fact that variations in the order of minutes do not have significant effects in the extraction efficiency.

A significance *t*-test [47] was used to determine whether variations have a significant effect on the result, and the calculated *t* values were compared with the corresponding critical *t* values ($n = 4$) at 5% significance level and three degrees of freedom. The results obtained indicated that the procedure can be considered robust against the considered factors for all the analysed compounds.

3.4. Environmental water sample analysis

The different water samples were selected taking into account the maximum variability with respect to provenance and matrix composition

First, the different water samples were submitted to the HF-LPME proposed procedure and analysed. None of the sulfonamides and their corresponding N^4-acetyl metabolites were detected in the water samples. In order to check the suitability of the pro-

posed procedure, spiked samples at three concentration levels: 100 (15 for SMX and NSMX), 500 and 5000 ng L^{-1}, were analysed. Results obtained are shown in Table 5. In the "more simple" water samples (RIVER1, RIVER2, LAKE and TAP), recoveries for all the compounds are within 93 and 101%. Wastewater samples are, in general, complex samples from the analytical point of view, that frequently require complex clean-up processes. The direct application of the proposed HF-LPME procedure to the different wastewater types analyses shows, in general, excellent results with recoveries within 99 and 101% for SMZ, NSMR and NSMX, however the rest of analysed compounds show variable recoveries within 33 and 90%. It is remarkable that, in general, recoveries slowly increase with the depuration process, showing the better values for the WWT sample with recoveries higher than 72% except for SMR (56–56%) and NSMZ (40–44%). Urban wastewaters have extremely high surfactants concentrations that could modify the supported liquid membrane behaviour [48] and that could lead to recovery decreases but remain low standard deviations. Despite the recovery decreases, the excellent preconcentration and clean-up obtained implies a great advantage over other sample treatment procedures which can justifies the use of the proposed HF-LPME procedure.

Figs. 2 and 3 show representative DAD and FLD chromatograms obtained from blank and spiked (500 ng L^{-1}) wastewater (WWR) and river (RIVER1) samples. These samples have been selected as the more complex wastewater (raw wastewater) and surface water. As it can be seen, RIVER1 blank chromatograms show excellent baselines that are a little poor for the WWR ones. Spiked RIVER1 chromatograms only show well defined peaks corresponding to
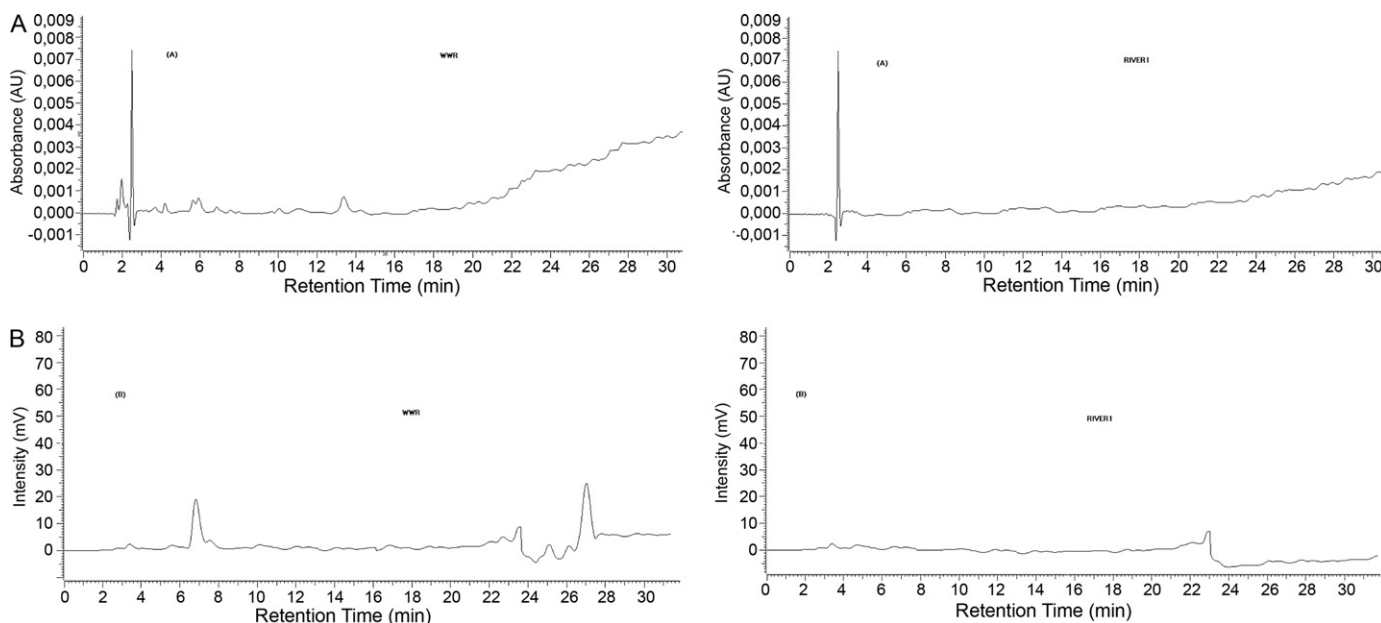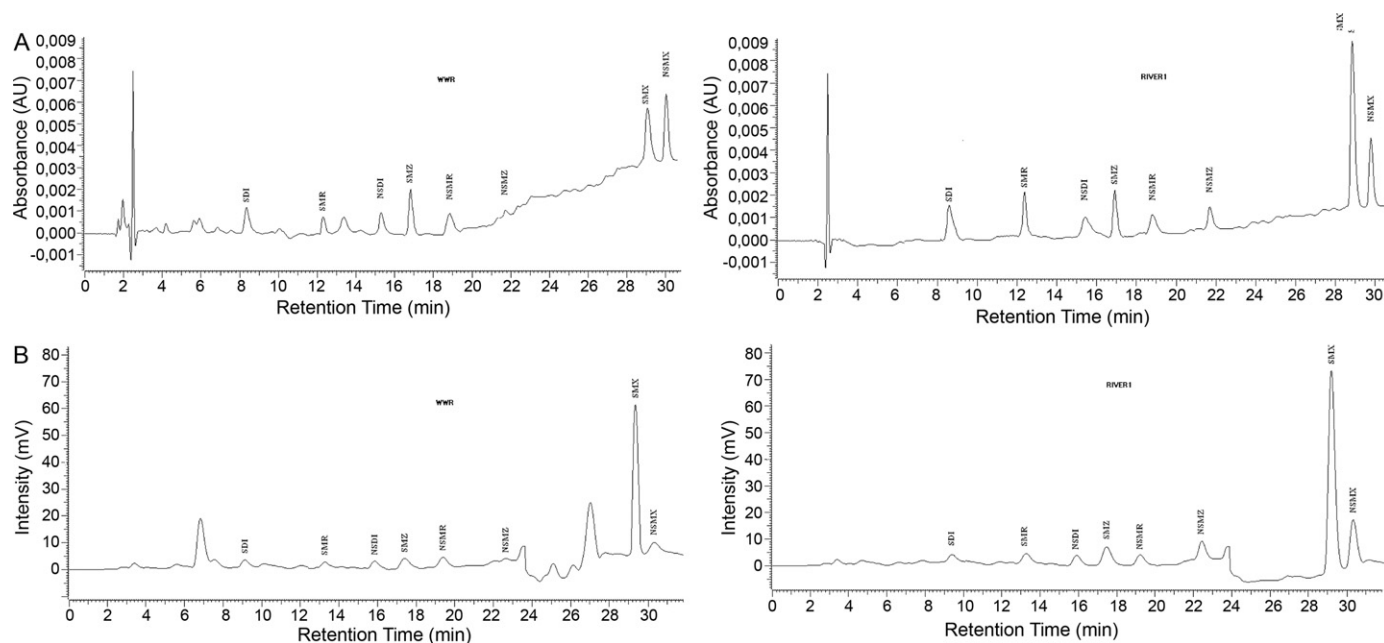

Fig. 2. DAD (a) and FLD (b) chromatograms of blank wastewater (WWR) and river (RIVER1) samples.

Table 5
Recoveries (%) using HF-LPME/HPLC from water spiked samples (average of three determinations ± standard deviation).

	Spiked level (ng L^{-1})	Water sample[a]							
		WWR	WW1	WW2	WWT	RIVER1	RIVER2	Lake	Tap
SDI	100	63.6 ± 2.2	68.7 ± 1.8	70.4 ± 1.8	78.2 ± 1.8	99.7 ± 0.6	98.6 ± 1.6	99.7 ± 2.2	99.9 ± 1.6
	500	66.7 ± 1.8	69.1 ± 1.8	71.0 ± 1.8	81.3 ± 1.2	99.8 ± 0.2	98.3 ± 0.4	100.2 ± 1.6	100.0 ± 1.6
	5000	71.3 ± 2.4	68.9 ± 1.6	70.9 ± 2.2	82.2 ± 1.2	100.0 ± 0.2	99.1 ± 0.4	99.9 ± 1.0	100.0 ± 0.8
SMR	100	32.6 ± 3.8	34.2 ± 2.2	35.2 ± 1.8	56.2 ± 3.0	99.9 ± 2.2	99.4 ± 1.2	99.6 ± 1.8	99.6 ± 1.6
	500	33.7 ± 3.0	35.2 ± 0.8	34.6 ± 1.6	58.8 ± 1.8	98.4 ± 1.8	99.6 ± 0.8	99.9 ± 0.8	99.3 ± 1.8
	5000	36.2 ± 1.6	35.1 ± 0.8	34.7 ± 1.6	58.3 ± 1.6	100.0 ± 1.0	99.9 ± 0.6	100.1 ± 0.8	100.0 ± 1.2
NSDI	100	51.9 ± 1.8	52.2 ± 1.6	54.5 ± 1.0	72.5 ± 1.6	99.6 ± 1.8	98.3 ± 0.6	100.30 ± 0.4	99.49 ± 0.8
	500	52.4 ± 1.6	52.4 ± 1.4	54.2 ± 0.8	72.1 ± 1.0	99.7 ± 1.6	98.7 ± 0.6	99.56 ± 0.2	100.21 ± 0.8
	5000	53.2 ± 0.4	52.7 ± 0.8	54.8 ± 0.2	74.2 ± 1.0	100.0 ± 1.6	99.1 ± 0.6	99.77 ± 0.2	100.16 ± 0.2
SMZ	100	98.9 ± 2.2	98.9 ± 1.6	98.6 ± 0.15	99.1 ± 3.2	99.4 ± 2.8	98.3 ± 1.4	99.7 ± 1.4	99.9 ± 2.2
	500	100.0 ± 1.2	100.0 ± 1.6	99.9 ± 1.2	100.0 ± 1.8	99.8 ± 1.6	98.1 ± 1.0	99.9 ± 0.6	101.1 ± 2.0
	5000	99.7 ± 1.6	99.6 ± 2.4	100.0 ± 1.2	100.1 ± 3.0	99.9 ± 1.2	98.3 ± 0.8	99.7 ± 0.2	100.0 ± 1.6
NSMR	100	97.5 ± 1.0	98.4 ± 1.2	99.9 ± 1.6	100.2 ± 0.8	99.6 ± 1.8	99.75 ± 1.8	100.1 ± 1.6	99.8 ± 1.2
	500	100.0 ± 1.0	99.6 ± 0.8	99.6 ± 0.8	99.9 ± 0.8	100.0 ± 1.0	101.01 ± 1.6	99.9 ± 1.6	99.0 ± 1.2
	5000	100.1 ± 0.6	100.6 ± 0.8	99.3 ± 0.6	99.47 ± 0.4	99.7 ± 1.0	99.89 ± 1.6	100.2 ± 0.6	99.8 ± 1.0
NSMZ	100	Detected	Detected	Detected	Detected	95.6 ± 1.0	93.21 ± 1.6	98.4 ± 2.2	99.2 ± 1.8
	500	33.1 ± 1.6	34.0 ± 1.8	38.2 ± 1.6	41.0 ± 2.2	95.4 ± 1.0	96.15 ± 1.6	99.5 ± 0.8	99.8 ± 1.8
	5000	35.2 ± 1.6	35.7 ± 1.2	38.8 ± 1.2	44.2 ± 1.8	99.8 ± 0.6	99.0 ± 0.8	100.2 ± 0.8	100.1 ± 1.2
SMX	15	62.5 ± 1.8	62.5 ± 1.8	83.2 ± 1.6	88.7 ± 1.6	100.0 ± 1.4	100.2 ± 2.2	100.0 ± 1.8	100.1 ± 2.4
	500	61.8 ± 1.8	61.8 ± 1.8	88.2 ± 1.6	90.0 ± 1.8	100.1 ± 1.0	100.1 ± 1.8	99.8 ± 1.6	100.0 ± 1.6
	5000	63.7 ± 0.2	63.7 ± 0.2	89.7 ± 1.2	91.0 ± 0.8	100.0 ± 1.4	99.9 ± 1.8	100.1 ± 1.6	99.9 ± 0.05
NSMX	15	100.0 ± 2.2	100.0 ± 1.6	99.84 ± 3.0	99.9 ± 2.2	100.2 ± 0.6	99.9 ± 0.1	99.8 ± 1.6	99.9 ± 1.8
	500	100.0 ± 1.8	99.9 ± 1.0	99.96 ± 0.8	100.2 ± 1.0	100.1 ± 0.6	99.7 ± 1.8	99.9 ± 1.0	100.2 ± 1.8
	5000	99.9 ± 1.2	100.2 ± 1.0	100.08 ± 0.8	100.0 ± 0.6	100.0 ± 0.2	100.4 ± 0.6	100.2 ± 1.0	100.0 ± 0.8

[a] Average recovery (%) ± standard deviation ($n = 3$).

Fig. 3. DAD (a) and FLD (b) chromatograms from spiked (500 ng L^{-1}) wastewater (WWR) and river (RIVER1) samples.

the added substances, and for WWR, the low peaks observed in the blank chromatograms do not interfere with the corresponding spiked ones.

4. Conclusions

This study presents a hollow fiber-based liquid phase microextraction (HF-LPME) method combined with an HPLC (DAD-FLD) determination using a high packing chromatographic column that allows a simple, low-cost, accurate, highly sensitive and selective methodology for the determination of four widely used sulfonamides and their corresponding N^4-acetyl metabolites. The proposed extraction procedure has a very low (few microlitres) organic solvent consumption. The excellent preconcentration and clean-up obtained implies a great advantage over other sample treatment procedures including a previously described HF-LPME method that uses a mixture ionic liquid/TOPO as supported liquid membrane, with high solvent cost; our procedure shows an

improvement in the detection and quantitation limits and it has been demonstrated their applicability to the analysis of the main sulfonamide metabolites.

The proposed procedure has been demonstrated adequate for the determination of the analytes in environmental samples including urban wastewaters that usually require tedious clean-up and preconcentration steps.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.12.006.

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