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Equilibrium sampling through membrane based on a hollow fiber for determination of naproxen and diclofenac in sludge slurry using Taguchi orthogonal array experimental design

Maryam Ezoddin[a,*], Jan Åke Jönsson[b], Anahita Kyani[c]

[a]*Department of Chemistry, Payame Noor University, P.O. BOX 19395-3697, Tehran, Iran*
Tel. +98 231 3374001; Fax: +98 231 3333485; email: maryams77@gmail.com
[b]*Center for Analysis and Synthesis, Department of Chemistry, Lund University, P.O. Box 124, Lund 221 00, Sweden*
[c]*Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 13145-1384, Tehran, Iran*

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ABSTRACT

A three-phase hollow fiber liquid phase microextraction (HF-LPME) method was evaluated for the extraction and preconcentration of naproxen and diclofenac using a polypropylene membrane followed by analysis using HPLC or LC/MS. In this technique, the drugs were extracted into di-*n*-hexyl ether immobilized in the wall pores of a porous hollow fiber from 50 mL of sludge slurry sample as a donor phase with pH 3, and then back-extracted into the acceptor phase located in the lumen of the hollow fiber. Experimental factors were studied in 16 trials using a Taguchi orthogonal array experimental design with an OA_{16} (4^5) matrix. The significance of these factors was investigated using analysis of variance. The extraction time was statistically demonstrated as the main factor for the extraction of naproxen and diclofenac, while ionic strength played the role of the second most important factor for HF-LPME extraction of diclofenac. The method permitted a detection limit of 0.2–0.7 ng g^{-1} with relative standard deviation values of 3–5%. Enrichment factors of 2,300 for naproxen and 1,400 for diclofenac were achieved. The method was applied to determine naproxen and diclofenac in sewage sludge from sewage treatment plant, Källby (Lund, Sweden).

Keywords: Hollow fiber liquid phase microextraction; Taguchi orthogonal array; Diclofenac; Naproxen; Liquid chromatography–mass spectrometry

1. Introduction

Pharmaceuticals are being used in large quantities in human and veterinary medicine, and many of these compounds are excreted without being entirely metabolized in the target organism, thus emerging as a new and important contamination factor in the aquatic environment. Acidic pharmaceuticals (e.g. nonsteroidal anti-inflammatory agents (NSAIDs)) have been largely studied because of their ubiquity in the environments due to high consumption. The following two pharmaceuticals from the class of NSAIDs were selected as model compounds in this work (see Fig. 1

*Corresponding author.

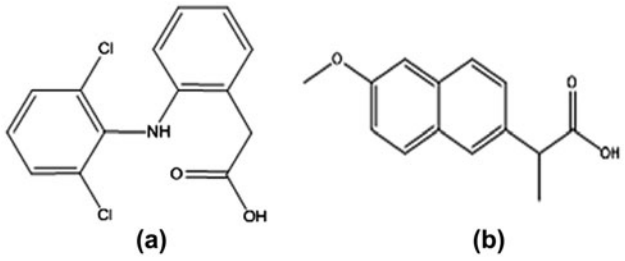

Fig. 1. Chemical structures of the target analytes, a: Diclofenac and b: Naproxen.

Table 1
Physical properties of the target compounds

Compound	Solubility in water (mg/L)	pK_a	$\log K_{o/w}$
Naproxen	16	4.15	3.18
Diclofenac	2	4.15	4.51

and Table 1): Diclofenac (2-(2,6-dichloroanilino) phenyl acetic acid), (a) and naproxen (2-(6-methoxy-2-naphthyl) propionic acid), (b). These are widely used [1] for the treatment of pain and fever and constitute the active ingredients in many common painkillers. These drugs have been extensively detected in sewage treatment plant (STP) influents and effluents, and in surface water samples [2]. In addition, they can also be detected in sewage sludge. Even though the concentrations are often low, there is a continuous input of these substances into the environment. There are indications that chronic effects from NSAIDs might occur at environmentally observed concentrations. For example, diclofenac has been shown to cause cellular damage in several organs at concentrations, as low as, $1\,\mu g\,L^{-1}$. It has been shown that toxicity of the NSAIDs is additive since the mode of action (inhibition of the cyclooxygenase enzymes; COX-1 and COX-2) is the same for all substances. Thus, even if NSAIDs occur below their no observed effect concentration, they can still together give rise to toxic effects. Studies also indicate that certain degradation products from diclofenac and naproxen seem to exhibit a higher toxic potency towards aquatic organisms than the parent compounds themselves [3]. Due to the complexity of the composition of the matrices and the trace amounts of pharmaceuticals occurring in sludge, an effective extraction/purification approach prior to final analysis is of vital importance for residue analysis of drugs regardless of the chromatographic method used. Generally, solid phase extraction (SPE) has been used for sample preparation of environmental aqueous samples containing different pharmaceuticals [4–7], since it is a relatively robust technique leading to low limit of detection (LOD) in the low $ng\,L^{-1}$ range using chromatographic techniques for the final analysis. However, this common technique is time consuming, because clean-up is often required. Several methods, such as ultrasonic solvent extraction (USE), microwave assisted extraction, and pressurized liquid extraction (PLE) [8–13] have been reported for the extraction of NSAIDs from solid matrices. In some of these techniques combined with SPE, matrix effects for NSAIDs are considerable [8,12,13]. Also, in the case of NSAIDs, being acidic drugs, adjusting pH to acidic values before SPE to obtain higher retention efficiency leads to the formation of some colloidal precipitation which makes it difficult to perform SPE in a reasonable loading time and it is necessary to filter the extract before performing SPE [14].

Hollow fiber liquid phase microextraction (HF-LPME) is an attractive and novel pretreatment method characterized by high enrichment factors, rapid analysis time, simple set-up, and low cost [15]. As an environmental-friendly technique, it has been successfully employed for the determination of a wide range of environmental and biological contaminants [16–18]. HF-LPME can be performed either in a two-phase or a three phase mode. In three-phase HF-LPME, which is applicable here, the analytes are extracted through an organic solvent immobilized in the pores of the hollow fiber wall and further into a second aqueous phase in the lumen [19] of the fiber (Fig. 2). Three-phase HF-LPME is mainly suitable for ionizable and charged compounds in aqueous samples [20–22] and it has been applied to extract various drugs from water. In a STP, it can be expected that, to some part, drugs will remain concentrated in the sludge. Therefore, it is important to find techniques for extraction of the NSAID compounds in sludge. In this work, a technique using HF-LPME to extract the drug from sludge slurry is optimized and studied. Generally, the HF-LPME analysis is influenced by several experimental variables, such as the pH of donor phase, salt addition, stirring

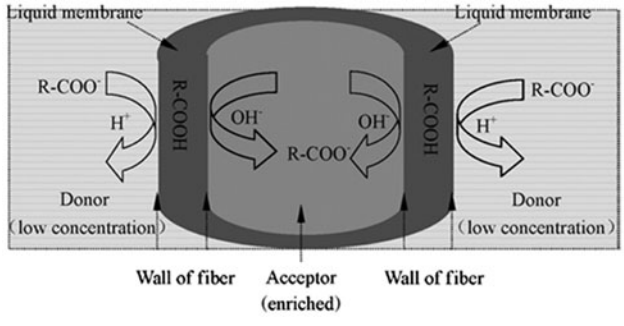

Fig. 2. Schematic representation of three-phase LPME extraction in a microporous hollow fiber.

rate, extraction time, etc. Previous studies were usually conducted as ''one factor at a time'' experiments to optimize analysis conditions, but such studies are time consuming and do not consider possible interactions among the factors. Response surface methodology, which can analyze the interactions of factors, needs a higher number of runs [23]. However, Taguchi's orthogonal array experimental design which takes into account several variables at a time, is a cost-effective approach and could be used to search for the optimal HF-LPME operational conditions. Taguchi design methods have been applied to analytical validations to minimize the determination variability and improve method robustness [24] for two decades. Furthermore, Taguchi methods have also been recently applied in other analytical development studies, including sample extraction, gas chromatography–mass spectrometry, and electrospray mass spectroscopy determinations of pharmaceuticals and chemicals [25–30].

In the current work, HF-LPME in combination with LC/MS was applied for the extraction and preconcentration of naproxen and diclofenac. Moreover, an orthogonal array design (OAD) Taguchi procedure with OA_{16} (4^5) matrix was applied to study the factors influencing the HF-LPME efficiency. The optimized conditions were then applied for the analysis of naproxen and diclofenac in sludge.

2. Experimental

2.1. Reagents and standards

HPLC-grade methanol, formic acid (>98% pure), ammonium carbonate (containing 30–33% NH_3), naproxen, and diclofenac (98% pure) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Sulfuric acid (18 mol L^{-1}) and di-*n*-hexyl ether (DHE; >97% pure) were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Sodium chloride was purchased from Merck (Darmstadt, Germany). Reagent water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The 50/280 Accurel PP polypropylene hollow-fiber membranes (50 μm wall thickness, 280 μm inner diameter, and 0.1 μm pore size) were obtained from Membrana GmbH (Wuppertal, Germany). The cartridges used for SPE were Oasis® HLB (200 mg, 6 mL) from Waters Corporation (Milford, MA, USA).

2.2. Standard solutions and real samples

Stock standard solutions of naproxen and diclofenac (100 μg mL^{-1}) were prepared by dissolving their salts in methanol. Mixed standard solutions of drugs (100 μg L^{-1}) were prepared by appropriate dilution of the stock solution in reagent water. All standard solutions were stored at 4˚C. Acceptor buffer solution was prepared by dissolving appropriate amounts of ammonium carbonate in water.

Sludge samples were collected during spring, 2009 in Källby STP. This plant is located in a Lund city suburb (in the south-western part of Sweden). The sewage treatment is made up of screen raking, sand catch, primary sedimentation, secondary biological treatment, and finally, tertiary treatment, where phosphate is chemically precipitated by iron. Some activated sludge from the secondary sedimentation unit is returned to the inlet of the primary clarifier. The remaining fraction of secondary sludge is combined with the primary and tertiary sludge and further treated (including, thickening, dewatering, and anaerobic digestion). The collected treated-sludge samples were transported to the laboratory in plastic buckets. The samples were stored refrigerated in closed bottles at 4˚C until analysis.

2.3. Chromatography

Separation and determination of the drugs were performed on a 1100 Series HPLC instrument from Agilent Technologies (Waldbronn, Germany) equipped with a quaternary pump, a vacuum degasser, an autosampler, column compartments, and UV/Vis detector. The injection volume was set to 5 μL. The HPLC data obtained were processed by the Agilent Chemstation software and further evaluated using Microsoft Excel. The chromatographic separation was carried out by a reverse-phase HPLC system using an Eclipse XDB-C_{18} column (150 mm × 4.6 mm × 5 μm particle size) from Agilent Technologies. The mobile phase consisted of methanol and 0.1% formic acid in water. Gradient elution was performed at a flow rate of 0.9 mL min^{-1}, starting with 70% methanol and increasing to 90% in 5 min followed by isocratic elution with 90% methanol for 3 min to wash out column and then it returned to the initial composition. Detection was carried out using the UV detector at a wavelength of 220 nm. Direct calibration of HPLC/UV was performed using standard solutions ranging 200–10,000 ng mL^{-1}.

2.4. LC–ES–MS system

LC analysis was performed using two Waters 515 pumps (Waters, Milford, MA, USA) equipped with a Waters Pump Control Module, an auto sampler Triathlon (Spark-Holland, Emmen, The Netherlands), and a vacuum degasser CSI 6150. The LC column

Table 2
LC electrospray conditions for the analysis of pharmaceutical residues by SIM acquisition in negative ion mode

Pharmaceuticals	Retention time (min)	Analytical ion $[M–H]^-$ (m/z)	Cone voltage (V)
Naproxen	7.94	229	10
Diclofenac	11.18	294	20

used was a HP column (100 mm × 2.1 mm, particle size 5 μm) with a octadecyl-bonded phase, ODS, C_{18}. The mobile phase consisted of methanol and water. For the analysis of drugs after extraction from sludge, a gradient elution was performed at a flow rate of 0.4 mL min^{-1} starting with methanol and water (40:60%). The injection volume of the sample was 10 μL and the total run time was 15 min. In this study, mass-selective detection was carried out with electrospray ionization in the negative ionization mode using a Waters CZQ single-quadruple mass spectrometer. Instrument control, data acquisition, and evaluation were done with Masslynx NT software (version 4.0) (Waters). The operating parameters were as follows: electrospray source block and desolvation temperature 150 and 325 °C, respectively, capillary voltage 3.5 kV, and desolvation gas flow 535 L h^{-1}.The optimal cone voltage for each compound chosen for Selective Ion Monitoring (SIM) experiments are listed in Table 2. Direct calibration of LC–ESI–MS was performed using standard solutions with concentrations ranging from 5 to 3,000 ng mL^{-1}.

2.5. Extraction procedure

Hollow fiber pieces of ~15 cm length were cut manually and connected to the needle of a BDM Micro-Fine syringe. The syringe was filled with ~0.5 mL of the acceptor solution and the plunger of the syringe was depressed to flush out acceptor to wash and fill the lumen of the hollow fiber. The fiber was dipped into DHE for 1 min to impregnate the pores of the hollow fiber. The lumen of the hollow fiber was flushed slowly with the rest of acceptor solution at a time, completely filling the lumen with acceptor without any air bubbles. The two ends were folded and closed with aluminum foil. After this preparation, the obtained sampling device has an effective fiber length of 11 cm with sampling phase (acceptor) volume of ~8μL. The whole sealed-fiber filled with acceptor was immersed in water for 30 s to wash out surplus organic solvent. After this, the hollow fiber sampling device was immersed in sludge slurry containing 0.5 g (dry weight) sludge in 50 mL of water, adjusted to pH 3. After stirring at 960 rpm for 5 h, the acceptor solution containing the extracted analytes was collected in a vial by pushing air through the fibers with BDM Micro-Fine syringe. An aliquot was injected into HPLC-UV or LC/MS.

2.6. Extraction efficiency and enrichment factor

The enrichment factor E_e was determined according to Eq. (1):

$$E_e = \frac{C_A}{C_S} \quad (1)$$

In this equation, C_A and C_S are the final concentration and initial concentration of the analyte in acceptor phase and donor phase, respectively. C_A of the extracted drug was calculated from the calibration curve. E_e will increase with time and eventually reach a constant equilibrium value. In this work, enrichment increased up 5 h, where after it started to decline, probably due to exhaustion of the buffer capacity of the acceptor, leading to pH changes. It was assumed that the system was at equilibrium after 5 h.

A related characteristic of the extraction is the extraction efficiency, i.e. the fraction of analyte extracted from the sample. The extraction efficiency was determined by using Eq. (2).

$$E = E_e \times \frac{V_A}{V_S} \quad (2)$$

2.7. Determination of C_{free} (free concentration) drugs in sludge

For the acidic drugs naproxen and diclofenac that are present in sludge sample, equilibrium between the freely dissolved form and the bound form is established. Naproxen and diclofenac absorb and bind to the surface of sludge particle. Therefore, the fraction of free concentration of drugs in water is reduced. The sludge – water partition coefficient (K_{SW}) based on Eq. (3) is defined as:

$$K_{sw} = \frac{C_{sludge}}{C_{free}} \quad (3)$$

C_{sludge} is the concentration of the drug that is present on the surface of sludge and C_{free} is the concentration of drugs in water.

3. Results and discussion

3.1. Organic solvent

The selection of extraction solvent is important in HF-LPME in order to obtain efficient analyte preconcentration, good sensitivity, precision, and selectivity in the extraction of the target compounds. Fast diffusion of the analytes and thereby, high enrichment is favored by a less-viscous membrane liquid, while the stability of the membrane is favored using a less-polar liquid with higher viscosity [3]. DHE with a medium polarity was shown in previous work to be suitable for the extraction of the actual analytes [3,31]. Therefore, DHE was selected for these experiments.

3.2. Optimization of the HF-LPME conditions using Taguchi design

The first step in the HF-LPME extraction is to optimize the operating conditions to obtain an efficient extraction of target compounds and avoid the co-extraction of undesired compounds. Since various parameters potentially affect the extraction process, the optimization of the experimental conditions is a critical step in the development of a HF-LPME method. Therefore, an OAD with an OA_{16} (4^5) matrix was employed to evaluate the effects of five factors, namely pH of the donor phase, concentration of the acceptor buffer phase, stirring speed, extraction time, and ionic strength (salt concentration) in the donor phase. Each factor was evaluated in four levels (Table 3). For increasing the precision of the optimization process, each experiment was repeated leading to a total of 32 experiments. The data obtained from orthogonal array experiments were evaluated by means of range analysis and analysis of variance (ANOVA).

The range analysis was visualized using main effects plots (Figs. 3 and 4) for naproxen and diclofenac. The plots indicate how the extraction efficiency changes when the level of each factor changes. These figures were obtained from the mean value of each response for the corresponding factors at each level.

ANOVA results for the calculated models are shown in Tables 4 and 5 for naproxen and diclofenac, respectively. The sum of squares (SS) for different variables and the error estimate of the experiments were calculated based on the method described in the literature [32]. The ANOVA results showed that all factors were statistically significant at $p < 0.05$ for both naproxen and diclofenac. Furthermore, from the relative contribution (Tables 4 and 5), it can be deduced that the most important factor contributing to the extraction efficiency was extraction time in the case of naproxen, and ionic strength in the case of diclofenac. Moreover, concentration of the acceptor buffer seems to be the least-important (although statistically significant) factor affecting the extraction of both pharmaceuticals.

Table 3
OA_{16} (4^5) experimental design for the optimization of HF-LPME extraction of naproxen and diclofenac

Trial no.	A	B	C	D	E
1	3	720	8	0.08	0
2	5	1,200	1	0.08	5
3	2	960	8	0.04	5
4	2	480	3	0.08	15
5	2	720	1	0.1	10
6	1.5	1,200	8	0.1	15
7	3	960	1	0.06	15
8	3	1,200	3	0.04	10
9	3	480	5	0.1	5
10	1.5	480	1	0.04	0
11	5	720	5	0.04	15
12	5	480	8	0.06	10
13	1.5	720	3	0.06	5
14	5	960	3	0.1	0
15	2	1,200	5	0.06	0
16	1.5	960	5	0.08	10

A (pH of donor phase), B (stirring speed, rpm), C (extraction time, h), D (concentration of acceptor phase, mol L^{-1}), (E (salt%, w/v).

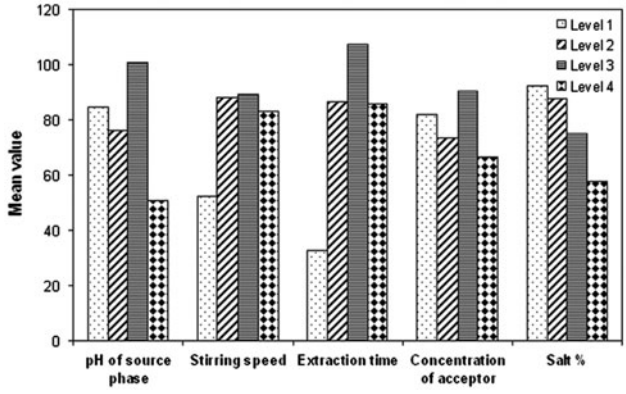

Fig. 3. The effect of pH of the donor phase, stirring speed, extraction time, concentration of acceptor buffer, and concentration of salt on HF-LPME of naproxen. Levels of the parameters are pH of donor phase: 1.5, 2, 3, and 5; stirring speed: 480, 720, 960, and 1,200 rpm; extraction time: 1, 3, 5, and 8 h; concentration of acceptor: 0.04, 0.06, 0.08, and 0.1 mol L^{-1}; salt concentration: 0, 5, 10, and 15% (w/v).

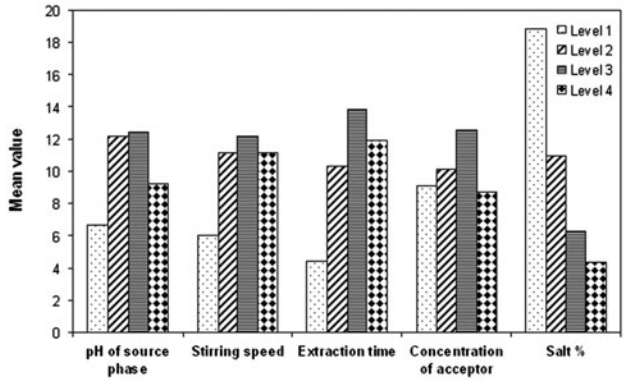

Fig. 4. The effect of pH of the donor phase, stirring speed, extraction time, concentration of acceptor buffer, and concentration of salt on HF-LPME of diclofenac. Levels of the parameters are pH of donor phase: 1.5, 2, 3, and 5; stirring speed: 480, 720, 960, and 1,200 rpm; extraction time: 1, 3, 5, and 8 h; concentration of acceptor buffer: 0.04, 0.06, 0.08, and 0.1 mol L^{-1}; salt concentration: 0, 5, 10, and 15% (w/v).

3.3. Selection of pH conditions

Naproxen and diclofenac are ionizable analytes and should be in their neutral form in the donor phase so that they can be transferred into the organic phase, while in the acceptor phase, they should exist in their ionized form and therefore, cannot be back-extracted into the organic phase. Generally, it is recommended that for acidic analytes, the pH in the sample should preferably be two units below the pK_a value. Therefore, the influence of donor phase pH was investigated by adjusting the sludge solution to different pH values. As seen in Figs. 3 and 4, when their ionization is inhibited (pH < 4), the enrichment factor and extraction efficiency increase significantly. The donor phase pH was adjusted to 3 in further experiments.

Ammonium carbonate buffer was used as acceptor phase because it is a volatile buffer suitable for ESI-MS and provides a suitable pH (9.5) for analytes to become deprotonated and trapped in the acceptor phase. As mentioned above, the concentration has little influence, and we selected 0.08 mol L^{-1} ammonium carbonate as acceptor buffer.

3.4. Influence of extraction time

In HF-LPME, the amount of extracted analyte over time is expected to increase until equilibrium between all phases is reached. Analyte extraction is controlled by the physico-chemical properties of the analyte, the sample matrix, and the organic and acceptor phases. To determine the optimum extraction time, 50 mL samples of sludge solutions spiked at concentration of 1 ng mL^{-1} of drugs were extracted from 1 to 8 h. When sufficient extraction time had elapsed for equilibrium to establish, a further increase in the time extraction showed a minimal effect on the extraction efficiency and enrichment factor. The enrichment factor increased with increasing exposure time up to 5 h (Figs. 3 and 4). At a higher extraction time than 5 h, the enrichment factor was reduced. Longer exposure time resulted in weak reproducibility and significant solvent dissolution. Thus, 5 h was used as extraction time for both naproxen and diclofenac.

3.5. Influence of stirring speed

The change of stirring speed plays a role in decreasing the thickness of the interfacial layer surrounding the hollow fiber and increasing the diffusion rate of drugs from donor phase to acceptor. Thus, it can enhance the enrichment factor and extraction efficiency. Stirring also decreases the equilibrium time. However, with too high stirring speeds, the contact area between sample and organic solvent is decreased and air bubbles are produced on the surface of the hollow fiber. Therefore, the effect of stirring rate for naproxen and diclofenac was examined from 480 to 1,200 rpm. As shown in Figs. 3 and 4, extraction efficiency was improved by increasing the stirring rate up to 960 rpm. Therefore, subsequent experiments were performed by stirring at 960 rpm.

3.6. Influence of salt

Salt is often added to the donor phase in order to increase the ionic strength and enhance the analyte extraction by increasing the salting-out power. With the change of the ionic strength, the viscosity will change and negatively affect the kinetics of the process. These two factors will alter the partition coefficient between the donor and the acceptor phase. Sludge is a complex matrix and its viscosity is high. In order to evaluate the effect of salt addition, different amounts of NaCl were added to the sludge sample with concentration 1 ng mL^{-1} of naproxen and diclofenac giving NaCl concentrations of 0–15% (w/v). As it can be seen from Figs. 3 and 4, a high concentration of salt has a negative effect on the enrichment factor.

3.7. Method validation and preconcentration of analytes in sludge sample

A number of performance parameters of the proposed HF-LPME method were calculated under optimized conditions described in the previous

Table 4
ANOVA results for the optimization of HF-LPME of naproxen

Source	DOF[a]	SS	Variance	*F*-ratio[b]	Pure SS	PC %[c]
pH of donor phase	3	10663.93	3554.64	28.79	10293.55	19.53
Stirring speed	3	7357.93	2452.64	19.87	6987.55	13.26
Extraction time	3	24386.43	8128.81	65.84	24016.05	45.57
Concentration of acceptor phase	3	2650.70	883.57	7.16	2280.32	4.33
Salt%	3	5667.54	1889.18	15.30	5297.16	10.05
Error	16	1975.35	123.46		3827.25	7.26
Total	31	52701.88				100.00

[a]Degrees of freedom. [b]$F_{critical}$ (3, 16; 0.05) = 3.24. [c]Percent contribution.

Table 5
ANOVA results for the optimization of HF-LPME of diclofenac

Source	DOF[a]	SS	Variance	*F*-ratio[b]	Pure SS	PC %[c]
pH of donor phase	3	180.72	60.24	68.45	178.08	9.70
Stirring speed	3	180.33	60.11	68.31	177.69	9.68
Extraction time	3	393.78	131.26	149.16	391.14	21.32
Concentration of acceptor	3	71.85	23.95	27.22	69.21	3.77
Salt%	3	993.76	331.25	376.42	991.12	54.01
Error	16	14.13	0.88		27.76	1.51
Total	31	1834.99				100.00

[a]Degrees of freedom. [b]$F_{critical}$ (3, 16; 0.05) = 3.24. [c]Percent contribution.

sections and shown in Table 6. The LOD and limit of quantification (LOQ) were determined as the minimum detectable amount of analyte with a signal to noise ratio (S/N) of 3 and 10, respectively. LOD and LOQ for naproxen and diclofenac were in the range of 0.2–0.7 and 0.7–2.5 ng g^{-1} in sludge samples, respectively. The precision of the proposed method was evaluated in terms of repeatability relative standard deviation (RSD% < 5, $n = 5$) for spiked sludge samples. Enrichment factors were evaluated for five sludge samples and the average values were calculated for each compound. The average enrichment factors were 2,300 and 1,400 for naproxen and diclofenac, respectively (see Table 6).

Table 6
Performance of the HF-LPME method with LC-MS in sludge sample

Analystes	LOD (ng g^{-1})	LOQ (ng g^{-1})	RDS[a] (%) (n = 5)	(r^2)	EF[b]
Diclofenac	0.7	2.5	3	0.9976	1,400
Naproxen	0.2	0.7	5	0.9949	2,300

[a]Diclofenac and naproxen concentration was 1 ng mL^{-1} for which R.S.D. was obtained. [b]Enrichment factor.

This method was applied for the determination of naproxen and diclofenac in sludge samples. A sludge sample was spiked with the studied analytes at concentration levels of 10 and 100 ng g^{-1} and submitted to the HF-LPME procedure described in Section 2. For evaluating the accuracy of the method, 0.5 g sludge was extracted by PLE using ASE 300-accelerated solvent extractor (Dionex, Sunnyvale, CA) equipped with 33 mL stainless steel extraction cells.

Table 7
Results obtained from analysis of sludge sample during the spring

Analystes	Concentration found in sludge (ng g^{-1})	Spiked level (ng g^{-1})	PLE + SPE Ra [a]	HF-LPME Ra
Diclofenac	35.0	10	85.5	87.3
		100	88.3	89.5
Naproxen	45.0	10	99.4	101.2
		100	102.1	100.4

[a]Relative recovery (average of three determinations).

Table 8
Comparison of the characteristics for the methods described in the literature with the proposed method for the determination of naproxen and diclofenac in sewage sludge samples

Analyets	Preconcentration method	Determination technique	Sample amount (g)	LOQ (ng g^{-1})	RSD (%)	References
Diclofenac	SPE-USE	LC–MS/MS	0.5	20	8	[10]
Diclofenac	DME[a]-SPE	GC–MS	0.5	22	15	[11]
Naproxen				15	13	
Diclofenac	SPE-PLE	LC–MS/MS	1	3.13	2	[12]
Naproxen				0.24	5	
Diclofenac	SPE-USE	LC–UV and LC–FLD	1.5	3.69	0.1 - 23	[9]
Naproxen				7.53		
Diclofenac	SPE-PLE	LC–MS/MS	1	96.3	1	[8]
Naproxen				65.2	3.5	
Diclofenac	HF-LPME	LC-MS	0.5	2.5	3	This work
Naproxen				0.7	5	

[a]Dispersive matrix extraction.

The sludge samples were spiked with a standard mixture of analytes to 10 and 100 ng g^{-1} concentrations. Optimization of extraction parameters and extraction method was followed according to the previously reported method [12]. The extraction method was established with the following parameters: 0.5 g of sample, temperature 100°C, methanol/water, 1/2 (v/v) as extraction solvent, temperature 100°C, a preheating period of 5 min, 3 static cycles, each lasting 5 min, and total flush volume of 100% of cell with 60 s of nitrogen purge. The extract obtained in PLE was diluted to 500 mL with HPLC water (methanol <5%) and processed by SPE. Finally, the compounds were eluted with 8 mL of methanol at a flow rate of 1 mL min^{-1}.The SPE extracts were evaporated under a nitrogen stream and reconstituted with 1 mL of methanol. Prior to the analysis, the samples were passed through 0.45 μm filters. The data obtained with the proposed PLE methods for real samples are presented in Table 7. The results of analysis of samples showed that the proposed method can be reliably used for the determination naproxen and diclofenac in different matrixes.

3.8. A comparison of analytical performance data

A comparison of the presented method with other reported preconcentration methods for the determination of naproxen and diclofenac in sewage sludge samples is given in Table 8. In comparison with other reported methods, HF-LPME has a low LOD, low RSD, and minimum use of organic solvent (a few microliters of DHE). The evaluation of the technique was done with few experiments because of using experimental design method.

4. Conclusions

A method based on HF-LPME sample pretreatment was successfully developed for the extraction and preconcentration of naproxen and diclofenac in sludge samples. Three-phase HF-LPME as a clean-up method decreased the matrix effect and produced relatively high enrichment factors in the extract of the sewage sludge. Taguchi OAD was employed to optimize the HF-LPME conditions for the determination of target analytes. OAD led to considerable time-saving and considered interactions among extraction conditions, which were not possible in a univariate approach. An OA_{16} (4^5) matrix was employed to evaluate the effects of the five factors in four levels. The data obtained from orthogonal array experiments were evaluated by means of range analysis and ANOVA. The ANOVA results showed that all factors were statistically significant at $p < 0.05$ for both naproxen and diclofenac. The proposed method showed good repeatability and low detection limits.

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