Determination of non-steroidal anti-inflammatory drugs in urine by hollow-fiber liquid membrane-protected solid-phase microextraction based on sol–gel fiber coating

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ABSTRACT

A new rapid, simple and effective cleanup procedure is demonstrated for the determination of ibuprofen, naproxen and diclofenac in urine samples by using hollow-fiber liquid membrane-protected solid-phase microextraction (HFLM-SPME) based on sol–gel technique and gas chromatography–flame ionization detector (GC–FID). In this technique, a sol–gel coated fiber was protected with a length of porous polypropylene hollow fiber membrane which was filled with water-immiscible organic phase. Subsequently the whole device was immersed into urine sample for extraction. Poly(ethylene glycol) (PEG) grafted onto multi-walled carbon nanotubes (PEG-g-MWCNTs) was used as extraction phase to prepare the sol–gel SPME fiber. Important parameters influencing the extraction efficiency such as desorption temperature and time, organic solvent, extraction temperature and time, pH, stirring speed and salt effect were investigated and optimized. Under the optimal conditions, the method detection limits (S/N = 3) were in the range of 0.03–0.07 ng mL^{-1} and the limits of quantification (S/N = 10) between 0.08 and 0.15 ng mL^{-1}. Relative standard deviations for intra-day and inter-day precisions were 4.8–9.0% and 4.9–8.1%, respectively. Subsequently, the method was successfully applied to human urine fractions after administration of ibuprofen, naproxen and diclofenac.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstay of drug therapy for the inflammation and pain associated with various forms of arthritis. In the case of long-term NSAIDs therapy, a high incidence of severe side effects, including gastrointestinal ulcer and nephrosis, has been reported, especially in rheumatoid arthritis therapy [1–3]. Hence, it is considered that the monitoring of NSAIDs is of importance in order to improve the toxicological management of long-term NSAID therapy.

Solid-phase microextraction (SPME) technique, introduced by Pawliszyn and coworkers [4,5], is a convenient and solvent-free extraction method which combines extraction, concentration and sample introduction in one step. This technique has important advantages over conventional extraction techniques due to its ease of use, being rather rapid, easy-to-automate, portable and solvent-free. Up to now, several SPME technique coupled with gas chromatography (GC) have been developed for the analysis of NSAIDs [6–11] and high performance liquid chromatography (HPLC) [12–14].

In spite of the advantages of this technique, the lack of proper chemical bonding of the extraction phase coating and the relatively high thickness of the conventional fibers seem to be responsible for some drawbacks of the commercial fiber such as: low thermal and chemical stability, the stripping of coating and short lifetime [15].

Sol–gel chemistry can overcome this problem by providing efficient incorporation of organic component into the inorganic polymeric structure in solution under very mild thermal conditions [16]. The porous structure of the sol–gel coating offers a high surface area; allowing high extraction efficiency and the coating composition can be altered with a relative ease to give different selectivity characteristics. Strong adhesion of the coating onto the support due to chemical bonding is a very important characteristic which increases the coating stability toward organic solvents and high desorption temperatures [15].

Compared with other materials used for SPME, nanomaterials offer a significant higher surface area-to-volume ratio that promises much greater extraction capacity and efficiency. Carbon nanotubes (CNTs), with large accessible specific surface area, the ability to establish π–π interactions, excellent chemical, mechanical and thermal stability, etc., are a new type of carbon nanomaterial first found in 1991 by Iijima [17]. Carbon nanotubes as a kind of effective sorbent nanomaterials have been successfully used as the SPME fiber coating for analyzing organic compounds [18–22].

In this work, poly(ethylene glycol) (PEG) grafted onto multi-walled carbon nanotubes (PEG-g-MWCNTs) were used as coating material. Polymer functionalization with PEG is adopted to improve the solubility of MWCNTs in organic solvents, selectivity and their viscosity, which could be good for coating.

The main drawback of silica based sorbents is the narrow range of pH stability. Under extreme pH conditions, silica-based materials become chemically unstable, and their sorptive properties may be compromised. Therefore, the development of alternative materials possessing superior pH stability and better mechanical strength should provide SPME with additional ruggedness and versatility. Recently, titania has attracted interest in separation science due to its superior pH stability and mechanical strength compared with silica. A number of reports have also recently appeared in the literature on the use of titania based hybrid organic–inorganic sol–gel sorbents in SPME [23–25].

The complexity of the sample can affect the recovery of the analytes, and also the analytical method precision, the accuracy, and the sample compatibility with a subsequent chromatographic technique [26]. Furthermore, for samples containing both non-volatile target analytes and high-molecular weight interfering compounds, the application of direct or headspace SPME may be challenging.

In 1999, Pedersen-Bjergaard and Rasmussen introduced an extraction technique that reduced solvent consumption, termed hollow-fiber liquid-phase microextraction (HF-LPME) [27]. HF-LPME combines extraction, concentration and sample clean-up in one step. 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, thus yielding very clean extracts. Additional advantages of HF-LPME are its tolerance to a wide pH range, as well as its application in assays that are not suitable for silica-based SPE or SPME.

For the first time, Basheer and Lee [28] proposed a SPME procedure with the polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber protected by a polypropylene hollow fiber membrane. The method was effective for protection of SPME fiber in "dirty" samples.

In a previous study [29], a silica-based sol–gel PEG-g-MWCNTs coated fiber was found to be very effective in extracting nonpolar analytes when coupled to GC analysis. In this study, we report the preparation of the titania based hybrid organic–inorganic sol–gel sorbents from a precursor, titanium(IV) isopropoxide, and a sol–gel-active polymer (PEG-g-MWCNTs). For the first time, we introduced a new method, which combines the advantages of SPME based sol–gel, CNTs and HF-LPME for extraction and preconcentration of ibuprofen, naproxen, and diclofenac in urine samples. This new method is termed hollow-fiber liquid membrane-protected solid-phase microextraction (HFLM-SPME). In this method, a titania based sol–gel PEG-g-MWCNTs coated fiber was protected by a hollow fiber impregnated with a water-immiscible organic phase. The performance of the proposed method was evaluated by comparing with the results of DI-SPME and HF-LPME.

2. Experimental

2.1. Chemicals and materials

Trifluoroacetic acid (TFA, 99%), poly(ethylene glycol) (PEG, MW 6000), tetrahydrofuran (THF), thionyl chloride (SOCl$_2$) and sodium chloride were purchased from Merck (Darmstadt, Germany). Titanium(IV) isopropoxide was obtained from Aldrich (Sigma–Aldrich,

Germany). The drugs, ibuprofen (99.9%), naproxen (99.9%), and diclofenac (99.9%), were obtained from Daana pharmaceutical Co. (Tabriz, Iran). These drugs were provided in powder form. The MWCNTs (outer diameter 10–20 nm, length range of 5–15 μm, purity >95%, specific surface area >40–300 m^2 g^{-1}) were purchased from Shenzhen Nanotech Port (Shenzhen, China). Hydrochloric acid (37% (w/w), Merck, Darmstadt, Germany) was used for modification of sample pH.

Methanol, 2-octanone and toluene with Suprasolv quality were obtained from Merck (Darmstadt, Germany). 1-Octanol was purchased from Fluka (Buchs, Switzerland). The porous membrane used to support the organic phase and for protecting the sol–gel fiber was an accurel Q 3/2 polypropylene hollow fiber membrane (Membrana, Wuppertal, Germany). The wall thickness of the fiber was 200 μm, the inner diameter was 600 μm, and the pore size was 0.2 μm.

2.2. Instrumentation

A Chrompack CP9001 gas chromatography system equipped with a split/splitless injector and flame ionization detector (FID) was employed for the SPME-GC experiments. Helium (99.999%, Sabalan Co., Tehran, Iran) was employed as carrier gas and its flow rate was adjusted to 1 mL min^{-1}. The separation was performed with a CP-Sil 24CB (50% phenyl, 50% dimethylsiloxane) capillary column, WCOT Fused silica, 30 m × 0.32 mm ID with 0.25 μm stationary film thickness (Chrompack, Middelburg, The Netherlands). The column temperature was programmed as follows: initial oven temperature 80 °C for 3 min, increasing to 260 °C at 20 °C min^{-1} then holding for 10 min. The optimum desorption temperature was selected as 280 °C and optimum desorption time was 5 min. The detector temperature was held at 300 °C.

The surface characteristics of the created sol–gel PEG-g-MWCNTs coating were studied by scanning electron microscopy (SEM) (LEO, model 1450VP, Germany).

A VELP Scientifica heating magnetic stirrer, model ARE (Milano, Italy) was employed for stirring and heating samples during the extraction. To mix various solution ingredients, a ultrasonic bath (Branson 1510, Branson Ultrasonics Co., Danbury, CT), was employed at a frequency of 42 kHz.

2.3. Fiber preparation

The titania based sol–gel PEG-g-MWCNTs coated fiber was prepared using the exact same procedure used for the sol–gel silica based PEG-g-MWCNTs coating [29]. However, instead of using methyltrimethoxysilane, titanium(IV) isopropoxide was used as the sol–gel precursor. Before being used, the MWCNT-g-PEG sol–gel fiber was conditioned at the GC injection port under helium gas, at 100 °C for 30 min, then 200 °C for 1 h, and finally 300 °C for 1 h.

2.4. Sample preparation

Stock standard solutions of ibuprofen, naproxen and diclofenac (100 mg L^{-1}) were prepared by dissolving proper amounts of each drug in methanol and stored in the dark at 4 °C. The urine samples were collected from drug-free healthy volunteers. When HFLM-SPME or HF-LPME was applied, do not require any further pre-treatment steps. But for DI-SPME, urine samples were filtered through a 0.22 μm membrane (Millipore) and diluted 10 times with deionized water.

The urine samples were stored at −20 °C prior to use. Spiked urine samples with ibuprofen, naproxen and diclofenac were prepared from the stock solutions freshly prior to analysis. When

higher concentrations (more than 400 ng mL^{-1}) of real samples were tested, the urine samples were diluted with deionized water.

2.5. HFLM-SPME procedure

The general photograph of the HFLM-SPME setup is illustrated in Fig. 1. The hollow fiber was cut into 2.5-cm segments for HFLM-SPME experiments. Each piece of the hollow fiber was employed only once to avoid any possibility of carryover. The hollow fiber segments were sonicated in acetone to remove any contaminants in the hollow fiber. After sonication, the hollow fibers were removed from acetone and the solvent was allowed to evaporate completely.

For each HFLM-SPME analysis, 3 mL of spiked urine sample was placed into a long-neck 3 mL glass vial without headspace. The vial was sealed with a rubber septa to prevent sample evaporation. Prior to extraction, sample solution was adjusted to pH 2 with hydrochloric acid solution. A Hamilton 10 μL microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was used to introduce the organic solvent into the hollow fiber. A 6 μL of the organic solvent was withdrawn into the microsyringe and its needle was inserted into the lumen of the hollow fiber. The fiber was immersed in an organic phase for 30 s in order to impregnate the pores of the hollow fiber with the organic solvent. The excess of organic phase was carefully removed by washing the outside of the hollow fiber with ultrapure water. Then, 6 μL of the organic solvent was injected into the lumen of hollow fiber and the end of the hollow fiber was sealed up by hot pliers. Subsequently, the membrane was then mounted onto the tip of the stainless steel protective tubing for the sol–gel coated SPME fiber. Then the SPME fiber was inserted into the lumen of the hollow fiber. The prepared fiber assembly was immersed in the sample solution. The extraction was performed at 30 °C and the sample was stirred at 800 rpm during the extraction (80 min). After extraction, the hollow fiber was discarded. The sol–gel coated fiber was gently wiped with soft tissue to remove the remaining solvent and inserted into the hot injection port of the GC at 280 °C for desorption. The fiber was cleaned everyday prior to the first extraction by leaving it in the injection port for 30 min at 300 °C to eliminate any carry-over of analytes from the previous extraction. Prior to starting a set of experiments, a blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber.

2.6. DI-SPME procedure

For each DI-SPME analysis, 3 mL of spiked urine sample was placed into a 3 mL glass vial without headspace. The vial was sealed with a rubber septa to prevent sample evaporation. Prior to extraction, each sample was adjusted to pH 2 with 1.0 mol L^{-1} hydrochloric acid solution. The extraction was performed by exposing of the titania based sol–gel PEG-g-MWCNTs coating fiber directly into the sample solution at 50 °C for 60 min under magnetic stirring (1000 rpm). After extraction, the fiber was then immediately inserted into the heated GC injection port for thermal desorption.

2.7. HF-LPME procedure

As a further comparison, urine samples were also processed by HF-LPME. Hollow fibers were cut into 2.5-cm pieces, washed with acetone in ultrasonic bath and dried. Each fiber was used only once to decrease the memory effect. For each experiment, 3 mL of the spiked urine sample (pH 2) was poured into a 3 mL sample vial having a magnetic stirring bar. A Hamilton 10 μL microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was used to introduce organic solvent (1-octanol) into the hollow fiber, to support the hollow fiber, and to serve as an injection device. The hollow fiber was then immersed in the organic solvent for 30 s to impregnate its pores with organic phase. Then 6 μL of the organic solvent was introduced into the hollow fiber with slow pushing of the microsyringe plunger. Then, the fiber was placed into the sample solution. The sample solution was stirred at 800 rpm during the extraction. After extraction for 60 min, the extraction solvent was withdrawn and 1.0 μL was injected into the GC–FID for analysis.
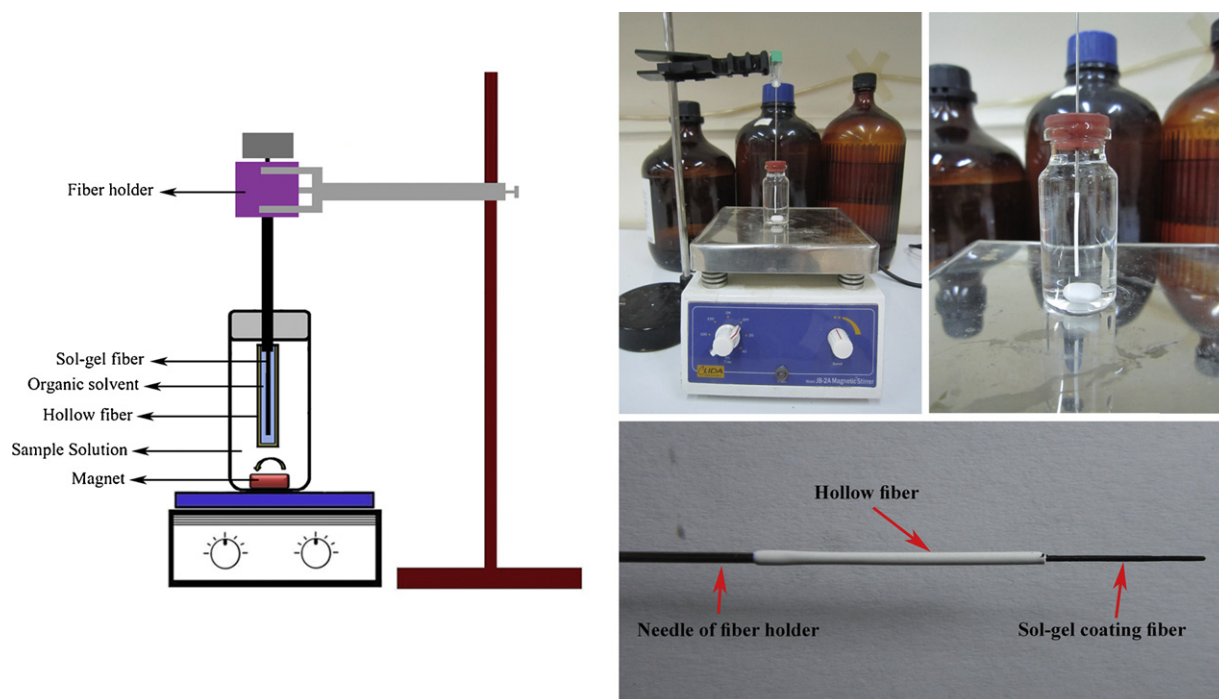

Fig. 1. Photograph of the hollow-fiber liquid membrane-protected solid-phase microextraction.
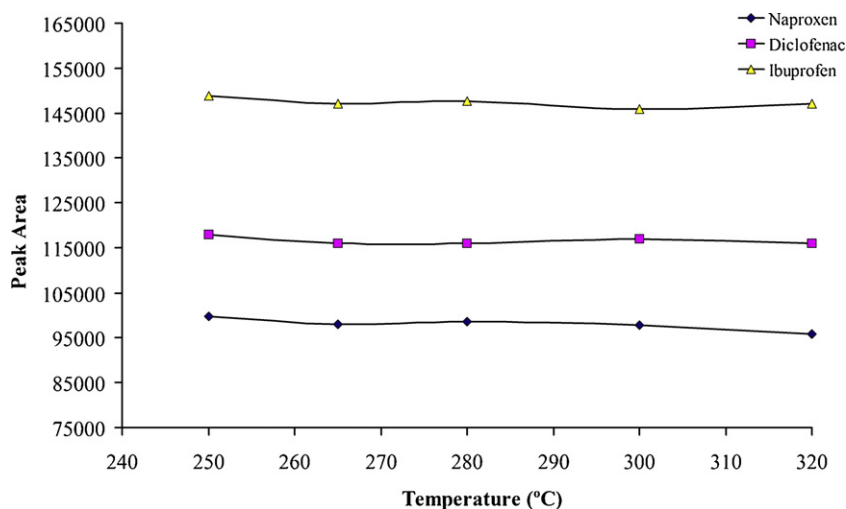
Fig. 2. Thermal stability of titania based sol–gel PEG-g-MWCNTs coated fiber.

3. Results and discussion

3.1. Coating properties

In sol–gel approach, the extraction phase coating is chemically bonded to the surface of the fiber under extremely mild thermal conditions. The chemical bonding between the coating and the surface of silica surely would enhance the thermal and chemical stability and lifetime of the fiber coating. In this study, titania iso-propoxide was used as the sol–gel precursor and PEG-g-MWCNTs as the sol–gel active polymer. The approximate film thickness of the titania based sol–gel PEG-g-MWCNTs coating on the fiber is estimated to be 20 μm.

3.2. Thermal stability and lifetime of the coating

Fig. 2 demonstrates the thermal stability of the titania based sol–gel PEG-g-MWCNTs coating fiber. As can be seen from the figure, the extraction efficiency of the analytes dose not significantly decrease after it was conditioned for 1 h at 250, 265, 280, 300 and 320 °C. Such a high operating temperature achieved is due to the thermal stability of MWCNTs and the strong chemical bonding provided by sol–gel technology. The enhanced thermal stability allowed the use of a higher injection port temperature for efficient desorption of semivolatile analytes and thus extended the range of

analytes. The fiber's lifetime was studied by monitoring the change of its extraction efficiency after it had been used for 30, 60, 90, 120 and 150 times at 280 °C. As can be seen in Fig. 3, the response has no obvious decline after being used for 150 times. Thus, the lifetime of this fiber is longer than that of commercial fibers. The fiber can be used over 150 times while all commercial fibers can only be used about 40–100 times [30,31].

3.3. Optimization of extraction and desorption conditions of HFLM-SPME

Experimental conditions, like extraction solvent, desorption time and temperature, extraction temperature and time, sample pH, the salting effect and agitation of the sample were optimized before validating the analytical method. All our experiments were carried out in triplicates.

3.3.1. Selection of organic solvent

Selection of the proper organic solvent used in HFLM-SPME is an important consideration for an efficient extraction. Because, it has two functions, the acceptor phase in the former liquid–liquid microextraction procedure and the extraction solvent in the latter liquid-coating fiber back extraction procedure [32]. The selected organic solvent has to satisfy the following requirements: (1) compatible with the hollow fiber to fill the pores of the fiber completely;
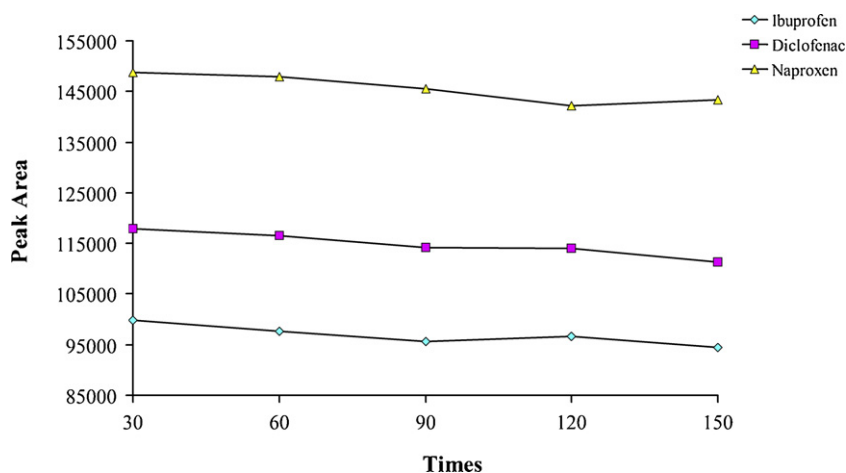

Fig. 3. Lifetime profile of titania based sol–gel PEG-g-MWCNTs coated fiber.

(2) good affinity for the target compound; (3) immiscible with water and urine to avoid dissolution and be nonvolatile to prevent solvent loss during the extraction; (4) compatible with the titania based sol–gel PEG-g-MWCNTs fiber coating. Considering the above factors, three organic solvents, including 1-octanol, toluene and 2-octanone were evaluated as the membrane solvents. Among the tested organic solvents, 1-octanol exhibited the highest extraction efficiency for the target analytes. In addition, 1-octanol has a low loss during extraction, and it could be easily immobilized in the pores of the hollow fiber. Thus, 1-octanol was selected as the extraction solvent for subsequent studies.

3.3.2. Carry-over

Carry-over was determined by analyzing the fiber blank directly after the initial injection and expressed as percentage of the initial peak area. The percent carry-over was measured by dividing the peak area from the second GC analysis by the sum of the peak areas from the first and second analysis. Carry-over of the titania based sol–gel PEG-g-MWCNTs fiber, after a desorption step of 5 min at 280 °C, were investigated in order to detect the presence species in the coating. The carry-over of ibuprofen, naproxen and diclofenac were 0.5, 0.7, and 1.2%, respectively. To minimize carry-over effects after each extraction, the fiber was kept in the injection port for 5 min more after starting the run with the injector in the split mode.

3.3.3. Effect of temperature

Temperature has a significant effect on both the kinetics and the thermodynamics of the extraction process. High temperature reduces the time required for reaching equilibrium by enhancing the diffusion of analyte toward the organic phase. On the one hand, temperature can alter distribution coefficients of analyte between the donor phase-organic solvent and organic solvent, thus influencing extraction yield. However, heating might also cause swelling in fibers and does not favor organic solvent immobilization in membrane pore [33]. Extraction temperature was studied at 20–60 °C. The experimental results show that extraction efficiency was maximum at 30 °C. Therefore, a temperature of 30 °C was selected as the extraction temperature.

3.3.4. Extraction time

SPME and LPME are not exhaustive processes but equilibrium processes, in which analytes are partitioned between the sample matrix, the organic solvent and the coating. The equilibrium time refers to the time after which the amount of extracted analyte remains constant and corresponds to the amount extracted after infinite time, within the limits of experimental error. The SEM

micrograph provided an estimated film thickness of 20 μm for the sol–gel coating. This thickness is almost five times smaller than the conventional 100-μm coating thickness used on commercial fused-silica fibers. The smaller coating thickness should help faster mass transfer during extraction as well as analyte desorption processes during sample introduction. But, due to the fact that there are two interfaces in this extraction system (i.e. sample solution-organic phase and organic phase-fiber coating) and existence of a membrane barrier between the sample solution and organic phase, solute molecules require a long time to pass through these interfaces.

The extraction time varied from 20 to 120 min for determination of the optimum extraction time (Fig. 4). It can be seen from Fig. 4 that extraction efficiencies for all compounds under investigation increased by increasing the extraction time up to 80 min. It also increased, but at slower rate, between the extraction times of 80 and 120 min. Considering there is a potential solvent loss with continuing prolongation of exposure time and it is not necessary to attain equilibrium if extracting conditions remain constant, 80 min was selected as extraction time for the subsequent experiments.

3.3.5. Salt concentration

The salting-out effect has been used extensively in different extraction methods and it has been reported that addition of salt can increase the extraction efficiency. The effect of salt on extraction efficiency was determined by adding sodium chloride at 0, 5, 10 and 15 (w/v). Based on the results obtained, the addition of salt led to a decrease in the extraction efficiency of the analyte. The negative effect is attributed to changes in the physical properties of the extraction film, which results in reducing the diffusion rate of the analytes from the sample into the organic phase [34]. Therefore, sample analysis in this work was performed without salt addition.

3.3.6. Stirring speed

Stirring rate affects the kinetics of the extraction. In fact it improves mass transfer in the source phase and induces convection in the membrane phase. Sample agitation enhances the extraction rate and reduces extraction time because the equilibrium can be achieved more rapidly. It also enhances the diffusion of the analyte from the source phase into the receiving phase. The effect of stirring rate in the range of 200–1000 rpm was evaluated. Extraction efficiency of the three analytes improved as the agitation rate increasing from 200 to 800 rpm. However, when it exceeded 800 rpm, instead of increasing, peak area decreased. This phenomenon can be accounted for the fact that although agitation could accelerate distribution equilibrium by facilitating mass
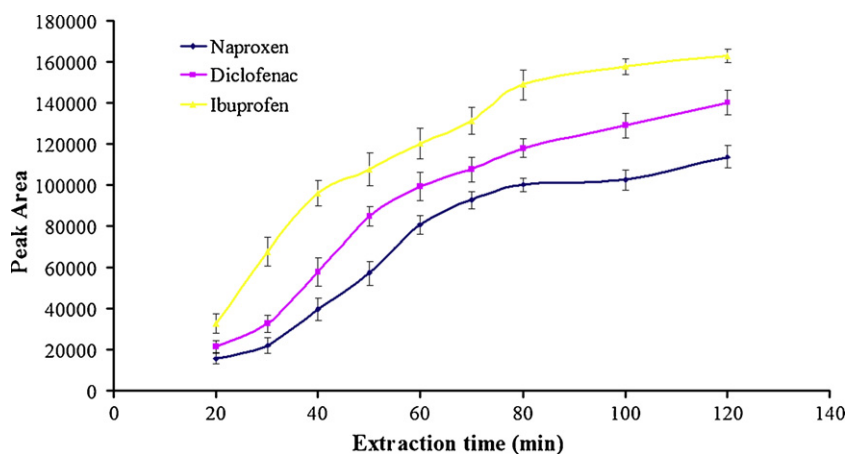

Fig. 4. Effect of extraction time on the extraction efficiency. Extraction conditions: sample volume, 3 mL; desorption temperature, 280 °C; desorption time, 5 min; extraction temperature, 30 °C; sample pH, 2; no adjustment of salt.

transfer, high stirring speed would generate air bubbles adhering on the surface of hollow fiber and promote solvent loss. Therefore, 800 rpm of stirring speed was selected.

4. Validation of the method

The analytical characteristics of the optimized HFLM-SPME method in terms of its linear range, limits of detection (LODs) and limits of quantitation (LOQs) were investigated to estimate the efficiency and the feasibility for application to the analysis of urine samples.

4.1. Linearity, LOD and LOQ

In the optimum conditions, calibration curves were constructed from the analysis of eight spiked urine samples containing all the analytes at different concentrations. For each point three replicate extractions were performed. The results are summarized in Table 1. Good linear relationship between the corresponding peak areas and the concentrations were obtained for all the analytes ($r > 0.9985$). The large linear range of concentrations for this method can be satisfactorily applied for therapeutic drug monitoring of ibuprofen, naproxen and diclofenac. This range can also be applied in pharmacokinetics studies of the drug, even when using sub-clinical doses.

The limits of detection (LODs) and quantification (LOQs), based on signal-to-noise ratio (S/N) of 3:1 and 10:1, were determined. Excellent results were obtained with LOD values in the low ng mL^{-1} range (Table 1), thus proving the potential of the method for the determination of NSAIDs compounds at trace levels. LODs and LOQs were obtained in the range of 0.03–0.07 ng mL^{-1} and 0.08–0.15 ng mL^{-1}, respectively.

The absolute recovery is defined as the amount extracted by the fiber from the total amount of analyte present in the sample vial × 100% and corresponds to the term recovery as defined in FDA guidelines [35]. Regulatory guidelines do not require absolute recovery to be 100% as long as the absolute recovery for a particular method is known and reproducible. In microextraction methods, absolute recovery is calculated by comparison of sample response to that of calibration curve obtained by direct liquid injection of analyte standards. Because SPME is a non-exhaustive extraction technique, recoveries are usually low. Therefore, for the determination of absolute recovery, urine samples spiked with ibuprofen, naproxen and diclofenac at two concentration levels (5.0 and 200 ng mL^{-1}) were analyzed, and peak areas were compared with those obtained by a splitless injection of 1 μL of a methanolic solution containing the same amount of the analytes. The absolute recoveries achieved by the method were between 8.1 and 12.1% (Table 1).

4.2. Method accuracy and precision

Method accuracy and precision were evaluated on spiked urine samples at three concentration levels: 0.2, 50, and 200 ng mL^{-1}.

The results of the intra-day and inter-day precision at three different concentration levels are presented in Table 2. The intra-day precision of the method was investigated on standard solutions by performing daily five replicates. The same solutions were analyzed three times each day for a period of 5 days for the inter-days precision evaluation. As it is illustrated, the intra-day RSD% ($n = 5$) and inter-days ($n = 3$ over 5 days) RSD% were 4.8–9.0% and 4.9–8.1%, respectively.

Accuracy (or relative recovery) is defined as the ratio of the concentration experimentally determined and true concentration × 100%, which corresponds to the term accuracy as defined in US Food and Drugs Administration (FDA) guidelines [35].
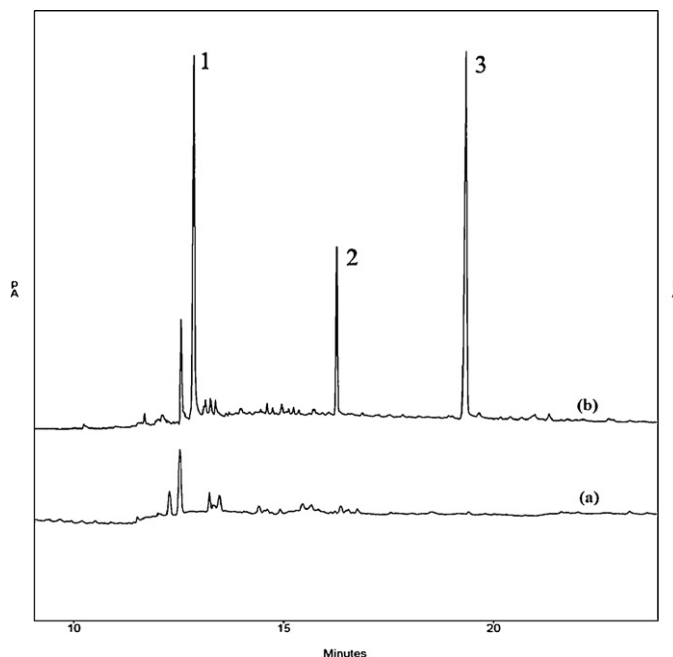

Fig. 5. Typical chromatograms of the healthy urine sample obtained by HFLM-SPME. (a) Blank urine sample and (b) urine sample spiked with 20 ng mL^{-1} of NSAIDs. Peak numbers correspond to (1) ibuprofen, (2) naproxen, (3) diclofenac.

In microextraction methods, such as SPME, relative recovery is obtained by comparing the response obtained from sample to the response of calibration standards which were extracted using the exact same conditions and from the same matrix as the samples.

Relative recovery was performed using spiked human urine samples from volunteers to obtain three concentration levels of ibuprofen, naproxen and diclofenac that were submitted to the HFLM-SPME and GC determination procedure described in Section 2. Table 2 shows a summary of accuracy results. Accuracies for ibuprofen, naproxen and diclofenac were found to be between 80.2% and 98.5% for the concentrations evaluated.

These results clearly demonstrate the absence of significant matrix effects on the efficiency of HFLM-SPME. Minor matrix effect on the HFLM-SPME method is probably attributed to the selectivity of the hollow fiber due to the pores in its wall. It is apparent that porous hollow fiber functions as a filter in dirty samples, since large molecules, which can also be soluble in the organic solvent, will not be extracted. In this way, this microextraction technique can be potentially used to extract complex matrixes, while preventing co-extraction of extraneous materials.

Fig. 5 shows typical chromatograms of HFLM-SPME extracts from both the spiked and blank urine samples.

5. Comparison of HFLM-SPME with HF-LPME and DI-SPME

Some statistical data of the proposed method were compared with HF-LPME and DI-SPME methods (Table 3) for determination of ibuprofen, naproxen and diclofenac in urine samples.

In DI-SPME, urine samples are generally diluted with a suitable buffer solution and filtered through a syringe filter to suppress matrix effects and to prevent contamination of the fiber. Interfering compounds or suspended particles can be adsorbed by fiber coatings during the DI-SPME technique [36].

The small pore size of the hollow fiber 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, thus yielding very clean extracts. It

Table 1
Figures of merit of the HFLM-SPME method.

Analyte	Regression equation	Linear range (ng mL^{-1})	Correlation coefficient (r)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Absolute recovery (%) ($n = 5$)	
						5 (ng mL^{-1})	200 (ng mL^{-1})
Ibuprofen	$y = 1313.5x + 1128.6$	0.08–400	0.9990	0.03	0.08	9.3	8.1
Naproxen	$y = 851.81x + 452.95$	0.15–400	0.9992	0.07	0.15	10.5	9.7
Diclofenac	$y = 1043.3x + 793.81$	0.1–400	0.9985	0.05	0.1	12.1	11.8

Table 2
The accuracy, intra-day and inter-day precisions of the HFLM-SPME method for the analysis of ibuprofen, naproxen and diclofenac in urine samples.

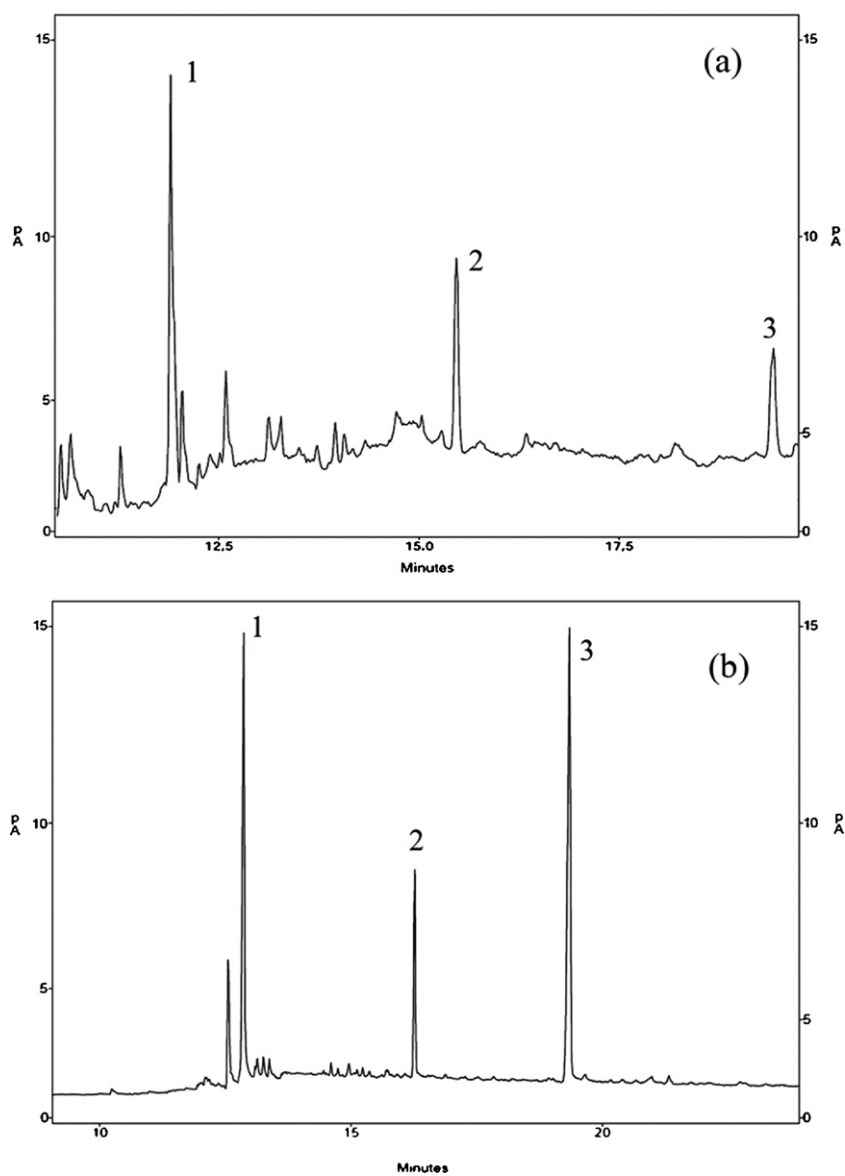| Urine concentration (ng mL^{-1})	Precision RSD (%)						Relative recovery (%)		
	Intra-day			Inter-day			Ibuprofen	Naproxen	Diclofenac
	Ibuprofen	Naproxen	Diclofenac	Ibuprofen	Naproxen	Diclofenac			
0.2	7.1	9.0	7.9	6.1	7.6	8.1	90.3	83.2	80.2
50	5.2	7.5	6.8	5.2	6.3	7.2	97.1	91.7	91.4
200	4.8	7.1	5.9	4.9	5.6	6.5	98.5	95.3	95.8

Fig. 6. Typical chromatograms of the healthy urine sample obtained by (a) DI-SPME and (b) HFLM-SPME.

Table 3
Comparison of HFLM-SPME with DI-SPME and HF-LPME for determination of ibuprofen, naproxen and diclofenac in urine sample.

Compounds	HF-LPME			DI-SPME			HFLM-SPME		
	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%) ($n = 5$)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%) ($n = 5$)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%) ($n = 5$)
Ibuprofen	5.0–1000	2.0	3.2	0.5–500	0.25	8.2	0.08–400	0.03	5.2
Naproxen	10.0–1000	4.0	3.9	1.5–500	0.8	10.5	0.15–400	0.07	7.5
Diclofenac	10.0–1000	5.0	4.1	0.7–500	0.3	7.9	0.1–400	0.05	6.8

Table 4
Concentration of the ibuprofen, naproxen and diclofenac in urine after the administration of oral doses.

Sample	Administration		Sampling time (min)[b]	Concentration (μg mL^{-1})
	NSAID	Dosage[a]		
Urine 1	Ibuprofen	200	180	2.51
Urine 2	Ibuprofen	200	300	1.74
Urine 3	Naproxen	500	180	3.85
Urine 4	Naproxen	500	300	2.90
Urine 5	Diclofenac	100	180	0.25
Urine 6	Diclofenac	100	300	0.12

[a] Amount of drug administrated via oral (in mg).
[b] Sampling time after administration (min).

can be seen from Fig. 6 that urine extracts from HFLM-SPME were remarkably clean when compared with extracts from DI-SPME.

Additional advantages of HF-LPME are its tolerance to a wide pH range, as well as its application in assays that are not suitable for silica-based SPE or DI-SPME. Although, the LODs obtained by the DI-SPME method is lower than HF-LPME method. This can be explained, taking into account that the whole extract is injected in DI-SPME, while only 1.0 μL (a fraction of the extract) was analyzed in HF-LPME. In addition, sol–gel coatings possess porous structures which should significantly increase the surface area availability on the fibers. Also MWCNTs with high surface area as a sorbent modifier will also be able to provide the enhanced adsorption efficiency for the analyte.

Under the optimum conditions, the repeatability, expressed as relative standard deviations (RSDs), was evaluated by five consecutive extractions at concentration of 50 ng mL^{-1} of the target compounds. The repeatability of HF-LPME was found to be better than DI-SPME method.

The combination of the SPME based sol–gel and HF-LPME essentially allows the proposed extraction method to integrate the advantages of both microextraction methods. It can be seen that HFLM-SPME has a lower LOD, satisfactory relative standard deviations and a relatively wide linear range. These advanced performances are mainly due to double preconcentration process (HF-LPME and SPME).

This approach allowed us to determine the trace level concentration of NASIDs in urine samples without further sample pretreatment. Logically, the sensitivity and therefore detection limits can be improved using other detectors like mass spectrometer.

In addition to excellent enrichment, a high sample clean-up potential was observed for HFLM-SPME regarding the biological samples.

6. Method application

Finally the proposed method was applied to the determination of the NSAIDs in urine samples. After administration of the NSAIDs included in this work to several volunteers, the urine sampling was accomplished in the interval of 180–300 min. The quantitative results of these urine samples are listed in Table 4.

7. Conclusion

In this work for the first time, hollow fiber-protected of chemically bonded titania based sol–gel PEG-g-MWCNTs coating were successfully applied for the analysis of NSAIDs in urine samples. Due to the unique properties of CNTs and the inherent advantageous features and also the performance of the sol–gel coating technology, this innovative fiber exhibited porous surface structure, good precision and accuracy, high sensitivity and high thermal stability. But in most analyses, due to suppress matrix effects and to prevent contamination of the fiber, the sample are diluted with a suitable buffer and filtered through a syringe filter. On the other hand, the small pore size of hollow fiber 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, thus yielding very clean extracts. The HFLM-SPME method, integrating the advantages of SPME based sol–gel coating and enrichment and sample cleanup capability of the HF-LPME into a single device, is a promising sample preparation method for complex samples.

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