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Application of dynamic liquid-phase microextraction and injection port derivatization combined with gas chromatography–mass spectrometry to the determination of acidic pharmaceutically active compounds in water samples

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ABSTRACT

A method has been established for the determination of four pharmaceutically active compounds (ibuprofen, ketoprofen, naproxen and clofibric acid) in water samples using dynamic hollow fiber liquid-phase microextraction (HF/LPME) followed by gas chromatography (GC) injection port derivatization and GC–mass spectrometric (MS) determination. Dynamic HF/LPME is a novel approach to microextraction that involves the use of a programmable syringe pump to move the liquid phases participating in the extraction so as to facilitate the process. Trimethylanilinium hydroxide (TMAH) was used as derivatization reagent for the analytes to increase their volatility and improve chromatographic separation. Parameters that affect extraction efficiency (selection of organic solvent, volume of organic solvent, agitation in the donor phase, plunger movement and extraction time) were investigated. Under optimal conditions, the proposed method provided good enrichment factors up to 251, reproducibility ranging from 3.26% to 10.61%, and good linearity from 0.2 to 50 μg/L. The limits of detection ranged between 0.01 and 0.05 μg/L ($S/N=3$) using selective ion monitoring. This method was applied to the determination of the four pharmaceutically active compounds in tap water and wastewater collected from a drain in the vicinity of a hospital.

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1. Introduction

In mainly developed countries, thousands of tonnes of pharmaceuticals are used to treat illnesses. Due to such high usage of these materials, dozens of pharmaceutically active compounds (PhACs) have entered the aquatic environment, mainly through human wastes by excretion of the parent compounds and/or their metabolites [1]. Many of these compounds are not subjected to degradation in sewage treatment plants, and are then introduced to receiving waters. The increased reuse (by reclamation) of water resource has raised concerns about the potential risk of these compounds to human life. As such, trace determination of PhACs in the aqueous environment has become an emerging issue for environmental and health authorities across the European Union (EU), in North America and elsewhere. In the past decade, these compounds have been widely detected in different environmental water samples including sewage water, surface water, groundwater and even drinking water with concentrations up to the μg/L range [2–12].

Clofibric acid, ibuprofen, naproxen and ketoprofen are four common acidic PhACs. Clofibric acid is the active metabolite of several blood lipid regulators (clofibrat, etofibrat and etofyllinclofibrat). It was the first reported PhAC in the aquatic environment [13] and remains the most widely reported PhAC in different receiving waters around the world [5–7,10,11,14–17]. Ibuprofen, naproxen and ketoprofen are anti-inflammatory drugs. They are among the most commonly consumed over-the-counter drug preparations in the world and are also widely detected in various receiving waters [5–7,9,10,12,14–17]. Solid-phase extraction (SPE) combined with high-performance liquid chromatography-ultraviolet detection (HPLC-UV) or liquid chromatography/mass spectrometry (LC–MS) has been the primary methods for the determination of these four acidic PhACs in aquatic samples [3,11,12,15]. Due to its high resolution and high sensitivity, gas chromatography–mass spectrometry (GC–MS) (with derivatization) has also been applied to the determination of these compounds in water samples as an alternative of the generally more costly LC–MS which may also suffer from signal suppression by the sample matrix [18]. Sample preparation methods often employed in GC determination of PhACs include SPE [16,17,19] and liquid-liquid extraction (LLE) [20]. However, the amount of organic solvent needed for SPE and LLE ranges from several to hundreds of milliliters, respec-

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Table 1
Chemical structures and physical properties of target analytes[a].

Analyte	Structure	CAS number	Log K_{ow}	pK_a	Water solubility (mg/L)
Ibuprofen		15687-27-1	3.97	4.91	21
Clofibric acid		882-09-7	2.57	n.a.	583
Ketoprofen		22071-15-4	3.12	4.45	51
Naproxen		22204-53-1	3.18	4.15	15.9

n.a.: not available.
[a] Values taken from Ref. [32].

tively, which is still considerable. In addition, LLE is time consuming and labor intensive. Although automated SPE is available, it is expensive.

In the past decade, microextraction techniques have set the trend of sample preparation methods in environmental analysis. Both solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) have been applied to the determination of some PhACs in environmental and biological samples [21–24]. However, most PhACs are polar chemicals and cannot be handled by GC directly. LPME combined with LC–MS, which has become an important analytical technique for polar compounds, has been applied to the determination of PhACs in water samples [25]. As far as is known, there has been no report on the combination of LPME and GC–MS for the determination of PhACs in water samples.

In the present study, dynamic hollow fiber-protected liquid-phase microextraction (HF/LPME) was coupled with GC–MS to determine PhACs in water samples. Dynamic HF/LPME was developed by Zhao and Lee. In this procedure, there is a degree of automation (relating to the movement of the microsyringe plunger and thus, the organic solvent held in the wall pores and channel of the HF, and also the sample solution brought into the HF). The movement, and thus, mixing of the sample and organic phases facilitated extraction [26]. The results show that this mode of LPME exhibited higher sample enrichment factors than static LPME [26,27]. Recently, some other modes of dynamic LPME techniques have also been developed and applied to environmental and biological analysis, such as continuous flow control assisted dynamic LPME [28,29], solvent cooling assisted dynamic LPME [30] and automatic multiple dynamic LPME [31]. In the present study, a simple syringe pump based dynamic HF/LPME of PhACs followed by injection port methylation of the analytes using trimethylanilinium hydroxide (TMAH) prior to GC–MS was developed and applied to water samples.

2. Experimental

2.1. Reagents, chemicals and materials

1-Octanol (>99.5%) was purchased from Merck (Darmstadt, Germany). Deionized water was produced on a Nanopure water purification system (Barnstead, Dubuque, IA, USA). TMAH was purchased from Sigma–Aldrich (St. Louis, MO, USA). The four PhACs studied were clofibric acid (purity 99%), naproxen (purity 99%), ibuprofen (purity 99%) and ketoprofen (purity 99%). They were purchased from Sigma–Aldrich. The structures and physical properties of the tested PhACs are shown in Table 1. Stock solutions containing all compounds (1000 μg/mL) were prepared in methanol and diluted with methanol to obtain working solutions at various concentrations. They were stored at 4 °C. Wastewater samples were collected from a drain at a hospital. Directly potable tap water samples were collected from a laboratory, after allowing the water to flow for about 3–4 min.

The Accurel Q3/2 polypropylene HF membrane (600 μm I.D., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). The HF was ultrasonically cleaned in acetone and air-dried. The fiber was then cut carefully into 1.3-cm lengths for the experiments.

2.2. Instrumentation

All analyses were performed on a Hewlett-Packard (HP) (Palo Alto, CA, USA) GC system model 6890 with a model 5973 MS detector. The GC system was fitted with a DB-5 column (30 m × 0.32 mm I.D., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1.7 mL/min. The following temperature program was employed: initial temperature of 60 °C for 2 min; increased at 10 °C/min to 260 °C, held for

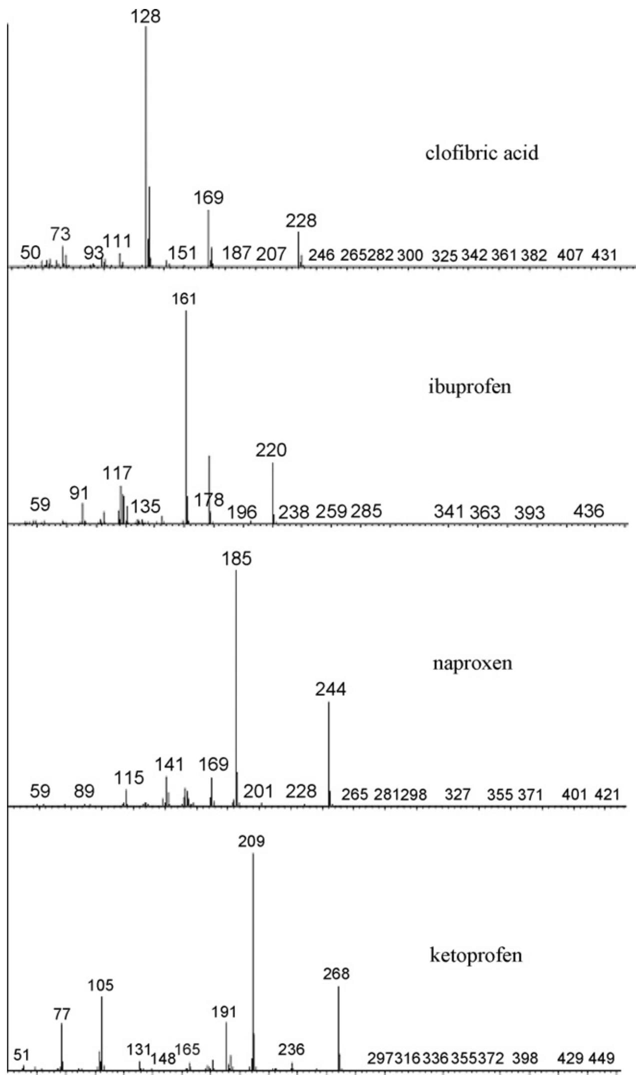


Fig. 1. Mass spectra of derivatives of the PhACs.

2 min. The injector temperature was 270 °C and all injections were made in the splitless mode. TMAH methylation of the four target drugs (which are all carboxylic acids) provided methyl esters, leading to a shift of 14 amu for the heaviest ion (adding $-CH_2$ to the carboxylic acid), which could be identified (see Fig. 1). To confirm drug ions tentatively identified by selected-ion monitoring (SIM), two characteristic ions of each derivatized compound were monitored: m/z 128, 169 (clofibric acid); m/z 161, 220 (ibuprofen); m/z 185, 244 (naproxen); m/z 209, 268 (ketoprofen). The mass spectra of derivatives of the PhACs are shown in Fig. 1.

The GC–MS interface temperature was set at 270 °C. The peak areas were calculated based on the respective molecular ions.

A Harvard Apparatus (Holliston, MA, USA) PHD 2000 programmable syringe pump was used to withdraw and discharge the extraction solvent into the hollow fiber at a prescribed rate, for the operation of dynamic HF/LPME.

2.3. Dynamic LPME with on-column derivatization

A 10 μL microsyringe (SGE, Sydney, Australia) with a cone tip was used both for extraction and for injecting the extracts together with derivatization reagent into the GC–MS. First, 2 μL of organic solvent was withdrawn into the microsyringe. The needle tip was inserted partially into a 1.3-cm long HF. The HF-syringe assembly was immersed in the organic solvent for about 5 s to impregnate the pores of the HF. The solvent in the syringe was then injected carefully into the HF. The microsyringe was subsequently placed in the groove of the syringe pump (placed vertically) and the HF-syringe assembly was immersed in the aqueous sample for extraction. The sample was stirred at 104 rad/s. HCl (final concentration of 0.001 M) and sodium chloride (final concentration of 2.5%) were added to the sample solution to enhance the extraction efficiency. The syringe pump was programmed thus: step (1), withdraw process: the pumping speed (e.g. 20 μL/min) and sampling volume (e.g. 2 μL) were set; step (2), pause pumping: dwell time 1 (e.g. 5 s) was set (the dwell time refers to the period when there is no movement of the plunger); step (3), infuse process: the pumping speed (e.g. 20 μL/min) and infusion volume (e.g. 2 μL) were set; step (4), pause pumping: dwell time (e.g. 5 s) was set; step (5), repeat steps (1)–(4). The syringe pump was then switched off. The extract (1 μL) was carefully withdrawn into the microsyringe (while ensuring the absence of air bubbles). The used HF was removed and discarded. Immediately, 1 μL of the derivatization reagent was withdrawn into the same microsyringe. The extract and the derivatization reagent were then injected directly into the GC–MS for derivatization and analysis.

3. Results and discussion

3.1. Optimization of dynamic LPME

3.1.1. Effect of extraction solvent

In dynamic HF/LPME, a syringe pump is used to automate the process of withdrawing and expelling an aliquot of sample solution into, and from, the HF channel, to facilitate the extraction process [26]. As the water sample is brought into the channel (replacing the organic solvent formerly occupying the space and which is simultaneously moved into the syringe needle), it interfaces with the film of organic solvent formed along the inside wall of the HF channel as the solvent is withdrawn. Efficient mass transfer of the analyte occurs from the water sample to this film which subsequently recombines with the organic solvent when the latter is forced back into the HF channel by the syringe pump plunger movement [26].

The choice of organic solvent is very important in dynamic LPME. The organic solvent used determines the partition coefficient between the extraction phase and donor phase and is also responsible for the thickness of the formed extraction film on the HF wall in dynamic LPME, both of which affect the extraction efficiency significantly. In addition, the extraction organic solvent also serves as the carrier for the subsequent derivatization procedure, with the consequence that injection port derivatization efficiency is at its most favorable. 1-Octanol, toluene, butyl acetate and hexane were evaluated in this work. The results are shown in Fig. 2. It can be seen that 1-octanol gives the highest analytical signals for the

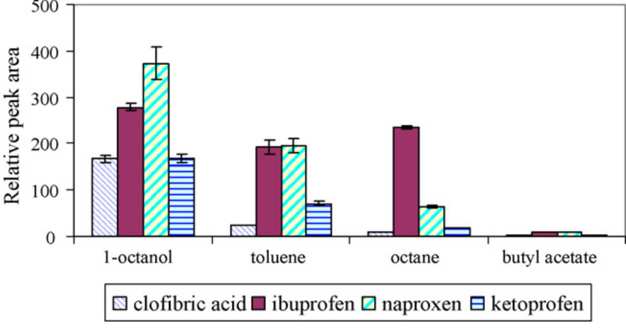


Fig. 2. Effect of extraction solvent on HF/LPME.

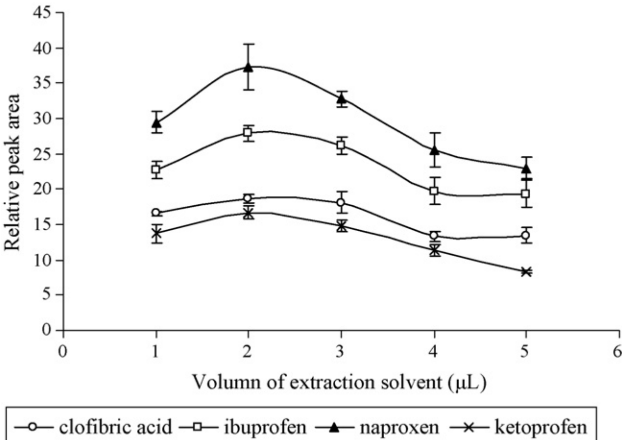


Fig. 3. Effect of volume of extraction solvent on HF/LPME.

four drugs and was chosen as the extraction solvent for subsequent experiments.

3.1.2. *Effect of volume of extraction solvent*

The extraction solvent volume was also investigated for optimization of the method. The results are shown in Fig. 3. It can be seen that 2 μL of 1-octanol was superior to 1 μL. There are several possible reasons. First, when 2 μL of 1-octanol was applied, a longer hollow fiber (1.3 cm) was required. Thus, the interfacial area between the donor phase and acceptor was increased. This helps to increase the mass transfer rate and thus the concentration of extracts. Second, when the extraction solvent was withdrawn into the microsyringe, there is dilution due to the existence of a small but finite volume of organic solvent contained at the tip of the microsyringe. Since a larger volume of extraction solvent is less likely to be affected by the dilution, this leads to a higher final concentration in the extract. On the other hand, it can also be seen that when the volume of extraction solvent was more than 2 μL, the analytical signals decreased. The reason may lie in the fact that a smaller volume of extraction phase accounts for higher enrichment factor, which is related to the final concentration in the extraction phase [33]. With a larger volume, the enrichment factor is lower. Based on the above results, it is likely that 2 μL is the optimal volume for the determination of the four target analytes.

3.1.3. *Effect of stirring of the sample solution*

Since agitation of the sample permits the continuous exposure of extraction surface to fresh aqueous sample, the extraction efficiency could be enhanced by enhancing the stirring of the sample. As seen from Fig. 4, stirring improved dynamic LPME efficiency.

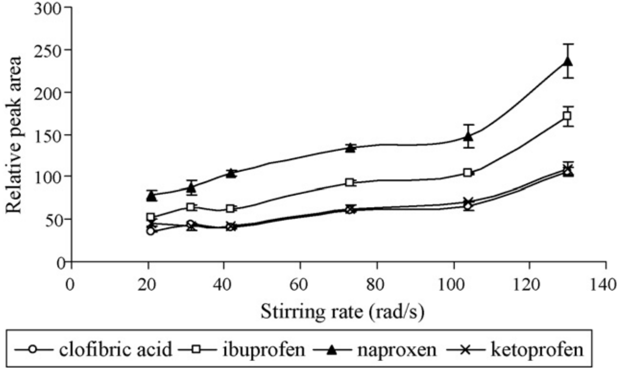


Fig. 4. Effect of stirring rate on HF/LPME.

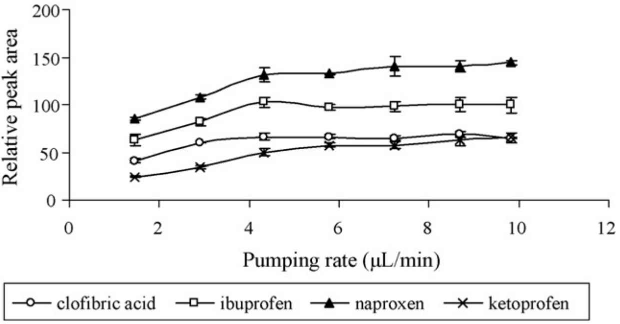


Fig. 5. Effect of syringe plunger withdrawal rate on HF/LPME.

However, with extraction at 130 rad/s stirring rate, excessive air bubbles at the HF were generated. These adhered to the HF surface leading to poorer extraction precision. Therefore, on the basis of these observations the lower stirring rate 104 rad/s was selected.

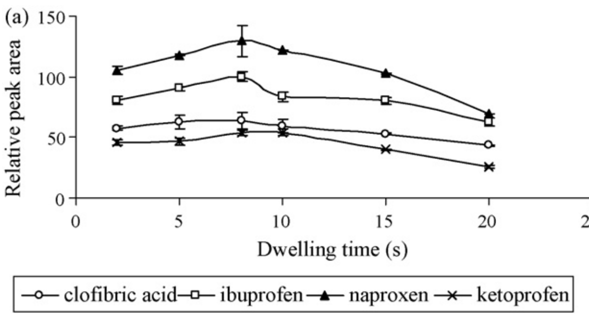
3.1.4. *Plunger movement*

The extraction efficiency of dynamic LPME greatly depends on the movement of the plunger [26,27]. In this study, different plunger withdrawal rates varying from 1.45 to 9.84 μL/min were investigated to determine their effects on extraction efficiency. The results are shown in Fig. 5. It was found that the extraction efficiency was relatively low when the plunger movement rate was set below 1.5 μL/min. This may be because when the plunger withdrawal rate was decreased, the time required for withdrawing the extraction solvent and expelling it was correspondingly increased. This leads to fewer extraction cycles over a given period of extraction time. According to a previous report of dynamic LPME [33], fewer extraction cycles led to lower enrichment factors. However, it can be seen that when the plunger withdrawal rate was set higher than 4.34 μL/min, the analytical signals did not change significantly with the increase of the plunger withdrawal rate. This may be attributed to the thicker organic film generated on the HF wall by a higher plunger withdrawal rate [34,35]. A thicker and thus larger volume of organic film leads to the reduction of the mass transfer rate [36]. It is possible that the effect of a thicker film generated by higher plunger rate was offset by the effect of more extraction cycles caused by the higher rate of plunger movement. Therefore, the analytical signals did not increase or decrease significantly when the plunger rate was set beyond 4.34 μL/min. However, it was found that for the compound giving the lowest peak area, ketoprofen (see Fig. 5), the highest plunger movement rate (9.84 μL/min) that could be achieved by the syringe pump gave the highest analytical signal. Thus, the rate of 9.84 μL/min was chosen in the subsequent experiments in order to focus more on increasing the analytical signal of ketoprofen.

Dwell time in plunger movement steps (1) and (2) is another factor that affects the extraction efficiency. Dwell times varying from 2 to 10 s were investigated. The results are shown in Fig. 6. A 8 s dwell time in step (1) and 10 s in step (2) were applied for the subsequent experiments, because these times gave the best peak area responses, respectively.

3.1.5. *Extraction time*

A series of extraction times was investigated to study the extraction process. The experiments were carried out on sample solutions containing 10 μg/L of each analyte. As shown in Fig. 7, the analytical signals for naproxen, ibuprofen, and to a lesser extent, ketoprofen, increased significantly with the increase of the extraction time even up to 60 min. For clofibric acid, the increase was more moderate after 30 min extraction. This may be attributed to the lower octanol–water coefficient (Table 1) of the clofibric acid (2.57) than that of the other three analytes (3.12–3.97). The results indicate



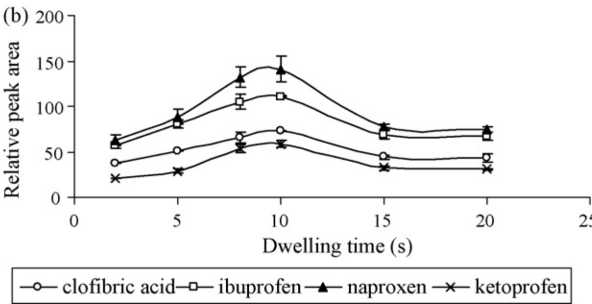


Fig. 6. Effect of dwell time: (a) dwell time in syringe pump programme step (2); (b) dwell time in syringe pump programme step (4).

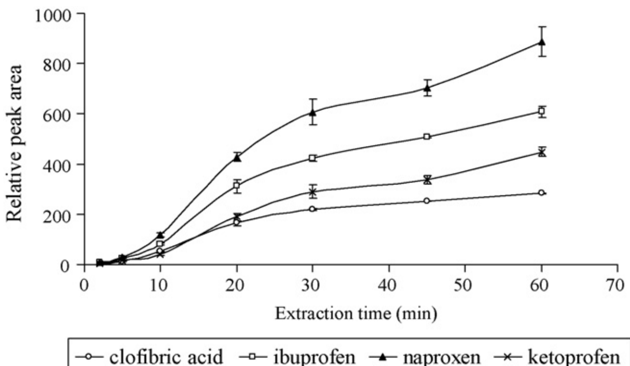


Fig. 7. Extraction time profile of HF/LPME.

that extraction for naproxen, ibuprofen and ketoprofen did not attain equilibrium in 60 min. Therefore, in order to increase the sample preparation throughput, a compromised extraction time other than equilibrium time was chosen as the optimal extraction time. An extraction time 20 min, which is a little shorter than the chromatographic analysis time, was adopted. Previous studies have shown that non-equilibrium LPME gave acceptable analytical results, indicating that this is a legitimate approach to microextraction [26,27,36].

3.2. Enrichment factors

Enrichment factors which are defined as the ratios of the final analyte concentrations in the acceptor phase and the initial concentrations of analytes within the sample, were assessed. The following conditions were employed to investigate enrichment factors: 2 μL 1-octanol as extraction solvent, TMPAH as derivatization reagent, 104 rad/s stirring rate, and 20-min extraction time. As shown in Table 2, HF/LPME combined with injection port derivatization provided high enrichment factors ranging from 130 to 251. To compare dynamic LPME with static LPME, the experiments were performed under the above conditions for both modes (except for the movement of the microsyringe plunger: for static LPME, no plunger movement was involved, of course). The results are also shown in Table 2. It can be seen that dynamic LPME provides much higher enrichment factors compared to static LPME.

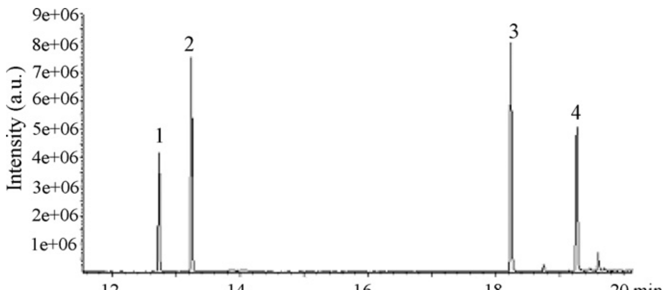


Fig. 8. Total ion chromatogram of four PhACs spiked into tap water samples after extraction by the proposed method: (1) clofibric acid; (2) ibuprofen; (3) naproxen; (4) ketoprofen.

3.3. Method evaluation

The optimized extraction conditions were employed to evaluate the HF/LPME procedure. Under these conditions, the reproducibility, linearity, and limits of detection (LODs) were measured. The results are shown in Table 2. The HF/LPME method exhibited good reproducibilities ranging from 3.26% to 10.61%. The good reproducibilities achieved may be attributed to the very simple and rapid extraction and derivatization procedure afforded by the current method. The calibration curve was linear in the range of 0.2–50 μg/L. Good coefficients of correlation (r) from 0.9962 to 0.9995 were achieved.

LODs, defined at a signal-to-noise (S/N) of 3, ranged from 0.01 to 0.05 μg/L. Compared to LODs obtained by SPME–GC–MS for the same analytes [23,24], the current method provides better sensitivity. The LODs were also lower that those obtained by another mode of dynamic LPME developed by Valcárcel and his coworkers [29], although the PhACs considered were not identical to those studied in this work. The LODs achieved in the present work are also in the range of concentrations reported for the PhACs in receiving waters [5–7,14–16], and are thus adequate for environmental analysis.

3.4. Tap water and wastewater analysis

The current method was applied to the determination of PhACs in tap water. However, as expected, none of the target analytes were detected. Therefore, these samples were spiked with 10 μg/L of each drug and then extracted by the procedure. The extracts were

Table 2
Quantitative results of dynamic HF/LPME of PhACs.

Analyte	Relative standard deviation (%)	Linearity range (μg/L)	Coefficient of correlation (r)	Limits of detection (μg/L)	Enrichment factor (dynamic HF/LPME)	Enrichment factor (static HF/LPME)
Clofibric acid	8.64	0.2–50	0.9967	0.02	151	96
Ibuprofen	4.96	0.2–50	0.9962	0.01	130	75
Naproxen	3.26	0.2–50	0.9995	0.01	161	100
Ketoprofen	10.61	0.2–50	0.9982	0.05	251	154

Table 3
Relative recoveries of wastewater samples by HF/LPME combined with injection port derivatization and GC–MS.

Analyte	Concentration in wastewater (μg/L)	Relative recoveries (%)	
		Tap water	Wastewater
Clofibric acid	0.77	105.5	97.6
Ibuprofen	0.21	101.5	97.3
Naproxen	0.26	103.3	98.2
Ketoprofen	0.48	105.2	96.9

derivatized in the injection port as described, then analyzed by GC–MS–SIM. Fig. 8 shows a typical chromatogram of the four PhACs after extraction. Very symmetrical peak shapes are obtained. The method was also applied to determine PhACs in wastewater from a hospital drain. Although none was expected, surprisingly, all four target compounds were detected. The results are shown in Table 3. The relative recoveries for the real samples are shown in Table 3. It can be seen that the relative recoveries, which are defined as the ratios of the GC peak areas of spiked real water extracts and the peak areas of spiked ultrapure water extracts, ranged from 97.3% to 105.5%. This suggests that the matrix has little, if any, significant effect on the current extraction method. The proposed method can therefore be an effective sample preparation method for the determination of PhACs in genuine water samples.

4. Conclusion

For the first time, dynamic hollow fiber-protected LPME, combined with a GC injection port derivatization technique was applied to the analysis of PhACs (clofibric acid, ibuprofen, naproxen and ketoprofen) in water samples. High extraction efficiency and enrichment factors were achieved. In addition, the method has as its distinct advantage, simplicity and much lower organic solvent consumption and derivatization reagent (in the microliter range), making it an environmentally friendly approach. Compared to other miniaturized extraction method such as SPME–GC–MS, the current method provides better sensitivity.

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