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Three liquid-phase microextraction of diclofenac and ibuprofen from water samples prior to high performance liquid chromatography

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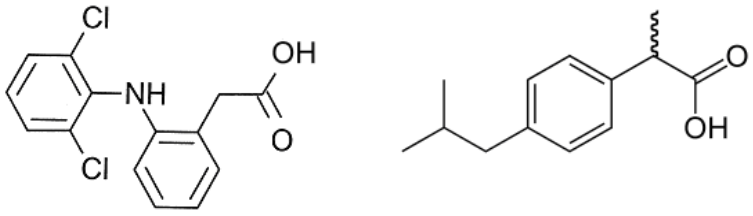
Abstract
A microextraction method using polypropylene membrane coupled with high performance liquid chromatography (HPLC) was developed for the extraction and determination of non-steroidal analgesic, anti-inflammatory drugs (NSAIDs) in water samples. Analytes including diclofenac and ibuprofen were extracted from acidic aqueous sample solution (donor phase) into a thin film of the organic solvent (o-xylene) then back extracted into the basic aqueous solution (acceptor phase). After extraction, 5 µL of the aqueous acceptor phase was withdrawn back into the syringe and injected directly into the HPLC system for further analysis. The parameters influencing the extraction efficiency including kind of the organic solvent and its volume, composition of donor and acceptor phases and the volume ratio between them, extraction time, stirring rate and pH were investigated and optimized. Under the optimal conditions, the obtained enrichment factors were more than 900. Dynamic linear ranges were 0.1-2000 and 0.5-2000 µg L^{-1} (r > 0.9971) and also the limits of detection (LODs) were 0.05 and 0.1 µg L^{-1} for diclofenac and ibuprofen, respectively.

Keywords: Liquid-phase microextraction method; Hollow fiber; High performance liquid chromatography; Diclofenac; Ibuprofen.

1. Introduction
Diclofenac (DIC) and ibuprofen (IBU) [Figure 1] are the most frequently administered non-steroidal analgesic, antipyretic and anti-inflammatory drugs (NSAIDs) with properties mainly used for the treatment of the rheumatic diseases or to relieve other pain. They are produced and used in great annual increasing volumes. This growth leads to a drastic fear about the effects of these compounds on the environment. In recent years, they have been found as environmental contaminants in sewage, surface, ground and drinking water samples [1–5]. Several methods have been described for the quantification of the NSAIDs in water samples, plasma and urine based on different extraction procedures then various analytical techniques such as high-performance liquid chromatography (HPLC) [6-10], capillary electrophoresis [11,12], thin-layer chromatography [13] and spectrofluorimety [14].
Pharmaceutical residues are usually present in environmental water samples in trace levels; therefore, a preconcentration step is generally required for determination of them as the pollutants. The most common sample preparation and preconcentration technique is solid phase microextraction (SPME) [15]. But this method has some disadvantages like; expensive SPME fibers have a limited lifetime and the polymer coating is fragile. Also, when SPME is coupled to HPLC, a special SPME–HPLC interface device has to be used for the solvent desorption. Because of these problems, another miniaturized sample preparation method, i.e., liquid-phase microextraction (LPME) was emerged for overcoming [16]. In LPME, only a small amount of the extracting solvent (microliter) is needed for concentrating of the analytes from the aqueous samples.
In recent years, several microextraction methods were used for the separation of diclofenac and ibuprofen in real samples (water, urine and plasma) such as single drop microextraction (SDME) [8], hollow fiber based liquid phase microextraction (HF-LPME) [9-11] and solid phase microextraction (SPME) [17].
In the present study, an HPLC method combined with prior HF-LPME was developed for the separation and preconcentration of diclofenac and ibuprofen from aqueous samples. In this purpose, the microporous hydrophobic hollow fiber membrane was used to separate the aqueous donor sample solution and the aqueous

acceptor phase. All the HF-LPME and HPLC parameters have been optimized in order to propose a rapid, simply and sensitive determination of those drugs as pollutants in environmental water samples.
This method can be compared with the other microextraction methods which were mentioned above. The obtained data show good advantages of the proposed method. For example, it presents lower limit of detection values, higher efficiency factors and also it requires lower extraction time.



a) Diclofenac b) Ibuprofen

Figure 1: Chemical structure of two analytes.

2. Materials and methods

2.1. Standards and Reagents

Diclofenac sodium salt and ibuprofen were from Daru-Pakhsh (Tehran, Iran). 1-octanol, toluene, *0*-xylene, methanol and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany) and were used without further purification. Also, Sodium hydroxide, hydrochloric acid and sodium chloride were obtained from Merck. A stock solution of 100 µg mL^{-1} of each analyte was prepared in methanol and stored at 4°C. Standard sample solutions which contain the two target compounds were provided daily at different concentrations by diluting the stock standard solutions with distilled water. The Q 3/2 Accurel PP polypropylene microporous hollow-fiber membrane (200 µm wall thickness, 600 µm inner diameter, 0.2 µm pore size, and 75% porosity) was obtained from Membrana (Wuppertal, Germany).

2.2. Apparatus

The HPLC system consisted of an Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD). Data acquisition and analysis were performed using software (Chem. Station Rev. A 10.01). A KNAUER C_{18} column (Berlin, Germany) was used for separation. The characteristic of this column was 125 mm length, 4.0 mm diameter and 5µm particle size. The column was at ambient temperature (22 ± 0.5 °C). The degassed mobile phase consisting of methanol-10 mM sodium hydrogen phosphate (pH 5.5) -acetonitrile (10:40:50, v/v) was delivered by a LC pump at 1.0 mL min^{-1}. The UV detection wavelength was set at 240 nm.

2.3. Extraction procedure

In the present work, we used hollow fiber which was cut into segments with a length of 2.5 cm with the internal volumes of 6 µL. These segments were placed for 5 min in acetone to remove any contaminants. After then, the fibers were removed from the acetone and the solvent was allowed to evaporate completely. These hollow fiber segments were used for subsequent extractions. A 10 µL flat-cut HPLC microsyringe was used to introduce the aqueous acceptor phase (6 µL of NaOH 1 mol L^{-1}, pH 13) into the hollow fiber. Then, two ends of the hollow fiber segment were heat-sealed. Sample solution was placed in a 25 mL beaker, along with a 7×3 mm stirring bar. A heating-magnetic stirrer (0-2000 rpm) was used to stir the extraction mixture. Organic solvent was added to the sample solution by a 50 µL syringe. Then the mixture was agitated for 90 s at 2000 rpm. Thereafter, the magnetic stirrer was switched off in order to gather the tiny drops of the organic solvent which were enriched by the analytes over the aqueous sample solution. Afterwards, the two-end sealed hollow fiber which was filled with the acceptor phase was placed in the centre of this organic solvent for impregnation of its pores. At the same time, the magnetic stirrer was switched on to start the extraction. An aluminum foil was used to cover the beaker during the extraction procedure to prevent the evaporation of

the volatile organic solvent. After a prescribed time, the magnetic stirrer was switched off and the hollow fiber was removed from the sample solution. The acceptor solution in the hollow fiber lumen was withdrawn back into the microsyringe and 5 µL of it was injected into the HPLC system for separation and identification.

3. Results and discussion

3.1. Optimization Method

To obtain the optimal extraction efficiency, various parameters that potentially affect sample extraction were studied which can be discussed respectively.

3.1.1. Selection of the organic Solvent

Five organic solvents with different viscosities and volatilities have been examined in this work. These extracting solvents were: 1-octanol, toluene, *o*-xylene, *n*-hexane and *n*-heptane. All of these solvents were easily immobilized in the pores of the hollow fiber. Among of them, the extraction efficiencies of *n*-hexane and *n*-heptane were not desirable. Therefore, as shown in Figure 2, *o*-xylene was selected as the organic solvent for further studies due to the highest analytes enrichment among the others.

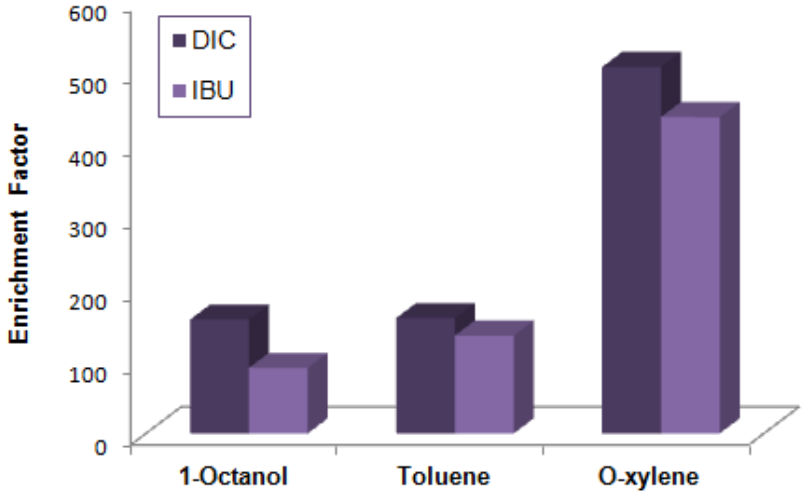


Figure 2: The effect of organic solvent on the enrichment factors.

3.1.2. Extraction time

In this work, we used *o*-xylene with low solubility in water as extractant. For extraction procedure, at first, the stirring speed for agitating the donor sample solution and the organic phase was fixed at 2000 rpm for the optimal extraction time of 90 s. Afterward; the mixture was allowed to be quiescent for few seconds to gather the tiny drops of the organic solvent in the surface of the sample solution. Therefore, a spot of organic solvent which was enriched by the analytes was produced and by placing the two-end sealed hollow fiber in it, the wall pores of the hollow fiber were filled entirely. After then, the back-extraction was occurred from the enriched organic solvent (*o*-xylene in the pores of hollow fiber) into the aqueous acceptor phase (inside the hollow fiber lumen), with a large rate constant. The enrichment factor (EFs) did not increase significantly after 20 min. Thus, the equilibrium time of back extraction was chosen 20 min (Figure 3).

3.1.3. Volume of donor and organic phase

In the current work, the volume of the donor phase was changed in the range of 5 to 11 mL whilst the volume of the acceptor phase was kept constant at 6 µL. As shown in Figure 4, by increasing the volume of donor phase up to 10 mL, the enrichment factors were increased. But, there was no significant increase in extraction efficiency by more increasing of the donor phase volume. Therefore, the volume of 10 mL was chosen as donor phase volume.

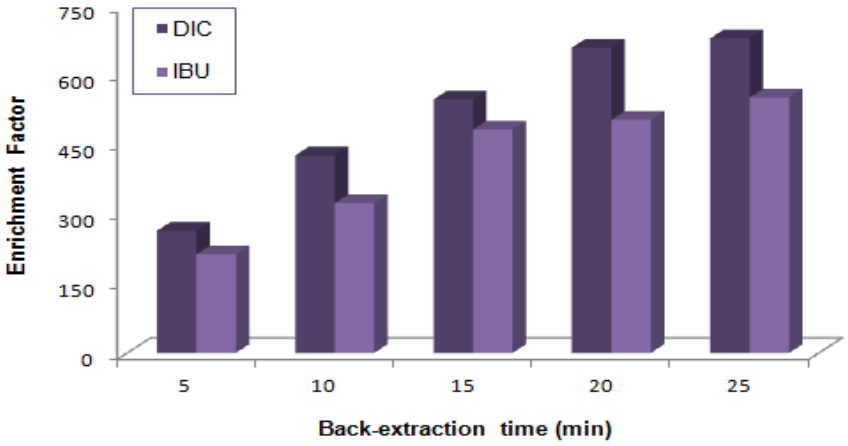


Figure 3: The effect of back-extraction time on the extraction efficiency.

The volume of the organic phase was too important and must be carefully optimized. The results indicate that the best volume of the organic solvent was found to be 30 µL (Figure 5). Lower volumes of the organic solvent tend to solvent loss during agitation because of its high volatility and higher volumes cause lower enrichment factor. Hence, a 30 µL volume of the organic solvent was chosen for the subsequent extractions.

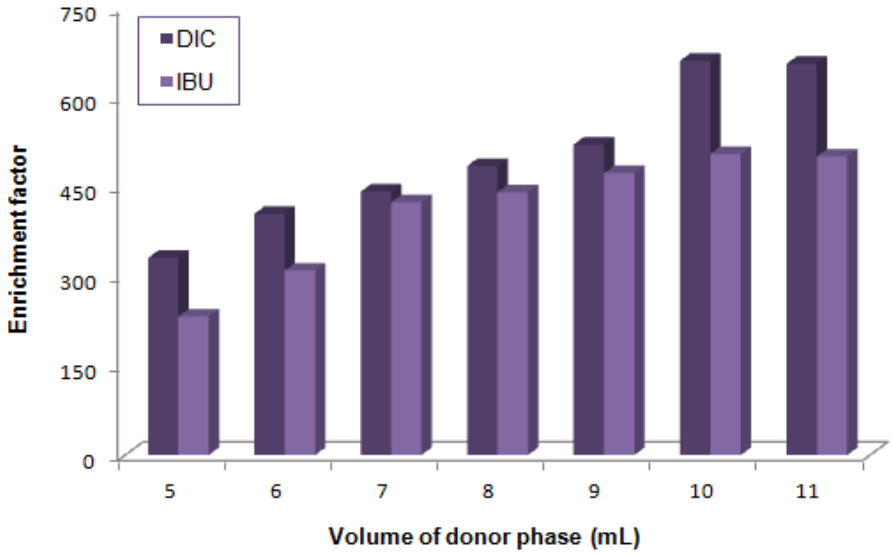


Figure 4: The effect of aqueous sample solution volume on the EFs.

3.1.4. Stirring rate

The effect of stirring speed on the extraction efficiency was also examined. Higher stirring speed causes an increase in the mass transfer process and also the kinetic rates. In the current work, the freely movement of the fiber will contribute to the mass transfer process. Therefore, the stirring speed was also optimized for better extraction, while back extraction performed. The stirring speed was in the range of 400-1000 rpm and the obtained results were shown in Figure 6. By increasing the speed of agitation, the extraction efficiency was improved but in very high speed (more than 1000 rpm) a vortex was created in the sample solution and the fiber stuck the wall of the beaker because of the centrifugal force. Consequently, the stirring speed was selected 900 rpm.

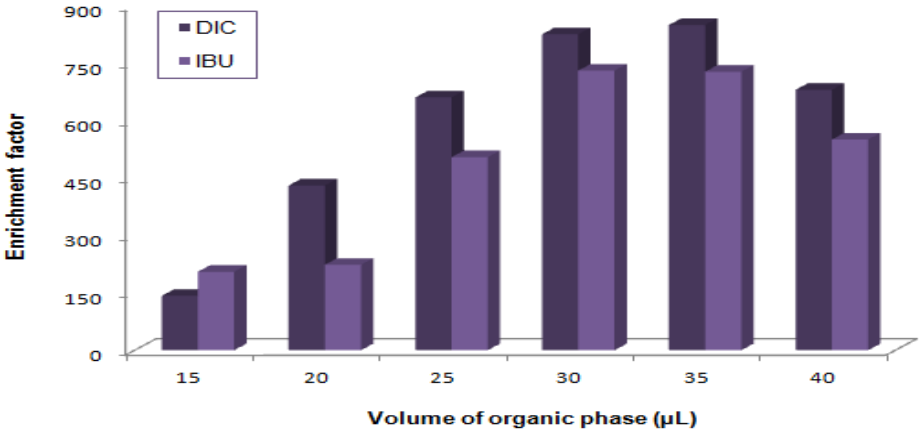


Figure 5: The effect of organic solvent volume on the obtained enrichment factors.

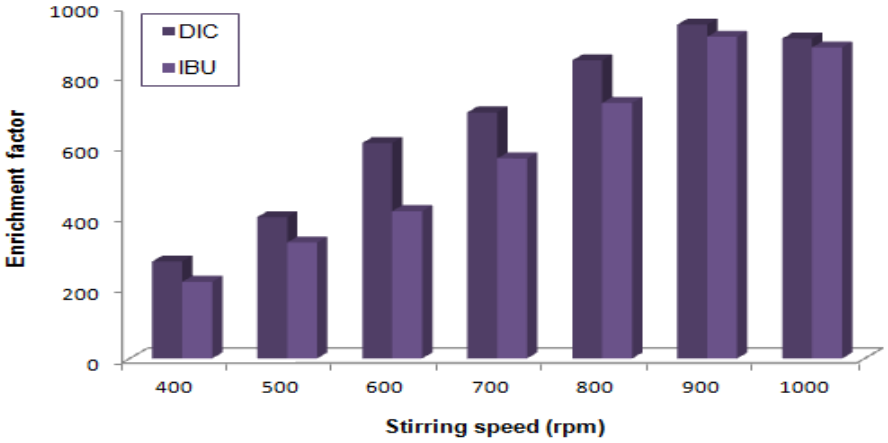


Figure 6: The effect of stirring rate on the extraction efficiency.

3.1.5. The pH of donor and acceptor phases

The difference in pH between the donor and acceptor phase is one of the major parameters which can promote the transfer of the analytes from donor to acceptor phase. The pH of the donor phase should be adjusted to deionize the analytes and the acceptor phase adjusted to ionize them. Since, the target compounds are weak acids {diclofenac (2-[(2,6-dichlorophenyl)amino]-benzeneacetic acid) (pK_a = 4.18), ibuprofen (2-[4-(2-methylpropyl)phenyl]propanoic acid) (pK_a = 5.2) [18]}; they are unionized in acidic, as well as neutral solutions. So, the pH of the sample solution is not a very critical factor. In this research, a 0.1 mol L^{-1} of HCl (pH 2) was used as donor phase. On the other hand, the extraction efficiency was more depended on the pH of the acceptor solution, which should be basic enough to ionize these weak acidic analytes by accepting of proton from them. Therefore, the NaOH concentrations were studied in the range of 0.001 to 0.1 mol L^{-1}. The results show that by increasing the NaOH concentrations in the aqueous acceptor solution, the enriching of the analytes are improved. Therefore, the pH of acceptor phase is a very important factor and influences the EFs. Finally, a 0.1 mol L^{-1} NaOH (pH 13) was used as acceptor phase.

3.2. Quantitative considerations

Under optimal extraction conditions, enrichment factors, repeatability, the linearity and the limits of detection were determined by utilizing standard solutions of two analytes in water. The repeatability in peak areas was studied for five replicate experiments. By plotting peak areas versus concentrations of the analytes in the sample solution, calibration curves were obtained which showed that correlation coefficient (r) were above 0.9971. The limits of detection (LODs at S/N=3), limits of quantitation (LOQs at S/N=10), enrichment factors and other analytical data are summarized in Table 1.

Table 1: Analytical performance of the proposed extraction procedure.

Analytes	RSD% (n=5)	Correlation Coefficient (*r*)	Limit of detection LOD (µg L^{-1})	Limit of quantitation (LOQ) (µg L^{-1})	Limit of linearity (LOL) (µg L^{-1})	Enrichment Factor (EF)
DIC	7.2	0.9989	0.05	0.1	2000	960
IBU	5.4	0.9971	0.1	0.5	2000	905

3.3. Real water analysis

Two real environmental water samples including tap and well water were studied using the proposed method. No target compounds could be detected in the samples; therefore, separate samples were spiked with 1 µg L^{-1} of each analytes and the relative recoveries were calculated. The obtained results for the spiked tap water were 98 % for both of the analytes. Also, for well water which was spiked with the same concentrations of the analytes, the results were 102 and 99 % for diclofenac and ibuprofen, respectively.

Conclusions

In the present study, a new mode of liquid-phase microextraction, using a microporous membrane was developed for the extraction of diclofenac and ibuprofen from water samples. The extraction was carried out by using of volatile organic solvent which has low viscosity that leads to increase of mass transfer and extraction efficiency along with a decrease in extraction time. On the other hand, this method is very easy and simple and the eluted analytes are directly determined with HPLC. Using this technique, the analytes can be extracted from water samples quantitatively with a good linearity and repeatability.

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