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Microextraction and Determination of Diclofenac in Biological Samples using Hollow Fiber Liquid Phase Microextraction Technique Coupled with HPLC-UV

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Abstract

In this study, hollow fiber liquid phase microextraction (HF-LPME) coupled with high-performance liquid chromatography (HPLC) with UV detection was applied for preconcentration and determination of Diclofenac sodium in biological fluids. Parameters affecting the extraction process including pH of donor phase and acceptor phase, type of extraction solvent, stirring rate, extraction time, and salt addition were studied and optimized. Under the optimized conditions donor phase pH=3, acceptor phase pH=11.7, extraction solvent *n*-octanol, stirring rate =750 rpm, extraction time=27 min and without the addition of salt, enrichment factors up to 170 were achieved and the relative standard deviation (RSD %) of the method was within the range of 2-3%. The method was successfully applied to determine the concentration of diclofenac sodium in the biological samples and satisfactory results were obtained.

Keywords:*Diclofenac, Liquid-phase microextraction, HPLC, Biological samples.*

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Introduction

Diclofenac (DIC) is the most frequently administered non-steroidal analgesic, antipyretic, and anti-inflammatory drug (NSAID) with properties mainly used for the treatment of the rheumatic diseases. In recent years, it has been found as environmental contaminants in sewage, surface, ground and drinking water samples [1, 2, 5, 9]. Several methods have been described for the quantification of the NSAIDs in biological samples, based on different extraction procedures and various analytical techniques such as high-performance liquid chromatography (HPLC) [3,4], capillary electrophoresis [6], and spectrofluorimetry [7]. Pharmaceutical residues are usually present in biological samples at trace levels; therefore, a pre-concentration step is generally required for determination of them. The most common sample preparation technique is solid phase microextraction (SPME).

When SPME is coupled with HPLC, an elaborate SPME–HPLC interface device has to be used for the solvent desorption. Due to these problems, another miniaturized sample preparation method, i.e., liquid-phase microextraction (LPME) was emerged for overcoming this issue. In LPME, only a small amount of the extracting solvent (microliter) is needed for concentrating the analytes from the aqueous and biological samples. In recent studies, several microextraction methods were used for the separation of diclofenac in real samples (water, urine, and plasma) such as single drop microextraction (SDME), and solid phase microextraction (SPME) [8].

In the present study, the HPLC method combined with prior HF-LPME was developed for the separation and preconcentration of diclofenac from biological samples. In this purpose, the microporous hydrophobic hollow fiber 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, simple, and sensitive method for determination of drug as pollutants in biological samples. This method was compared with the other microextraction methods which were mentioned above. The obtained data showed good advantages of the proposed method. For example, it presented lower limit of detection values, higher enrichment factor. Also, it required lower extraction time. The aim of this work was to develop a simple, sensitive, and effective method for the determination of DIC, which can be easily applied to the plasma. DIC was extracted from plasma and urine by HF-LPME and various parameters on extraction were optimized and determined by HPLC-UV.

Experimental

Standards and Reagents

Diclofenac sodium salt was donated from food and drug organization laboratory (Tehran, Iran). n-octanol , methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany) and used without further purification. Also, Sodium hydroxide, hydrochloric acid, and sodium chloride were obtained from Merck (Darmstadt, Germany). A stock solution of 100 mg L^{-1} of each analyte was prepared in methanol. Standard sample solutions were provided daily at different concentrations by diluting the stock standard solutions with distilled water. The polypropylene microporoushollowfiber membrane (200 μm wall thickness, 600μm inner diameter, 0.2μm pore size, and 75% porosity) was obtained from Membrana (Wuppertal, Germany).

Apparatus and chromatographic conditions

Chromatographic separations were carried out using a Youngling HPLC equipped with a YL 9110 quaternary pump HPLC, a 10 μL sample loop, and a YL 9120 UV–VIS detector. Chromatographic data were recorded and analyzed using a Youngling Autochro-3000. The chromatographic separation was carried out at room temperature (about 25 °C) on a C18 column (150 mm × 4.6 mm, with 5 μm particle size) with a C18guard column (4.0 mm × 10 mm, 5 μm) from Teknokroma (Barcelona, Spain). The degassed mobile phase consisting of methanol-potassium hydrogen phosphate (pH 2.5) (70:30, v/v) was delivered by a Quaternary pump at 0.9 mL min^{-1}. The UV detection wavelength was set at 254 nm. All of the pH measurements were performed with a GpHR 1400A pH meter(Germany). AnMR Hei-Standard magnetic stirrer by Heidolph Company (Germany) and syringe (25 μL) model 702 NR from Hamilton (Bonaduz, Switzerland) were also used. The ultrapure water was obtained from a MilliQ Ultrapure water purification system (Millipore, Bedford, MA, USA).All extractions were carried out using a Q3/2 Accrual polypropylene hollow fiber membrane from Membrana (Wuppertal, Germany) with a 0.2 μm pore size, 600 μm internal diameter and 200 μm wall thickness.

Extraction procedure

In the present work, we used hollow fiber which was cut into segments with a length of 4.5 cm with the internal volumes of 10μL. These segments were placed for 5 min in acetone and put in an ultrasonic bath for 10 min to remove any contaminants. 7.5 mL of the aqueous Diclofenac

solution as a donor phase (DP) with exact concentration was transferred into a 30 mL glass vial containing a magnetic stirring bar; the vial was then put on a magnetic stirrer. 15 µL of the acceptor phase (AP) was injected by a 25 microliter Hamilton microsyringe into the cleaned hollow fiber and the fiber was submerged in the organic solution for 10 s and then into the reagent water for 3 s so that the extra organic solution would be washed from the outer surface of the fiber. After that, the fiber was bent into a U-shape and was submerged in the glass vial of the sample solution for different lengths of time. The vial was covered with ParaFilm and stirred for a prescribed time period. At the end of the extraction time, the hollow fiber was removed from the sample solution, and the acceptor phase was withdrawn into the syringe. Finally, 10 µL of the acceptor phase was injected directly into the HPLC system for analyzing the Diclofenac.

Results and discussion

Optimization Method

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

Selection of the organic Solvent

Four organic solvents with different viscosities and volatilities have been examined in this work. These extracting solvents were: isobutyl methyl ketone, Dodecan-1-ol, *n*-octanol, and *n*-octan. All of these solvents were easily immobilized in the pores of the hollow fiber. Among them, the extraction efficiencies of isobutyl methyl ketone, Dodecan-1-ol and *n*-octan were not desirable. Therefore, *n*-octanol was selected as the organic solvent for further studies due to the highest analytes enrichment among the others.[16,17]

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 be a promoting factor for transferring the analytes from donor to acceptor phase. The pH of the donor phase should be adjusted to demonize 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) (p*K*a = 4.0 ± 0.2), it is unionized in acidic, as well as neutral solutions. So, the pH of the sample solution is the very premier factor. In this research,

(pH= 3) was used as a 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 aproton from them. The results showed that by increasing the NaOH concentrations in the aqueous acceptor solution, the enriching of the analytes was improved. Therefore, (pH= 11.7) was selected as the pH of acceptor phase. The pH of acceptor phase is a very important factor [13].

Effect of stirring rate

In this work, different stirring speeds ranging from 250 to 1000 rpm were tested to determine their effects on the extraction efficiency of the drug. The results showed that extraction efficiency was improved by increasing the stirring rate to 750 rpm. However, very high stirring rates (up to 750) would lead to the production of excessive air bubbles and loss of solvent that could affect the precision. Therefore, 750 rpm was chosen as a suitable stirring rate for future experiments.

Effect of extraction time

The impact of extraction time on extraction efficiency was studied in the range of 20–40 min. At the extraction times higher than 45 min, the extraction efficiency reduced. This could be due to solvent evaporation and bubble formation on the fiber wall. Therefore, in order to obtain high sensitivity, the extraction time of 27 min was chosen as the optimum time for the subsequent experiments.[18,19]

Influence of temperature

In this step, extraction temperatures in the range of 20-45˚ C were investigated. The results show that increasing the temperature up to 40˚ C reduced extraction efficiency. On the other hand, the solvent loss may occur due to increased solubility of the organic solvent at higher temperatures, leading to decline in extraction efficiency. the optimum extraction temperature of 40˚C was selected for further experiments.

The effect of volume ratio of donor phase to acceptor phase

Pre-concentration factor (PF) in three phase HF-LPME was calculated with $PF=V_dR/V_a.100$ where V_d is the volume of the donor phase and V_a is the volume of the acceptor phase and R is the extraction efficiency. Thus, in this method, the optimization of the volume of DP and AP is very important on the extraction efficiency. In this study, the volume of the acceptor phase was kept constant (20 µL) and the volume of the donor phase was changed and its effect on extraction rate was investigated. 15 ml of DP was selected as the optimum volume for subsequent experiments (Table 1).

Table1. Optimum conditions of HF-LPME of DIC.

Volume of DP (mL)	T (°C)	Time (min)	stirring rate (rpm)	pH of AP	pH of DP	Solvent
15	40	27	750	11.7	3	n-octanol

Quantitative considerations

Under optimum extraction conditions, enrichment factors, repeatability, the linearity and the limits of detection were determined by utilizing standard solutions of analyte in plasma and urine. By plotting peak areas versus concentrations of the analyte in the sample solution, calibration curves were obtained which showed that correlation coefficient (*r*) were above 0.9971. The limits of detection (LOD), limits of quantitation (LOQs), enrichment factors and other analytical data are summarized in Table 2.

Table 2. Analytical characteristics of the proposed HF- LPME method.PF: pre-concentration factor; LOD: limit of detection; LOQ: limit of quantification; R^2: correlation coefficient; DLR: dynamic linear range; RSD%: relative standard deviation.

PF	LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)	R^2	DLR ($ng\ mL^{-1}$)	%RSD Intra-day	%RSD Inter-day
178	2.8	8.4	0.99	50-2000	2.7	3.8

Analysis of real samples

Under the optimized conditions, the developed HF-LPME-HPLC technique was applied to preconcentration and determination of diclofenac in plasma and urine samples. The pH of the real samples was adjusted to 3 by adding of 0.1M NaOH to plasma and H_3PO_4 to urine solution. Prior to the spiking and extraction procedure of the target drug, the plasma samples were diluted 1:1 with water. Figure1 is the typical chromatograms for the spiked plasma sample obtained by HF-LPME-HPLC and Figure 2 is the typical chromatograms for the urine sample obtained by HF-LPME-HPLC.

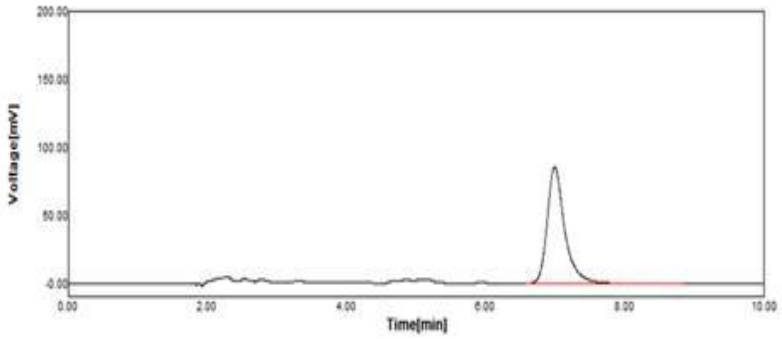

Figure 1. Chromatograms obtained after applying HF-LPME to plasma sample spiked with 3 ng mL^{-1} of Diclofenac under the optimum conditions(pH of donor phase:3; pH the acceptor phase:11.7; stirring rate:750 rpm; extraction time:27 min; temperature:40 °C).

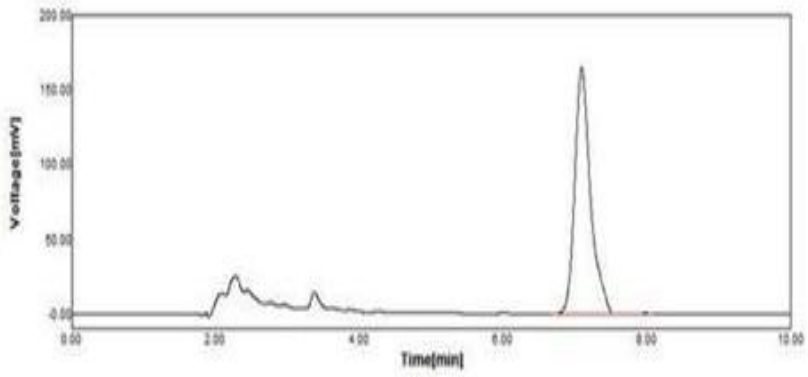

Figure 2. Chromatograms obtained after applying HF-LPME to urine sample spiked with 3 ng mL^{-1} of Diclofenac under the optimum conditions(pH of donor phase:3; pH the acceptor phase:11.7; stirring rate:750 rpm; extraction time: 27 min; temperature:40 °C).

The present method was also compared with other studies in terms of method of extraction, validation, and precision. As can be deducted, the method is quite comparable to those mentioned in Table 3. As can be seen, the LODs of this method are comparable with those obtained in the previous studies and even lower than those reported in the literature. In addition, due to the simplicity and low cost of the extraction device, the hollow fiber can be discarded after each extraction to avoid carryover and cross-contamination (Tables 3-5).

Table 3. The results of diclofenac analysis in plasma sample in optimum condition of HF-LPME (n=5).

Sample	C_{Added} (ng mL^{-1})	C_{Found} (ng mL^{-1})	RSD%	Recovery (%)
Plasma1	3.0	3.1	3.6	103
Plasma2	10.0	9.7	3.1	97

Table 4. The results of diclofenac analysis in urine sample in optimum condition of HF-LPME (n=5).

Sample	C_{Added} (ng mL^{-1})	C_{Found} (ng mL^{-1})	RSD%	Recovery (%)
Plasma1	3.0	3.0	3.9	100
Plasma2	10.0	9.9	3.5	99

Table 5. Comparison of the proposed method with other developed methods for determination of DIC in biological samples.

Instrumentation	Sample preparation	Sample	DLR ng mL^{-1}	LOD ng mL^{-1}	RSD%	Reference
HPLC	LLE[2]	Water	500-2000000	100	7/2	[15]
HPLC/UV	S.P.E[1]	Urine	-	280	3/56	[12]
HPLC-UV	−	Urine	100-2000	12.03	-	[14]
LC-ESI-MS	HF-LPME[3]	Sewage	-	1-3.9	-	[20]
HPLC	CFHF-LPME	Sewage	-	0.25	-	[21]
HPLC	-	Urine	-	40	-	[11]
LC/MS	-	Drugs mixture	50-300	0. 5-4	8-9	[10]
HF-LPME	HF-LPME	Urine and plasma	50-2000	2.8	2.7	This work

[1]Solid-phase extraction
[2]Liquid–liquid extraction
[3]Hollow fiberliquid phase microextraction

Conclusions

In the present study, a new method of liquid-phase microextraction, using a microporouspolypropylene was developed for the extraction of diclofenac from urine and plasma samples. The extraction was carried out by using avolatile organic solvent which has alow viscosity that leads to the increase of mass transfer and extraction efficiency along with a decrease in the extraction time. This method was very practical and simple and the eluted analytes were directly determined by HPLC. Using this technique, the analytes can be extracted from water samples quantitatively with a good linearity and repeatability.

References

[1] W. Ahrer, E. Scherwenk, W. J. Buchberger, *J. Chromatogr. A*, 910, 69 (2001).

[2] S. Öllers, H. P.Singer, P. Fässler, S.R. Müller, *J. Chromatogr. A*, 911, 225 (2001).

[3] A. Panusa, G. Multari, G. Incarnato, L. Gagliardi, *J. Pharm. Biomed. Anal.*, 43, 1221 (2007).

[4] L. Kaphalia, B. S. Kaphalia, S. Kumar, M. F. Kanz, M. Treinen-Moslen, *J. Chromatogr. B,* 830, 231 (2006).

[5] T. Heberer, K. Reddersen, A. Mechlinski, *Water. Sci. Technol*., 46, 81 (2002).

[6] M. Villar Navarro, M. Ramos Payán, R. Fernández-Torres, M. A. Bello-López, M. Callejón Mochón, A. Guiráum Pérez, *Electrophoresis,*32, 2107 (2011).

[7] J. A. Arancibia, M. A. Boldrini, G. M. Escandar, *Talanta*, 52, 261(2000).

[8] A.Sarafraz-Yazdia, A. H. Amiri, G. H. Rounaghi, H. Eshtiagh-Hosseini, *J. Chromatogr. B,* 908, 67 (2012).

[9]T. Heberer, K. Reddersen, A. Mechlinski, *Water Sci. Technol*., 46, 81(2002).

[10] H. Hamza, *Biomed. Anal*., 24, 587 (2001).

[11] M. Ramos, M.A. Bello Lopez, *J. Chromatogr B*, 879, 197(2011).

[12] D. Hermawan, L. Hui Ling, W. Ibrahim, M.M Sanagi, *J. Fundamental Applied Sci*., 9, 1(2013).

[13] K.E Rasmussen, S. Pedersen-Bjergaard, *J. Chromatogr. A*, 1, 132 (2008).

[14] K. Laxman, J. Millership, *Talanta*, 85, 1948 (2011).

[15]Sarafraz Yazdi, A.; Eshaghi, Z.*Chromatographia*, 67, 49 (2008)

[16] E. Psillakis, D. Mantzavinos, N. Kalograkis, *Anal. Chim. Acta*, 501, 13 (2004).

[17] S. Palmarsdottir, E. Throdarson, L.E. Edholm, *J. Anal. Chem*., 69, 1732 (1997).

[18] F. Barahona, A. Gjelstad, S. Pedersen-Bjergaard, K. EinarRasmussen, *J. Chromatogr. A.*, 1217 ,13,1989 (2010).

[19] Y.Y. Yang, J. Chen, Y.P. Shi, *J. Chromatogr. B.*, 878, 2811 (2010).

[20] A. Saleh, E. Larsson, Y. Yamini, J. A. Jonsson, *J. Chromatogr.A*, 1218, 1331 (2011).

[21] N. Larsson, E. Petersson, M. Rylander, *J. Anal. Methods*, 1, 59 (2009).

[22] W. Jin, J. Zhang, *J. Chromatogr. A*, 868, 101 (2000).