Molecularly Imprinted Stir Bar Sorptive Extraction Coupled with High-Performance Liquid Chromatography for Trace Analysis of Diclofenac in Different Real Samples

Hashemi, Sayyed Hossein*+; Zahra Monfaredzadeh

Department of Marine Chemistry, Faculty of Marine Science, Chabahar Maritime University,
P.O. Box 98617-85553 Chabahar, I.R. IRAN

ABSTRACT: *A novel molecularly imprinted polymer coated stir bar has been used to selectively extract diclofenac (DFC) directly from real samples. DFC was used as template molecule for preparation of MIP coating. The effect of different parameters on the extraction efficiency were studied and the optimum conditions were established as: the absorption and desorption times were fixed at 10 min, stirring speed was 600 rpm, pH was adjusted to 5.1, amount of NaCl was 0.35 mol/L and extraction process was performed at a temperature of 45 °C. Under the optimum conditions, the linear range of method was 0.5- 500.0 µg/L for DFC and the detection limit was calculated to be 0.15 µg/L with an enrichment factor of 242 folds. The technique was successfully applied for the analysis of trace amounts of DFC in seawater and commercial tablet samples. The mean recoveries of spiking real samples with DFC at 10.0 µg/L level were between 94.2-100.0 % with a mean RSD of 0.7-4.6%.*

EYWORDS: *Diclofenac; Molecularly imprinted polymer coated stir bar; Seawater analysis; Sample preparation; Pharmaceutical analysis.*

INTRODUCTION

Diclofenac (DCF), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid monosodium salt, is one of the most widely available non-steroidal anti-inflammatory drugs, by annual sales of approximately 100 metric tons [1, 2]. DCF is often used for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and sports injuries [2]. Due to its extensive use, this analyte has been considered as one of the most frequently detected pharmaceutical residues in water bodies thus far. DCF impact on water life revealed some adverse effects on rainbow trout exposed to water concentrations of 1.0-500 µg/L for 28 days [3, 4]. For these reasons, the pre-concentration and determination of DFC are very important for medical and pharmaceutical practical aspects where it is applied for the treatment of different diseases. So, the developments of new methods for quantifying trace amounts of DFC are required [2]. Different methods such as High Performance Liquid Chromatography (HPLC) [4-8], gas chromatography/mass spectrometry (GC/MS) [4], High Performance Liquid chromatography/MS (HPLC/MS) [4], Capillary Electrophoresis (CE) [5], potentiometry [9], fluorimetry [10]

and spectrophotometry [11] have been proposed for the determination of trace amounts of DFC in different samples.

However, the direct determination of DFC at a very low concentration level is challenging. This is mainly due to its low concentration or severe matrix interferences in real samples such as seawater. Employing extraction before chromatographic analysis, can overcome these disadvantages, because it can pre-concentrate the analytes and also eliminate the interfering elements at the same time [12]. For DFC pre-concentration, Magnetic Molecularly Imprinted Polymer (MMIP) [2], Molecularly Imprinted Polymers (MIP) [3, 4, 13], Hollow Fiber-Based Liquid Phase MicroExtraction (HF-LPME) [7] and activated carbon [14] were used. Solid Phase MicroExtraction (SPME) is a relatively recent microextraction method that typically uses conventional chromatographic stationary phases coated on fibers to extract analytes [15]. The simplest way of combining SPME technology with MIPs was introduced by *Mullet et al.* in 2001 [16] that the method involved packing a capillary with the MIP particles for in-tube SPME. Nevertheless, this technique requires the use of extra instrumentation and has other important drawbacks such as the lack of compatibility between the solvent needed to desorb template (analytes) from the MIP and the mobile phase applied [17,18].

A sorptive extraction procedure using a stir bar coated with polydimethylsiloxane (PDMS) was first introduced in 1999 derived from SPME and called stir bar sorptive extraction (SBSE). SBSE is based on the same methods as those of SPME. However, the amount of sorptive coating for stir bars are 25-125 µL, which is 50-250 times higher than that on a SPME fiber, causing in better extraction efficiency and sample capacity [19-21]. Since only the PDMS is available as extraction phase on commercial stir bar which shows good affinities only to nonpolar and weakly polar analytes, attempts have been made for applying other coatings especially for more polar compounds [17, 22, 23].

Molecularly imprinted polymers (MIPs) are rapidly developing methods for the preparation of polymers having specific molecular recognition properties [24-28]. Recently, this technique involved as new selective sorbents for solid-phase extraction of organic and inorganic compounds such as methylene blue, copper, iron, di (2-ethylhexyl) phthalate and polycyclic aromatic hydrocarbons in complex matrices due to its stability, ease of preparation, low cost and specific recognition to the target template (target molecules) [29-33].

Knowing that high selectivity can be achieved by MIPs, we tried to develop a simple, fast and highly selective MIP-coated stir bar (MIPSB) for the extraction of DFC from different real complex samples followed by HPLC determination. MIP was synthesized using a combination of 4-vinylpyridine (VP) as the functional monomer, ethylene glycol dimethacrylate (EDMA) as the cross-linker and DFC as the template molecule.

EXPERIMENTAL SECTION
Materials

Diclofenac sodium salt, ibuprofen sodium salt, naproxen sodium salt, 4-vinylpyridine (VP), ethylene glycol dimethacrylate (EDMA) and 2, 2'-azobisisobutyronitrile (AIBN) were all obtained from Sigma-Aldrich (St. Loius, MO, USA). All salts, acids and organic solvents were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany) and used as received. The HPLC grade methanol, water, toluene and acetonitrile were also obtained from the same company. Milli-Q® water (18.3 MΩ/cm) was used throughout all experiments after filtering through 0.22 mm Nylon membrane. Carbamazepine (CBZ) were purchased from Fluka AG, Switzerland and utilized to check the selectivity of MIPSB. A stock solution of DFC (500 mg/L) and CBZ (500 mg/L) was separately prepared in Millipore water and methanol: water (1:1 v/v) mixture, respectively, and stored at 4 ^{0}C. Work solutions were prepared daily by suitable dilution of them.

Instruments

A Knauer HPLC (Germany) was employed for all analysis which was consisted of an EA4300F smart line pump, fitted with a smart line auto sampler 3950, a spectrophotometric detector (used at fixed wavelength of 272 nm) and a 250×4.6 mm Eurospher 100-5 C18 column, guarded by the same pre-column. ChromGate V3.1.7 software was applied for chromatographic data handling. The injection volume was 10 µL. The pH was determined by a model 630 Metrohm (Switzerland) pH meter with combined glass-calomel electrode. A Fourier Transform InfraRed (FT-IR) spectrometer Perkin-Elmer (Bucks, UK) was employed for qualitative spectra interpretations

as well as for structure elucidation. An S 360 scanning electron microscope (England) was used to investigate the coating surface.

Preparation of the stir bar

A home-made glass bar (0.5 cm length × 2 mm i.d) was applied for the preparation of the sorptive stir bar. It was consisted of a magnetic iron bar which was inserted inside a Pyrex® glass tube and sealed by flame. To make its surface active for salinization and subsequent polymerization, after its cleaning by distilled water, it was treated with 1.0 mol/L sodium hydroxide for 8 h at room temperature. After rinsing with water, it was soaked in 1.0 mol/L hydrochloric acid for a few minutes and rinsed again by water and dried for 1 h in an oven (150 °C). Double bonds were formed on the glass of the bar with putting it for 3 h in a 25% (V/V) 3- (methacryloxy) propyltrimethoxy silane solution in acetone at room temperature. Finally, the stir bar was washed by methanol and dried at a stream of nitrogen [14, 34].

Coating of the stir bar with MIP

The procedure for the coating of the stir bar with MIP has been explained in one of our previous reports [30]. Briefly, pre-polymer solution for the preparation of MIP was obtained with mixing 0.20 g of DFC, 0.27 mL of functional monomer 4-VP, 2.62 mL of cross-linker EGDMA and 40 mg of initiator AIBN dissolved in 8 mL of porogen toluene. Next, the silylated stir bar was immerged and deoxygenized by a nitrogen stream for 5 min and sealed in the glass vial. Subsequently polymerization was carried out at 60 ^{0}C over a period of around 6h in a water bath. Finally, the MIPSB was removed from the bath and sonicated in methanol/acetic acid solution (9:1, v:v) to remove the template from it. As a reference, the Non-Imprinted Polymer (NIP), which did not contain the template, was prepared in parallel by the MIP using the same synthetic protocol without adding DFC.

These covalent attachments of the MIP coating to the glass surface of the bar via double bonds were formed. Double bonds on the glass surface of the stir bar were created using 3- (methacryloxy) propyltrimethoxysilane as a coupler prior. The preparation scheme is showed in Fig. 1.

The scanning electron microscopy (SEM) images of the MIPSB coatings are shown in Fig. 2 which were used to characterize the microscopic surface texture.

Procedure

In a typical assay, the MIPSB was inserted in a 10 mL aqueous solution containing suitable amount of DFC and the pH was adjusted to 5.1 by drop wise addition of either 1 mol/L of sodium hydroxide or hydrochloric acid in the same concentration. After that, 0.21 g of NaCl was added and stirred at 600 rpm for 10 min. After extraction was completed, the MIPSB was taken out and inserted in a glass vial containing 5 mL of 1:2 mixture of methanol: acetic acid and stirred at 600 rpm for 10 min at 50 °C to remove the template. This solution which contains desorbed target compound were collected and dried under a gentle nitrogen stream. The residues were reconstituted in 1 mL of methanol for HPLC analysis.

Chromatographic conditions

For doing HPLC, several different mobile phases were investigated including methanol, acetonitrile, water and different mixtures of them in both isocratic and gradient elusion modes. Finally, eluent A was selected to be methanol and eluent B was water with 0.1% acetic acid. The gradient was held at 75% A for 5 min and increased to 90% A in 5 min and held for 5 min, then reset to initial conditions within 5 min and held for another 5 min. The flow rate was set constant at 0.35 mL/min. The column temperature was fixed at 30 °C and the mobile phase was degassed with a stream of helium prior to use. Samples were filtered through a 0.45 μm Nylon filter before injection into HPLC.

RESULTS AND DISCUSSION
Infrared spectra

The Infrared spectra of leached and unleached DFC imprinted polymers particles and stir bar were characterized using KBr pellet method (Fig. 3). As is obvious, in the region of 1638-1648 cm^{-1}, no absorption band is present which shows the absence of vinyl groups in the polymer particle. Lack of this absorption confirms the complete polymerization of VP [30]. The N–H band of the free DFC, shifted from 3323 cm^{-1} to 3430 cm^{-1} in MIP due to the interaction between template and VP, which indicates the presence of DFC in MIP. Moreover, it can be seen that in Fig. 3b, the characteristic peak at 3323 cm^{-1} is disappeared and the intensity of the strong peaks are decreased after desorption, confirming the complete removal of DFC
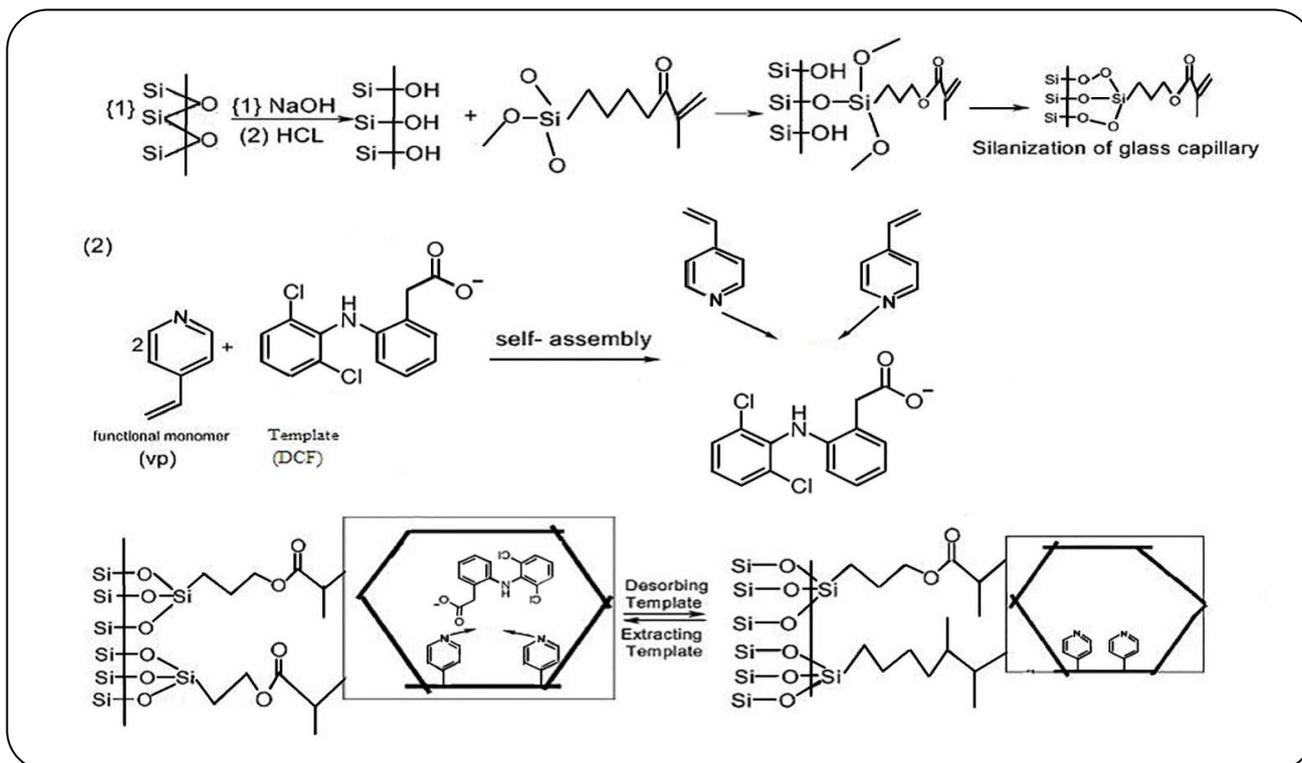
Fig. 1: Schematic diagram of the preparation of MIP-SBSE coating using DFC as the template molecule.
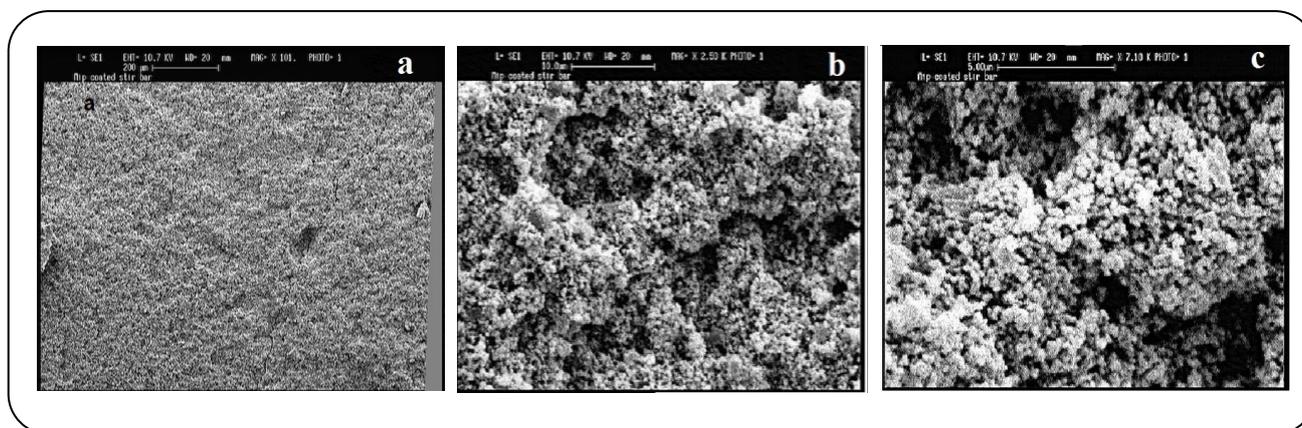*(1) Silanization of stir bar and (2) coating of diclofenac MIP-coated stir bar.*

Fig. 2: Scanning electron micrographs of the MIP coated stir bar. The magnifications were 100 (a), 2500 (b) and 7000 (c).

from polymer. Meanwhile, there is distinct difference between the IR spectra of the leached and unleached polymer.

Optimization of MIPSB for DFC extraction

In order to optimize the MIPSB stirring extraction operating conditions for DFC analysis, several parameters that could influence the MIPSB extraction were studied and optimized in 500 µg/L of a standard of DFC in aqueous media.

Effect of pH

The effect of the pH value on the absorption of DFC from water samples was studied in the range of 2.0 to 7.5. A set of solutions containing 5.0 µg of DFC in 10 mL solution were taken in a vial. The pH of the solution was adjusted to the required value and extraction was performed. The recovery was at the highest point at a pH of 5.1. Hence, in subsequent studies this pH was used (Fig. 4).
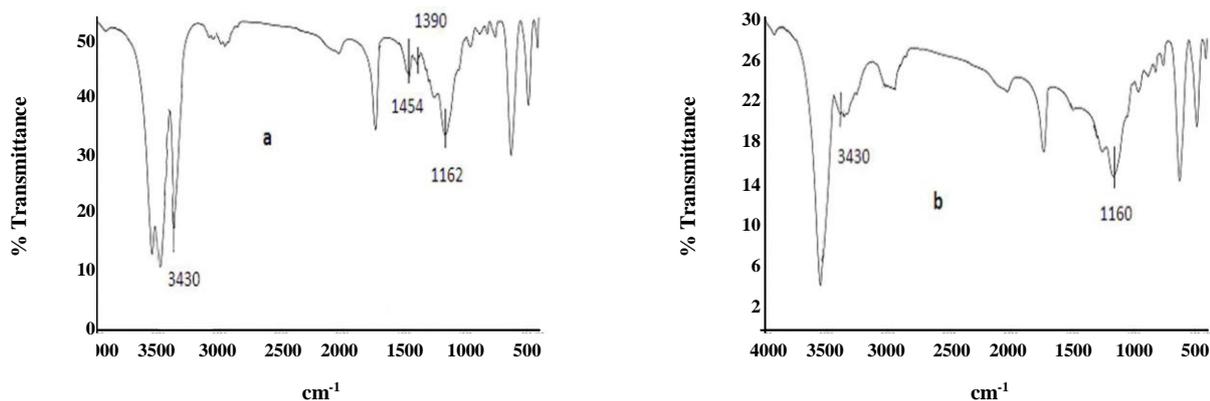
Fig. 3: FT-IR spectra of unleached (a) and leached (b) DFC imprinted polymer materials.
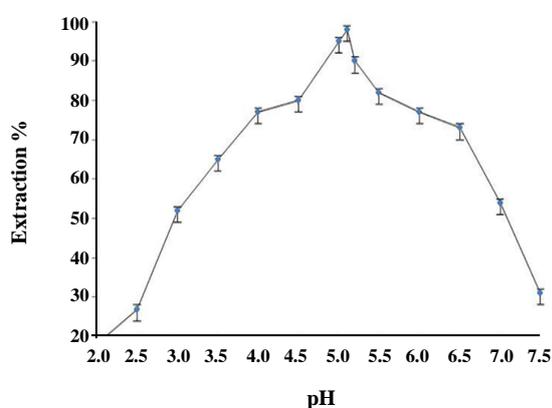

Fig. 4: Effect of pH on extraction efficiency of DFC. Conditions: sample solution, 10 mL; elution solvent, methanol/acetic acid (1:2); absorption and desorption times, 10 min; amount of NaCl, 0.21 g; stirring speed, 600 rpm; temperature, 45 °C.

Choice of the desorption solvent

The effect of the kind of desorption solvent on the analysis of DFC was investigated. 2.5 mL of different elution solvents such as acetonitrile, methanol: H_2O (1:1), acetic acid, ethanol, methanol: acetic acid (1:1), methanol: acetic acid (9:1), methanol: acetic acid (2:1) and methanol: acetic acid (1:2) were tested. It was found that 2.5 mL of a mixture of methanol/ acetic acid (1:2) can accomplish the quantitative elution of DFC from the MIPSB.

Effect of adsorption and desorption time

The percent extraction of DFC increases as the adsorption time increases and reaches a maximum after 10 min. DFC imprinting particles generate binding cavities that are complementary to the original DFC

in both shape and functionality. Quantitative desorption of DFC from MIPSB sorbent also occurred after 10 min at methanol: acetic acid (1:2) solution. Therefore, adsorption and desorption times of 10 min were selected for further works.

Effect of ionic strength

The salting-out effect is widely used in traditional liquid– liquid extraction because it decreases the solubility of analytes in the aqueous phase, thus more analytes can enter into the extracting phase. Here, the effect of salt on the extraction efficiency is studied by adding various amounts of sodium chloride (NaCl) to the solutions from 0.1 to 0.4 g/mL. It was found that the extraction of DFC is quantitative while solution is saturated with NaCl at ~ 0.4 g/mL. Therefore, subsequent extraction experiments were performed while the samples were saturated by NaCl.

Effect of stirring rate

The effect of stirring rate on the extraction efficiency of DFC was also investigated. The experimental results showed that the extraction efficiency increases with increasing the stirring rate up to 600 rpm and then remains constant. Therefore, a stirring rate of 600 rpm was selected for subsequent studies.

Effect of temperature

Desorption of DFC from the MIPSB significantly increased by increasing temperature up to 45 °C and became constant after then. This might be due to the breaking of adsorptive forces between the active sites of the imprinted polymer and DFC (Fig. 5).
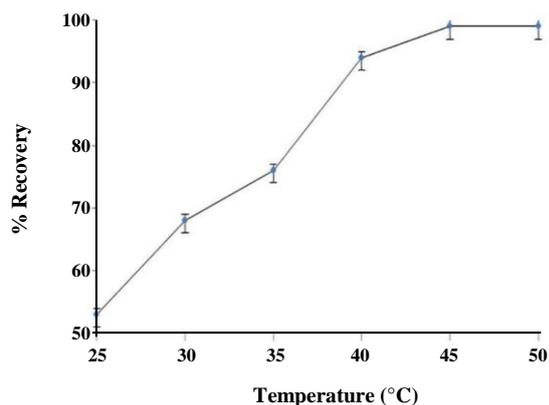
Fig. 5: Effect of temperature on the recovery. Conditions: pH, 5.1; sample solution, 10 mL; elution solvent, methanol/ acetic acid (1:2); absorption and desorption times, 10 min; amount of NaCl, 0.21 g; stirring speed, 600 rpm.

Analytical performance

Linearity, limit of detection and enrichment factor

Under the optimum conditions, the calibration curve was linear over a concentration range of 0.5-500.0 µg/L for DFC and the least square equation at above the dynamic linear range is:

$$A = 1.531C(\mu g/L) + 0.601 \quad \text{with} \quad r^2 = 0.998$$

Where C and A are the concentrations of DFC and HPLC response, respectively.

The percent extraction (E %) of DFC was obtained from the eq. 1.

$$E\% = 100(C_B/C_A) \quad\quad\quad (1)$$

In that, the concentrations of DFC in solution before and after extraction are presented by C_A and C_B, respectively.

Under the optimal experimental conditions, the limit of detection (LOD) of the proposed technique (3 S_d/m), defined as three times of the standard deviation (S_d) of 7 consecutive measurement of blank signal intensity to the slop of the calibration curve (m) was calculated to be 0.15 µg/L. Blank signal was measured by highest to lowest response in the noise over the retention time of DFC. In order to obtain a high pre-concentration factor, the influence of the sample volume on the extraction efficiency of DFC on MIPSB was investigated in the range of 10–500 mL. The results showed that the recovery of DFC was very efficient (>97%) in a sample

volume range of 10–250 mL. After that recovery was decreased. By considering the final elution volume of 1 mL and the sample volume of 250 mL, an enrichment factor (EF) of 250 was expected to be achievable which was closed to the EF experimentally determined (242 folds). Table 1 compares the characteristic data of the present technique with those reported in the literature. As can be seen in Table 1, the MIPSB has lower detection limits, good extraction recovery and higher linear range compared with the other techniques recently introduced. The method showed shorter linear range in comparison with the MIP coupled with HPLC-UV which is due to the usage of higher amounts of MIP in DFC extraction. These results show that the proposed method is simple and rapid with high extraction capability. The coating is very stable, while it needs just a small volume of the sample. Furthermore, it is economical and have high sensitivity.

Sensitivity

In order to further investigate the recognition ability of MIPSB, the same DFC solution was extracted using both MIPSB and Non-Imprinted Stir Bar (NIPSB). As can be seen in Fig. 6, enhancement of sensitivity of MIP-coated stir bar is obvious.

Selectivity of diclofenac MIPSB

The selectivity of MIPSB for the adsorption of DFC was evaluated by using CBZ, ibuprofen and naproxen as interfering compound because their chemical molecular structures are similar to DFC and it is also widely coexists by DFC in many matrices. Aliquots of 10 mL of aqueous solution containing 5.0 µg of each of DFC, ibuprofen, naproxen and CBZ were taken into the solutions and the recommended procedure was followed. The results showed that there was no significant interference.

Determination of DFC in real sample

In order to evaluate the performance of the developed method, extraction and determination of the analytes in different real sample such as seawater samples and DFC tablets was tested. No salt was added for seawater samples, since it is salt saturated by itself. Seawater samples were taken from four stations beside Chabahar Bay (southern east of Iran). To investigate the effect of sample matrices on extraction efficiency, these real

Table 1: Comparison of the published methods for DFC determination with the proposed technique in this work.

Number	Method	Detection method	LOD (μg/L)	Linear range (μg/L)	Recovery (%)	Ref.
1	MMIP	spectrophotometer	0.23	1.0-50.0	95.3- 103.3	2
2	MIP	HPLC (UV)	10	100-1000	≥94	3 and 4
3	SPE (Solid phase extraction)	HPLC (UV)	0.4	1.0–200	93.8–102.0	35
4	SPME	HPLC (UV)	1.5	4.0–50.0	117.4	36
5	SPE	HPLC (UV)	1.2	5.0–80	94-101	37
6	MIPSB	HPLC (UV)	0.15	0.5-500.0	94.2-100.0	This work
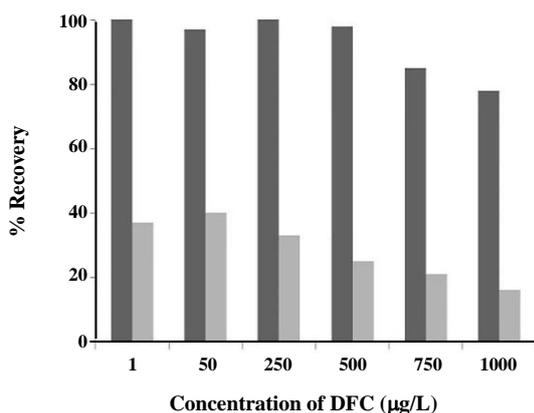
Fig. 6: Behavior of the MIP-coated stir bar (a) and NIP-coated stir bar (b) for DFC uptake at various concentrations of diclofenac.

samples were spiked at the concentration of 10 μg/L by DFC. Sample chromatograms for station 4 are shown in Fig. 7. As can be seen, DFC could not be quantitatively extracted when NIPSB was used. The NIPSB possessed extraction capability much lower than that of the MIPSB, leading to lower sensitivity. Employing MIP-coated stir bar (chromatogram b, Fig. 7), however, enables us to accurately analyze DFC, because of the high pre-concentration occurs after MIP extraction. Moreover, MIP-coated stir bar could eliminate the matrix interferences successfully. The recoveries for 4 stations are shown in Table 2. Reproducibility of the technique as percent of relative standard deviation (RSD %) was found to be in the range of 0.7 to 4.6%. These results indicate that the developed technique can be successfully applied for the determination of DFC in complicated matrices such as seawater samples. The technique was used for the determination of DFC by known concentration in selected pharmaceuticals. The results showed that at 95% confidence level there is no significant difference between the amount found (24 mg per tablet, 96% recovery) and the labeled value (25 mg

per tablet). This indicates the high accuracy of this technique for this type of sample. Also at 95% confidence level, our results are in consistent with a previously published work which showed recovery of 96.8 for DFC ampoule [2]. To validate the recoveries of MIPSB, the commercial PDMS coated stir bar (PDMS-SB) was also used for the same extraction under similar conditions. A chromatogram of Chabahar Bay water sample taken from station 4 (Beheshti) after pretreatment with PDMS- SB is showed in Fig. 7d which indicates that it extract the analyte successfully. Also the results showed that the PDMS-SB limits of quantification, precision, accuracy and extraction capability are much lower than that of the MIPSB. Using CBZ, ibuprofen and naproxen as interfering compound, again, it was observed that the selectivity of MIPSB is much higher than the commercial PDMS.

The synthesized MIPSB could be applied 60 times without apparent damage and were kept in dried air for at least 9 months without a reduction of extraction ability. The repeatability between stir bars (n=5) was obtained to be better than 9.2 %.

CONCLUSIONs

In this research, the applicability of the MIPSB prepared by precipitation polymerization for the selective enrichment of DFC in different real samples was confirmed. This method showed that the proposed MIPSB for the separation of DFC has the potential to be a new type of carrier. The MIPSB could be applied for many extractions of DFC (at least 60 times) without apparent damage and were kept in dried air for 9 months without a reduction of extraction ability. The linear ranges had a wide concentration and the MIPSB could selectively extract DFC for HPLC analysis even at lowconcentration. MIPSB extraction has the advantages of ease of preparation, stability, simplicity of operation with high extraction capability, repeatability and rapid separation, low cost and repeatability [18, 38].

Table 2: Recovery results for real samples obtained from different locations of Chabahar Bay (Iran) and DFC tablet.
Conditions: pH, 5.1; sample solution, 10 mL; elution solvent, methanol/acetic acid (1:2); absorption and desorption times, 10 min;
stirring speed, 600 rpm; temperature, 45 °C.

Sample	Recovery % at spiked level of 10 (µg/L)	DFC found (µg/L)	RSD (%) [b]
Station1, Chabahar Maritime University [a]	-	1.72	1.56
Station1, Chabahar Maritime University	100.0	11.72	2.6
Station 2, Tis [a]	-	2.27	2.4
Station 2,Tis	99.5	12.22	2.03
Station 3, Kalantary [a]	-	3.18	2.6
Station 3, Kalantary	94.2	12.60	1.9
Station 4, Beheshti [a]	-	3.29	0.7
Station 4, Beheshti	95.8	12.87	3.4
DFC tablet [a]	-	50.00	2.5
DFC tablet	96.0	59.60	4.6

a) No spiking,   b) RSD, relative standard deviation, for seven replicate measurements
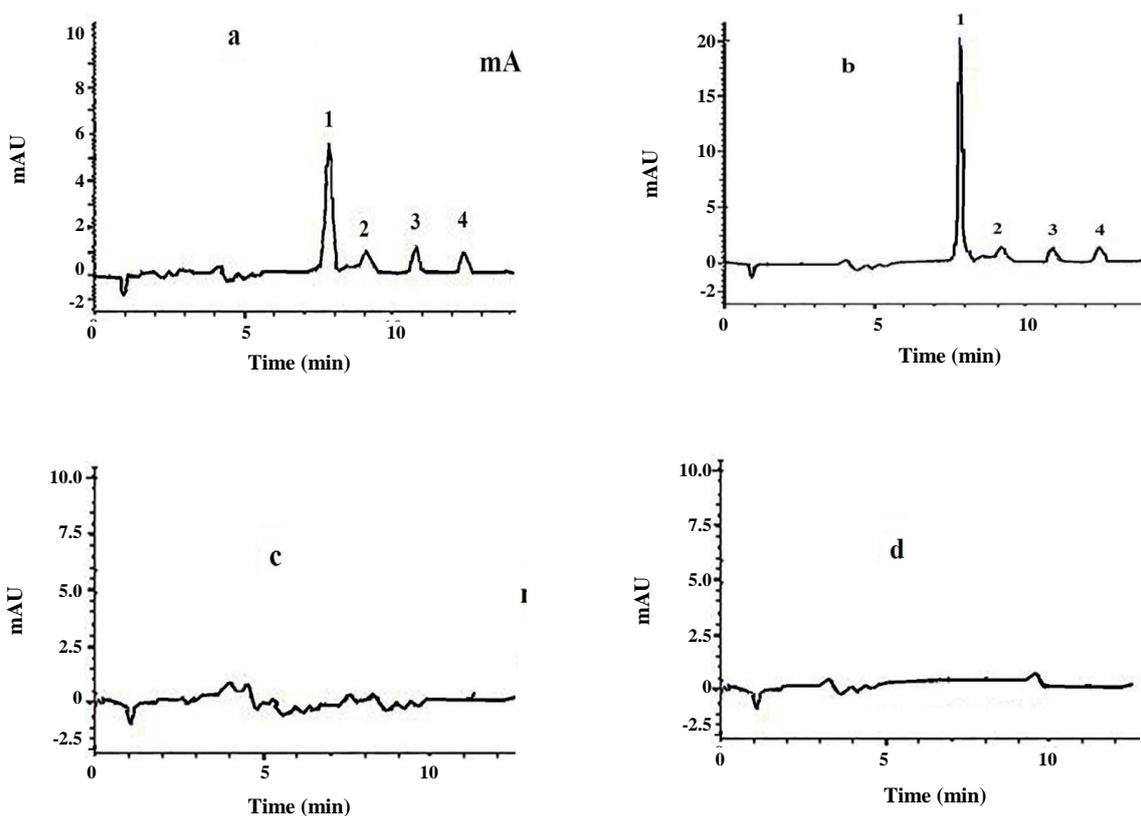

Fig. 7: Sample HPLC chromatograms of Chabahar Bay water sample taken from station 4 (Beheshti) (a) MIP-coated stir bar sorptive extraction without sample spiking (b) MIP-coated stir bar sorptive extraction of 10 µg/L spiked sample (c) NIP-coated stir bar sorptive extraction of 10 µg/L spiked sample (d) PDMS--coated stir bar sorptive extraction of 10 µg/L spiked sample (1) DFC (2) 2,4- dinitrophenol (2,4-DNP) (3) 4- nitrophenol (4-NP) (4) ph. Conditions: pH, 5.1; sample solution, 10 mL; elution solvent, methanol/acetic acid (1:2); absorption and desorption times, 10 min; stirring speed, 600 rpm; temperature, 45 °C.

Research Article

So, our results suggest that the MIP provided has enough reliability and efficiency to be used for enrichment of low concentrations of DFC in various real samples.

REFERENCES

[1] Perisic D.J., Gilja V., Stankov M.N., Katancic Z., Kusic H., Bozic A.L., Stangar U.L., Dionysiou D.D., Removal of Diclofenac from Water by Zeolite-Assisted Advanced Oxidation Processes, *J. Photochem. Photobiol*., **321**: 238-247 (2016).

[2] Pebdani A.A., Shabani A.M.H., Dadfarnia S., Selective Separation and Determination of Diclofenac *via* Magnetic Molecularly Imprinted Polymer and Spectrophotometry, *J. Iran. Chem. Soc*., **13**: 155-164 (2015).

[3] Dai C.M., Geissen S.U., Zhang Y.L., Zhang Y.J., Zhou X.F., Selective Removal of Diclofenac from Contaminated Water Using Molecularly Imprinted Polymer Microspheres, *Environ. Pollut*., **159**: 1660-1666 (2011).

[4] Dai C.M., Zhou X.F., Zhang Y.L., Liu S.G., Zhang J., Synthesis by Precipitation Polymerization of Molecularly Imprinted Polymer for the Selective Extraction of Diclofenac from Water Samples, *J. Hazard. Mater.*, **198**: 175-181 (2011).

[5] Aurora-Prado M.S., Steppe M., Tavares M.F.M., Kedor- Hackmann E.R.M., Santoro M.I.R.M., Comparison Between Capillary Electrophoresis and Liquid Chromatography for the Determination of Diclofenac Sodium in a Pharmaceutical Tablet, *J. Aoac. Int*., **85**: 333-340 (2002).

[6] Gonzalez L., Yuln G., Volonte M.G., Determination of Cyanocobalamin, Betamethasone, and Diclofenac Sodium in Pharmaceutical Formulations, by High Performance Liquid Chromatography, *J. Pharm. Biomed. Anal*., **20**: 487-492 (1999).

[7] Payan M.R., Lopez M.A.B., Torres R.F., Bernal J.L.P., Mochon M.C., HPLC Determination of Ibuprofen and Diclofenac and Salicylic Acid Using Hollow Fiber-Based Liquid Phase Micro Extraction, *Anal. Chim. Acta*, **653**: 184-190 (2009).

[8] Hajkova R., Solich P., Pospisilova M., Sicha J., Simultaneous Determination of Methylparaben, Propylparaben, Sodium Diclofenac and its Degradation Product in a Topical Emulgel by Reversed-Phase Liquid Chromatography, *Anal. Chim. Acta*, **467**: 91-96 (2002).

[9] Pimenta A.M., Araújo A.N., Montenegro M.C.B.S.M., Simultaneous Potentiometric and Fluorimetric Determination of Diclofenac in a Sequential Injection Analysis System, *Anal. Chim. Acta*, **470**: 185-194 (2002).

[10] Carreira L.A., Rizk M., Shabrawy Y.E., Zakhair N.A., Tonbar S.S., Europium (III) Ion Probe Spectrofluorometric Determination of Diclofenac Sodium, *J. Pharm. Biomed. Anal*., **13:** 1331-1337 (1995).

[11] Souza R.L.D., Tubino M., Spectrophotometric Determination of Diclofenac in Pharmaceutical Preparations, *J. Braz. Chem. Soc*., **16**: 1068-1073 (2005).

[12] Nassiri M., Kaykhaii M., Hashemi S.H., Sepahi M., Spectrophotometric Determination of Formaldehyde in Seawater Samples after in-situ Derivatization and Dispersive Liquid-Liquid Microextraction, *Iran. J. Chem. Chem. Eng. (IJCCE)*, Articles in Press.

[13] Sun Z., Schussler W., Sengl M., Niessner R., Knopp D., Selective Trace Analysis of Diclofenac in Surface and Wastewater Samples using Solid-Phase Extraction with New Molecularly Imprinted Polymer, *Anal. Chim. Acta*, **620**: 73-81 (2008).

[14] Larous S., Meniai A.H., Adsorption of Diclofenac from Aqueous Solution Using Activated Carbon Prepared from Olive Stones, *Int. J. Hydrogen Energy*, **41**: 10380–10390 (2016).

[15] Kaykhaii M., Dicinoski G.W., Haddad P.R., Solid-Phase Microextraction for the Determination of Inorganic Ions: Applications and Possibilities, *Anal. Lett*., **43**: 1546-1555 (2010).

[16] Mullett W.M., Martin P., Pawliszyn J., In-Tube Molecular Imprinted Polymer Solid-Phase Microextraction for the Selective Determination of Propranolol, *Anal. Chem*., **73:** 2383-2389 (2001).

[17] Hashemi S.H., Kaykhaii M., Tabehzar F., Spectrophotometric Determination of Four Naphthalene Sulfonates in Seawater after Their Molecularly Imprinted Stir Bar Sorptive Extraction, *J. Chil. Chem. Soc*., Accepted for publication.

[18] Hashemi S.H., Kaykhaii M., Tabehzar F., Molecularly Imprinted Stir Bar Sorptive Extraction Coupled with High Performance Liquid Chromatography for Trace Analysis of Naphthalene Sulfonates in Seawater, *J. Iran Chem. Soc.*, **13**: 733-741 (2016).

[19] Xu Z., Hu Y., Hu Y., Li G., Investigation of Ractopamine Molecularly Imprinted Stir Bar Sorptive Extraction and its Application for Trace Analysis of Beta 2-Agonists in Complex Samples, *J. Chromatogr. A*, **1217**: 3612-3618 (2010).

[20] Hu C., He M., Chen B., Zhong C., Hu B., Sorptive Extraction Using Polydimethylsiloxane/metal-Organic Framework Coated Stir Bars Coupled with High Performance Liquid Chromatography-Fluorescence Detection for the Determination of Polycyclic Aromatic Hydrocarbons in Environmental Water Samples, *J. Chromatogr. A,* **1356**: 45-53 (2014).

[21] David F., Sandra P., Stir Bar Sorptive Extraction for Trace Analysis, *J. Chromatogr. A*, **1152**: 54-59 (2007).

[22] Lei Y., Xu G., Wei F., Yang J., Hu Q., Preparation of a Stir Bar Coated with Molecularly Imprinted Polymer and its Application in Analysis of Dopamine in Urine, *J. Pharm. Biomedical Analysis*, **94**: 118-124 (2014).

[23] Qiu X., Wu Y., Chen D., Lai L., Liang Y., Guo H., Fabrication of Novel Stir Bar Sortive Extration Coating Based on Magnetic Molecularly Imprinted Polymer Through Atom Transfer Radical Polymerization for Tace Analysis of Estrogens in Milk, *J. Nanoscience and Nanotechnology*, **16**: 12374-12381 (2016).

[24] Hashemi S.H., Kaykhaii M., Khajeh M., Molecularly Imprinted Polymers for Stir Bar Sorptive Extraction: Synthesis, Characterization, and Application, *Anal. Lett.,* **48**: 1815-1829 (2015).

[25] Khajeh M., Bohlooli M., Hashemi H., Imprinted Polymer Particles for Aluminum Uptake: Synthesis and Analytical Applications, *J. Macromol. Sci.*, **46**: 1-7 (2009).

[26] Kaykhaii M., Khajeh M., Hashemi S.H., Magnetic Molecularly Imprinted Polymer Nanoparticles for Selective Extraction of Copper from Aqueous Solutions Prior to its Flame Atomic Absorption Determination, *J. Anal. Chem.*, **70:** 1318-1329 (2015).

[27] Amiri A., Ramazani A., Jahanshahi M., Moghadamnia A.A., Synthesis and Evaluating of Nanoporous Molecularly Imprinted Polymers for Extraction of Quercetin as a Bioactive Component of Medicinal Plants, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **35:** 11-19 (2016).

[28] Khajeh M., Kaykhaii M., Mirmoghaddam M., Hashemi H., Separation of Zinc from Aqueous Samples Using a Molecular Imprinting Technique, *J. Environ. Anal. Chem.*, **89**: 981-992 (2009).

[29] Khajeh M., Afzali Moghaddam S., Bohlooli M., Ghaffari-Moghaddam M., Application of the Artificial Neural Network and Imperialist Competitive Algorithm for Optimization of Molecularly Imprinted Solid Phase Extraction of Methylene Blue, *E-Polymers*, **16**: 243–253 (2016).

[30] Hashemi H., Khajeh M., Kaykhaii M., Molecularly Imprinted Stir Bar Sorptive Extraction Coupled with Atomic Absorption Spectrometry for Trace Analysis of Copper in Drinking Water Samples, *Anal. Methods*, **5**: 2778-2783 (2013).

[31] Khajeh M., Kaykhaii M., Hashemi H., Mirmoghaddam M., Imprinted Polymer Particles for Iron Uptake: Synthesis and Analytical Applications, *Polym. Sci. Ser. B*, **51:** 344-351 (2009).

[32] Chen F., Yang Z., Tang Y., Wang X., Selective Extraction and Determination of Di (2-ethylhexyl) Phthalate in Aqueous Solution by HPLC Coupled with Molecularly Imprinted Solid-phase Extraction, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **36**: 127-136 (2017).

[33] Khajeh M., Sharifirad M., Bohlooli M., Ghaffari-Moghaddam M., Magnetic Molecularly Imprinted Polymers–Silver Nanoparticle Based Micro-Solid Phase Extraction for the Determination of Polycyclic Aromatic Hydrocarbons in Water Samples, *RSC Adv.*, **6:** 54702-54708 (2016).

[34] Hu Y., Li J., Hu Y., Li G., Development of Selective and Chemically Stable Coating for Stir Bar Sorptive Extraction by Molecularly Imprinted Technique, *Talanta*, **82**: 464-470 (2010).

[35] Rezaei F., Yamini Y., Moradi M., Ebrahimpour B., Solid Phase Extraction as a Cleanup Step Before Microextraction of Diclofenac and Mefenamic Acid Using Nanostructured Solvent, *Talanta*, **105**: 173-178 (2013).

[36] Candioti L.V., García M.D.G., Galera M.M., Goicoechea H.C., Chemometric Assisted Solid-Phase Microextraction for the Determination of Anti- Inflammatory and Antiepileptic Drugs in River Water by Liquid Chromatography-Diode Array Detection, *J. Chromatogr. A*, **1211**: 22-32 (2008).

[37] Azzouz A., Souhail B., Ballesteros E., Determination of Residual Pharmaceuticals in Edible Animal Tissues by Continuous Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry, *Talanta*, **84**: 820-828 (2011).

[38] Hashemi S.H., Kaykhaii M., Developments in Methods of Analysis for Naphthalene Sulfonates, *Crit. Rev. Anal. Chem*., **47**: 127-137 (2016).