



## ORIGINAL ARTICLE

# Molecularly Imprinted Polymers (MIP) for Selective Solid Phase Extraction of Celecoxib in Urine Samples Followed by High Performance Liquid Chromatography

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**KEYWORDS**

Celecoxib;  
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**ABSTRACT:** In this study, for the analysis of human urine samples, a novel method explained for the determination of celecoxib, a nonsteroidal anti-inflammatory drug (NSAID), using molecularly imprinted solid-phase extraction (MISPE) coupled with high-performance liquid chromatography (HPLC). The synthesis of the MIP was performed by precipitation polymerization in methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), chloroform, 2,2'-azobisisobutyronitrile (AIBN) and celecoxib as the functional monomer, cross-linker monomer, solvent, initiator and target drug, respectively. The celecoxib imprinted polymer was utilized as a specific sorbent for the solid phase extraction (SPE) of celecoxib from samples. The molecularly imprinted polymer (MIP) performance was compared with the synthesized non-molecularly imprinted polymer (NIP). Scanning electron microscopy (SEM), FT-IR spectroscopy, UV-VIS spectrophotometry and thermogravimetric analysis (TGA/DTG) were used for characterizing the synthesized polymers. Moreover, the MISPE procedure parameters such as pH, eluent solvent flow rate, eluent volume and sorbent mass that probably influence the extraction process have been optimized to achieve the highest celecoxib extraction efficiency. The relative standard deviation (RSD %), recovery percent, limit of detection (LOD) and limit of quantification (LOQ) of this proposed method were 1.12%, 96%,  $8 \mu\text{g L}^{-1}$  and  $26.7 \mu\text{g L}^{-1}$ , respectively. The proposed MISPE-HPLC-UV method can be used for the separation and enrichment of trace amounts of celecoxib in human urine and biological samples.

**INTRODUCTION**

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide, a nonsteroidal anti-

inflammatory drug (NSAID), belongs to a class of agents that selectively inhibit cyclooxygenase-2

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(COX-2) enzymes” [1]. The presentation of this first specific COX-2 inhibitor in the pharmaceutical business markets has many advantages such as relief of the signs and symptoms of osteoarthritis (OA), rheumatoid arthritis (RA), and ankylosing spondylitis for the management of acute pain in adults and primary dysmenorrhea treatment [2], and is applied for its chemopreventive activity in case of colon carcinogenesis, UV light induced skin cancer and breast cancer [3]. Of course, celecoxib affects the renal function and cardiovascular system which these influences are discussed controversially. “Celecoxib exhibits anti-inflammatory, analgesic, and antipyretic activities by selective inhibition of cyclooxygenase-2 (COX-2), the inducible isoform of cyclooxygenase, involved in the prostaglandin synthesis, and does not inhibit platelet aggregation” [3]. In contrast, other nonselective NSAIDs for example naproxen, aspirin, and ibuprofen prevent both cyclooxygenases isoforms (COX-1 and COX-2). After about 2 h from oral administration of celecoxib, it is absorbed by gastro-intestinal tract. The liver acts a principal role in celecoxib metabolism by the cytochrome P450 and elimination of celecoxib mainly occurs as metabolites in the faeces and urine.

Till now, several methods for the quantification of celecoxib in pharmaceutical preparations [4] and biological samples [5] have been described utilizing HPLC with UV detection [6, 7], fluorescence detection [8], liquid chromatography mass spectrometry [9] and densitometric methods [10]. Traditionally, liquid-liquid extraction (LLE) was one of the most preferred techniques often used for sample clean-up. However LLE resulted in relatively clean extracts with good recoveries and time-consuming, but at the same time, large quantities of expensive, toxic and environmentally unfriendly organic solvents were utilized at this technique which often harmed environmental and health. Because of the low concentration level of drugs in human samples, this extraction method cannot be used in low concentration levels of analytes and complex matrices.

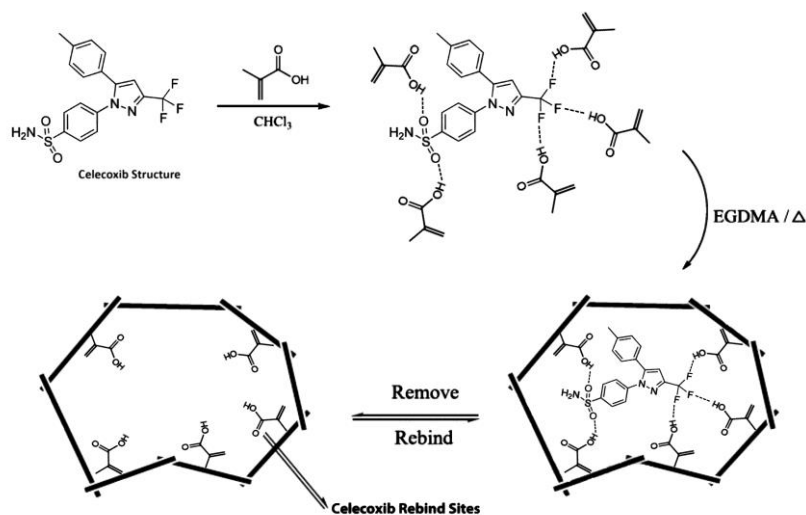
In order to solve these problems, the solid phase extraction (SPE) which is one of the powerful and im-

portant preconcentration methods with satisfactory recoveries, cost effective and quite fast, has been generally applied. In the recent years, one of the most preferred technical and applicable extraction methods, based on utilize of imprinted synthesized polymers, is molecularly imprinted solid-phase extraction (MISPE) procedure. The molecularly imprinted polymers (MIPs) have been enormously used to the selection extraction or to clean up of analytes from various complex samples such as environmental and biological samples [11, 12], food and pharmaceuticals [13, 14]. Many studies on the extraction of a drug by MIPs from various samples such as tablets [15], plasma [16], serum [17], or from urine [18] have been considered in the pharmaceutical field. The MIPs applications for chiral separation [19], biomimetic sensors [20], SPE [21], and drug release have been proven useful, and their utilization as sorbent materials for SPE procedure [22] has turned into a compelling method. Mainly when a selective extraction process must be considered, the utilization of molecularly imprinted polymers in solid phase extraction has many specific advantages. According to these features, the MISPE method, not only used to preconcentrate of the analyte but also is applied to remove other compounds present in the sample matrix.

The polymerization procedure of a solution comprising a functional monomer, a cross-linker and a template made a molecularly imprinted polymer that can be used in many processes. Some chemical bonds such as hydrogen, polar, hydrophobic and/or ionic bonds [23] cause to interaction among the functional. Monomer with the template before polymerisation. The next step after polymerization procedure is washing process. At this stage, the template molecules are removed and final material has cavities that can selectively bind to compounds very analogous in structure, with consider to functional groups and conformation, to the template used. Figure 1 illustrates the schematic representation of the imprinting process and the removal of celecoxib from the imprinted polymer. Thus, the imprinted polymer can specifically rebind the analyte (e.g. the template) when a sample is loaded onto a

MIP in a typical SPE procedure. After these steps, the analyte is eluted from the SPE cartridge ideally free of co-extracted compounds. In this approach, the non-covalent imprinting interactions of template-monomer complex are obtained. The non-covalent imprinting, as

well as called as a self-assembling approach, is specified by weak interactions between the template molecule and the functional monomers, such as hydrogen bonds, Van der Waals forces, ion or hydrophobic interaction [24].



**Figure 1.** A scheme for the synthesis of celecoxib imprinted polymer particles.

In this study, based on best previously optimized operational circumstances (1:4:20 ratio of template: monomer: cross linker molar ratio) [25], both of the molecularly imprinted polymer (MIP) and the nonimprinted polymer (NIP) were synthesized for comparing the extraction recovery (ER %). At this condition, a highly selective MIP as a novel sorbent for SPE procedure was prepared which cause to increase in the selectivity of the method for determination of trace quantity of celecoxib in MISPE method. To the best of author's knowledge, no study utilizing MISPE-HPLC-UV technique have been accomplished for extraction of celecoxib from urine samples.

## MATERIALS AND METHODS

### Materials and chemicals

All chemical materials were used without further purification and were of the highest grade commercially available. Celecoxib was purchased from Arasto Pharmaceutical Chemicals Inc. (Saveh, Iran). 2,2-Azobisisobutyronitrile (AIBN) was from Sigma Aldrich (St. Louis, MO, USA). All other chemicals such as methacrylic acid (MAA, 98%), ethylene glycol

dimethacrylate (EGDMA, 98%), chloroform, acetonitrile, methanol, acetic acid, hydrochloric acid, and sodium hydroxide were of HPLC qualities and were obtained from Merck (Darmstadt, Germany).

### Instruments

The morphology of polymers was evaluated by a scanning electron microscope (SEM, Philips, XL-30, and Almelo, Netherlands) instrument. FT-IR spectra of the samples were recorded on a Bruker-TENSOR27 (Germany) spectrometer. Linseis STA PT 1600 thermal analyzer was used to determine the thermal properties of synthesized polymers. A ZIS-TECH NANO-P566 peristaltic pump was used for pumping a variety of solvent amounts into SPE cartridge. A digital pH meter, BANTE Instrument, equipped with a combined glass calomel electrode was applied for the pH adjustments at  $25 \pm 1^\circ\text{C}$  temperature. Ultrapure water was prepared using a WaterPro water system (Millipore, Direct Q3, and China) and used throughout the experiments. For the polymer synthesis, the employed apparatus comprised soxhlet and a heater unit, a nitrogen supply system, an ultrasonic

shaker (JINYVANBAO), an oven (Memmert, Germany) and a magnetic shaker (HEIDOLPH-MR Hei-Standard). A digital balance (AND DJ-VSSOA) was utilized for the weight measurement of the reagents (milligram quantities or less).

### **Synthesis of Molecularly Imprinted Polymers**

A non-covalent molecular imprinting procedure was followed to prepare the MIP and the nonimprinted polymer (NIP). The monomer MAA (0.8 mmol), celecoxib (0.2 mmol) and 20 mL of chloroform were located in a glass sample vial. Then cross-linker EGDMA (4 mmol) was added. The blend was uniformly interspersed by sonication. After sonication, it was purged with N<sub>2</sub> for 15 min and the glass tube was sealed under this atmosphere. Then the reaction initiator AIBN (0.1 mmol) was added to the mixture. The polymerization was carried out for 24 h in a water bath 60°C. The synthesized polymer particles, after the polymerization procedure and drying, were washed with methanol and acetic acid for three times and with distilled water for two times using soxhlet extraction. After washing step, the complete elimination of template molecules from the structure network was evaluated by spectrophotometer of UV until no celecoxib spectra not seen in the analysis. The optimum ratio of template molecule to functional monomer to cross-linker was 1:4:20. So to ascertain that maintenance of template was because of molecular recognition and not to nonspecific binding, a control (nonimprinted polymer) was prepared by the same procedure, including washing, but with the elimination of the target particles, celecoxib.

### **Preparation of standard solutions**

Celecoxib is dissolvable in organic solvents, for example, methanol, ethanol, DMSO and acetonitrile with high stability (two years) based on storage at -20°C. A stock solution (100 mg L<sup>-1</sup>) of the celecoxib was prepared by dissolving 10 mg celecoxib in 100 mL of 75:25 methanol: water mixture. The stock solution was preserved from light and stored at 4°C and con-

veyed to surrounding temperature just prior to use.

### **Preliminary MIP-SPE procedure**

The extraction procedure includes several stages, which include packing, conditioning and loading, washing, eluting, and regenerating. Extraction of celecoxib molecules from modeling solutions and real samples is followed by two stages; the first step is sorption and the second is desorption. In the sorption step, pH of the sample solution was adjusted by dropwise addition of sodium hydroxide or hydrochloric acid solutions. The dry molecularly imprinted polymer/nonimprinted polymer was placed in empty SPE cartridges among two polyethylene cribriform plates. After the packing process the MIP-SPE cartridge was firstly rinsed with 10 mL methanol and then with 10 mL deionized water to eliminate possible adsorbed materials; followed by loading sample solution with optimize pH and flow rate in the condition of negative pressure. In the next step, when the sample loading finished, the cartridge was washed with 10 mL of deionized water to eliminate the impurities and eluted with acetic acid in methanol (10:90 v/v %) solvent with optimize volume and flow rate to desorb celecoxib. The washing solutions are able to interrupt Van der Waals interactions and, presumably, a part of the hydrogen bonds because of their hydrogen bond donor properties. At the end, the sample was analyzed by HPLC.

### **FT-IR analysis**

FT-IR analysis was used to examine the interaction among MIP and celecoxib. During a run, the FT-IR spectra of the MIP were determined first. Then the MIP saturated with celecoxib solution was determined. A comparison of the FT-IR spectra of MIP before and after celecoxib saturation was made and it provided information on the functional groups of the MIP and its interaction with celecoxib. All spectra were determined at 25°C.

### *Thermal behavior of polymers*

In this study, the MIP and NIP particles were investigated by means of simultaneous thermogravimetry/differential thermogravimetry (TGA/DTG). For this purpose, the weight loss of MIP and NIP particles was measured up to 600°C. The outcomes permitted us to gain data concerning these compounds in the solid state, including their thermal stability and thermal decomposition. Thermogravimetry (TGA) and differential thermogravimetry (DTG) were carried out using a Linseis STA PT 1600 thermal analysis, applying the heating rate of 10°C min<sup>-1</sup> in a temperature range of 30–600°C, under a nitrogen atmosphere with the flow rate of 50 mL min<sup>-1</sup>. The sample mass used was about 3 mg.

### *HPLC Analysis*

All measurements were performed using a Yong Lin HPLC system (YL9100, South Korea), equipped with an SP930D pump, an UV30D detector, and a Kromasil C<sub>18</sub> column (250 mm × 4.6 mm, 5 μm) that the flow rate and UV wavelength were set at 1.5 mL min<sup>-1</sup> and 255 nm, respectively. All injections were carried out manually with a 10 μL sample loop.

### *Extraction procedure for urine samples*

To research on the ability of the MISPE method to extract celecoxib particles from biological samples, urine samples were acquired from two volunteers (25 and 55 yr old). One of them was healthy and with drug-free urine (sample A) and the other was constantly taking celecoxib (sample B). The analysis was performed by using the standard addition technique. They were spiked with celecoxib at a concentration of 0.8 mg L<sup>-1</sup> and then percolated through the MIP after conditioning. After a washing step, celecoxib was recovered with methanol-acetic acid solvent. The blank samples were prepared similarly to the spiked sample except that no celecoxib standard substances were added. All experiments were performed in triplicate and results reported here were the mean values.

## RESULTS AND DISCUSSION

### *Characterization of MIP and NIP*

The synthesized polymers morphological structures were investigated by scanning electron microscopy (SEM). Figure 2 presents the SEM images of the MIP and NIP under the magnifications of 30,000. It was clear that both MIP and NIP were consistently distributed with pore diameter and size. These observations show that the formation celecoxib imprinted particles were performed effectively and it can be utilized as a specific solid phase for extraction of trace amounts of celecoxib.

FTIR spectroscopy is an appropriate strategy to determine the functional groups and sorts of bonds presence in the polymers. Therefore FT-IR was performed to testify the successful preparation of the imprinted polymer. The infrared spectra of celecoxib, MIPs before and after depletion of celecoxib and NIP are illustrated in Figure 3. The stretching vibration of OH bonds attributed physical and morphological characterization to the hydroxyl groups of MAA molecules, corresponded to the broad absorption band at 3448 cm<sup>-1</sup>. Two stretching vibration methyl C–H and C=O bonds could be attributed to the bands observed at 2900 cm<sup>-1</sup> and 1732 cm<sup>-1</sup>, respectively which demonstrated the MAA was well polymerized with EGDMA. The absorption peak around 1637 cm<sup>-1</sup>, attributed to the stretching vibration of residual vinylic C=C bonds and intensity of this vibration band are weak due to overlapping with C=O absorptions, while this peak was discovered on all of the polymers. This absorption peak demonstrated that only a few remained unlinked and most MAA were cross-linked with EGDMA. Curve d in Figure 3 was the IR spectrum of MIP before eluting template. The differences between curve c and curve d were the N–H stretching vibration bond of –SO<sub>2</sub>NH<sub>2</sub> group at 3422 cm<sup>-1</sup> and S=O symmetric and asymmetric stretching bonds at 1269 and 1325 cm<sup>-1</sup>, respectively. The presence of IR absorption changes demonstrated that celecoxib might have formed hydrogen binds with MAA in the reaction with MAA in

the imprinted material. The experimental results illustrated that the polymerization procedure had happened successfully and are reasonable because both NIP (Figure 3b) and MIP after eluting templates (Figure 3c) have the similar chemical spectrums.

In order to indicate the difference of decomposition stages between monomer, MIP and NIP particles and to evaluate the stability of the synthesized polymers at the high-temperature condition, the TGA/DTG thermograms were used. Figure 4 depicts the TGA/DTG plots of the MIP and NIP particles. The thermogram of celecoxib MIP and NIP have shown a similar kind of

degradation pattern. Regarding the MIP particles, the mass loss ( $\Delta m = 76.5\%$ ) is in the range of 245–360°C, which occurs in a single step, assigned to the decomposition of the free monomer and the cross-linker, and celecoxib decomposition as the melting point of celecoxib is 183°C. Considering to the NIP particles, the mass loss ( $\Delta m = 71.5\%$ ) is in the range 190–340°C, which occurs in a single step, attributed to the cross-linker and free monomer decomposition. It indicates NIP particles have lower thermal stability compared to MIP particles and the rigidity of the MIP particles is more than NIP.

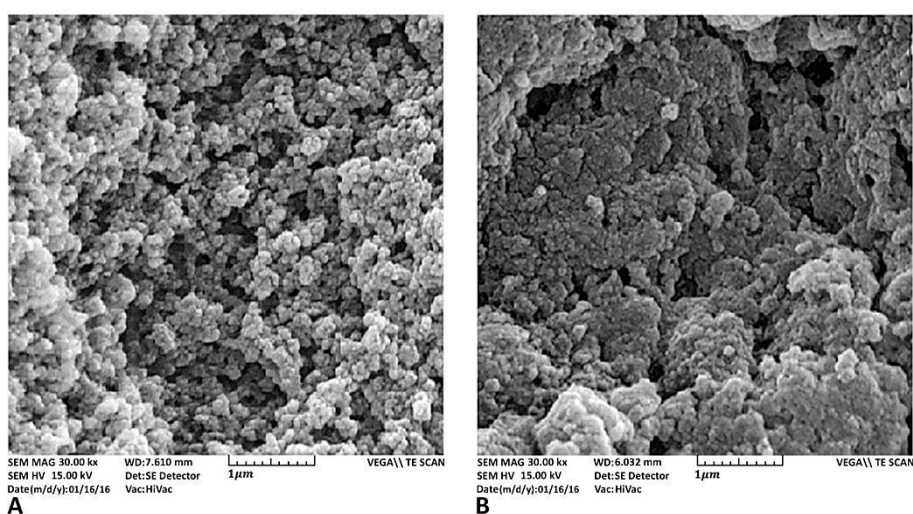


Figure 2. SEM images of celecoxib-MIP and NIP; (magnification of 30,000). Key: (A) celecoxib-MIP and (B) NIP.

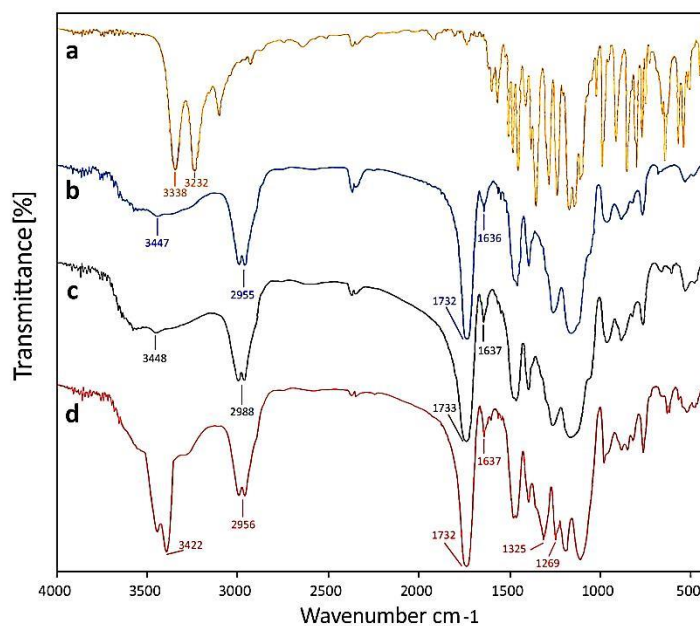


Figure 3. FTIR of the polymers. Key: (a) celecoxib; (b) NIP; (c) MIP after the depletion of celecoxib and (d) MIP before the depletion of celecoxib

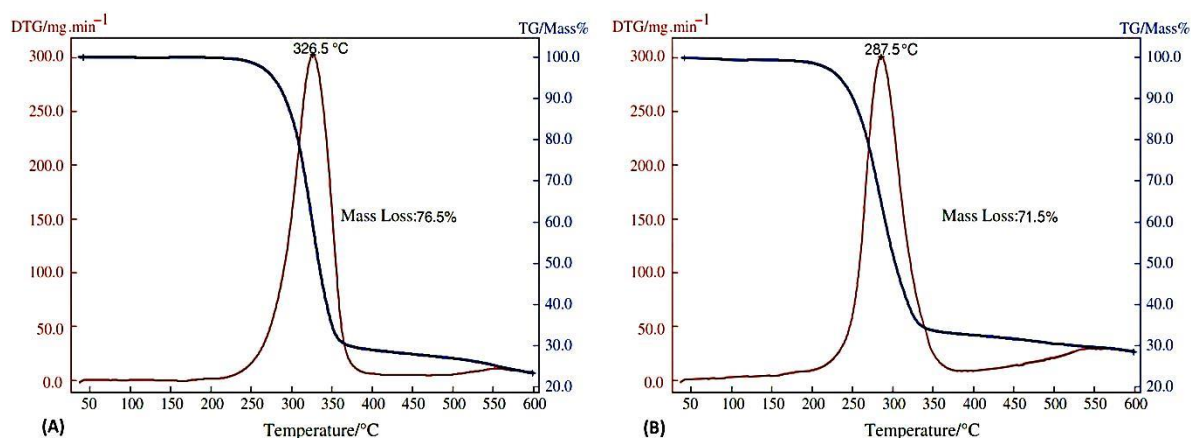


Figure 4. TGA/DTG curves for (A) Celecoxib-MIP and (B) NIP particles. Sample mass 3 mg; heating rate  $10^{\circ}\text{C min}^{-1}$ ; nitrogen atmosphere.

### Optimization of extraction conditions

In spite of the amicability in the survey articles within the previous decades, the selectivity of MISPE instruments, mechanisms, and their levelheaded control has not completely been identified. In this manner, there is a need to enhance the MISPE extraction system in more points of interest. Since all the conditioning, loading, washing, and elution step parameters (both type and amounts) have a great effect on the entire MISPE implementation in terms of affinity, selectivity, loading capacity, etc. In this survey, solid phase extraction (SPE) using the imprinted polymers (MIPs) has been optimized for celecoxib optimum determination. Factors that most likely influence the extraction procedure, for example, pH, eluent solvent flow rate, eluent volume and sorbent mass, were appraised to obtain the greatest extraction affinity.

### Desorption composition

The eluting solvent has a significant role to elute the caught analyte in MISPE procedure. In this condition, different elution solvents were considered to get the highest celecoxib recovery percent. In this study, several solvents such as methanol: acetic acid (90:10 v/v %), acetonitrile: acetic acid (90:10 v/v %), methanol: distilled water: acetic acid (45:45:10 v/v %), methanol and distilled water were used in elution process. To avoid halogenated solvents, the selected solvents were in accordance with the principles of green chemistry. Acetic acid hinders the formation of hydrogen bonding between celecoxib and functional monomer and leads to easier removal of celecoxib. As it is shown in Table 1, among different eluents, satisfactory recovery was obtained using methanol: acetic acid (90:10 v/v %) as eluent solvent.

Table 1. Recovery of celecoxib from MIPs cartridge using different elution solvents.

Eluent solvent	Recovery %
Methanol: Acetic acid (90:10 v/v %)	96
Acetonitrile: Acetic acid (90:10 v/v %)	82
Methanol: Distilled Water: Acetic acid (45:45:10 v/v %)	58
Methanol	75
Distilled Water	15

### Effect of pH

The influence of pH on the extraction recovery of celecoxib from the MIP-SPE was researched at differ-

ent pHs in the range of 3 to 9 (utilizing universal buffer). In sample loading, the optimum pH ought to be

used for a superior position of the analyte on the polymer network due to its chemical structure. In these conditions, appropriate hydrogen bonding with polymer was obtained. Figure 5A demonstrates the influence of solution's pH on extraction recovery. The extraction recovery was improved with growing the pH from 3 to 5 and was recessed with reducing the pH from 5 to 9. In the pH of 5, cationic and impartial portions are available and the initial species is an impartial molecule of celecoxib which binds to the imprinting places of MIP by hydrogen bonds. So the quantity of celecoxib adsorbed on the MIP grew, which brought resulted in the high recovery. Although, when the pH is more than 8, the predominant species is anionic. Therefore no interaction among the analyte and the polymer happens, which leads to the low extraction recovery. Thus, the pH of 5 was selected as optimized pH, for quantitative adsorption of celecoxib on the synthesized MIP in the future experiments.

#### ***Effect of amount of MIP***

To evaluate the optimum quantity of MIP on the quantitative extraction of celecoxib, as it can be seen in Figure 5B, by varying amount of MIP, which is in the range of 0.1 to 0.7 g, the extraction was conducted.

The highest celecoxib recovery was obtained at the MIP mass of 400 mg. Thus, the MIP mass of 400 mg was applied as the optimum quantity for the extraction processes.

#### ***Effect of Eluent volume***

The effect of eluent volume on the extraction recovery of celecoxib was assayed at various volumes (acetic acid in methanol, 10:90 v/v %) in the range of 2–20 mL. Based on the outcomes (Figure 5C), it can be illustrated that 5 mL of eluent solvent was sufficient to elute analyte from the MIP. Because of the extraction recovery remained nearly steady with the enhancement of eluent volume, further enhancement the eluent volume was not preferred and therefore 5 mL of eluent solvent was selected for further study.

#### ***Effect of Eluent Flow Rate***

So various flow rates in the range of 1.92–4.22 mL min<sup>-1</sup> was applied to assessment of the eluent flow rate effect on recovery of the celecoxib particles which were trapped in imprinted polymer network. Based on what has presented in the Figure 5D, the outcomes demonstrated that the utmost sign was acquired at the flow rate of 1.92 mL min<sup>-1</sup>. This flow rate of was afterward chosen as an ideal eluent flow rate for the further examination.



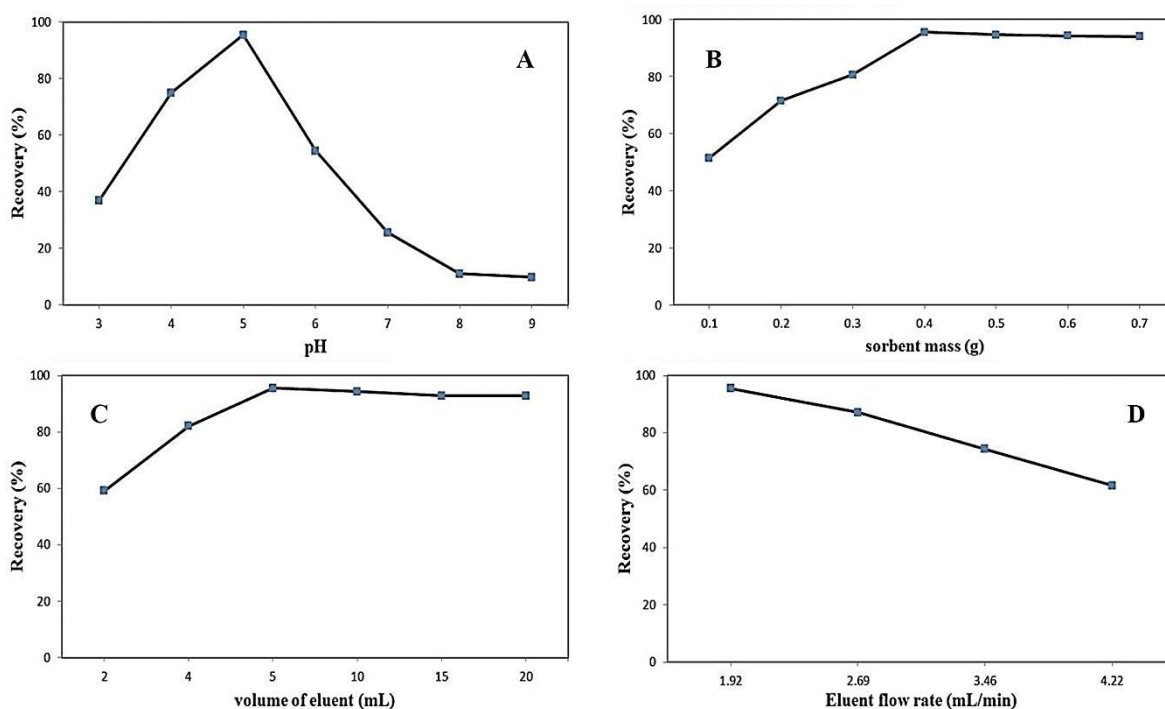


Figure 5. Effects of (A) pH value, (B) sorbent mass, (C) eluent volume and (D) eluent flow rate on the recovery of celecoxib.

### Comparison of MIP and NIP

To the examination of MIP and NIP contrast, two extractions by both MIP and NIP in water sample were evaluated under the optimal circumstances. As it has demonstrated in the previous sections and because of the high capacity of MIP for extraction of celecoxib in contrast with NIP, the extraction recovery of MIP (96%) is rather than of NIP (13.51%). The acquired outcomes can assert the effective engineering building of cavity for celecoxib in the construction of synthesized MIP and NIP. To assess the utmost adsorption capacity, the contrast among the concentration of the solution before and after extraction was computed.

### Assessment of Selectivity

To evaluate the selectivity, two other medicines, i.e. sumatriptan and valdecoxib were selected. The proof for the choice of these medicines was that they afford many similar functional groups which can bind to MIP. The chemical constructions of these medicines are shown in Fig. 6. The primary concentrations of these medicines were separated by 400 mg of MIP in

the optimum circumstance. The outcomes demonstrated that adsorption recovery for celecoxib, valdecoxib and sumatriptan were acquired 96, 43.33 and 16.41 percent, respectively. This experiment unfolds that binding to the MIP is not because of surface adsorption at all (surface adsorption <1.0%), rather, it is for the most part because of the template selectively with appropriate hole.

The polymers are normally assessed to check their recognition properties for an objective analyte. With a specific end goal to quantify the selectivity of these polymers, the primary concentrations of these drugs were separated by 400 mg of MIP and NIP in the optimized circumstance. As can be seen in Table 2, distribution ratio ( $K_D$ ), selectivity coefficient ( $k_{sel}$ ) and relative selectivity coefficient ( $k'$ ) of both MIP and NIP for these drugs were obtained in competitive experiments. The distribution ratio ( $\text{mL g}^{-1}$ ) of celecoxib among the MIP particles and aqueous solution was defined by following equation:

$$K_D = (C_i - C_f) V / C_f m \quad (1)$$

Where V is the initial solution volume, m is the MIP mass,  $C_i$  is the primary concentration and  $C_f$  is the final concentration. Selectivity coefficients for celecoxib relevant to foreign compounds are defined as:

$$K_{VMP/j}^{sel} = K_D^{VMP} / K_D^j \quad (2)$$

Where  $K_D^{VMP}$  and  $K_D^j$  are the distribution ratios of celecoxib and foreign compound, respectively. The

above MISPE evaluations were applied additionally to these medicine molecules on NIP particles. The relative selectivity coefficient ( $k'$ ) was also defined by the following equation:

$$k' = k_{MIP} / k_{NIP} \quad (3)$$

According to the results in Table 2 and comparing outcomes of selected drugs, with using MIP-SPE method for celecoxib determination, a quantitative extraction, and an excellent MIP/NIP selectivity were obtained.

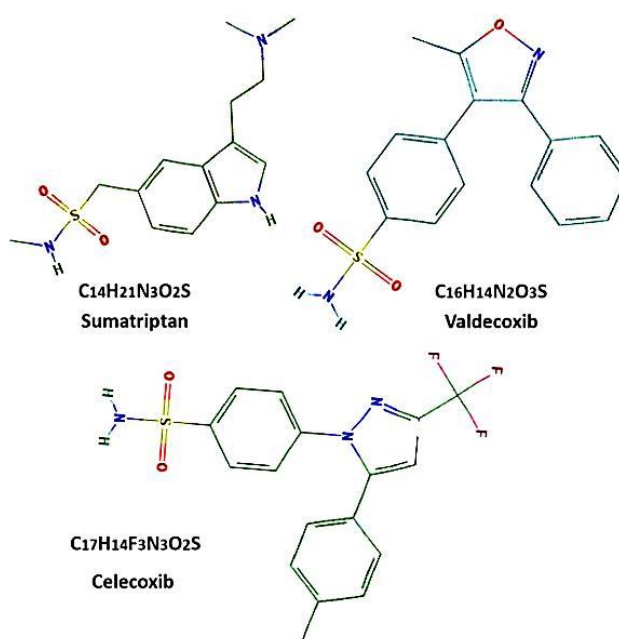


Figure 6. Chemical structures of celecoxib, sumatriptan and valdecoxib.

Table 2. Distribution ratio ( $K_D$ ), selectivity coefficient ( $k^{sel}$ ) and relative selectivity coefficient ( $k'$ ) values of MIP and NIP material for different drugs.

Drug	$K_D$ (MIP) (mL g <sup>-1</sup> )	$K_D$ (NIP) (mL g <sup>-1</sup> )	$k^{sel}$ (MIP)	$k^{sel}$ (NIP)	$k'$
celecoxib	7500	55.5	---	---	---
valdecoxib	238.65	45.1	31.43	1.23	25.55
sumatriptan	61.30	29.1	122.34	1.91	64.05

### Validation of the method

By a series of experimental and analytical parameters including linear range, limit of detection (LOD), the method relative standard deviation (RSD %), correlation coefficients ( $R^2$ ), and limit of quantification (LOQ), the validation of the suggested method was evaluated. The outcomes illustrated that a good linearity was obtained in the range of 0.05–100 mg L<sup>-1</sup> with  $R^2$  of 0.997 for celecoxib and the linear regression equation was  $Y=30.99X+3.0086$ , where Y is peak area and X is the concentration of the analyte. In addition, the LOD, LOQ, and RSD % were 8 µg L<sup>-1</sup>, 26.7 µg L<sup>-1</sup> and 1.12%, respectively.

### Real sample analysis

The application of the polymers to particular adsorption of celecoxib in urine samples was studied. The offered SPE method was effectively used to measure of celecoxib in urine samples. The investigation was performed by utilizing the standard addition method. The outcomes are epitomized in Table 3 that the three

replicate analysis results of every genuine sample got by the method are in great concurrence with the spiking quantities. Good recoveries in all samples were acquired. This implies that the suggested method can be appropriate to the investigation of this and other comparable liquids containing celecoxib.

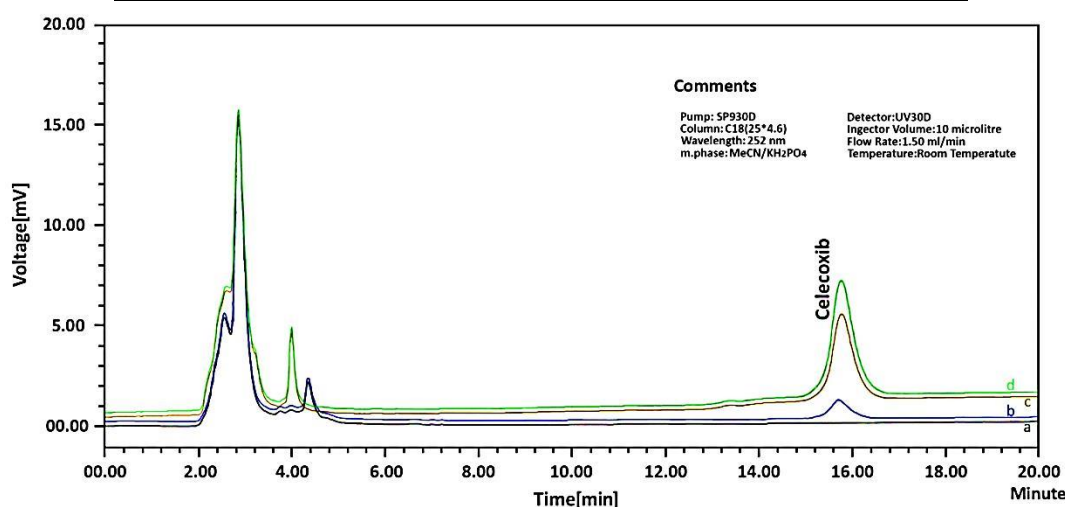
The repeatability of the technique was exhibited by the mean relative standard deviation (RSD %). As well as, the relative recovery is obtained from the following equation:

$$\text{Recovery \%} = (C_{\text{founded}} - C_{\text{real}}) / C_{\text{added}} \times 100 \quad (4)$$

In this equation,  $C_{\text{founded}}$ ,  $C_{\text{real}}$ , and  $C_{\text{added}}$  related to the concentration of the analyte after addition of known quantity of standard in the real sample, the concentration of the analyte in the real sample and the concentration of known quantity of standard which was spiked to the real sample, respectively. The HPLC chromatograms for determination of celecoxib in spiked urine samples were illustrated in the Figure 7.

**Table 3.** Recoveries and precision of celecoxib in spiked urine samples

Sample	$C_{\text{added}}$ (mg L <sup>-1</sup> )	$C_{\text{founded}}$ (mg L <sup>-1</sup> )	Recovery (%)	RSD (%) (n=3)
Sample A	0	0	---	---
	0.8	0.71	89	2.24
Sample B	0	1.53	---	2.16
	0.8	2.30	96	1.85



**Figure 7.** Chromatograms of both kinds of human urine samples with HPLC analysis. Key: (a) urine sample A just with dilution; (b) urine sample A spiked with 0.8 mg L<sup>-1</sup> celecoxib solution; (c) urine sample B just with dilution; and (d) urine sample B spiked with 0.8 mg L<sup>-1</sup> celecoxib solution.

## CONCLUSIONS

In this study, a non-covalent molecular imprinting procedure was used to synthesize celecoxib MIP particles as a solid phase sorbent for the selective extraction and preconcentration of this drug from human urine samples. To obtain the highest extraction efficiency and to get the favorable and optimized circumstance, different effective parameters on the extraction method of MIP were precisely assessed. Because of the intense productivity of the MISPE-HPLC-UV method, the analytical characteristics, for example, great exactness and specific recognition ability, high quantities of recovery, wide dynamic linear range and low detection limit were obtained through this method. Also, other advantages of suggested method are simplicity, high selectivity, environmentally friendly, safe and excellent reusability that can be useful for the extraction. These outcomes caused to trace amount of celecoxib at ppm and ppb levels can be determined and separated by this imprinted polymers which were due to relatively high preconcentration factor and selective interactions with specific cavities. In conclusion, this method can be generally utilized and has a great potential to clinical application for checking and determining celecoxib in patient urine.

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