Journal of Pharmaceutical and Biomedical Analysis 249 (2024) 116336

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Development of a new magnetic solid-phase extraction method prior to HPLC determination of naproxen in pharmaceutical products and water samples

Ayşenur Karataş [a], Tülay Oymak [a,*], Ahmet Çelik [b,*]

[a] Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Analytical Chemistry, Sivas 58140, Turkey
[b] Ankara Yildirim Beyazit University, Faculty of Engineering and Natural Sciences, Department of Chemical Engineering, Ankara, Turkey

ARTICLE INFO

Keywords:
Naproxen
Magnetic biochar
Pumpkin peel
Magnetic solid-phase extraction
Pharmaceutical contamination
HPLC

ABSTRACT

In this study, a new magnetic solid phase extraction based on magnetic composite modified with biochar obtained from pumpkin peel was developed for the enrichment and extraction of Naproxen in lake water, tablet and urine samples. The effects of main parameters such as pH, extraction time, amount of adsorbent and sample volume, which affect magnetic solid phase extraction, were investigated. Under optimal conditions, intraday and interday precision values for naproxen were below 5.9, with accuracy (relative error) better than 7.0 %. The detection limit and preliminary concentration factor were 12 ng/mL and 10, respectively. The method proposed here can be used for routine analysis of naproxen in lake water, urine and tablets.

1. Introduction

In recent decades, a significant number of active pharmaceutical compounds, including lipid regulators, nonsteroidal anti-inflammatory drugs, anticancer, antibiotics, and chemotherapy agents, have been discovered in surface water, wastewater effluent, and, particularly, certain drinking water sources. These pharmaceutical substances consist mostly of non-biodegradable components, and their presence in water sources poses significant risks to human health and potentially threatens the environment[1]. Conventional wastewater treatment methods have proven ineffective in completely removing pharmaceutical residues [2, 3]. Although pharmaceutical concentrations detected in environmental samples are relatively low, ranging from ng/L to μg/L, they can still be capable of causing toxic effects [4].

Naproxen (also known as (S)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid) (NAP) is a non-steroidal anti-inflammatory drug commonly prescribed for the treatment of rheumatic diseases like rheumatoid arthritis and osteoarthritis. It is also used to alleviate acute painful conditions, including migraines, headaches, and postoperative pain, particularly in gynecological procedures. The dissolution of this drug is constrained by its rate [5]. Studies have indicated that naproxen is found in a single crystal form, and the structure of its crystallographic has been identified as monoclinic[6]. Before starting naproxen therapy, it is crucial to carefully evaluate both the potential advantages and drawbacks of this treatment, as well as explore alternative therapeutic options [7]. It is recommended to use the lowest effective dose and the shortest treatment duration that aligns with the patient's treatment objectives [7]. There are various methods which are liquid chromatography, capillary electrophoresis, gas chromatography, and fluorescence spectroscopy to determine naproxen. Among these, high-performance liquid chromatography stands out as the most frequently utilized method [8].

However, due to the trace levels of naproxen in samples and the complicate matrix in biological samples, direct determination of NAP is not possible with available instruments. Thus, an extra step of sample preparation is required to before analysis [9]. Solid phase extraction (SPE) is a commonly employed method for sample preparation. Among the various methods of sample preparation, solid-phase extraction (SPE) stands out as a prominent technique because it has capability to separate diverse analytes from complex matrices, offers high extraction recovery, uses less solvent volume, and provides convenience. The selection of the adsorbent in the SPE method significantly influences the effectiveness of the adsorption process [10]. In SPE, the sorbent serves a crucial role in the extraction of an analyte by selectively and effectively interacting with it. Thus, the preparation or selection of an adsorbent has a critical impact on the prosperity of the analyte extraction process, particularly

* Corresponding authors.
E-mail addresses: tulayoymak@cumhuriyet.edu.tr (T. Oymak), acelik@aybu.edu.tr (A. Çelik).

https://doi.org/10.1016/j.jpba.2024.116336
Received 24 April 2024; Received in revised form 1 July 2024; Accepted 3 July 2024
Available online 4 July 2024

when dealing with real samples [11]. In recent years, biochar (BC) derived from biomass has many advantages such as good performance, various resources, low cost, etc. Moreover, biochar is increasingly used because of its exceptional ability to adsorb heavy metals and organic pollutants in aqueous solutions. Nonetheless, separating powdered BC from an aqueous medium typically involves centrifugation and filtration processes. This requirement imposes limitations on the application of biochar in adsorption [12]. Hence, it is imperative to address the limitations associated with biochar in adsorption applications. Magnetic biochar presents a solution as it facilitates easy and rapid removal from aqueous media using magnetic separation, effectively addressing the bottleneck problem associated with biochar. Notably, the magnetic biochar is suitable for the removal of pollutants from aqueous solutions due to its combination of properties with a magnetic material [13,14]. Numerous studies have documented that magnetic biochar serves as an efficient adsorbent to remove heavy metals and organic pollutants from wastewater, as well as from water contaminated with nuclear waste. Thus, the MBC preparation from biomass represents a win-win strategy that addresses the limitations of BC in various applications as extending the utilization of BC as an adsorbent [15].

The use of unnecessary agricultural wastes for sorption purposes also serves a crucial role in addressing environmental problems through environmentally sustainable processes [16]. Pumpkin is recognized as a significant vegetable in agricultural production and is widely grown as a commercial plant [17]. Pumpkin peel, an agricultural waste, was selected based on its structural characteristics, water insolubility, chemical stability, and local accessibility [18]. Some adsorbents derived from pumpkin waste, such as pumpkin and pumpkin seed peels,have been reported in the literature [19,20]. According to our literature research, no study has been found in the literature on magnetic biochar obtained from pumpkin peel and the extraction and pre-concentration of naproxen.

In this study, MSPE method combined with HPLC was used for the extraction, enrichment, and NAP determination. Magnetic nanocomposite was synthesized using biochar produced from pumpkin peel modified with KOH and its applicability was examined. The influence of various parameters such as pH, extraction time, and sample volume affecting MSPE was extensively examined and optimum conditions were chosen. Direct application of HPLC to the detection of NAP in complex matrices such as milk and urine samples is limited due to the large number of interferences from both mutual and correlated base components. Also, due to the low concentrations of NAP in water samples, a separation/enrichment procedure must be applied before detection. In this study, the same samples were analyzed with direct analysis by HPLC and MSPE-HPLC and the results were compared.

Experimental results clearly showed that a separation/enrichment procedure should be applied before detection, especially due to the matrix effect in urine samples and the low concentration of NAP in lake water. The optimized method was successfully applied for the determination of NAP in lake water, tablet, and artificial urine samples.

2. Experimental

2.1. Materials and methods

Naproxen sodium was obtained from TCI; $FeSO_4.7\ H_2O$;H_3PO_4 were acquired from Sigma Aldrich (Steinheim, Germany); acetonitrile and KH_2PO_4 were purchased from Carlo Erba(Cornaredo, MI); $FeCl_3.9\ H_2O$ was from Merck (Darmstadt, Germany).; methanol was from Honeywell (Seelze, Germany); and NH_3 was achieved from Isolab (Eschau, Germany). SEM images from the Tescan Mira III (Brno, Czech Republic), were utilized for the analysis of the surface morphology of MBC. X-ray diffraction spectrometry (XRD) was conducted by the Panalytical-XRD device (Almelo, Holland), employing Cu kα anode X-ray radiation within an angle range of 10–70° (X-rays with a wavelength of 1.54056 A). The magnetization curves of MBC were determined by a vibrating sample magnetometer (Ohio, USA). BET analyses were performed using a Quantachrome Novatouch LX4 (Quantachrome,USA) instrument at a temperature of 77 K, employing liquid nitrogen (N_2). The specific surface area was acquired and computed utilizing the Brunauer-Emmett-Teller (BET) method.

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was utilized to analyze biochar and magnetic biochar's functional groups and structural changes. Spectra were recorded using BRUKER TENSOR II brand (Billerica, MA) ATR-FTIR in the range between 450–4000 cm^{-1}.

2.2. Production of biochar from pumpkin peel

In this study, the pumpkin peels (PP) were obtained from a local market in Sivas, Turkey. Pumpkin peels (PP) underwent a washing process using deionized water to eliminate any dust. Following this, they were dried in an oven at 80°C for 24 hours and subsequently finely ground into small pieces. The mixture of KOH and pumpkin peel powder (3:1 w/w) was placed in a porcelain crucible and heated in a muffle furnace at 500°C for 2 hours. The biochar obtained as a result of this process was washed several times with deionized water, and then dried in the oven at 80°C for 24 hours. dried [21].

2.3. Preparation of magnetic biochar

Magnetic biochar was synthesized using the coprecipitation method. 1.0 g of biochar was introduced into 100 mL of deionized water and shaken in an ultrasonic shaker for 1 h. 6.1 g of $FeCl_3.6\ H_2O$ was dissolved in 100 mL of water, and upon adding 4.2 g of $FeSO_4.7\ H_2O$, a few drops of 37 % HCl were introduced. The mixture was then heated with stirring. Once the temperature reached 80°C, 20 mL of 26 % ammonia and the dispersed biochar solution were introduced, maintaining the temperature at 80°C while stirring for half an hour [22,23]. After this duration, the prepared magnetic biochar was separated from the aqueous phase using a magnet. It underwent several washes with water and ethanol. Following the final wash, the Magnetic Biochar (MBC) was dried in an oven at 60°C and was subsequently ready for use in the experimental stages.

2.4. Chromatographic conditions

Chromatographic analyses were carried out using a Waters Alliance 2695 series high liquid chromatography system (Milford, MA USA) equipped with a quaternary pump, autosampler, and UV- detector (Waters Alliance 2487 Detector). This device was linked to an Empower 2 software that was utilized for data collection and analysis.

All separations were performed using an analytical SilUR / USil (250 mm,4.6 mm, 5 μm,; Kayseri, Türkiye) The mobile phase was prepared with 20 mM ammonium acetate solution(pH 7) and methanol 40;60, v/v). A flow rate of 1.0 mL/min and column temperature of 25°C were maintained. The measurement wavelength of the detector was set at 230 nm. The injection volume was 10 μL. Retention time and total analysis time were 4.7 and 7 min respectively. A chromatogram of 5 μg/mL Naproxen is given in Figure S1.

2.5. Separation- preconcentration of NAP by magnetic solid-phase extraction

Initially, 100 mg MBC was washed with deionized water and buffer solution. Then, 30 mL of solution containing 0.4 μg/mL NAP buffered to pH 3.0 was added and shaken at 50 rpm for 5 min. The sorbent was separated with an external magnet and the supernatant was decanted. 3.0 mL of 5 % NH_3-methanol was added onto MBC and the mixture was vortexed at 1000 rpm for 3 min for desorption. The MBC was held to one side of the tube using a strong magnet, the eluent solution was transferred to a clean vial, and the eluent solution was subjected to analysis

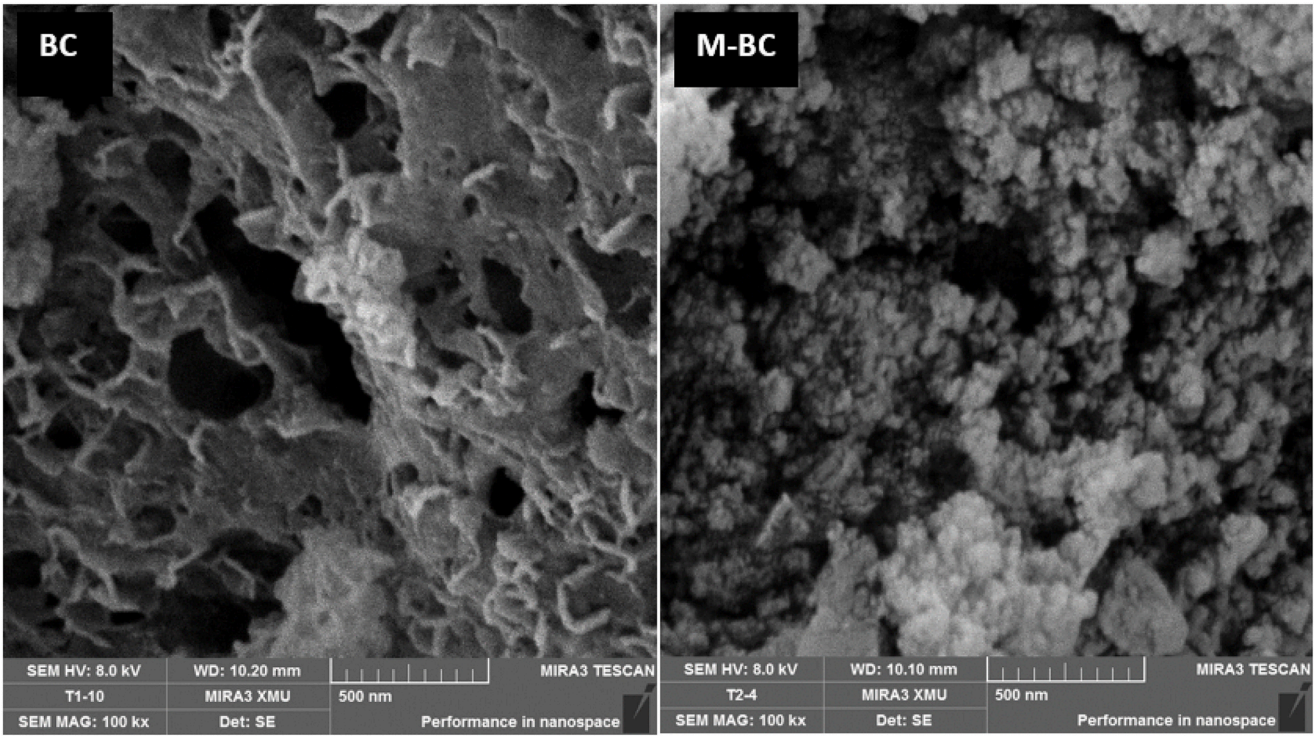

Fig. 1. SEM images of BC and MBC.

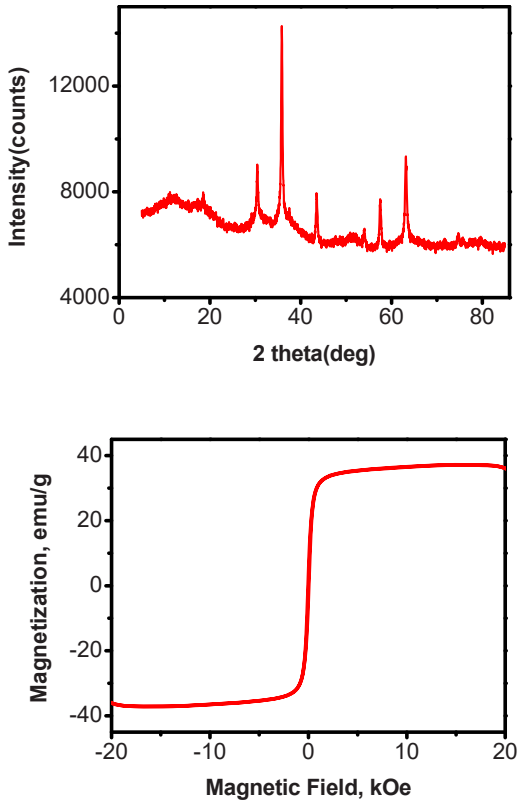

Fig. 2. a) XRD pattern of MBC b) M-H curve of MBC.

by HPLC. Each experimental parameter was repeated three times and the results are given with the mean value and standard deviations of these experiments.

3. Results and discussion

3.1. Characterization results

Analyzing SEM images and conducting Energy Dispersive Spectrometry (EDS) measurements is crucial in assessing both elemental composition and topological information. This approach is commonly employed in the characterization of magnetic materials. It can be seen in Fig. 1 that the surface structure of BC exhibits a porous structure. Following the modification, the iron oxide particles became embedded into the BC structure as seen in Fig. 1. The EDS spectrum of BC reveals that it contains 34.45 % C and 42.01 % O elements as the main components. EDS analysis results of MBC show that it contains 15.84 % C and 31.81 % O elements and 49.11 % Fe. This findings indicate that the magnetic nanocomposite was successfully synthesized. EDS spectra and results are given in Figure S2.

In Fig. 2a, the XRD pattern of exhibits several discernible diffraction peaks at 2θ positions of 30.4, 35.8, 43.4, 54.0, 57.6, and 63.2. These peaks align closely with the diffraction planes of 220, 311, 400, 422, 511, and 440, respectively. XRD spectrum indicates that the synthesized magnetic biochar contains Fe_3O_4 (magnetite) [24].

The saturation magnetization of MBC was measured with a vibrating sample magnetometer. The results show that the hysteresis curve of MBC is symmetric and passes through the origin and has obvious superparamagnetism. MBC saturation magnetization (Ms) is up to 37.2 emu/g.

The BET analysis results display that BC and MBC have surface areas of 125.185 m^2/g and 153.667 m^2/g, respectively. The increase in specific surface area can be explained by the loading of iron oxide

Table 1
Surface area and pore characteristics of BC, and MBC.

	BET Surface Area m^2/g	Pore volume cm^3/g	Pore radius, nm
BC	125.185	0.192	1.88
MBC	153.667	0.289	2.91

Table 2
Parameters of kinetic for the adsorptions of NAP on MBC.

	the model of PFO			the model of PSO		
NAP Concentration (mg/L)	q_e (mg/g)	k_1 (1/min)	R^2	q_e (mg/g)	k_2 (g/mg.min)	R^2
20	2.19	0.0193	0.9476	5.87	0.022	0.9997
30	3.21	0.0139	0.9654	7.87	0.010	0.9985

nanoparticles onto the surface. The larger pore volume and pore radius of MBC compared to BC indicate its greater suitability for the adsorption of macromolecules [14]. BET analysis results are summarized in Table 1.

ATR-FTIR analysis was employed to elucidate and compare BC and MBC functional groups.In Figure S3, it can be seen that the spectrum of biochar is different from the spectrum of magnetic biochar. This indicates that magnetic particles are loaded into biochar and the chemical structure changes.

The existence of the Fe-O group is indicated by the peaks between 400–700 cm^{-1}. The peak at 559 cm^{-1} in the FTIR spectrum of MBC shows the Fe-O group. This peak is not present in the FTIR spectrum of BC [23]. The band at 987 cm^{-1} and 3269 cm^{-1} was attributed to the OH and C═O stretching vibration of the hemicelluloses and cellulose respectively [25]. The peak at 987 cm^{-1} in the FTIR spectrum of BC is remarkably reduced in MBC.

The zero charge point (pHpzc) of MBC was determined according to methods reported in the literature. The pH of the solution was adjusted to a range of 2–10 using solutions of 0.1 M HNO_3 and 0.1 M NaOH. 100 mg MBC was added to the prepared 30 mL solutions. Following 24 hours of shaking at room temperature, the solution pH was determined. ΔpH, which is the difference between the final pH and the initial pH of the solutions, is graphed as a function of the initial pH. (Figure S4) [26]. By identifying the point where the curve intersects the x-axis, the pH_{PZC} of MBC was found to be pH = 5.8.

3.2. Adsorption kinetics

Data on the adsorption of NAP were utilized to investigate the kinetic behavior of the adsorption process. Pseudo-first and pseudo-second-order kinetic models were employed for this investigation. The equation of pseudo-first-order (PFO) suggests that adsorption occurs at one active site on the adsorbent surface of an adsorbate molecule, whereas the model of pseudo-second-order suggests that an adsorbate molecule is adsorbed at two active sites. The model of PFO (1) and the model of PSO (2) equations are given below.

$$\log(q_e - q_e) = \log q_e - \frac{k_1}{2.303}t \quad (1)$$

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \quad (2)$$

Here; qe and qt: Amount of substance adsorbed at equilibrium and time t (mg/g)

k_1: defined as first-order adsorption rate constant (1/min), k_2: second-order adsorption rate constant (g/mg.min).

In the model of PFO, the values of k_1 and q_e were determined by calculating the slope and intercept of the linear relationship derived by plotting the natural logarithm of ($q_e - q_t$) against time t. In the model of PSO, the values of q_e and k_2 were determined based on the slope and intercept of the linear relationship established by plotting the t/qt against time t [27].

In the comparison of the correlation coefficients (R^2) derived from two adsorption kinetic models, it was found that the PFO model exhibited a superior fit for describing the adsorption kinetics of naproxen on MBC, surpassing the PSO model which had the highest correlation coefficient. (Figure S5,Figure S6. and Table 2). This finding

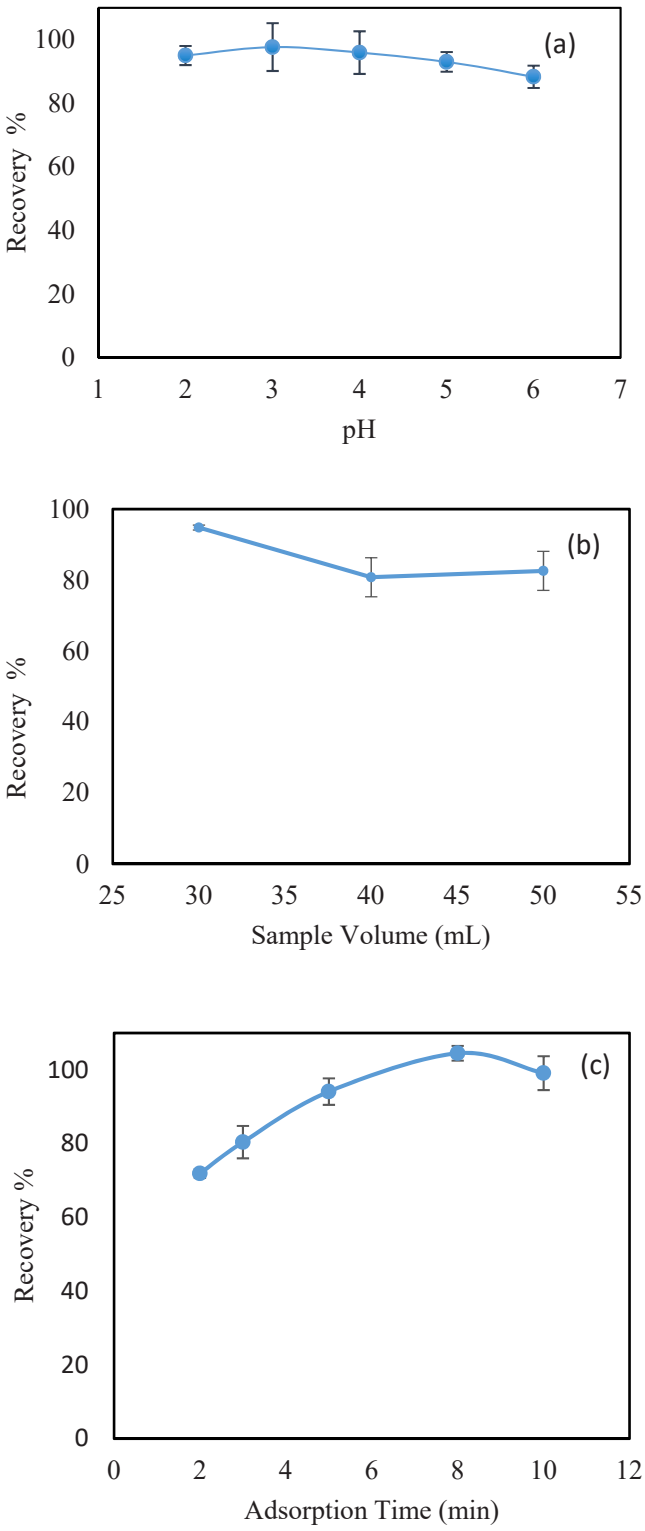

Fig. 3. a. Effect of pH on the recovery of NAP b. The effect of the sample volume c. The effect of the adsorption time (MBC of amount 100 mg; 0.4 μg/mL NAP; *n*=3).

Table 3
Confidence parameters of method; intra-day and inter-day precision and accuracy for determination of naproxen (n=3).

	Intra-day (n=3)			Inter-day (n=5)		
Added (ng/mL)	Found ($\bar{X}$±SD, ng/mL)	Precision (RSD%)	Accuracy (RE%)	Found. ($\bar{X}$±SD, ng/mL)	Precision (RSD%)	Accuracy (RE%)
100	100±2	2.3	0.0	102±2	2.0	2.0
200	186±5	2.5	-7.0	187±7	3.7	-6.5
400	393±23	5.9	-1.8	420±7	1.7	5.0

confirms that the primary mechanism responsible for the adsorption of naproxen onto MBC is chemisorption.

3.3. Optimization of MSPE conditions

3.3.1. pH influence

The pH is an important factor in the extraction process as it impacts the adsorbent stability, the surface charges present on it, and the charges of the analytes involved. Model solutions of 30 mL, each containing 0.4 μg/mL of naproxen, were prepared using 100 mg of MBC. The solutions pH was prepared utilizing buffer solutions ranging from 2.0 to 7.0, as illustrated in Fig. 3a. The extraction efficiency of naproxen exhibited an increase within the pH range of 2–4, whereas a decline was observed within the pH range of 6–8. Therefore, pH 3 was chosen as optimum, which exhibited the highest recovery of naproxen within the pH 2–4 range.

3.3.2. The mass of MBC influence

The mass of MBC is a significant factor affecting the efficiency of extraction. To find the optimum adsorbent mass for naproxen recovery, model solutions with 0.4 μg/mL naproxen were prepared, and 50, 100, and 150 mg of MBC were separately added into 30 mL of these solutions. The pH was then adjusted to 3, and the mixture was shaken at 50 rpm for 10 min. The adsorbent used at amounts of 50, 75, and 100 mg of MBC was performed, recoveries were 70.9 ±3.5, 79.7 ±2.4, and 99.1± 4.6 respectively. Further experiments were conducted utilizing 100 mg of MBC.

3.3.3. Sample volume effect

The volumes of sample are essential to determine the enrichment factor. MSPE was employed on model solutions of varying volumes: 30, 40, and 50 mL, each containing 100 mg of MBC and naproxen at a 0.4 μg/mL. Based on the findings presented in Fig. 3.b, the naproxen recovery was highest at a volume of 30 mL, but it decreased in volumes of 40 and 50 mL. Therefore, subsequent experiments were performed in a sample volume of 30 mL.

3.3.4. The effect of adsorption and desorption times

The effect of adsorption time was examined between 2 and 10 min with solutions containing 0.4 μg/mL NAP in a volume of 30 mL at pH 3. NAP recovery reached the % 95 at 5 min adsorption time. The optimum adsorption time was chosen as 5 min. The experiment results are given in Fig. 3.c.

Solutions containing 0.4 μg/mL NAP in 30 mL model solution pH 3 were adsorbed for 5 min and then the effect of elution time was examined between 2 and 5 min. Recovery values are 84.7±3.4 %, 98.1 ±4.1 % and 100.5±7.6 % for 2, 3 and 5 min, respectively. A desorption time of 3 min was used for further experiments.

3.4. Analytical performance

The calibration curve demonstrated linear behavior in the concentration between 0.5 and 30.0 μg/mL. The calibration equation was determined as $Y = 153783\ C + 41283$ (where C represents the naproxen concentration in μg/mL), with a corresponding correlation coefficient of 0.9991 ($n = 3$). The pre-concentration factor is described as the ratio of sample volume to extraction solvent, and the pre-concentration factor of the optimized method was found to be 10.

Table 4
System suitability results.

Parameters	Result	Recommendation
Theoretical plates (N)	2085	> 2000
Capacity factor (k)	1.89	> 2.0
Tailing factor (TF)	1.03	≤ 2
Retention time (RT)	4.7	

Capacity factor (k') $= \frac{t_R - t_0}{t_0}$; Theoretical plate number (N) $= 16(\frac{t_r}{W_T})2$ Abbreviations: t_R: retention time of the analyte peak; t_0: retention time of mobile phase peak; W_T: peak width

The detection limit (DL), and the quantitation limit (QL), were conducted by according to ICH guidelines [28]. DL was calculated by preparing 10 blank solutions, each comprising 30 mL of 100 ng/mL naproxen, subjecting these solutions to the developed MSPE method. The DL was calculated using the following equation $DL = \frac{3.3s}{m}$

Here, s represents the standard deviation of the response, and m denotes the calibration curve slope.

Considering the pre-concentration factor (10), the calculated detection limit was 12.0 ng/mL.

QL was determined by dividing ten times the standard deviation of the peak areas by the calibration line slope from measurements of 10 solutions with 100 ng/mL naproxen. Accounting for the the pre-concentration factor, the QL for naproxen was calculated to be 37.0 ng/mL.

The reproducibilities of MBC on MSPE were investigated intra-day and inter-day. The intra-day reproducibility of naproxen solutions containing 100, 200, and 400 ng/mL was measured five times at various intervals within the same day. Additionally, the inter-day reproducibility of naproxen solutions containing 100, 200, and 400 ng/mL was determined for three sequential days. Table 3 presents the averages, standard deviations(SD), and relative standard deviations (RSD). The intra-day and inter-day reproducibilities of the method were presented as the RSD. The intra-day reproducibility for naproxen ranged from 2.3 % to −5.9 %, while the inter-day reproducibility ranged from 1.7 % to 3.7 %.(Table 3) These findings indicate that the method has good reproducibility.

The test results of the system suitability study are given Table 4.

3.5. Selectivity

The selectivity of the described MSPE method for NAP was tested with 30 mL model solutions containing amoxicillin (AMX), sulfadiazine (SDZ), and ibuprofen (IBU).Naproxen recovery values in solutions containing SDZ, AMX, and IBU were 94.7±1.3 %, 96.6±1.1 %, and 84.6 ±1.5 % respectively. The chromatograms in Figure S7 are indicated that MBC has good selectivity for NAP. As depicted in Figure S7, the described method effectively separated NAP from the other antibiotics.

3.6. Reusability of the MBC

In this study, the reusability of MBC for the determination of NAP was investigated by adsorption and desorption cycle experiments. The

Table 5
Recovery results for determination of naproxen for artificial urine and lake water.

Sample	Method	Added	Founded	Recovery %
Artificial Urine	Direct analysis	0	<DL	
		2[a]	0.78±0.02	39.2
		5[a]	1.39±0.07	27.8
	MSPE	0	<DL	
		2[a]	1.93±0.10	96.5
		5[a]	4.94±0.21	98.8
Lake Water	Direct analysis	0	<DL	
		100[b]	<DL	
		200[b]	<DL	
	MSPE	0	<DL	
		100[b]	100±0.6	100.0
		200[b]	193±1.4	96.5

a: μg/mL **b:** ng/mL

results show that NAP recovery is quantitative over 35 cycles. The average recovery value with a standard deviation of NAP for 35 studies was determined to be 98.6 ± 4.1 %. This result shows that the adsorbent is mechanically stable and has good reusability.

3.7. Determination of NAP in real samples

The extraction and preconcentration of NAP from lake water were investigated to assess the applicability of the tested technique on real samples. Samples were obtained from Beyşehir Lake in the Türkiye. To examine the effect of sample matrix on recovery, lake water sample was spiked at concentrations of 100 and 200 ng/mL of NAP. The NAP concentration in the lake water sample was determined both with and without using the described method. The findings are summarized in Table 5.

The developed procedure was utilized for an artificial urine sample.

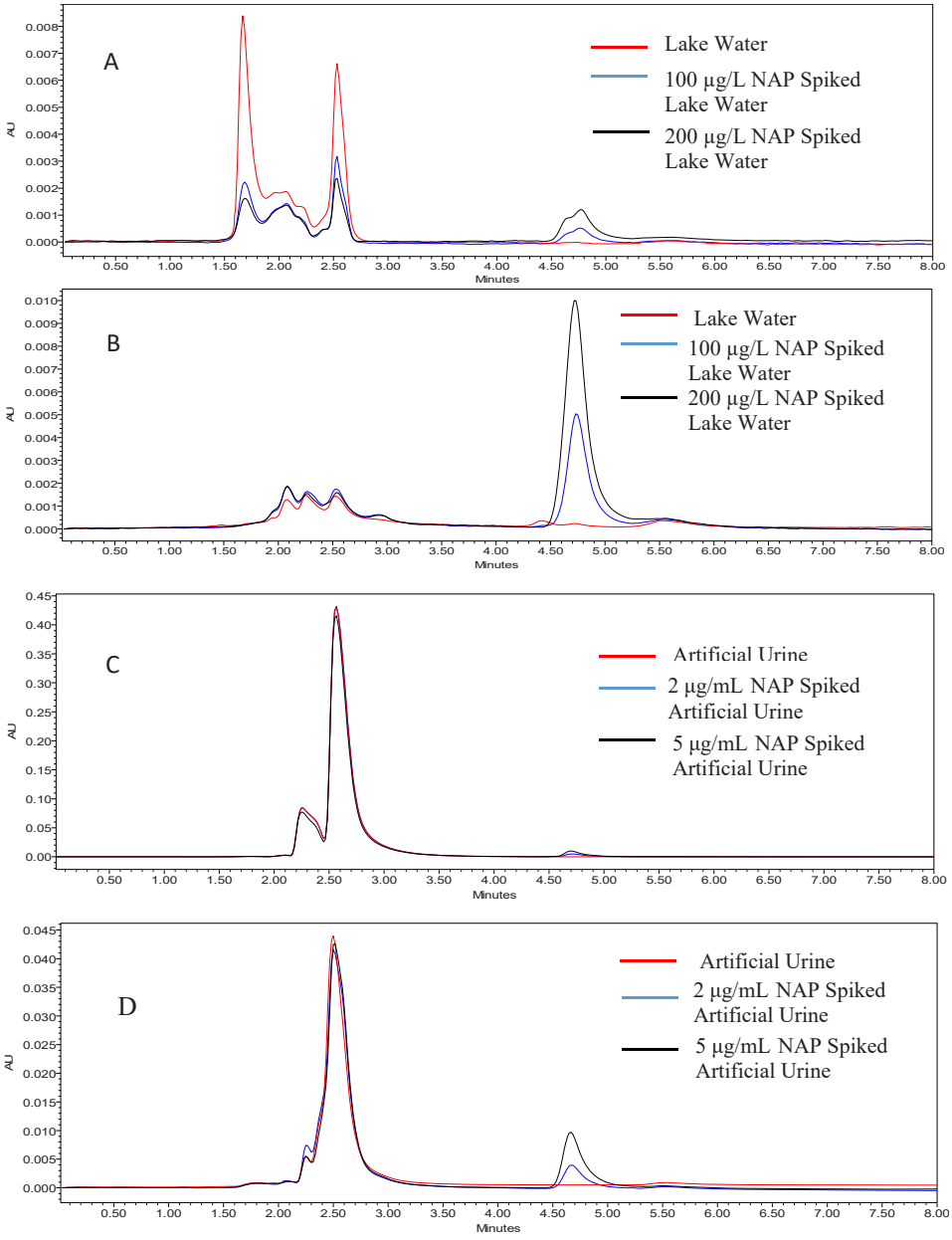

Fig. 4. a. Direct analysis chromatogram samples of lake water spiked with 100 and 200 ng/mL naproxen and without spiked b. Chromatogram samples of lake water spiked with 100 and 200 ng/mL naproxen and without-spiked after applying the MSPE method c. Direct analysis chromatogram samples of urine spiked with 2 and 5 μg/mL naproxen and without spiked d. Chromatogram samples of urine spiked with 2 and 5 μg/mL naproxen and without spiked after applying the MSPE method.

Table 6
Results of assay and recovery studies.

Pharmaceutical formulation	Labeled amount (mg)	Amount found (mg)	Recovery %
Apranax^{R} 275	275	288±13	105±5

Table 7
Recovery results for determination of naproxen for milk sample.

Sample	Added (μg/mL)	Founded (μg/mL)	Recovery %
Milk	0	<DL	
	2[a]	2.05±0.15	102
	5[a]	4.3±0.48	86

Artificial urine constituents and their concentrations were prepared based on previous studies documented in the literature on artificial urine [29]. To investigate the sample matrix effect on recovery, artificial urine sample was put at concentrations of 2 and 5 μg/mL of NAP. The spiked artificial urine (1.0 mL) was diluted with deionized water, adjusted to pH 3 using 1 M HCl, and then applied developed MSPE method. The eluate was carefully transferred to a new vial and analysied with HPLC. Table 5 shows that the described method has better recovery values than direct determination in the urine sample. Table 5 shows the recovery results for artificial urine and lake water the chromatograms are given in Fig. 4.

The commercial pharmaceutical formulation, ApranaxR containing 275 mg Naproxen Sodium was analyzed using the proposed method. 5 tablets were accurately weighed and powdered in a mortar. A sample weighing 15.4 mg was dissolved in a solution of methanol and water (1:1 v/v) in a 50 mL calibrated flask then centrifuged at room temperature for 30 min at 5000 rpm. The supernatant was transferred to a new conical tube and stored at 4 C. Tablet solutions were diluted to the concentration range of 2 μg/mL for NAP in a 50 mL-calibrated flask. The solution was acidified at pH 3 with 1 M HCl and then applied the developed method. The experimental findings regarding a pharmaceutical dosage form are displayed in Table 6. The results of the suggested method closely match the labeled value of the commercial pharmaceutical dosage form. These results show that the MSPE can be acceptably used for the detection of NAP in pharmaceutical tablets.

The milk samples have been prepared on the previously reported method with a slight modification. The milk samples were bought from local supermarket. 500 μL of the milk sample was diluted with 1.25 mL deionized water then it was placed into the 15.00 mL PTFE (polytetrafluoroethylene) tube. 250 μL of 10 % (v/v) $HClO_4$ solution was introduced to precipitate protein in the mixture. The mixture was centrifuged for 30 min at 4000 rpm. Finally, the developed procedure was applied to the supernatant of the resulting solution.

Preparing milk samples were spiked at concentrations of 2 and 5 μg/mL of NAP. The spiked milk (1.0 mL) was diluted with deionized water

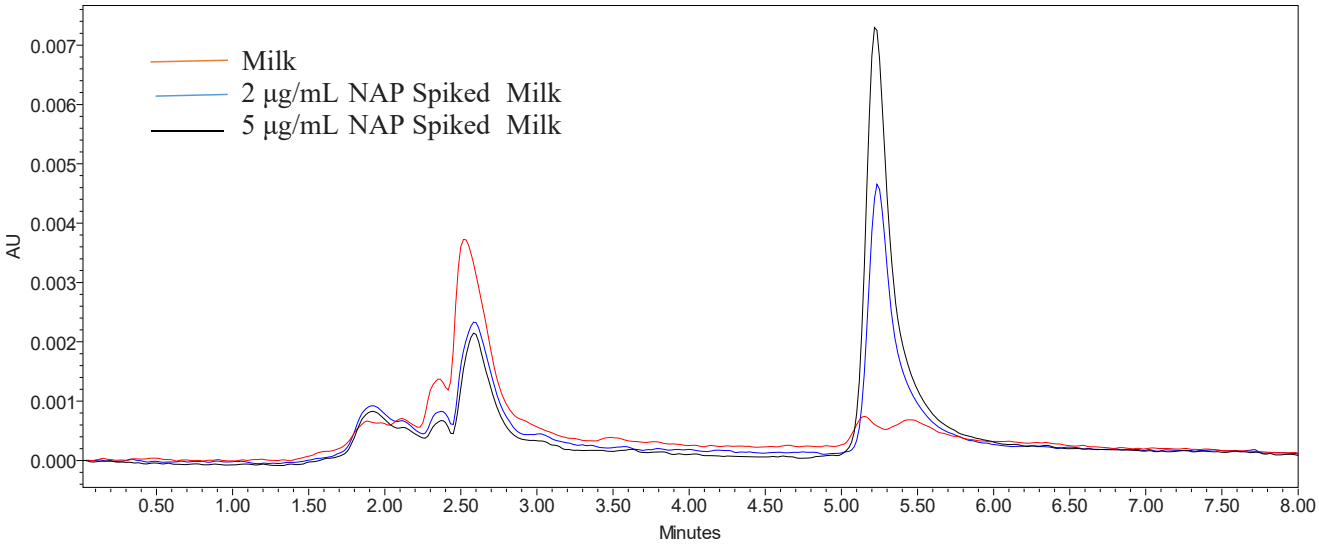

Fig. 5. Chromatogram samples of milk sample spiked with 2 and 5 μg/mL naproxen and without spiked after applying the MSPE method.

Table 8
Comparison of MSPE –HPLC-UV with other methods for determination of NAP.

Method	Sample	LOD (ng /mL)	Linear range (ng/ mL)	RSD (%)	References
HF-LPME-HPLC/FLD	Milk, water, wastewater, urine, and plasma	1.3	9.0–300		[8]
MWCNTs-HPLC	Water samples	0.008	0.03–500	4.3	[9]
CIM–SSF	Pharmaceuticals	110	500–20,000	<12	[30]
PANI-Fe_3O_4–FL	Human urine and plasma	17	40–1000	2.34	[1]
LDH-PS-μSPE-HPLC-UV	Human urine	5.0	25–1000	4	[31]
MFA-SPE - HPLC-UV	Human urine	0.4	1–400	7.1	[32]
SPME–LC–UV	Urine	30	200–20, 000	4.5	[33]
nano-SiO_2-HSA-UV-L5S	Urine	2.3	5–150	3.7	[34]
MHSPE-HPLC-UV	Plasma and urine	0.07	0.5–500	<2.1	[35]
MSPE-HPLC-UV	Pharmaceuticals,Lake water,milk and artificial urine	12	500–3000	≤5.9	This study

CIM: complex imprinted membrane; SSF: solid surface fluorescence, LDH-PS-μSPE:Micro-solid phase extraction by packed sorbent layered double hydroxide, MFA-SPE: magnetic field assisted solid phase extraction, SPME: solid-phase micro extraction, nano-SiO_2-HSA: human serum albumin functionalized silicon dioxide nanoparticles UV-L5S: ultraviolet spectrophotometer, MHSPE magnetic mixed hemimicelles solid phase extraction, MSPE: Magnetic Solid-Phase Extraction, HPLC-UV: high-performance liquid chromatography with ultra-violet dedector.

and it was acidified at pH 3 with 1 M HCl and then applied the suggested MSPE method The eluate was carefully transferred to a new vial and analyzed with HPLC. The recovery results in the milk sample are summarized in Table 7 and the chromatograms are given in Fig. 5.

3.8. Comparison with other methods

Comparisons of the developed MSPE-HPLC-UV method for the determination of NAP with different analytical techniques in the literature are given in Table 8. In comparison with other reported methodologies, the developed method suggested here shows low LOD, good anti-interference ability, rapid adsorption, and elution time, and it is suitable for the determination of trace NAP in real samples.

4. Conclusion

The issue of antibiotic waste poses a notable environmental challenge because of its resistance to decomposition leading to prolonged presence in the environment. The new MSPE method has been developed for the determination of NAP. Optimization of factors influencing MSPE efficiency was carried out and the method validation was confirmed. To examine the behavior of NAP in different matrix environments, a recovery study was conducted in lake water and artificial urine and how it affected the quantitative measurement was calculated. The low recovery values in direct analyses indicate that a separation method is required before determination. Experimental results have showed that the developed MSPE method can be applied in the extraction and enrichment of NAP in lake water and urine samples.

CRediT authorship contribution statement

Ayşenur Karataş: Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Tülay Oymak**: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Ahmet Çelik**: Writing – review & editing, Visualization, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) under the Programme called “2209-A University Students Research Projects Support Program”. The HPLC used in this study was donated by Sanofi. Therefore, we would like to thank all the staff at Sanofi for their support and their collaboration.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2024.116336.

References

[1] P. Rafighi, M.R. Yaftian, B. Haghighi, Magnetic nanofibrous polyaniline nanocomposite for solid-phase extraction of naproxen from biological samples prior to its spectrofluorimetric determination, J. Iran. Chem. Soc. 15 (2018) 1209–1221, https://doi.org/10.1007/s13738-018-1319-x.
[2] I. Villaescusa, N. Fiol, J. Poch, A. Bianchi, C. Bazzicalupi, Mechanism of paracetamol removal by vegetable wastes: the contribution of Π-Π interactions, hydrogen bonding and hydrophobic effect, Desalination 270 (2011) 135–142, https://doi.org/10.1016/j.desal.2010.11.037.
[3] I. Cabrita, B. Ruiz, A.S. Mestre, I.M. Fonseca, A.P. Carvalho, C.O. Ania, Removal of an analgesic using activated carbons prepared from urban and industrial residues, Chem. Eng. J. 163 (2010) 249–255, https://doi.org/10.1016/j.cej.2010.07.058.
[4] M. Gros, M. Petrović, A. Ginebreda, D. Barceló, Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes, Environ. Int. 36 (2010) 15–26, https://doi.org/10.1016/j.envint.2009.09.002.
[5] H.A. Panahi, B. Tahmouresi, E. Moniri, M. Manoochehri, Synthesis and characterization of polymer brushes containing β-cyclodextrin grafted to magnetic nanoparticles for determination of naproxen in urine, Anal. Lett. 47 (2014) 2929–2938, https://doi.org/10.1080/00032719.2014.928885.
[6] J.S. Song, Y.T. Sohn, Crystal forms of naproxen, Arch. Pharm. Res. 34 (2011) 87–90, https://doi.org/10.1007/s12272-011-0110-7.
[7] H.A. Panahi, A. Feizbakhsh, S. Khaledi, E. Moniri, Fabrication of new drug imprinting polymer beads for selective extraction of naproxen in human urine and pharmaceutical samples, Int. J. Pharm. 441 (2013) 776–780, https://doi.org/10.1016/j.ijpharm.2012.10.004.
[8] M. Asadi, S. Dadfarnia, A.M. Haji Shabani, B. Abbasi, Hollow fiber liquid phase microextraction method combined with high-performance liquid chromatography for simultaneous separation and determination of ultra-trace amounts of naproxen and nabumetone in cow milk, water, and biological samples, Food Anal. Methods 9 (2016) 2762–2772, https://doi.org/10.1007/s12161-016-0449-y.
[9] H. Akhlaghi, M. Ghorbani, N.A. Lahoori, A. Shams, O. Seyedin, Preconcentration and determination of naproxen in water samples by functionalized multi-walled carbon nanotubes hollow fiber solid phase microextraction—HPLC, J. Anal. Chem. 71 (2016) 641–647, https://doi.org/10.1134/S1061934816070091.
[10] R. Karami-Osboo, F. Ahmadpoor, M. Nasrollahzadeh, M. Maham, Polydopamine-coated magnetic Spirulina nanocomposite for efficient magnetic dispersive solid-phase extraction of aflatoxins in pistachio, Food Chem. 377 (2022) 131967, https://doi.org/10.1016/j.foodchem.2021.131967.
[11] M. Varak Neshin, R. Sanavi Khoshnood, D. Sanavi Khoshnoud, Surfactant assisted magnetic dispersive micro solid phase extraction-HPLC as a straightforward and green procedure for preconcentrating and determining Caffeine, Lidocaine, and Chlorpromazine in biological and water samples, Int. J. Environ. Anal. Chem. 00 (2022) 1–18, https://doi.org/10.1080/03067319.2021.2014473.
[12] Y. Yi, Z. Huang, B. Lu, J. Xian, E.P. Tsang, W. Cheng, J. Fang, Z. Fang, Magnetic biochar for environmental remediation: a review, Bioresour. Technol. 298 (2020), https://doi.org/10.1016/j.biortech.2019.122468.
[13] H. Chakhtouna, H. Benzeid, N. Zari, A. el kacem Qaiss, R. Bouhfid, Functional CoFe2O4-modified biochar derived from banana pseudostem as an efficient adsorbent for the removal of amoxicillin from water, Sep. Purif. Technol. 266 (2021) 118592, https://doi.org/10.1016/j.seppur.2021.118592.
[14] H.M. Qasim, Z.N. Abudi, L.A. Alzubaidi, Cobalt ion removal using magnetic biochar obtained from Conocarpus erectus leaves, Biomass Convers. Biorefinery 13 (2023) 16865–16875, https://doi.org/10.1007/s13399-022-02307-5.
[15] Q. Zhao, T. Xu, X. Song, S. Nie, S.E. Choi, C. Si, Preparation and application in water treatment of magnetic biochar, Front. Bioeng. Biotechnol. 9 (2021) 1–10, https://doi.org/10.3389/fbioe.2021.769667.
[16] M. Hassan, S. Wook, High - performance liquid chromatography for determining a mixture of nonsteroidal anti - inflammatory drugs, Electron. Mater. Lett. 17 (2021) 414–420, https://doi.org/10.1007/s13391-021-00306-8.
[17] A. Çelekli, B. Bozkuş, H. Bozkurt, Development of a new adsorbent from pumpkin husk by KOH-modification to remove copper ions, Environ. Sci. Pollut. Res. 26 (2019) 11514–11523, https://doi.org/10.1007/s11356-017-1160-2.
[18] E. Šabanović, M. Memić, J. Sulejmanović, J. Huremović, Sorption of metals on pulverized pumpkin (Cucurbita pepo L.) peels, Anal. Lett. 49 (2016) 2446–2460, https://doi.org/10.1080/00032719.2016.1152580.
[19] Ç. Özer, M. İmamoğlu, Isolation of Nickel(II) and Lead(II) from Aqueous Solution by Sulfuric Acid Prepared Pumpkin Peel Biochar, Anal. Lett. 56 (2023) 491–503, https://doi.org/10.1080/00032719.2022.2078981.
[20] D. Bal, Ç. Özer, M. İmamoğlu, Green and ecofriendly biochar preparation from pumpkin peel and its usage as an adsorbent for methylene blue removal from aqueous solutions, Water Air. Soil Pollut. 232 (2021), https://doi.org/10.1007/s11270-021-05411-w.
[21] J. Wang, S. Kaskel, KOH activation of carbon-based materials for energy storage, J. Mater. Chem. 22 (2012) 23710–23725, https://doi.org/10.1039/c2jm34066f.
[22] C. He, J. Qu, Z. Yu, D. Chen, T. Su, L. He, Z. Zhao, C. Zhou, P. Hong, Y. Li, S. Sun, C. Li, Preparation of micro-nano material composed of oyster shell/fe3o4 nanoparticles/humic acid and its application in selective removal of hg(II), Nanomaterials 9 (2019), https://doi.org/10.3390/nano9070953.
[23] A.H. El-Sheikh, F.S. Nofal, M.H. Shtaiwi, Adsorption and magnetic solid-phase extraction of cadmium and lead using magnetite modified with schiff bases, J. Environ. Chem. Eng. 7 (2019) 103229, https://doi.org/10.1016/j.jece.2019.103229.
[24] M. Jafari Eskandari, I. Hasanzadeh, Size-controlled synthesis of Fe3O4 magnetic nanoparticles via an alternating magnetic field and ultrasonic-assisted chemical co-precipitation, Mater. Sci. Eng. B 266 (2021) 115050, https://doi.org/10.1016/j.mseb.2021.115050.
[25] X. Liu, Y. Jiang, S. Yang, X. Meng, X. Song, M. Wu, Effects of pectinase treatment on pulping properties and the morphology and structure of bagasse fiber, BioResources 12 (2017) 7731–7743, https://doi.org/10.15376/biores.12.4.7731-7743.
[26] S. Bakhshi Nejad, A. Mohammadi, Epoxy-triazinetrione-functionalized magnetic nanoparticles as an efficient magnetic nanoadsorbent for the removal of malachite green and Pb(II) from aqueous solutions, J. Chem. Eng. Data 65 (2020) 2731–2742, https://doi.org/10.1021/acs.jced.0c00063.

[27] H.B, M.T. Seyedeh Soghra Mosavi, Ehsan Nazarzadeh Zare, 2023, , Remov. Amoxicillin Antibiot. Polluted Water a Magn. Bionanocomposite Based Carboxymethyl.
[28] Ich, ICH Topic Q2 (R1) validation of analytical procedures: text and methodology, Int. Conf. Harmon. 1994 (2005) 17.
[29] M. Pietrzyńska, A. Voelkel, Stability of simulated body fluids such as blood plasma, artificial urine and artificial saliva, Microchem. J. 134 (2017) 197–201, https://doi.org/10.1016/j.microc.2017.06.004.
[30] H. Lian, Y. Hu, G. Li, Novel metal ion-mediated complex imprinted membrane for selective recognition and direct determination of naproxen in pharmaceuticals by solid surface fluorescence, Talanta 116 (2013) 460–467, https://doi.org/10.1016/j.talanta.2013.07.022.
[31] S. Seidi, S.E. Sanàti, Nickel-iron layered double hydroxide nanostructures for micro solid phase extraction of nonsteroidal anti-inflammatory drugs, followed by quantitation by HPLC-UV, (2019).
[32] H. Bagheri, P. Khanipour, S. Asgari, Magnetic field assisted μ-solid phase extraction of anti-inflammatory and loop diuretic drugs by modified polybutylene terephthalate nanofibers, Anal. Chim. Acta 934 (2016) 88–97, https://doi.org/10.1016/j.aca.2016.06.003.
[33] A. Aresta, F. Palmisano, C.G. Zambonin, Determination of naproxen in human urine by solid-phase microextraction coupled to liquid chromatography, 39 (2005) 643–647. ⟨https://doi.org/10.1016/j.jpba.2005.04.017⟩.
[34] Q.L. Wang, J. Xie, J. Liang, G.T. Dong, L.S. Ding, P. Luo, L.Sen Qing, Competitive protein binding assay of naproxen by human serum albumin functionalized silicon dioxide nanoparticles, Molecules 24 (2019), https://doi.org/10.3390/molecules24142593.
[35] K. Pourghazi, M. Amoli-Diva, Magnetic nanoparticles solid phase extraction based on the formation of supramolecular mixed hemimicelle aggregates for the determination of naproxen in biological fluids using high-performance liquid chromatography-UV, Micro Nano Lett. 9 (2014) 577–581, https://doi.org/10.1049/mnl.2014.0170.