Regular Article

ANALYTICAL
AND BIOANALYTICAL
CHEMISTRY
RESEARCH

Published by the
Iranian Chemical Society

***Anal. Bioanal. Chem. Res.**, Vol. 10, No. 2, 169-182, April 2023.*

Assessment and Selective Extraction Megestrol Drug by Molecularly Imprinted Polymers Method in Human Fluid Samples Using Liquid Chromatography

Elham Pournamdari[a,*] and Alireza Geramizadegan[b]

[a]*Department of Chemistry, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*

[b]*Department of Chemistry, Dashtestan Branch, Islamic Azad University, Dashtestan, Iran*

(Received 28 August 2022, Accepted 15 November 2022)

In this study, a molecularly imprinted polymers-solid phase extraction-liquid chromatography method to isolate megestrol drugs in human fluid samples. The molecularly imprinted polymers use methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the crosslinking monomer, and 2,2-azoisobutyronitrile as an initiator for polymer preparation. To evaluate the applicability of the molecularly imprinted polymers used as a selective sorbent, general parameters: pH, the amount of solvents, washing solution, and eluent, and time, were optimized following a step-by-step approach. Under the optimum conditions, the detection limit linear range was obtained (0.02 to 2.0 μg l^{-1}). The relative standard deviation of (± 2.0%) and the detection limit of the method (0.02 μg l^{-1}) were obtained. The recoveries up to approximately 97.0% from spiked human fluid samples could be obtained. The maximum adsorption capacity for the determination of megestrol drugs was 6.8 mg g^{-1}. The proposed molecularly imprinted polymers-solid phase extraction-liquid chromatography method could be applied to the direct determination of megestrol drugs in human fluid samples.

Keywords: Megestrol drug, Molecular imprinted polymer, Solid phase extraction, High-performance liquid chromatography, Human fluid, Determination

INTRODUCTION

Megestrol Drug, 17-(acetyloxy)-6-methyl-progen-4,6-din-3,20-dione, a synthetic steroid progesterone, is an effective treatment for improving appetite and increasing body weight in patients with cancer-associated anorexia, some characteristics of the studied megestrol drug are given in (Table 1) [1-3]. Mammography and other screening techniques, along with effective adjuvant therapies, have allowed most women with breast cancer to survive long and tumor-free [4,5]. Nowadays, surgery, chemotherapy, and radiotherapy are the best way to treat breast cancer, but they all lead to hypoxia to varying degrees. It is necessary to target anti-cancer drugs. So, they only affect cancer cells and also use the minimum concentration of drugs so that the toxic effects of the drug on normal cells are reduced [6-8]. Considering the existing risks, it seems necessary to develop methods for analyzing and determining the amount of drugs in biological and environmental samples and comparing the results with current standards [9-12]. But even with advanced analysis equipment, there are problems such as disturbing compounds in the sample matrix, lack of sufficient sensitivity to detect small amounts of some analytes by joint detectors, or the lack of such equipment in standard laboratories, the necessity of developing selective methods and the precision of sample preparation is felt for the concentration and purification of samples of all kinds of compounds methods [13,14]. Due to the disadvantages of traditional methods of sample preparation, there are trends towards the development of fast and cheap methods with the ability to recover and the possibility of automation, such as separation using solid

*Corresponding author. E-mail: elhampurnamdari@gmail.com

Table 1. Characteristics of the Studied Megestrol Drug

Drug	Megestrol
Appearance	White
Brand	Megestrol Acetate
Chemical formula	$C_{22}H_{30}O_3$
Molecular weight (g mol^{-1})	342/47
IUPAC name	17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione.
Floor	Clinical

phase extraction (SPE) and separation using solid phase microextraction (SPME) has increased [15-18]. Also, the efforts made to improve the selectivity in the mentioned methods have led to the emergence of new adsorbents with specific action based on the immune mechanism of living organisms and molecularly imprinted polymers (MIPs). The molecular template polymers are unique, and particular adsorbents that show remarkable efficiency and high stability and resistance in different conditions [19-22]. By using a three-dimensional polymer network according to the shape and size of the template molecule (analyte) during the synthesis process, these adsorbents have enabled the specific absorption and recovery of the desired compounds from all kinds of samples [23-26]. Unlike complex analytical techniques, HPLC and UV-spectrophotometric techniques for megestrol drug quantification are low-cost, effective, easy to use, and on-site. In order to ensure the safety and effectiveness of drugs in various matrices, qualitative and quantitative analysis is crucial. As a result, developing HPLC and UV-Spectrophotometric methods that display selective. The point of this work is for extraction and measurement of megestrol drug in human fluid samples with examination design molecularly imprinted polymer and HPLC and Spectrophotometric.

EXPERIMENTAL

Chemicals and Reagents

Megestrol drug (98.0%) from (Cipla, Indian Company), methacrylic acid (MAA) (98%) as the functional monomer, ethylene glycol dimethacrylate (EDMA) (98%) as a side linker, and 2,2-azobisisobutyronitrile (AIBN) (98%) as radical reaction initiator from (Merck, Germany). Solvents of toluene, chloroform, methanol, acetic acid, hydrogen chloride, and sodium hydroxide from Merck, Germany.

Apparatus

Spectrophotometer model 1601 PC (Shimadzu Company, Japan). Analysis of all samples using a high-performance liquid chromatography device made by Knauer, Germany, equipped with a high-pressure pump (K-1001, ultraviolet detector (K-2006), and the reverse phase C_{18} column (250 mm × 4.6 mm, the particle diameter of 5 µm), was carried out under optimized conditions including a uniform mixture of acetonitrile and water at a ratio of 80:20 as the mobile phase, a flow rate of 1.5 ml min^{-1} and a wavelength of 290 nm.

Molecularly Imprinted Polymers Synthesis Process

In general, for the synthesis of molecularly imprinted polymers, various factors such as the ratio of template to monomer, the ratio of cross-linker to monomer, and the type of solvent play an important role. According to the study of previous works done in the field of molecularly imprinted polymer synthesis, the amount of extraction with chloroform solvent is more than other solvents. Therefore, chloroform solvent was used for synthesis. Also, different ratios of the template to monomer were investigated for the synthesis of megestrol drug molecularly imprinted polymer. For the preparation of the megestrol-imprinted polymer, the template (megestrol drug, 0.072 g, 0.3 mmol) was dissolved in the porogen (chloroform, 15 ml) in a 25 ml thick-walled glass tube. The functional monomer (MAA, 0.15 ml, 1.80 mmol), the crosslinking monomer (EGDMA, 1.1 ml, 6.0 mmol), and the initiator (AIBN, 0.08 g, 0.51 mmol) were then added. The resultant solution was cooled on an ice bath and degassed with oxygen-free nitrogen for 5 min before being sealed under nitrogen. The polymerization was allowed to proceed at 60 °C for 24 h in a water bath. After this period, the glass tube was broken and the monolith obtained was ground mechanically and wet sieved using acetone to obtain regularly sized particles with diameters between 25 µm and 50µm suitable for the MISPE evaluations, as shown in (Fig. 1). The values of template, monomer, crosslinker, initiator, and solvent for synthesized ratios separately, are

Table 2. The Ratio of Template, Monomer, Cross-linker, Initiator, and Solvent Values for the Synthesis of Molecularly Imprinted Polymers of Megestrol Drug

MIP	Molecularly imprinted polymers to monomer ratio	Monomer (MAA) (mmol)	Megestrol drug (mmol)	Crosslinking monomer EGDMA) (mmol)	Initiator (AIBN) (mmol)	Chloroform solvent (ml)
MIP_1	1:4	1.8	0.450	9.0	0.163	15
MIP_2	1:6	1.8	0.300	9.0	0.163	15
MIP_3	1:8	1.8	0.225	9.0	0.163	15

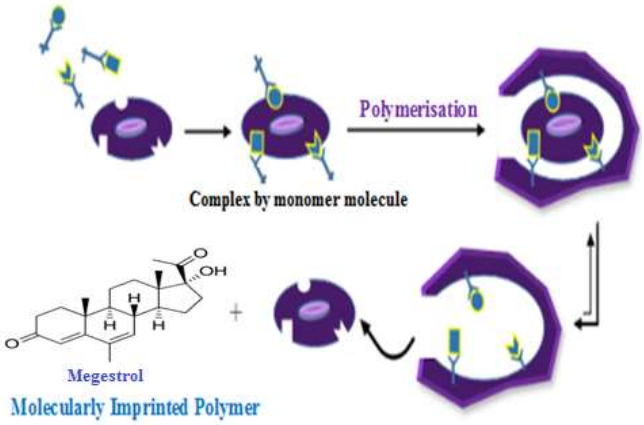

Fig. 1. Molecular imprinting process reproduced.

shown in (Table 2) [22,23].

Pretreatment of Actual Samples

In a 50 ml beaker, a 10 ml urine sample (or a spiked urine sample) obtained from the hospital was treated using 10 ml of the mixture of concentrated HNO_3 (63%) and $HClO_4$ (70%) with their ratio of 2:1 and then covered with a watch glass. Then, the treated sample was heated on a hot plate at 100 ºC for 15 min or at 150 ºC for 10 min. Next, by removing the watch glass, the acid was evaporated to dryness at 150 ºC. After that, 3 ml of $HClO_4$ was added to the resulting white residue, and the mixture was heated at 160 ºC to dryness. The whole heating process was done under a hood with the necessary safety precautions. Upon adding 5 ml of 1 M H_2SO_4, the mixture was heated at 150 ºC for 1 min, and then the volume was made up to the mark in a 50 ml volumetric flask [4,10].

In the presence of an oxidizing agent and 10 ml of concentrated HNO_3, 20 ml of a homogenized human blood sample obtained from the hospital was digested in a 200 ml flask. Then, 2 ml of 70% $HClO_4$ was added, and the sample was heated for 1 h. In all real and synthetic samples, the megestrol drug amount was found by the standard addition method. The performance of the optimized MISPE procedure was evaluated for trace analysis of the megestrol drug in urine and blood sample hospital and spiked with megestrol drug (0.5, 1.0, and 1.5 µg l^{-1}), and diluted in a volumetric flask [10].

Procedure of MIP-SPE-HPLC

In order to extract and determine of megestrol drug by MIP-SPE-HPLC procedure was carried out using this imprinted polymer as an enrichment sorbent. First, columns small with a length of 50 mm and an inner diameter of 6.5 mm were filled with 100 mg of molecular mold polymer with a size between 25 and 50 µm and molecular non-mold polymer. MIP-SPE-HPLC cartridges with 2 ml WD (deionized, water), and 1 ml of methanol to activate the sorbent before the enrichment procedure. 10 ml of bentazon solution was uploaded onto the preconditioned cartridge. Eluting step was performed using 1 ml of methanol/acetic acid (9/1, v/v) mixture solution, as shown in (Fig. 2) [17, 25,27].

Procedure Detection and Measurement

The synthesis of molecular the template polymer particles by sedimentation method, and using a ratio of 1:6:20 of the template molecule, functional monomer, and lateral linker in the 10 ml of chloroform demonstrated a special fondness and

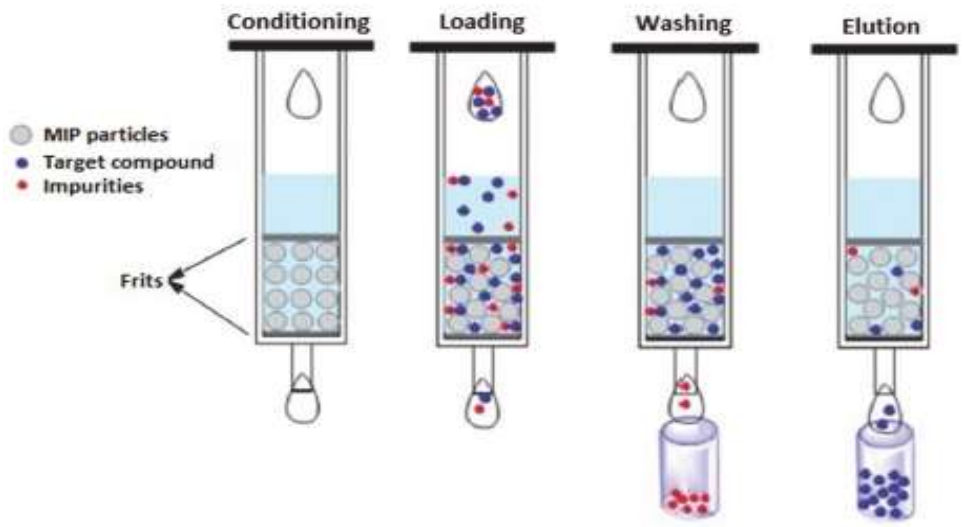

Fig. 2. Schematic representation of MIP-SPE-HPLC process reproduced.

selectivity and were picked as synthetically states of megestrol-MIP. In this regard, C_B is the amount of recovered concentration, C_A is the initial concentration, V_B is the volume of the recovered solution, and V_A is the volume of the initial solution, (Eqs. (1), and (2)) [25,28].

$$\text{Recovery extraction } (\%) = \frac{C_A - C_B}{C_A} \times 100 \quad (1)$$

$$\text{Recovery } (\%) = \frac{C_B \times V_B}{C_A \times V_A} \times 100 \quad (2)$$

The amount of analyte adsorption by polymer (Tb) was calculated. In this regard, V is the volume of the initial solution (ml), C_i is the concentration of the initial solution (µg l^{-1}), and C_f (µg l^{-1}) is the concentration of the solution after adsorption [25,29].

$$Tb\ (\mu g) = V(C_i - C_f) \quad (3)$$

The amount of analyte adsorption per gram of polymer using Eq. (4).

$$C_{MIP}\ (\frac{\mu g}{g}) = T_b/m \quad (4)$$

Also, molecular molding factor (IF) was used to evaluate molding effects. The molecular molding factor was calculated according to (Eq. (5)). In this regard, C_{MIP} and C_{NIP} respectively indicate the amount of analyte absorption per gram of mold and non-mold polymers [22].

$$IF = C_{MIP} - C_{NIP} \quad (5)$$

RESULTS AND DISCUSSION

MIP Characterization

MIP recognition before and after the adsorption of the megestrol drug was investigated by FTIR, and SEM methods. The MIP after the adsorption of the megestrol drug, confirming that the range of approximately 1200-1300 cm^{-1}, determines the C-0 group and a strong peak around the group. The two strong peaks observed, in addition to the C-H peak at 800-1100 cm^{-1}, confirmed the presence of MIP by the presence of the methacrylamide monomer. It is worth mentioning that the MIP is not destroyed after adsorption and the spectral variation is observed at approximately 2956 cm^{-1}, determining the C-H group methylene group strong peak around the group as shown in (Fig. 3).

Scanning electron microscopy images in MIP confirmed the presence of empty cavities and then, after adsorbing as shown in (Fig. 4). The empty spaces are filled with the megestrol drug ions. The results obtained by mentioned analyses confirm the formation of MIP and its effect on the absorption of the megestrol drug.

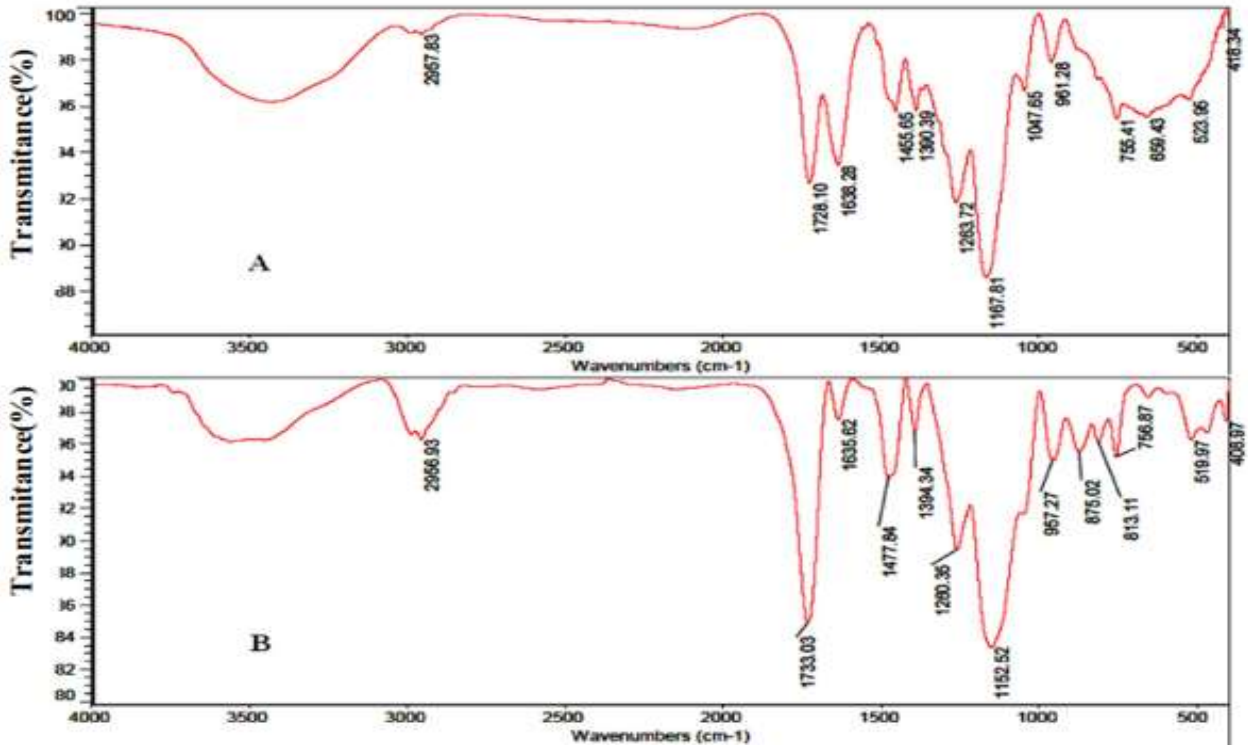

Fig. 3. (A) FTIR of the MIP before adsorption (B) FTIR of the MIP after adsorption.

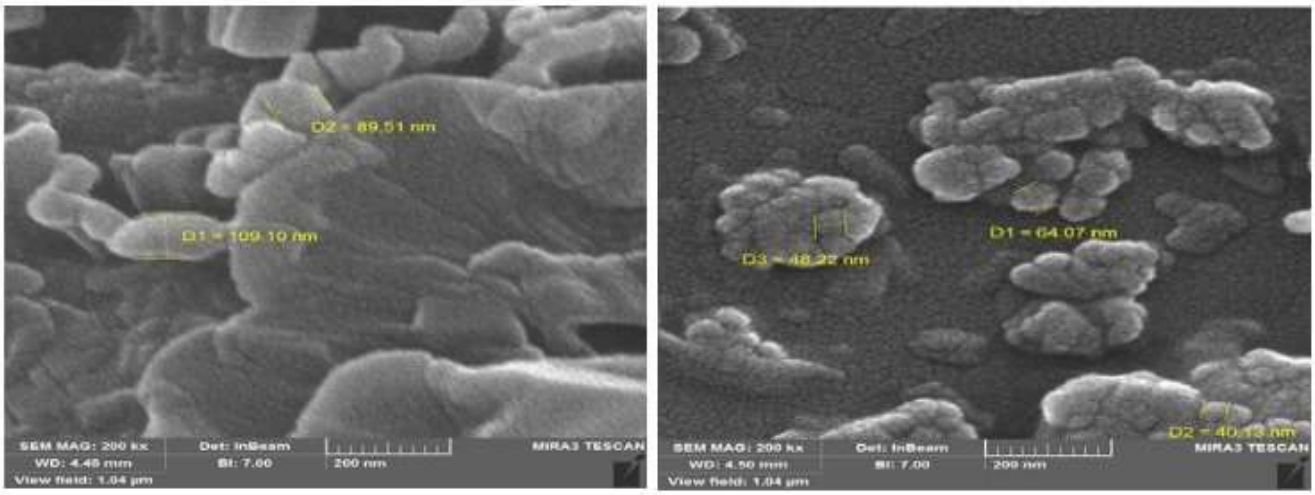

Fig. 4. (A) SEM micrograph of MIP before adsorption (200 kx) (B) SEM micrograph of MIP after adsorption (200 kx).

Optimization of the Ratio of Molecularly Imprinted Polymers to Functional Monomer

An important parameter that is effective on the ability of the synthesized molecularly imprinted polymers is the appropriate ratio of the molecularly imprinted polymers to the monomer, which causes the high selectivity of the polymer. Different ratios of molecularly imprinted polymers to monomer were investigated for the synthesis of megestrol

drug molecular template polymer. According to the results in (Table 3), the ratio of molecularly imprinted polymers to monomer 1:6 showed the highest percentage of extraction. The formation of the pre-polymer complex between the molecularly imprinted polymers and the active monomer is subject to a balance; Therefore, increasing the concentration of monomer causes the balance to shift towards the complex formation, and the result is that the number of final binding sites that were expected to form in the polymer increases, and the selectivity and capacity factors of the polymer increase. Increases. In ratios higher than 1:6, the amount of extraction decreased; In fact, with an excessive increase in the amount of active monomers that are placed freely in the polymer tissue, the number of non-selective absorptions increases and causes a decrease in the extraction efficiency [17,22].

Table 3. The Extraction of Megestrol Drug by Molecular Template Polymer with Different Ratios of Molecularly Imprinted Polymers to Monomer

Sorbent	Molecularly imprinted polymers to monomer ratio	Recovery (n = 3) (Mean ± SD)
MIP_1	1:4	88/7 ± 3/1
MIP_2	1:6	95/2 ± 2/9
MIP_3	1:8	78/8 ± 2/8
NIP	-	55/6 ± 2/5

Table 4. Determination of Adsorption Capacity of Megestrol Drug Mmolecularly Imprinted Polymers, and Non-Molecularly Imprinted Polymers

Polymer	Megestrol		
	Tb (µg)	C (mg g^{-1})	IF
MIP	1980	9.9	6.8
NIP	620	3.1	

Determination of Adsorption Capacity and Enrichment Factor of MIP

To calculate the adsorption capacity, and molding effects of megestrol drug molecularly imprinted polymers, and non-molecular template polymer, Various concentration of megestrol drug in the range 0.1-1.0 µg l^{-1} at the same volume (50 ml) was passed through the column for measuring retention capacity. After analyte elution with 2.0 ml methanol/acetic acid (9/1, v/v), HPLC analysis was carried out and the results show that the 50 ml of megestrol drug solution with the concentration of 0.5 µg l^{-1}. The retention capacity (mg adsorbed megestrol drug/g of sorbent) was obtained to be 6.8 mg g^{-1}, as see been in (Table 4) [22,28].

Reusability of Synthesized MIP-SPE Column

The reusability of the MIP-SPE column after every extraction is a promising aspect that enhances its utility for end application, therefore To reuse the polymers after every extraction, a short recovery step was performed by washing with 2 ml of methanol/ acidic corrosive (9/1, v/v). The outcomes demonstrated that the MIP could be utilized commonly and kept up their adsorption limit at relatively steady esteem as shown in (Fig. 5), which describes the reusability up to five cycles of the extraction process [22,26].

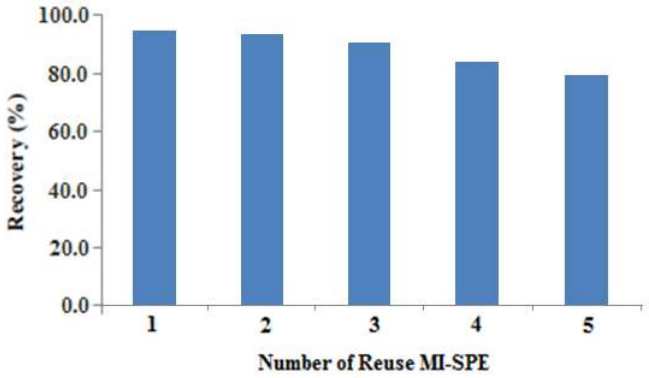

Fig. 5. Relation the reuse MI-SPE column and recovery of megestrol drug solution.

Optimization of the Effect of Different Parameters on Megestrol Drug Extraction

At first, according to the results of previous studies and experiments, practical factors in the recycling of megestrol drug by molecularly imprinted polymers, including the amount of adsorbent used, sample solution volume, pH, and flow rate of the sample, the volume of the extraction solvent, and the percentage of acid present in to be optimized.

Optimization of Sample Solution Volume

The absorption efficiency and, consequently, the recovery efficiency decreases with the increase of the sample volume due to the limited absorption capacity of the solid phase or the volatilization of the desired analyte when passing through the adsorbent. If we want to measure a sample with an extremely low concentration of analyte, it is necessary to pass a large volume of the sample through the column (solid phase) to keep the analyte in it on the solid bed. Then wash this inhibited analyte by the minimum volume of washing solution from the column and measure. Therefore, pre-concentration and analyte measurement will be done with high sensitivity. To obtain the maximum volume of sample solution, volumes of 50-300 ml of bentazone solution were evaluated. For optimization, a fixed amount of analyte was added to the samples. As the sample volume increased, the concentration of the solutions decreased. Sample volumes up to 250 ml were able to pass through the absorber without a significant decrease in recovery efficiency. This volume, in turn, can have a significant effect on the concentration of dilute samples. In volumes greater than 250 ml, a drop in recovery efficiency was observed, which is caused by the washing of megestrol molecules while passing through the absorbent (Fig. 6), the effect of sample volume on the amount of megestrol recovery is presented schematically [30].

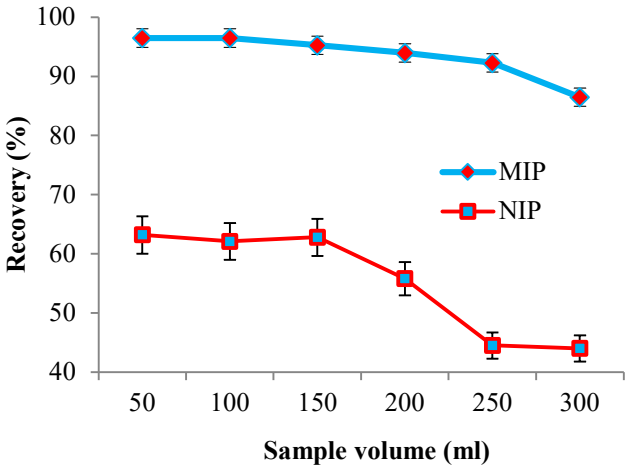

Fig. 6. The effect of sample solution volume on the recovery of megestrol drug by molecularly imprinted polymers, and non-molecularly imprinted polymers conditions: different volumes of 0.5 µg l^{-1} megestrol solution at pH, with 2 ml of methanol-acetic acid washing solution (1: 9).

Effect of pH

The effect of pH on the sorption of the megestrol drug was investigated by varying the solution pH from 1.5 to 14. Based on the results obtained from among the selected range to examine the effect of sample pH in the extraction process. Under acid conditions, chemical adsorption is dominant. The carboxyl of megestrol drug undergoes ion and/or proton exchange reactions with the reactive sites of molecularly imprinted polymers, with hydroxyl groups. In neutral and weak alkaline conditions, the megestrol drug exhibits substantial proton loss the chemical adsorption to some extent. As a result, the percentage of megestrol drug removal at pH above 7.0 to 8.5 is higher than in the strongly acidic environment shown. Finally, pH = 8.5 was determined as the optimal value to achieve the maximum absorption efficiency of the megestrol drug as shown in (Fig. 7a) [22,30]. In general, due to the nature of hydrogen bonds, it is expected to improve the efficiency of the adsorption process in a neutral and acidic range, at the opposite point of occurrence of the hydrolysis process at alkaline pH, the ability of the absorbent functional groups to establish an effective bond with the compound will follow the target [31,32].

Elution Solvent and Its Volume

The influence of elution solvent on the megestrol drug desorption from the adsorbent surface by three common solvents, including H_2O, methanol, H_2O/methanol (9:1, 7:3 and 5:5, v/v), methanol/acetic acid (9:1, 7:3 and 5:5, v/v), ethanol/acetic acid (9:1, 7:3 and 5:5, v/v), Fig. 7b, shows methanol/acetic acid (9:1) has the maximum elution power than other solvents. Thus, methanol/acetic acid (9:1) was used as an elution solvent for subsequent experiments [32,33].

The volume of elution solvent plays a vital role in extraction efficiency. The effect of elution solvent volume was investigated in the range of 1.0-5.0 ml, as can be seen from (Fig. 7b). Maximum peak area using 2.0 ml of methanol/acetic acid (9:1). Therefore, 2.0 ml as the optimum elution solvent volume [33].

Effect of Absorbent

The higher the amount of solid phase in the column, the higher the absorption capacity of the column, and the possibility of increasing the sample volume and,

subsequently, the concentration of the sample, and on the other hand, the volume of the washing solvent to separate the analyte from the solid phase will increase, which hurts it will show the concentration of the sample. In this context, it is necessary to balance the two variables. In order to obtain the most appropriate amount of absorbent, 25-300 mg of molecularly imprinted polymers were investigated. As the amount of adsorbent increases, the recovery percentage does not change much, because, at higher values, empty spaces and holes remain unused. In amounts, less than 100 mg, reducing the amount of active sites to form a bond between the analyte and the polymer causes the column capacity to decrease and the recovery percentage to fall (Fig. 7c), shows the effect of adsorbent amount on megestrol drug recovery rate [21,30].

Extraction and Desorption Times

In order to investigate the effect of the sample passage time on the absorption efficiency of megestrol drug by molecularly imprinted polymers, according to the results of similar studies, optimization tests of the absorption stage were carried out in the range of 0.5 to 2 ml min^{-1} for sample loading, and finally, the results of data modeling the experimental effect showed the effective absorption of megestrol drug at a time of 1.0 ml min^{-1}. Therefore, it seems that in line with the results of some similar studies. Reducing the time of passing the sample to a specific value can improve the absorption efficiency of the analyte by the adsorbent, as shown in (Fig. 7d) [30-34].

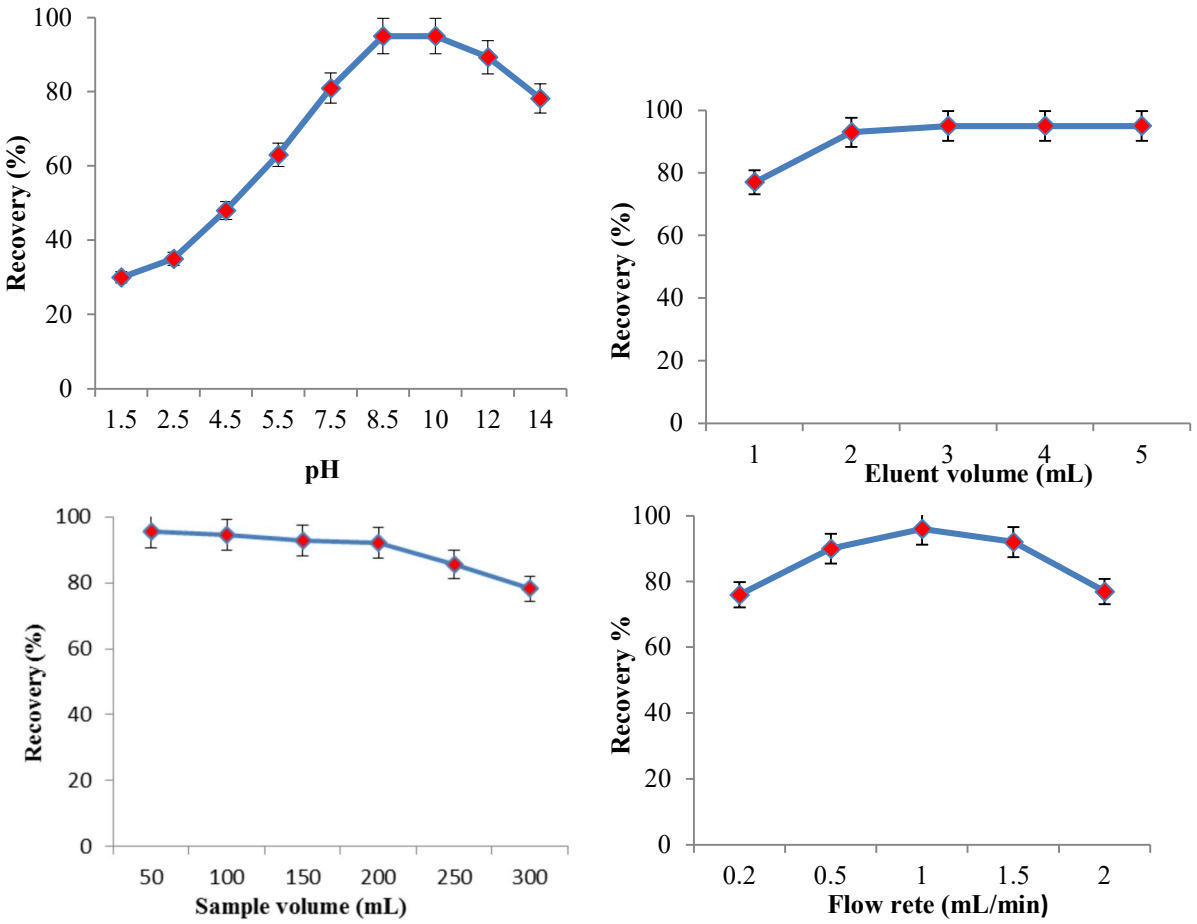

Fig. 7. (a) The effect of sample pH (b), The effect of Elution solvent and its volume (c), The effect of adsorbent (d), The effect of Flow rate and desorption times on the recovery of megestrol drug by molecularly imprinted polymers, conditions: 50 ml solution of 0.5 µg l^{-1} megestrol at different pH, with 2 ml of methanol-acetic acid washing solution (1: 9).

Determination of λ_{max}

First, the spectrophotometer was calibrated to zero. Then the maximum absorption wavelength of the megestrol drug solution (0.5 µg l^{-1}) was determined by scanning in the range of 200 and 500 nm.

Spectrophotometric Method

The spectrum of a megestrol drug solution in water (0.5 µg l^{-1}) against a blank has been shown in (Fig. 8). The most intense absorbance peak (λ) was observed at 290 nm. Several assays were carried out, and the best results have been achieved when using the amplitude from the valley at a wavelength of 290 nm to the zero baselines. The overlay spectrum of megestrol drug standard solutions and spectrum of sample solution [35].

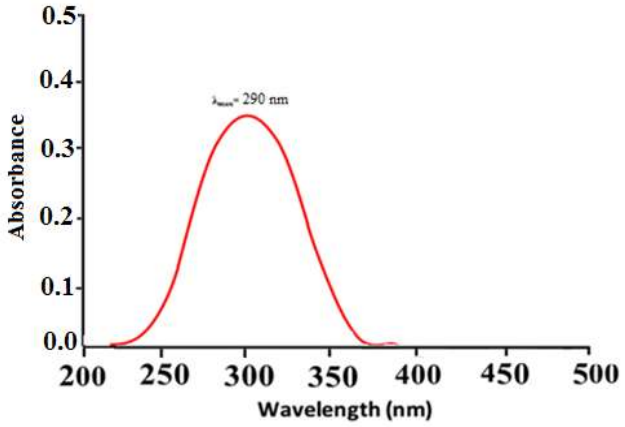

Fig. 8. Spectrum of standard for megestrol drug the response of spectrophotometric method.

Analytical Application

The response of the MIP-SPE-HPLC method was schemed, against the concentration of this compound (Fig. 9), and the calibration curve equation was built up by the least-squares method [22,30]. Into a sequence of ten volumetric flasks with a 50 ml capacity, megestrol drug (0.5 µg l^{-1}) volumes were transferred to prepare in the range of (0.02 to 1.0 µg l^{-1}). 10 µl of each one was injected in ten replicates and the average peak area was recorded to evaluate the developed method's linearity range.

Both methods (spectrophotometric and HPLC) sensitivities were established with the LOD and the LOQ. The LOD and LOQ values were computed with the help of a calibration curve, following the equations given below:

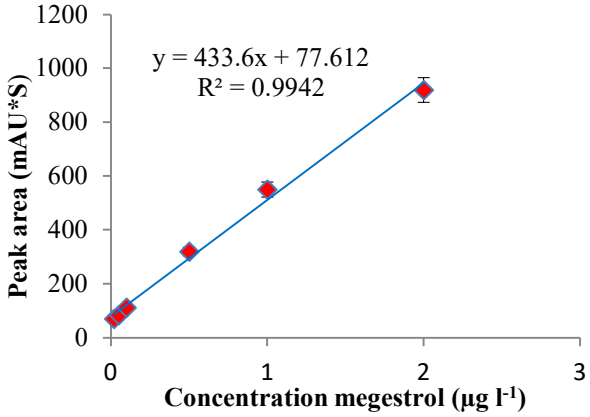

Fig. 9. Calibration graph for megestrol drug.

$$LOD = 3.3 \times S_0/m, \text{ and } LOQ = 10 \times S_0/m$$

Where S_0 = standard deviation of the y-intercept of a regression

Line: m = Slope of the calibration curve.

The precision of the method was evaluated by performing ten replicate measurements of megestrol solutions. The relative standard deviations (RSD) for these determinations were (±2.0%). The limit of detection (LOD) was 0.02 µg l^{-1}, respectively [34,35].

Optimum Values of Parameters

The analytical performance of the suggested pipette-tip extraction coupled with the MIP-SPE-HPLC method experiment was evaluated, and the results are summarized in (Table 5).

LOD, was based on $3S_b/m$ criterion for 10 blank measurements; RSD, standard deviation, for 5 replicate the measurements of 0.5 µg l^{-1} of each analyte Limit of detection (LOD) was obtained based on a signal-to-noise ratio of 3. The linearity range was studied by varying the concentration of the relative standard solution from (0.02 to 2.0 µg l^{-1}).

Preparation of Molecularly Imprinted Polymers

The preparation procedures for MIPs of megestrol drug were described in (Fig. 10). The functional monomer initially formed complexes with the template molecules. Then, their

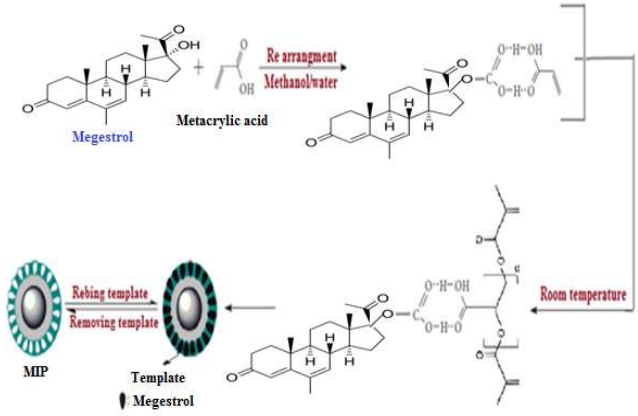

Fig. 10. Process for preparing MIPs of megestrol drug.

functional groups were held in position by the highly cross-linked polymeric structure after polymerization. The MIPs were finally grafted onto the surface of the Methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EDMA). After template removal, specific binding sites were left in the polymer material [22].

Interference Studies

To investigate and prove the specific performance of the synthesized polymer adsorbent in adsorbing and recycling megestrol, a solution with a concentration of 5.0 µg l^{-1} and a volume of 50 ml of this drug was prepared. The drugs (acetaminophen, amoxicillin, and ampicillin), were as interfering agents, and the concentration of the megestrol was added to the prepared solution [4,36].

Selectivity. To investigate the selectivity of the synthesized polymer, in order to isolate the megestrol was selected and the separation rate of these compounds with molecularly imprinted polymers, and non-molecularly imprinted polymers were investigated. The distribution coefficient for each of these compounds was calculated.

$$K_d = \frac{(C_i - C_f)V}{C_f m} \quad (6)$$

In the above relationship, V is the volume of the initial solution, m is the mass of the polymer, C_i is the initial concentration, and C_f is the final concentration of the solution.

The relative selectivity coefficient was calculated by the ratio between the selectivity of the molecularly imprinted polymers, and the non-molecularly imprinted polymers for each of the compound.

$$\acute{K} = \frac{K(MIP)}{K(NIP)} \quad (7)$$

This indicates non-specific surface absorption of compounds on non-molecularly imprinted polymers. The results and calculating the selectivity coefficient for megestrol drug (K_d (MIP) = 7833.0, and K_d (NIP) = 690.5). The results and calculation of the selectivity coefficient for megestrol, after the steps of absorption and recovery of each of the samples containing interfering agents, were carried out under optimal conditions and the analysis of the extracted solution from the phase separation column solid using a liquid chromatography device and the amount of analyte recovery in the presence of interfering agents was calculated. Based on the results mentioned in (Table 6), the company of interfering factors up to 100 times the concentration of megestrol could not have a significant effect on the recovery percentage of this poison [37].

Table 5. Analytical Feature of Merit for Pipette-tip Extraction of Megestrol Drug

Parameter	Analytical feature
Dynamic range	(0.02-2.0 µg l^{-1})
Correlation coefficient (R^2)	(0.9942)
pH	(8.5)
Detection limit (LOD)	(0.02 µg l^{-1})
Relative standard deviation (RSD)	(± 2.0%)
Advantages	High repeatability, sensitivity, selectivity, wide linear range

Table 6. The Extraction Efficiency of Megestrol Drug by Molecularly Imprinted Polymers in the Presence of 50 and 100 Times Concentrations of other Pesticides as Interfering Species

Conc. Foreign species	Recovery (%) SD (± Average)	Tolerance limit (μg l^{-1})
Acetaminophen, Amoxicillin, and Ampicillin	94.8 ± 2.2	100
Acetaminophen, Amoxicillin, and Ampicillin	91.2 ± 2.4	50

Table 7. The Recovery Efficiency of the Megestrol Drug in Urine and Blood Samples Spiked with Molecularly Imprinted Polymers (n = 3)

Conc. megestrol drug	0.1 (μg l^{-1})	0.5 (μg l^{-1})	1.0 (μg l^{-1})	1.5 (μg l^{-1})
Urine	93.0 ± 2.4	94.4 ± 3.6	95.1 ± 1.5	95.6 ± 2.6
Blood	94.04 ± 1.8	94.6 ± 1.9	95.1 ± 2.1	96.2 ± 2.8

N.D: Megestrol drug was not detected in the elution solvent.

Table 8. The Recovery Efficiency of Megestrol Drug for Megestrol Acetate Tablet Sample Analysis with Molecularly Imprinted Polymers (n = 3)

Sample	Homogenate (μg l^{-1})[a,b]	Spiking AA (μg l^{-1})[a,b]	Homogenate + Spiking AA (μg l^{-1})[a,b]	Recovery (%)
Megestrol acetate	98.0 ± 2.0	4.8 ± 2.0	12.1 ± 0.6	102.0
(tablet)		15.1 ± 0.9	21.7 ± 1.8	98.0

[a]Amount of AA. [b]The results are expressed as mean ± S.D.

Analysis Actual Sample

To evaluate the efficiency of the proposed MIP-SPE-HPLC method procedure for trace analysis of megestrol drug to the final concentration of the sample volume 50 ml, of (0.5, 1.0, and 1.5 μg l^{-1}), in urine and blood samples were utilizing standard addition method for determining of megestrol experiment 3, replicates measuring section. Obtained percentage percentiles in (Table 7), indicate that the prepared MIP-SPE-HPLC method has excellent performance for extraction of the megestrol drug in urine and blood samples [37,38].

Application of these Methods to Pharmaceutical Preparations

Test results for a tablet containing megestrol drug sold in pharmacies were presented in (Table 8). Concentration of megestrol drug was determined in pharmaceutical preparation, under the optimized experimental conditions using MIP-SPE-HPLC. We determined the concentration of the megestrol drug as (98 ± 2.0 mg) per tablet in a pharmaceutical preparation called Megestrol Acetate. The manufacturer of this preparation declares the amount of AA as 100 mg per tablet. The recovery of the amount of AA added into the sample was 102% for the lower addition of

AA (4.8 µg l^{-1}) and 98 % for the higher addition of AA (15.0 µg l^{-1}); for more details see Table 8. Results are very close to the amounts indicated on the label of the tablets. The MIP-SPE-HPLC methods recommended in this report can be applied appropriately for the analysis of megestrol drugs in pharmaceutical preparations.

Pipette-tip Solid Phase Extraction

Pipette-tip, solid phase extraction, based on bulk polymerization for the separation and preconcentration of megestrol followed by high-performance liquid chromatography for the extraction of megestrol in human fluid samples. Due to very high surface areas and fast diffusion rate, high adsorption capacities can obtain in a concise time. The optimized method is fast, economic, sensitive, accurate, and simple shown (Fig. 11) [17,39].

Comparison of Presented Work with Different Methods

The proposed method illuminates the applicability and efficiency of molecularly imprinted polymers, in this work, the results are compared with those of some of the recently reported methods for the determination of megestrol drug (Table 9). Obviously, measurement of the megestrol drug by molecularly imprinted polymers, shows the best limit of detection (LOD), and the least time for the determination of drugs in comparison with other methods.

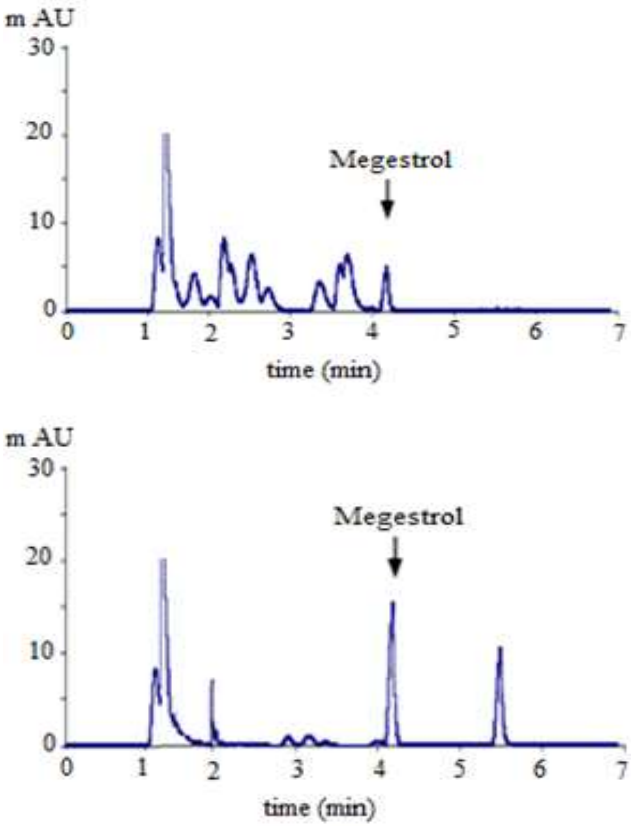

Fig. 11. MIP-SPE-HPLC method obtained from the extraction of (a) water sample spiked by (0.5 µg l^{-1}) of megestrol drug (b) After extraction of megestrol drug with MI-SPE-HPLC.

CONCLUSIONS

In this study, the synthesis of molecularly imprinted megestrol drug in human fluid samples. Various parameters affect the performance of these polymer absorbents, and

Table 9. Comparisons of the Proposed Method with other Methods for Extraction and Detection of Megestrol Drug

Samples	Extraction and detection method	LOD (µg l^{-1})	Linear range (µg l^{-1})	RSD (%)	Time (min)	Ref.
Megestrol	*Origanum majorana*-capped AgNPs (UV-Vis)	0.023	0.02-10.0	1.8%	8.0	[4]
Megestrol	*Albizia Lebbeck Leaves*-capped AgNPs (UV-Vis)	0.2	0.1-10.0	3%	7.0	[10]
Megestrol	Polymer MASDs (HPLC)	1.4	1.4-2.6	2.2%	15.0	[7]
Megestrol	Biopolymer Rofam 70 ((HPLC)	2.0	0.02-10.0	3%	15.0	[12]
Megestrol	MIP-SPE-HPLC	0.02	0.02-2.0	2%	1.0	Present study

according to the studies conducted, the most critical factors were investigated and optimized. The results showed the very high ability of the synthesized molecularly imprinted polymers in the selective separation and determination of the amount of the desired herbicide in optimal conditions. It also obtained successful results for separating small amounts of the desired analyte (megestrol drug), in actual samples. Among the advantages of using molecularly imprinted polymers as solid phase, specific adsorbents can be low cost, have high stability and resistance of these adsorbents in different conditions, have no need for increased amounts of solvent, and have particular action with efficiency and high speed. Finally, it to investigate the applicability of molecularly imprinted polymers to prepare samples of occupational and environmental toxins and drugs in other biological media for future studies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of this work by the Islamic Azad University, Branch of Islamshahr, Iran.

REFERENCES

[1] K.P. Sylvestre, M.C. Tang, A. Furtos, G. Leclair, M. Meunier, J.C. Leroux, J. Contr. Rel. 149 (2011) 273.

[2] E. Cho, W. Cho, K.H. Cha, Int. J. Pharm. 396 (2010) 91.

[3] Y.P. Shiu, Y.M. Tang, X.F. Zhang, J. Ge, Z.C. Pu, Banglad. J. Pharma. 10 (2015) 383.

[4] Z. Kazemi, F. Marahel, T. Hamoule, B. Mombeni Goodajdar, J. Appl. Chem. Res. 16 (2022) 30.

[5] S.M. Hejazi, M. Erfan, S.A. Mortazavi, Iran. J. Pharm. Res. 12 (2013) 239.

[6] K. Jang, S. Yoon, S.E. Kim, Drug Des. Devel. Ther. 8 (2014) 851.

[7] S.W. Hong, B.S. Lee, S.J. Park, Arc. Pharm. Res. 34 (2011) 127.

[8] H.S. Wen, X. Li, Y.Z. Cao, C.C. Zhang, F. Yang, Y.M. Shi, L.M. Peng, J. Chemother. 58 (2012) 461.

[9] Y. Pu, F. Cai, Z. Wu, D. Wang, X. Wen, Y. Li, J.F. Chen, X. Chen, N.R. Foster, J. Chem. Eng. Data. 62 (2017) 1139.

[10] L. Niknam, F. Marahel, J. Phys. Theor. Chem. 18 (2021) 37.

[11] F. Lu, H. Li, M. Sun, Anal. Chimica Acta 718 (2012) 84.

[12] M. Lachowicz, M. Kolodziejczyk, M. Lukosek, P. Olszewska, P. Szymanski, Molecules 21 (2016) 197.

[13] N.A. El Gohary, A. Madbouly, R.M. El Nashar, B. Mizaikof, Biosensors and Bioelectro. 65 (2015) 108.

[14] K. Kamari, A. Taheri, J. Taiwan Inst. Chem. Eng. 86 (2018) 230.

[15] A. Ostovan, M. Ghaedi, M. Arabi, Talanta 176 (2018) 760.

[16] M. Gomar, H. Ahmad Panahi, E. Pournamdari, Chem. Select. 3 (2018) 2571.

[17] S. Berijani, M. Sadigh, E. Pournamdari, J. Chromatogr. Sci. 54 (2016) 1061.

[18] M. Safari, Y. Yamini, Talanta 221 (2021) 121648.

[19] F.A. Aly, N. EL-Enany, H. Elmansi, A. Nabil, Chem. Centr. J. 11 (2017) 99.

[20] Y. Hua, D. Kukkar, R.J.C. Brown, K.H. Kim, Environ. Sci. Technol. 52 (2022) 1.

[21] S. Gul, N. Shah, M.B. Arain, N. Rahman, T. Rehan, M. UlIslamd, M.W. Ullah, G. Yang, Polymer Testing. 75 (2019) 262.

[22] A. Geramizadegan, D. Ghazanfari, A. Amiri, Int. Environ. Anal. Chem. 102 (2022) 1.

[23] R. Keçili, H.C. Mustansar, Int. J. Anal. Chem. 2018 (2018) 1.

[24] W. Zhang, X.H. She, L.P. Wang, H.J. Fan, Q. Zhou, X. Huang, J.Z. Tang, Materials. 10 (2017) 475.

[25] Z. Bahiraee, S. Fathi, M. Safari, Environ. Prog. Sustamia. Energy. 41 (2022) 13971.

[26] M. Samimi, M. Safari, Environ. Prog. Sustamia. Energy 41 (2022) 13859.

[27] T. Madrakian, F. Fazl, M. Ahmadi, A. Abhami, New J. Chem. 40 (2016) 122.

[28] N. Sun, Y. Han, H. Yan, Y. Song, J. Chromatogr. B 951 (2014) 104.

[29] X. He, X. Mei, J. Wang, Z. Lian, L. Tan, W. Wu, Marine. Pollut. Bull. 102 (2016) 142.

[30] A. Geramizadegan, D. Ghazanfari, A. Amiri, Int. J. Farm. Alli. Sci. 4 (2016) 485.

[31] L. Guzzella, N. Casatta, A. Dahchour, C. Baggiani, F. Pozzoni, J. Environ. Sci. Health B. 54 (2019) 702.

[32] G. Sheykhaghaei, M. Hossainisadr, S.

Khanahmadzadeh, M. Seyedsajadi, A. Alipouramjad, J. Chromatogr. B 1011 (2016) 1.

[33] J.L. Urraca, J.F. Huertas-Perez, G.A. Cazorla, J. Gracia Mora, A.M. Garcia-Campana, M.C. Moreno-Bondi, Anal. Bioanal. Chem. 408 (2016) 3033.

[34] Z. Zhang, X. Chen, W. Rao, H. Chen, R. Cai, J. Chromatogr. B 965 (2014) 190.

[35] H. Rahman, S.K.M. Haque, Turk. J. Pharm. Sci. 19 (2022) 267.

[36] M. Soleimani, M. Ahmadi, T. Madrakian, A. Abhami, Sensors and Actuators B: Chem. 254 (2018) 1225.

[37] I. Bulduk, E. Akbel, J. Taibah Univ. Sci. 15 (2021) 507

[38] Z.B. Zhang, Z.G. Shen, J.X. Wang, H. Zhao, J.F. Chen, J. Yun, Ind. Eng. Chem. Res. 48 (2009) 8493.

[39] F. Omidi, M. Behbahani, S.J. Shahtaheri, H. Sadeghi Abandansari, A. Sedighi, J. Environ. Health Sci. Eng. 12 (2014) 137.