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Application of a Molecularly Imprinted Polymer for Pipette Tip Micro Solid Phase Extraction of 6-Mercaptopurine in Seawater and Body Fluids samples before its Spectrophotometric Analysis

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This research investigates the usage of pipette tip micro solid phase extraction for the separation of 6-mercaptopurine of complicated matrices before its spectrophotometric detection. To overcome the non-selectivity of spectrophotometry, an ethylene glycol dimethacrylate-based molecularly imprinted polymer was prepared and applied as an adsorbent, which enabled selective and fast extraction of the analyte. To improve the transfer of the analyte to the adsorbent, a salting-out effect was employed by the addition of 300 mg of NaCl to the sample before performing microextraction. Variables affecting the microextraction of the protocol were investigated utilizing two ways one-factor-at-a-time and response surface methodology, which showed good consistency with each other. Extraction parameters were optimized as pH of sample = 9.0, sample volume = 10 ml, amount of adsorbent = 2.0 mg, eluent = 250 µl of methanol:acetonitrile (1:5 v/v), and extraction and elution cycles of 10 and 12 times, respectively. The dynamic linear range of the protocol was 1.0-1000.0 µg l^{-1}, with a limit of detection of 0.25 µg l^{-1}. The method was compared with extraction by a non-imprinted polymer. The extraction efficiency of the analyte was obtained from 96.0% to 99.8%, by relative standard deviations better than 5.3%. The suggested technique was employed to determine 6-mercaptopurine in seawater and body fluid samples, and the results were validated by comparing them to a standard HPLC method. The whole analysis time, including microextraction, was about 25 min and to perform this method, the sole instrument required is a conventional spectrophotometer.

Keywords: 6-Mercaptopurine, Pipette-tip micro solid-phase extraction, Molecularly imprinted polymer, Response surface methodology

INTRODUCTION

6-Mercaptopurine ($C_5H_4N_4S$, 6-MP) is an established chemotherapeutic agent that is usually used for the treatment of different types of cancer and inflammatory bowel diseases including ulcerative colitis and Crohn's disease. The compound can inhibit purine metabolism and expansion of the tumor cells. But there are some serious side effects associated with 6-MP which limit its usage. Prior investigations showed patients who were therapied with the same amount of 6-MP, the concentration of the drug in their plasma was highly different, as a result, its response to keep therapy is also different. For this reason, 35% of acute lymphoblastic leukemia patients relapsed after treatment [1-4]. This means that continuous monitoring of 6-MP in patient blood plasma is important to track how well the drug is working to treat cancer or multiple sclerosis. If the level is too low, the treatment may not be effective. If the level is too high, the patient may be at risk of side effects such as heart damage. For the patients who use medicines containing

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6-MP, the concentration of this drug can be varied from 400 to 1000 μg l^{-1} in the urine and blood plasma [5]. Because of the low metabolism and degradation of 6-MP drugs, they can be entered into the environment through the urine of patients, drug manufacturing facilities, or human disposal and can penetrate into the environmental waters, including seawater. The amount of 6-MP in environmental water samples was reported up to 0.22 μg ml^{-1} [6]. By regular water treatments, it cannot be removed from drinking water and therefore can cause public health concerns, such as allergic reactions and antibiotic resistance [4-10]. Therefore, the development of analytical protocols for the determination of 6-MP in various samples is still required.

For the analysis of 6-MP, various techniques based on instruments such as chemiluminescence [7], high-performance liquid chromatography (HPLC) [8], Raman spectroscopy [11], electrochemical protocols [12,13] and spectrophotometry [14] were used. However, because of the low amount of this drug in real samples with complicated matrices, direct determination of 6-MP by analytical instruments is not possible in many instances, and the application of an extraction/preconcentration step before its determination is necessary. For this purpose, extraction methods such as molecularly imprinted polymer (MIP) reinforced with ZnO graphene-capped quantum dots [1], metal-organic frameworks and quantum dots [4], modified silver nanoparticles [7], and modified graphite by polypyrrole/functionalized multiwalled carbon nanotubes [13] were suggested and developed.

Pipette tip micro solid-phase extraction (PT-μSPE) is a miniaturized version of solid-phase extraction and has been applied recently for the preconcentration of a variety of analytes from different samples. It uses a pipette tip as the separation column which results in the need for only a tiny amount of adsorbent, sample solution, and eluent which is consistent with green analytical chemistry protocols [15,16].

Molecular imprinting is an important method for obtaining recognition patterns of molecules with various shapes and sizes. MIPs were applied as an analytical tool due to their good binding, easy synthesis, selectivity, excellent performance, and good stability. By spiking analytes into the polymer, recognition cavities would be generated in the MIP while it is cured which can be used as selective extraction sites of the analyte presented in the sample. Therefore, MIP has a high selectivity and recognition capability toward a specific analyte [17,18].

In this research, an accurate, rapid, and easy-to-perform method is introduced for the analysis of 6-MP in various samples. In order to separate and preconcentrate the analyte, a microextraction step based on PT-μSPE was performed. Before the PT-μSPE, NaCl salt was added to the samples to improve the extraction of the target analyte through the salting-out effect. Extracting phase was a molecular imprinted polymer made of ethylene glycol dimethacrylate which can selectively adsorb 6-MP in very complicated matrices such as seawater. This method can be called MIP-PT-μSPE. Variables affecting the efficiency of the developed method were evaluated and optimized by one factor-at-a-time (OFAT) and response surface methodology (RSM).

EXPERIMENTAL

Materials

Analytical grades of 6-MP, ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), and 2, 2-azoisobutyronitrile (AIBN), were purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents were of analytical grade and were obtained from Merck KGaA (Darmstadt, Germany). A stock standard solution of 1000 mg l^{-1} of 6-MP was prepared by dissolving 100 mg of the solid in 20.0 ml of 0.020 M NaOH and then diluted to 100.0 ml in ultrapure water and stored at 4 °C in a dark place. All necessary standard solutions were obtained by suitable dilution of this stock solution.

Apparatus

Photometric determinations of samples were performed applying a Steroglass model Matricola 2016 Uplab spectrophotometer (Italy) at a wavelength of 210 nm. Two 300 μl quartz microcells (model q-01701, Starna, UK) were applied for placing samples into the spectrophotometer. pHs were determined by a 630 Metrohm pH meter (Switzerland). For qualitative spectral interpretation and structural elucidation, a Fourier transform infrared (FTIR) spectrometer (Perkin-Elmer, Bucks, UK) was utilized. The prepared MIP and non-imprinted polymer (NIP) were evaluated with a field emission scanning electron microscope (Sigma VP, Zeiss, Germany). A Knauer (Germany) high-performance liquid chromatography (HPLC) by UV-Vis system was employed to investigate the accuracy of the μSPE. For the injection of

samples and chromatographic data handling, an EA4300F Smartline autosampler 3950 and ChromGate V3.1.7 software were applied, respectively.

Synthesis of MIP and NIP Polymers

MIP was synthesized utilizing the following polymerization process (Fig. 1). 0.5 mmol of 6-MP as the target, MAA (2 mmol) as the monomer, EGDMA (20 mmol) as the cross-linker and 80 mg of AIBN as initiator were dissolved in 6.0 ml CH_3OH. The mixture was deoxygenated by passing nitrogen gas for 7 min. For polymerization, the vial was immersed for 2 h in a water bath, thermostated at 65 °C. The obtained solid MIP was washed 3 times by methanol dried in the ambient air and then ground in a mortar. For removal of the remaining analyte from the MIP, the polymer was washed with 0.5 M HCl until no 6-MP remained in the eluent (traced by UV-Vis). Finally, the MIP was washed three times by methanol and allowed to be dried. NIP was prepared in the same way but without 6-MP.

Procedure of MIP-PT-µSPE

To make a miniaturized extraction column, two polypropylene pipette tips (200 µl and 1000 µl) were washed with distilled water and methanol, and subsequently dried at 25 °C. 200 µl pipette tip was packed with 2.0 mg of MIP and some cotton was put at its end to avoid loss of sorbent after that, the tip of the bigger pipette was cut off to allow for insertion into the smaller tip. Before using this miniaturized extraction column, it was washed with 1 ml methanol and H_2O. The tip was mounted on a 10 ml glass syringe and then, 1.0 ml of the sample (the pH of 10 ml of the sample was adjusted to 9.0) was sucked through the tip and dispensed back to the sample. This extraction was carried out for 10 times. In order to elute the adsorbed analytes, 250 µl of the eluting solvent, *i.e.* CH_3OH:acetonitrile (1:5 v/v) was added into the pipette tip and purged into the microcell of the spectrophotometer. This solvent was sucked back again into the tip. The process was performed 12 times and then the microcell was transferred to a spectrophotometer for detection (Fig. 2).

HPLC Analysis

For validating the proposed µSPE, real samples were also analyzed by HPLC. The instrument was set to the following conditions. A C_{18} column was used with isocratic elution at a

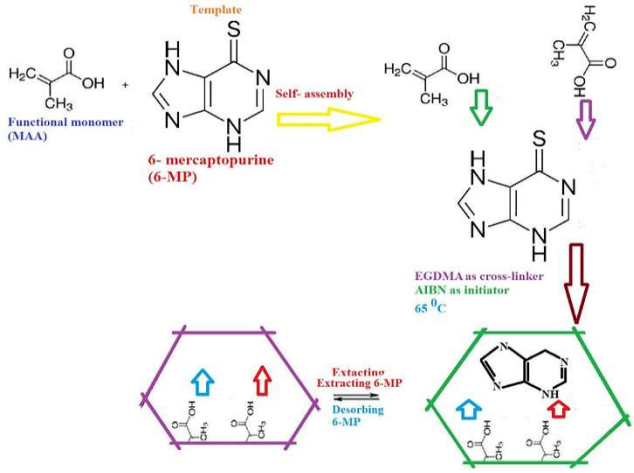

Fig. 1. Schematic diagram of MIP synthesis.

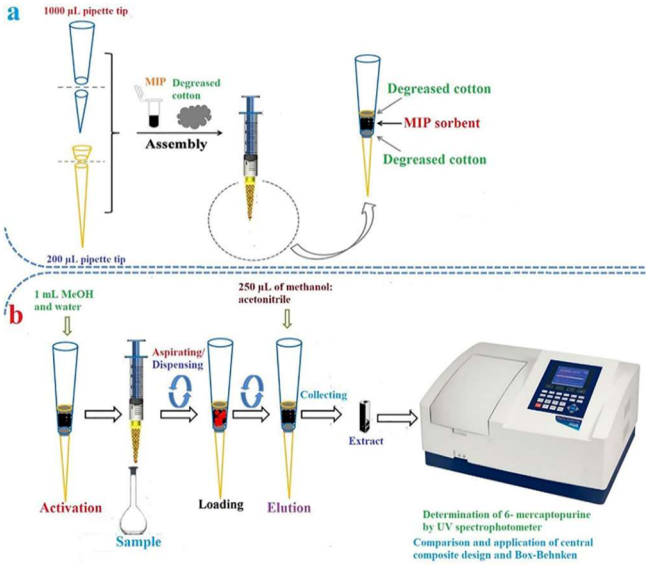

Fig. 2. The assembly (a) and procedure (b) of MIP-PT-µSPE.

flow of 1 ml min^{-1} of the mobile phase. The mobile phase was 0.2 M sodium formate buffer (pH 4.8): acetone (89:11, v/v). The formate buffer was prepared by pH adjustment of a 0.1 M solution of formic acid by 10 M sodium hydroxide solution. The detection wavelength was 210 nm.

RESULTS AND DISCUSSION

Characterization of MIP and NIP

The prepared MIP and NIP were studied by utilizing

field emission scanning electron microscopy. Images (Fig. 3) indicated that both sorbents have irregular shapes but MIP is more homogeneous and is denser with more cavities and higher surface area which facilitates adsorption of 6-MP. To prove that no 6-MP remained on the surface of leached MIP, FTIR spectra of NIP and MIP were compared (Fig. 4). Similarity of their spectrum verifies this claim. For example, main peaks at 1452 cm^{-1}, 1320 cm^{-1} and 1343 cm^{-1} correspond to the symmetric C-O-C stretches of monomers, 1296 cm^{-1}, 1144 cm^{-1}, 1721 cm^{-1}, and 2982 cm^{-1} are related to OH stretching of MAA which are almost the same as in NIP. The absence of the peaks of C=C at around 1585 cm^{-1} shows that the polymer is properly formed because double bands are entered into the polymer framework.

Optimization of MIP-PT-µSPE

To evaluate satisfactory recovery, affecting variables on enrichment were investigated and optimized. Generally, this type of optimization can be performed in two different ways OFAT and by experimental design methods, including RSM. OFAT is easy to perform and interpret, but it consumes more amounts of reagents and time, and interaction between factors is considered. On the other hand, RSM is effective in decreasing the number of experiments that are performed mathematically by a response function. However, it needs previous knowledge of the process to obtain a statistical model, predict the response, and test the adequacy of the model [17,18]. As a result, in this study, the amount of MIP and the type of eluent were optimized by OFAT, while the pH and volume of eluent, extraction, and elution cycles were evaluated by RSM. In order to find the proper RSM, two methods of central composite design (CCD) and Box-Behnken design (BBD) were evaluated and their results were compared together.

The amount of MIP. Similar to any other extraction by adsorbent, in this work, the mass of MIP placed into the pipette tip is a significant parameter. Taking into account that the lowest amount of MIP is consumed and at the same time a suitable response is achieved. The packing mass was changed at the amounts between 0.5 to 2.5 mg and was optimized at 2.0 mg. Because the instrument's response was improved with increasing of MIP amount up to 2.0 mg (because of the increase of sorbent surface) and then decreased due to the passing of sample and eluent it was difficult to further increase in MIP loading. Moreover, the presence of more packing material also prolongs the time required for their passage. Therefore, 2.0 mg was packed as the optimal mass for the rest of the experiments.

Type of the eluent. The desorption efficiency of 6-MP from the adsorbent is directly related to the polarity and dielectric constant of the eluent solvent. Various solvents, including acetonitrile, methanol, acetone, HCl (0.5 and 1.0 M), ethanol, and acetic acid, and their mixture in different ratios were examined. Among them, methanol:acetonitrile (1:5 v/v) showed the best signal and therefore was selected as the optimal desorption solvent.

Type and amount of salt. With the increase of salt in the sample or standard solution, the recoveries were improved (due to the salting-out effect). So, the effect of three salts such as NaCl, KCl, and Na_2SO_4 on the extraction of 6-MP was investigated. The observations proved that NaCl has the best

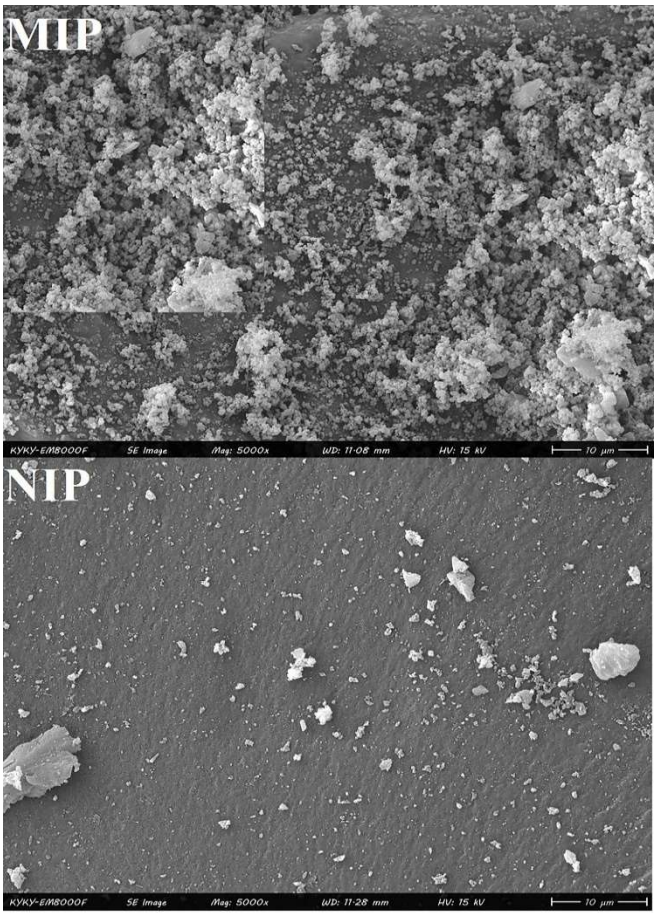

Fig. 3. Images of field emission scanning electron microscopy of synthesized MIP and NIP.

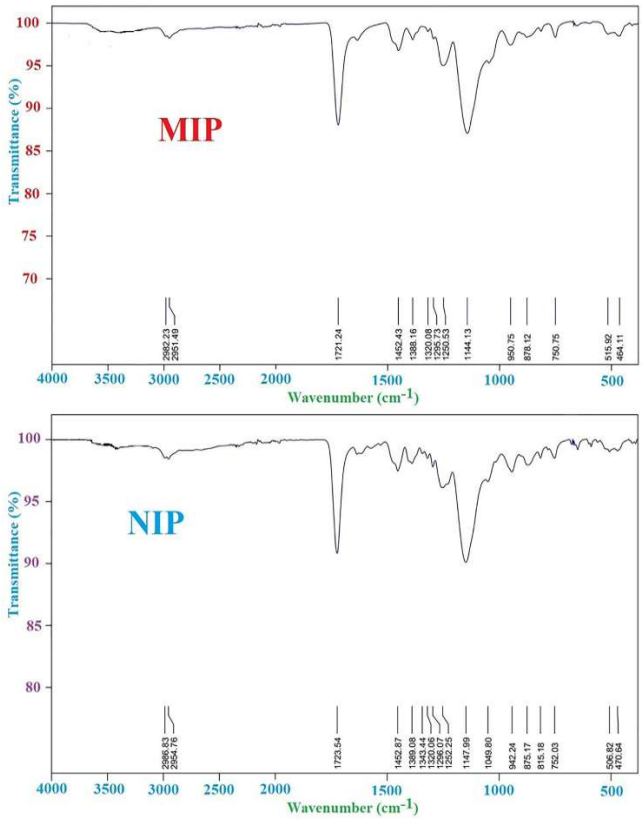

Fig. 4. FTIR spectra of MIP and NIP.

response and is selected as the optimal salt, which is due to the more solubility of it in the aqueous media. The amount of NaCl on the signal was optimized and experiments showed that 300 mg of the salt has the best absorbance. Subsequently, 300 mg of NaCl was considered as the optimum mass of NaCl in further experiments.

Response surface methodology. For this research, the significant factors investigated using RSM were pH (X_1 or A), volume of the eluent (X_2 or B), number of extraction cycles (X_3 or C), and number of elution cycles (X_4 or D). The low, middle, and high levels of each factor were expressed as -1, 0, and +1, respectively.

In a system including four important parameters, *i.e.* X_1, X_2, X_3, and X_4, the mathematical equation of the signals on the parameters can be indicated utilizing a quadratic polynomial equation (Eq. (1)).

$$Y \text{ (predicted signal)} = \beta_0 \text{ (constant)} + \sum\beta_i \text{ (linear effect) } X_i + \sum\beta_{ii} \text{ (quadratic effect) } X_{ii} + \sum\beta_{ij} \text{ (coefficient of the interaction variable) } X_i X_j + \varepsilon \text{ (random error)} \quad (1)$$

A multiple regression analysis was done to calculate the coefficients and the equation that could be employed to predict the response. The actual design of experiments is depicted in supplementary Tables SI1 and SI2. Eqs. (2) and (3) expressed the relationship between the four selected parameters and obtained signals.

$$Y \text{ (for CCD)} = -40.90373 + (5.32270 \times A) + (0.078581 \times B) + (0.85862 \times C) + (1.43959 \times D) + (6.09891 \times 10^{-4} \times A \times B) + (2.35199 \times 10^{-3} \times A \times C) + (1.17441 \times 10^{-3} \times A \times D) - (2.57595 \times 10^{-4} \times B \times C) + (6.84099 \times 10^{-7} \times B \times D) - (5.82037 \times 10^{-4} \times C \times D) - (0.30644 \times A^2) - (1.61372 \times 10^{-4} \times B^2) - (0.040054 \times C^2) - (0.059579 \times D^2) \quad (2)$$

$$Y \text{ (for BBD)} = (\text{Sin } (-29.84176 + (4.83630 \times A) + (0.036528 \times B) + (0.31391 \times C) + (0.55659 \times D) - (7.26795 \times 10^{-4} \times A \times B) - (7.43521 \times 10^{-5} \times A \times C) - (5.63459 \times 10^{-3} \times A \times D) - (1.76684 \times 10^{-4} \times B \times C) - (6.95720 \times 10^{-5} \times B \times D) + (3.08642 \times 10^{-4} \times C \times D) - (0.25590 \times A^2) - (5.47266 \times 10^{-5} \times B^2) - (0.013346 \times C^2) - (0.020264 \times D^2))\text{^}2 \quad (3)$$

The optimum point in the response was achieved utilizing solving equations for $\partial Y/\partial A = 0$, $\partial Y/\partial B = 0$, $\partial Y/\partial C = 0$ and $\partial Y/\partial D = 0$. The calculated data are pH (A) = 9.0 (for CCD) and 8.96 (for BBD), volume of eluent (B) = 252.46 µl (for CCD) and 250.05 µl (for BBD), extraction cycles number (C) = 10.08 (for CCD) and 10.22 (for BBD) and elution cycles number (D) = 12.12 (for CCD) and 12.13 (for BBD). A summary of the analysis of variance is shown in Tables SI3 and SI4.

The F-value of 349.60 (for CCD) and 37.48 (for BBD) showed that both of the models are suitable with only 0.01 change due to noise. Values of "Prob > F" less than 0.05 explained also that the model items are proper. In this case, B, D, AB, BC, A^2, B^2, C^2, D^2 (for CCD) and A, AB, A^2, B^2, C^2, D^2 (for BBD) are significant. The values of adjusted R^2 (0.9941 for CCD and 0.9463 for BBD) expressed that 0.59% and 5.37% of the variations were not demonstrated utilizing the models, respectively. The data of coefficient of determination (R^2 = 0. 9969 for CCD and 0.9722 for BBD) indicates a good relation between the experimental and predicted data. Predicted R^2 (0. 9828 for CCD and 0.8604 for BBD), shows that there is a good agreement between predicted values. The lack-of-fit (LOF) shows the failure of the model to values in the regression. Moreover, the LOF

F-value of 1.41 (for CCD) and 2.42 (for BBD) shows that the LOF F-value is not proper relative to the pure error. There is a 36.97% (for CCD) and 17.03% (for BBD) chance that a large lack of fit F-value could be created due to noise. The precision experiments were shown utilizing a low amount of the coefficient of variation (CV = 0.71% for CCD and 2.77% for BBD). As a result, the CCD has better results and so, in further works, CCD results can be applied. The response surface as a function of two parameters versus the amount of two variables is shown in Fig. 5.

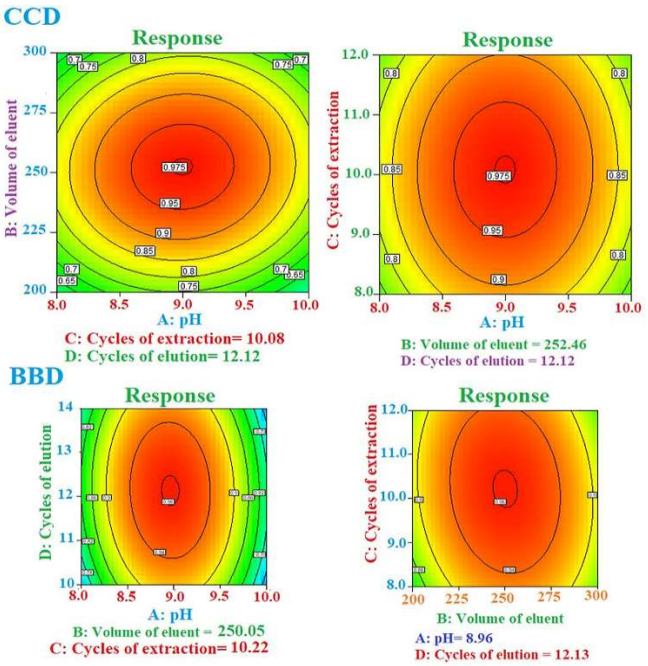

Fig. 5. Two-dimensional response surface as a function of two parameters at the center level of other factors.

Analytical Performance

Validation of MIP-PT-µSPE. The methodology of MIP-PT-µSPE was evaluated by determination of its analytical features consisting of precision, linearity, repeatability, limit of detection, and enrichment factor (EF). The external calibration curve was obtained using the signals of enhancing standard solutions in the range of 1.0-1000.0 µg l^{-1} (Table 1). Suitable linearity was achieved in this range with determination coefficients (R^2) of 0.9941 by a calibration equation of A = 1.4771C + 0.1541 (C = concentration of 6-MP (mg l^{-1}), A = instrument signal). The precision of the MIP-PT-µSPE was investigated by five replicate measurements of the spiked samples on the same day at three various levels and calculated as 1.7 to 2.6%. Inter-day precision was calculated to be 4.1 to 5.6%. The limit of detection (LOD) and limit of quantification (LOQ) of the method for 6-MP, were calculated according to $3S_d$ (S_d = standard deviation of 10 consecutive determination of blank solution) and $10S_d$ criteria and were found to be 0.25 and 0.75 µg l^{-1}, respectively. The EF of the MIP-PT-µSPE was obtained as the ratio of calibration curve slopes after MIP-PT-µSPE enrichment and without performing extraction which was achieved as 146 fold.

Where C_{found}, C_{real}, and C_{added} are 6-MP concentrations after analysis of a spiked sample, its concentration before spiking, and the concentration of the standard solution used for spiking of the sample, respectively.

In Table 1, the observations achieved for the analysis of Recovery percent (R%) for all solutions was calculated according to Eq. (4). Samples were spiked by a known volume of a standard solution of 6-MP.

$$R\% = (C_{found}-C_{real})/C_{added} \quad (4)$$

Table 1. Comparison of the Developed MIP-PT-µSPE by Similar Research Works

Sample matrix	Preconcentration method	Instrument	Linear range	LOD	Ref.
Pills, human plasma, and urine	MIP reinforced by ZnO graphene-capped quantum dots	Cyclic voltammetry, differential pulse voltammetry	0.01-700.00 µM	5.72 µM	[1]
Human urine	Not mentioned	HPLC with fluorescence detector	0.0615-2.40 µg l^{-1}	0.05 µg l^{-1}	[8]
Pharmaceutical samples	Fe(III)-potassium ferricyanide system	Spectrophotometer	412-2884 µg l^{-1}	12	[14]
Seawater, pills, human blood plasma, and urine	MIP-PT-µSPE	Spectrophotometer	1.0-1000.0	0.25	This work

6-MP concentration by the MIP-PT-µSPE technique are compared with other techniques existing in the literature. As can be seen, despite the weakness of the low sensitivity of spectrophotometry, the developed has a proper linear range and low LOD and utilizes a lower amount of adsorbent than the other protocols.

Evaluation of the sensitivity of MIP. The sensitivity of MIP in comparison with NIP for the preconcentration of 6-MP was evaluated. An extraction was performed on standard solutions with a range of concentration under the same optimum conditions with both MIP and NIP (Fig. 6). MIP showed on average 46% higher adsorption signal. This is because of the higher porosity of MIP which makes a larger adsorptive surface area and also because of the higher selectivity of it for the target molecules. While NIP can only adsorb the drug due to the surface absorption.

Effect of interferences. Interferences that usually co-exist with 6-MP in real samples are levofloxacin and mitoxantrone. To investigate their effect on the determination of the target analyte by the developed method, a 10 ml sample of 500 µg l^{-1} of 6-MP was prepared, including the same amount of the interfering compounds, and analyzed by MIP-PT-µSPE. Because of the high selectivity of prepared MIP toward the analyte, no interferences were observed.

Real samples pretreatment and analysis. Seawater, 6-MP pills, human blood plasma, and urine were samples which were selected to investigate their 6-MP content with this method, to study the accuracy of it. Seawater samples were achieved at two stations of Chabahar Bay, located in the southeast of Iran (Oman Sea). The only pretreatment on the seawater samples performed was their centrifugation at 5000 rpm for 10 min to delete any suspended particles. Also, no salt was added to these samples because they were already saturated with many salts. Sample pretreatment of human blood plasma was performed by mixing 250 µl of plasma with 100 µl of 6 M NaOH and placed in a water bath (60 °C for 0.5 h) and then reached 2 °C and 500 µl of 20% w/v trichloroacetic acid was mixed. The solution was mixed (for 1 min) and subsequently left for 10 min. After that, it was centrifuged (at 10,000 rpm for 10 min at 4 °C). The deproteinized supernatant was transferred into a flask and diluted to 10 ml by H_2O and subsequently determined [16]. Urine samples were obtained from a fasting healthy volunteer and from a patient who had taken 6-MP pills and were stored in a freezer. For its pretreatment, a method proposed by Ma and Row in 2019 was used [19]. The frozen urine samples were placed in a water bath at 65 °C for 1 h and then were centrifuged (at 10,000 rpm for 10 min). 150 µl of 1 M NaOH was mixed into them in a 10 ml test tube. Again samples were centrifuged at 10,000 rpm for 3 min and filtered through a 0.45 µm nylon membrane. In the case of blood plasma and urine samples taken from the patient, they were diluted to fit in the almost middle of the calibration curve. A 50 mg pill of 6-MP was also chosen as another real sample to be examined for its 6-MP content. It was dissolved in 10 ml methanol:water (1:1) solvent and filtered. Next, it was diluted 1×10^5 times to result in a concentration of approximately 50 µg l^{-1} of 6-MP. No more steps were necessary.

All of the samples were also spiked by 6-MP at concentration levels of 10, 25, 50, and 500 µg l^{-1} of the analyte, and the recoveries were obtained. Results are shown in Table 2. Recoveries were obtained from 96% to 99.8% by

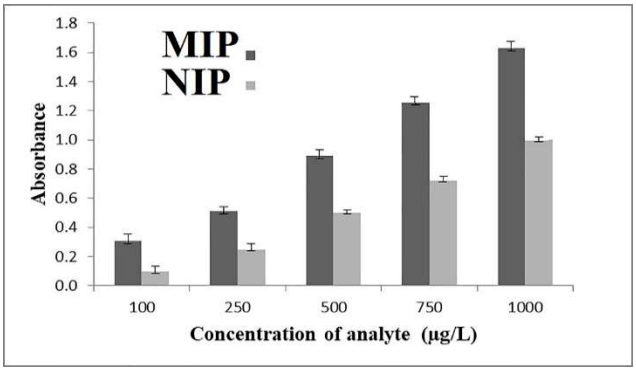

Fig. 6. Evaluation of the sensitivity of MIP.

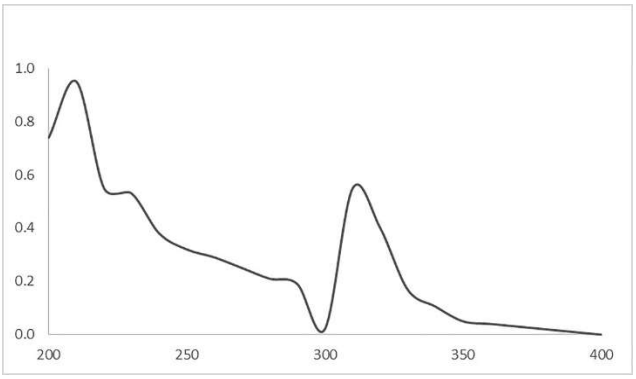

Fig. 7. Spectrum of 6-MP against blank solution after MIP-PT-µSPE of a seawater sample spiked with 500 µg l^{-1} of the analyte.

RSDs less < 5.3%. As an example, Fig. 7 depicts the spectrophotometric spectrum of 6-MP against a blank solution after MIP-PT-µSPE of a seawater sample spiked using 500 µg l^{-1} of the drug. This observation proves that the MIP-PT-µSPE with spectrophotometric detection had good selectivity, precision, and accuracy for the detection of 6-MP in complicated matrices.

Accuracy of the method. To study the validity of the suggested protocol, analysis of real samples spiked by 10 mL of 10 µg l^{-1} standard solutions of 6-MP was carried out by the MIP-PT-µSPE method and compared with a standard method of HPLC (Section of "HPLC analysis"). A Student's t-test at a 95% confidence limit is shown and statistically, there is no important difference between the results.

Table 2. Results of the Analysis of Real Samples

Sample	Analyte added (µg l^{-1})	Analyte found (µg l^{-1})	Recovery (%)	RSD% (n = 3)
Seawater (taken from Tis coast)	0	not detected	-	0.5
	10	9.9	99.0	1.6
	20	19.8	99.0	4.1
	50	49.8	99.6	2.7
	500	496.0	99.2	5.1
Seawater (taken from Haft Tir coast, Persian Gulf)	0	Not detected	-	-
	10	9.7	97.0	2.2
	20	19.9	99.5	2.9
	50	49.8	99.6	3.7
	500	490.0	98.0	3.3
Urine (sample 1)	-	Not detected	-	-
	10	9.6	96.0	1.7
	20	19.6	98.0	2.8
	50	48.7	97.4	4.1
	500	495.0	99.0	3.9
Urine (sample 2, patient)	0	490.0	98.0	3.2
	10	499.6	96.0	3.2
	20	509.6	98.0	4.1
	50	539.4	98.8	2.7
	500	984.0	98.8	3.9
6-MP pills (500 mg)	0	49.9	99.8	2.5
	10	59.6	97.0	2.4
	20	69.3	97.0	4.3
	50	99.3	98.8	2.8
	500	543.0	98.6	4.0
Human blood plasma (sample 1)	0	Not detected	-	-
	10	9.8	98.0	3.7
	20	19.7	98.5	3.1
	50	49.1	98.2	4.4
	500	488.0	97.6	4.1
Human blood plasma (sample 2, patient)	0	497.3	99.5	2.1
	1			
	10	506.9	96.0	2.6
	20	517.2	99.5	4.3
	50	546.1	97.6	4.4
	500	986.0	97.7	5.3

CONCLUSION

A new methodology for the analysis of 6-MP in several samples with complex matrices is developed in this paper. A MIP was synthesized and applied as the adsorbing media for the efficient enrichment of 6-MP. MIP guaranteed the selectivity of the method toward the target analyte, as a result, a simple spectrophotometer could do the detection job. Preparation of MIP is very fast and is low cost and only a few mg of it is needed for the extraction. Also, each PT column could be used 10 times. The whole analysis time, including extraction and spectrophotometry was taken in less than 15 min. This miniaturized method also has advantages including low consumption of solvents, wide linear range, and low LOD presenting the potential and prospective to extract and determine trace amounts of 6-MP.

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