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Application of MIL-53(Al)-NH_2 as a Dispersive Microsolid-Phase Extraction Material for Determination of Cyclophosphamide in Urine by High-Performance Liquid Chromatography

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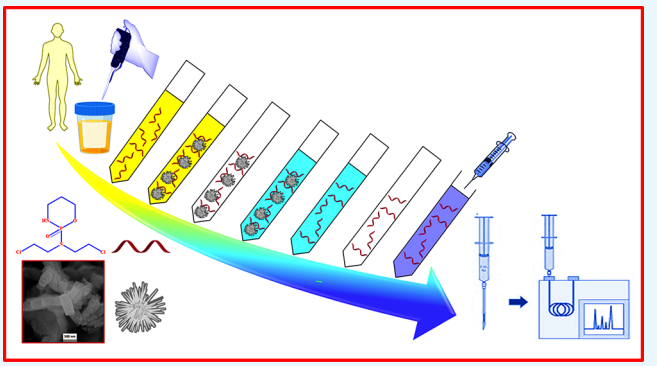
ABSTRACT: In this paper, an aluminum-based metal−organic framework (MIL-53(Al)-NH_2) was synthesized and employed as a well-known and efficient dispersive microsolid-phase extraction (Dμ-SPE) sorbent for reliable determination of cyclophosphamide in urine samples by the high-performance liquid chromatography (HPLC) technique. The synthesized MIL-53(Al)-NH_2 was characterized by FT-IR, PXRD, FE-SEM, and EDS for more details. Then, the effective parameters of the preconcentration and extraction of urinary cyclophosphamide including the amount of the solid sorbent, the pH of the sample, sample volume, extraction and desorption time, and the type and volume of elution solvent were thoroughly investigated and optimized. According to the results, a linear dynamic range of 0.14−120 μg mL^{-1} with a good correlation coefficient (R^2 = 0.998) and a limit of detection (LOD) of 0.05 μg mL^{-1} were obtained with intra- and interday relative standard deviations (n = 9) of 3.13 and 3.99% in optimized conditions, respectively. Furthermore, the absolute recovery of urinary cyclophosphamide at three concentrations (0.5, 50.0, and 100.0 μg mL^{-1}) was 94.0%. Finally, the optimal condition of the developed method was successfully applied to the extraction and analysis of cyclophosphamide from the real urine samples with satisfactory recovery (94.0−97.0%) and acceptable precision (<4.1%). The findings proved that MIL-53(Al)-NH_2 can be utilized as a suitable adsorbent for highly reliable extraction of cyclophosphamide in biological matrices.



1. INTRODUCTION

Cyclophosphamide is one of the most common and high-risk cytostatic drugs in chemotherapy centers, which is prescribed in the treatment and management of solid tumors, autoimmune diseases, hematological malignancies, and extra-articular manifestations of rheumatoid arthritis (such as pulmonary, ocular, and vacuity). This drug binds to a DNA molecule structure through an alkyl group (C_nH_{2n+1}) (unspecific alkylation) to prevent DNA replication. The protein binding of this drug in the body is more than 60.0%, its metabolism is hepatic, and its excretion is often through urine. On the other hand, chronic exposure to cyclophosphamide can cause carcinogenic, mutagenic, embryotoxic, and teratogenic effects in humans.[1,2] The International Agency for Research on Cancer (IARC) and the National Institute of Occupational Safety and Health (NIOSH) have listed cyclophosphamide in "group 1" and "dangerous drugs", respectively.[3−5]

So far, many studies have been performed to investigate occupational exposure to chemotherapy drugs among chemotherapy staff. Previous studies have exhibited that skin exposure (cleaning contaminated surfaces, tools, and equipment) and inhalation exposure (inhaling vapors and aerosols produced during drug preparation) are the most important routes for occupational exposure to chemotherapy drugs. Therefore, for an accurate evaluation of occupational exposure to cyclophosphamide, biological monitoring and determination of this compound in the urine sample are recommended. In several studies, the urine matrix and wastewater have been used as biological media for the biological monitoring of cytotoxic drugs in healthcare workers[6−9] (4).

In the literature review, the solid-phase extraction (SPE)[10,11] and microextraction by packed sorbent (MEPS)[12] methods have been utilized for the extraction of cyclophosphamide from the urine sample. Despite the widespread utilization of the SPE method, this extraction technique has limitations such as solvent loss, low extraction efficiency, high back-pressure, and

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large secondary wastes, is time-consuming, and requires a large amount of adsorbent.[13] Dispersed solid-phase extraction (DSPE) is an alternative method with improved factors instead of the conventional SPE method. In this technique, the small amount of the solid adsorbent is dispersed directly into the sample matrix without the need for sample manipulation.[14,15] This method is called dispersed micro-solid-phase extraction (Dμ-SPE) because it uses a small amount of solid adsorbent (a few mg). Dispersion of the solid adsorbent in the sample matrix increases the interface of the solid adsorbent and the target analyte, which leads to enhanced extraction efficiency and selectivity.[16]

In the Dμ-SPE method, the target analyte is extracted from the sample by the dispersed solid adsorbent. So, the important effective parameters such as selectivity, surface area, chemical stability, and nonreactivity of the solid sorbent should be considered.[15] To date, various solid sorbents have been used in the Dμ-SPE method to extract different analytes from the liquid matrices.[17−19] In this regard, the metal−organic framework (MOF) materials have been exploited on the Dμ-SPE method for extraction of chlorinated insecticides, herbicides, hormones, polyaromatic hydrocarbons, parabens, and sulfonamides.[20−23] MOFs are versatile porous materials consisting of metal clusters or ions and organic ligands, which have a repeatable crystal structure and controllable chemical structure.[24,25] These materials with eye-catching features such as high surface area, porosity, tunable and uniform pores, tunable morphologies, and fully assessable active sites have been used in various research studies.[26−29] Previous research of this group has focused on the sampling, extraction, and determination of different environmental pollutants from air and urine conditions by utilizing various MOFs.[30−33] In this work, MIL-53(Al)-NH_2 as a well-known and reliable solid sorbent with high surface area, high chemical stability, high thermal resistance, and high porosity was utilized in the extraction of the cyclophosphamide compound from the urine samples.[34−36] It is worth noting that the presence of an amine functional group in MIL-53(Al)-NH_2 can be affected by the enhanced extraction efficiency through the hydrogen bonding of NH_2 with the NH group of the cyclophosphamide compound.[37,38]

According to the literature survey, a rapid, reliable, and validated analytical method is still needed for the biological monitoring of cyclophosphamide with the aim of the safety of the chemotherapy operators handling this drug. To the best of our knowledge, there is not any study on the use of MIL-53(Al)-NH_2 MOF for microextraction and determination of cyclophosphamide compounds from the urine samples by the Dμ-SPE method. Herein, this study develops a Dμ-SPE analytical method for the fast, simple, sensitive, and accurate extraction and determination of trace levels of cyclophosphamide in urine samples. In this way, the MIL-53(Al)-NH_2 adsorbent was synthesized, characterized, and then used in the Dμ-SPE method. The key parameters (amount sorbent, pH of sample, sample volume, extraction and desorption time, and type and volume of elution solvent) were investigated and optimized by central composite design through the response surface methodology to find the optimal condition of the proposed method. Finally, the proposed method under optimal conditions was implemented for the extraction and determination of cyclophosphamide in the real urine samples. The achieved results indicated that the proposed Dμ-SPE technique coupled with HPLC can be used as a fast-response, sensitive, and efficient drug test procedure for the trace analysis of the cyclophosphamide compound in the urine matrix.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Reagents. Cyclophosphamide (99.3%), sodium phosphate buffer, sodium hydroxide (97.0%), sodium chloride (99.0%), hydrochloric acid (36%), methanol (MeOH: 99.4%), dimethylformamide (DMF), acetonitrile (ACN: 99.2%), and acetone (AC: 99.3%) were purchased from Sigma-Aldrich (Buchs, Switzerland) and consumed as received without any purification. Also, *N*,*N*-2-amino-1,4-benzene dicarboxylic acid (NH_2-BDC) and aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$) were obtained from Merck (Darmstadt, Germany) for the synthesis of MOF materials. Deionized water was provided by a Milli-Q system (model 1700; Schwalbach, Germany). All solutions were prepared at room temperature.

2.2. Instruments. The liquid chromatography analysis was performed by an Agilent HPLC system equipped with an ultraviolet−visible detector (infinity two variable), a C_{18} reverse phase column (100 × 4.6 mm, 5.0 μm, 100 Å; NUCLEOSIL 100-5) at ambient temperature, a binary pump, and a dual pump to separate and analyze cyclophosphamide. An Agilent 1260.0 UV−Vis diode array detector was set at 195 nm. Also, the volume injection of the sample was 20.0 μL. A mixture solution of acetonitrile:sodium phosphate buffer (70:30) (pH 4.0) was applied as an isocratic mobile phase at a flow rate of 1.0 mL min^{-1}.

MIL-53(Al)-NH_2 was characterized by a Mira instrument (TESCAN, Czech Republic) field emission scanning electron microscope, Perkin Elmer series spectrum 65 Fourier transform infrared (4000−400 cm^{-1}) spectrometer (PerkinElmer, USA), and Rigaku Smartlab X-ray diffractometer with Cu K radiation (Rigaku, Japan) to investigate the surface morphology and physicochemical properties of synthesized MIL-53(Al)-NH_2.

The sample and solutions were sonicated with a Tecno-Gaz ultrasonic bath (Parma, Italy) and homogenized with a ROTOFIX 32A centrifuge (Westphalia, Germany) and a vortex mixer SA8 purchased from Stuart Company (Padova, Italy). A digital pH meter (Mettler Toledo, Greifensee, Switzerland) was supplied to adjust the acidity of urine samples.

2.3. Synthesis of MIL-53(Al)-NH_2. The metal−organic framework (MIL-53(Al)-NH_2) was synthesized using the hydrothermal method according to the following process.[32] Initially, 3.0 mmol of BDC-NH_2 ligand was dissolved in 20.0 mL of deionized water using a magnetic stirrer (solution A). Also, the amount of 3.0 mmol of $AlCl_3{\cdot}H_2O$ salt was dissolved separately in 10.0 mL of deionized water (solution B). After complete dissolution, solution B was added to solution A drop by drop under stirring. The resulting solution was placed on a magnetic stirrer for 1 h. The achieved mixture was then transferred into a steel autoclave with Teflon and heated at 150 °C for 5 h. The obtained yellowish compound was cooled overnight at ambient temperature and then washed three times in deionized water after separation by a centrifuge. The obtained product was dispersed in 20.0 mL of DMF for 24 h under stirring to remove unreacted raw materials and open the pores. After separating the product, the yellow powder was dispersed in 20.0 mL of methanol solvent for another 72 h. To remove the solvent molecules from the pores and cavities, the final MOF was aged in a vacuum oven for 24 h at 70 °C. Also,

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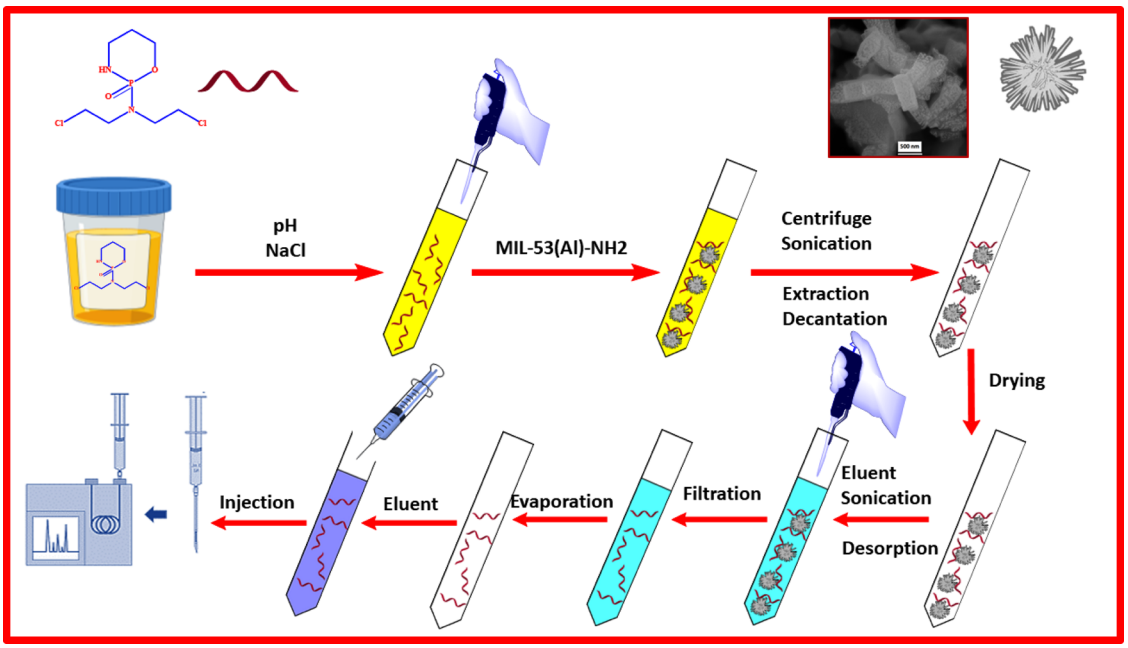

Figure 1. Schematic diagram of the Dμ-SPE procedure (this is a free domain; this figure was drawn by S.A.).

the resulting powder was activated at 200 °C for 4 h before use in the process.

2.4. Dispersive Microsolid-Phase Extraction Procedure. First, the spiked urine sample (50.0 μg mL^{-1}) was homogenized by a centrifuge at 1200 rpm for 2.0 min and filtered by a Millipore membrane (0.45 μm). Then, the pH of the sample was adjusted to 7.0 by 1.0 N NaOH or HCl solution. Also, the ion strength of the solution was set by sodium chloride (20.0% w/v). Next, by default, 15.0 mg of the MIL-53(Al)-NH_2 adsorbent was dispersed in a 10.0 mL glass vial containing 5.0 mL of the pretreated urine sample. The extraction step was performed by vortexing and sonication of the solution for 2.0 and 3.0 min, respectively. After extraction, the solution of the sample was slowly decanted and the dispersed MOF sorbent was separated and dried by a nitrogen gentle flow. The dried solid sorbent was sonicated for a few minutes (default: 2.0 min) by 2.0 mL of elution solvent (default: methanol) for the desorption of cyclophosphamide from the pores. Finally, the elution solvent containing cyclophosphamide was dried under a gentle nitrogen stream at 30 °C. The dried residue was reconstituted in 200.0 μL of elution solvent (default: methanol) and injected (20.0 μL) into the HPLC/UV−Vis system for further analysis. Figure 1 illustrates a schematic diagram of the Dμ-SPE procedure for the extraction of cyclophosphamide from the urine samples.

2.5. Experimental Design. The surface response methodology based on the central composite design (RSM-CCD) was applied to find the optimal point of effective parameters on the efficiency of the proposed method such as the pH of the sample, sample volume, the amount of sorbent, extraction and desorption time, and the type and volume of elution solvent. The above-mentioned independent parameters were assessed at five levels (−2, −1, 0, +1, and +2). The parameters included pH (2.0−10.0), sample volume (0.5−10.0 mL), salt amount (0−30.0% w/v), vortex time (0.5−5 min), sorbent mass (5.0−35.0 mg), desorption time (0.5−5 min), eluent solvent (methanol, acetonitrile, acetone, methanol:acetonitrile (1:1), methanol:acetone (1:1), and acetonitrile:acetone (1:1)), and elution volume (0.5−10.0 mL).

First, three standard solutions at concentrations of 0.5, 50.0, and 100.0 μg mL^{-1} (low, medium, and high) were injected directly into the HPLC system to determine the extraction recovery (ER%) of urinary cyclophosphamides using the optimized Dμ-SPE method. Then, the same three standard solutions were injected into the HPLC system after the preparation, extraction, desorption, and preconcentration of the targeted analyte under optimized conditions of the Dμ-SPE method. The ER% was calculated based on the following equation:

$$\mathrm{ER\%} = \mathrm{EF} \times \frac{V_{el}}{V_{ur}} \times 100$$

where EF is the enrichment factor, and V_{el} and V_{ur} are the volumes of elution solvent in the reconstitution step and initial urine sample solvent, respectively. The enrichment factor (EF) is a means of quantifying the enrichment of a potentially contaminant-derived analyte in a complex matrix relative to a user-defined background composition. The enrichment factor was obtained based on the slope calibration curve of the analyte after obtaining the preconcentration diving slope calibration curve of direct injection of a standard solution of the target analyte into the HPLC system. The enrichment factor for the target analyte was calculated at the concentration of 50 μg mL^{-1} by the following equation

$$\mathrm{EF} = \frac{\left(\frac{C_x}{C_{ref}}\right)_{\mathrm{Final}}}{\left(\frac{C_x}{C_{ref}}\right)_{\mathrm{Initial}}}$$

where C_x and C_{ref} are the concentrations of the target analyte (cyclophosphamide) and reference analyte (2-methyl hippuric acid) after extraction and initial sampling (ng mL^{-1}), respectively. All of the experiments were repeated three times.

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3. RESULTS AND DISCUSSION

3.1. Characterization of MIL-53. In the first step, Fourier transform infrared (FT-IR) spectrometry, powder X-ray diffraction (PXRD), and field emission scanning electron microscopy (FE-SEM) techniques were employed for investigating the physicochemical features of the prepared MIL-53(Al)-NH_2 absorbent. The FT-IR analysis was performed to check the bonding and functional groups of the synthesized absorbent. Figure 2 presents the FT-IR spectrum of the

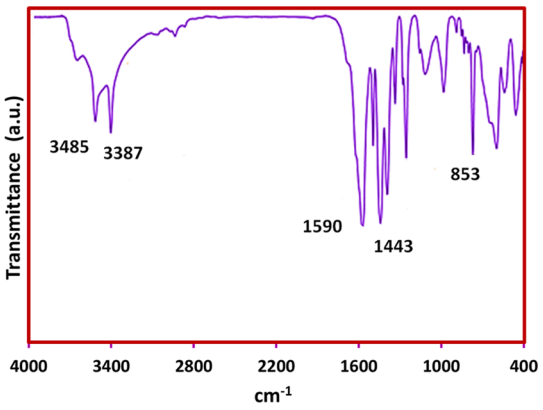

Figure 2. FT-IR spectrum of MIL-53(Al)-NH_2 MOF.

synthesized MIL-53(Al)-NH_2 MOF. According to the figure, the obtained spectrum is compatible with the previously reported patterns.[32,39] The disappeared broad hydroxyl peaks at 3098−2858 cm^{-1} and a free carbonyl group peak at 1733 cm^{-1}, the appeared amino functional group peaks at 3387.0 and 3485.0 cm^{-1}, and the observed stretching vibration of C═O at 1590−1395 cm^{-1} can be assigned to the contribution of *N*,*N*-2-amino-1,4-benzene dicarboxylic acid (NH_2-BDC) in the configuration of the final MOF structure. Also, the presence of Al−O peaks in the range of 1000−1100 cm^{-1} can be related to the coordination of ligand to the Al^{3+} cations in the structure of MIL-53(Al)-NH_2.

Also, the crystallinity and purity of the synthesized absorbent were determined by the PXRD analysis. The featured diffraction peaks at the recorded pattern illustrated the high purity and sufficient crystallinity structure of the obtained MOF, which is consistent with the previously reported patterns[32,39] (Figure 3).

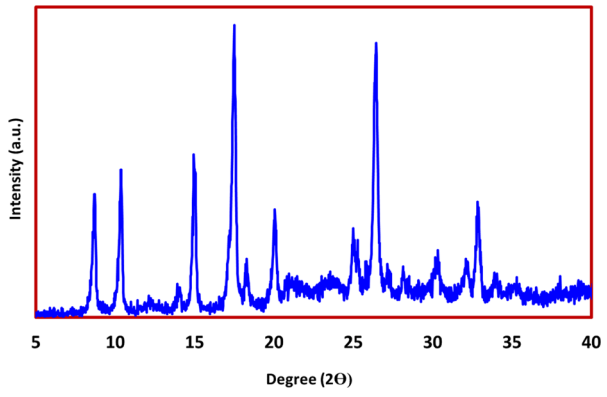

Figure 3. PXRD pattern of MIL-53(Al)-NH_2 MOF.

Finally, the morphological properties of synthesized MIL-53(Al)-NH_2 crystals were evaluated by the FE-SEM technique. Figure 4 presents the uniform and homogeneous cubic cylindrical crystals with an average size of around 500.0 nm.

3.2. Optimization of the Dμ-SPE Procedure. In this study, we tried to develop a fast, simple, inexpensive, and reliable sample preparation method (Dμ-SPE) for the extraction and analysis of cyclophosphamide in the urine sample by the HPLC/UV−Vis system. All of the optimization steps were performed using the spiked standard concentration of cyclophosphamide (50.0 μg mL^{-1}). One of the most important parameters that can affect the analyte desorption of the solid sorbent is the eluent solvent. To determine the optimal conditions for extraction of cyclophosphamide from the urine media using the MIL-53(Al)-NH_2@Dμ-SPE method, first, the type of elution solvent was optimized. For satisfactory desorption of the target analyte from the solid sorbent, a strong eluent solvent should be chosen and optimized according to the chemical structure of the analyte and the solid sorbent.[40,41] In the present study, to determine the best eluent solvent, other parameters affecting the efficiency of the Dμ-SPE method at the default state were considered to be unchanged and the effect of different eluent solvents such as methanol, acetonitrile, acetone, methanol:acetonitrile (1:1 v/v), methanol:acetone (1:1 v/v), and acetone:acetonitrile (1:1 v/v) on the extraction efficiency was investigated. Figure 5 shows the results of the ER% of cyclophosphamide from the solid sorbent by using different elution solvents. According to the obtained ER% of cyclophosphamide in the presence of different eluent solutions, the methanol:acetone (1:1 v/v) solvent was selected as the most suitable elution solution. It should be noted that the obtained result is consistent with the other similar studies.[5,12,42] It is worth noting that utilizing the polar solvents (such as methanol and acetone) can provide acceptable performance for the desorption of polar compounds (such as cyclophosphamide) from the MOF adsorbent through the electrostatic interactions.

Then, to optimize other parameters of the Dμ-SPE method in the extraction of cyclophosphamide using the MIL-53(Al)-NH_2 adsorbent, Design-Expert software and Central Composite Design (CCD) model were used. In this model, seven quantitative parameters encompass the following: sample pH, sample volume, sample ion strength, extraction and desorption time, the volume of elution solvent, and the amount of sorbent were optimized. Table 1 presents the type and levels of variables considered in the CCD model.

Figure 6 shows the ER% of cyclophosphamide from the urine sample following a change in sample pH, sample volume, sample ion strength, extraction and desorption time, the volume of elution solvent, and the amount of sorbent. The initial pH of the sample is an important parameter that can be effective on the ER%. On the other hand, the ionic charges of a urine sample can be impressive on the ER% through the protonation state and surface charge of the functional groups of the MIL-53(Al)-NH_2 sorbent and also on the ionic form cyclophosphamide as a polar compound.[29,30] The results showed that the highest extraction recovery of cyclophosphamide in urine samples was obtained at pH 5.0 (Figure 6). In the MIL-53(Al)-NH_2 structure, there are hydroxyl and carboxyl functional groups that can offer cationic−anionic charge transfer, π−π, and hydrogen interactions to cyclophosphamide.[25,26,28]

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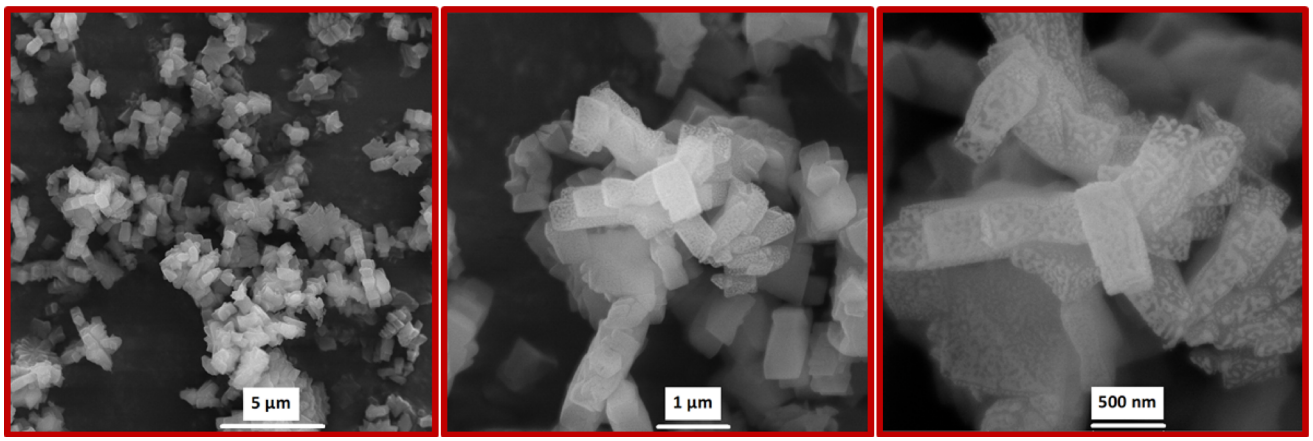

Figure 4. FE-SEM images of MIL-53(Al)-NH_2 MOF crystals.

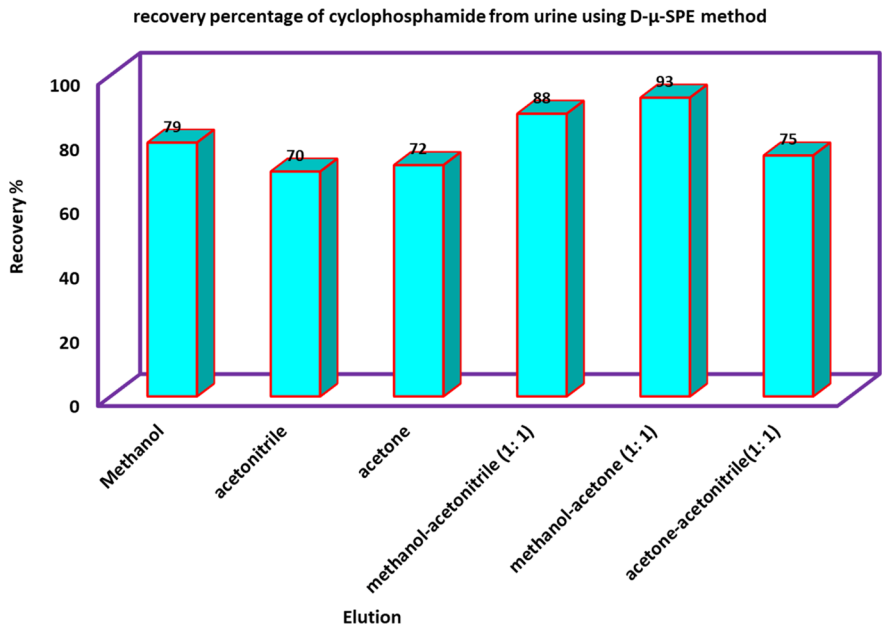

Figure 5. Effect of several eluent solutions on the recovery of cyclophosphamide by MIL-53(Al)-NH_2@Dμ-SPE: amount of sorbent, 15.0 mg; pH, 6.0; sample volume, 5.0 mL; extraction time, 5.0 min; desorption time, 3.0 min; elution volume, 2.0 mL (methanol); cyclophosphamide concentration, 50.0 μg mL^{-1}. Mean recovery percentage: methanol, 79 ± 1.2; acetonitrile, 70 ± 1.3; acetone, 72 ± 1.6; methanol:acetonitrile, 88 ± 1.2; methanol:acetone, 93 ± 1.1; acetone:acetonitrile, 75 ± 1.5.

Table 1. ANOVA Analysis of Extraction Variables in the Extraction of Urinary Cyclophosphamide by the Dμ-SPE-HPLC Technique

the optimal point of effective parameters							ANOVA analysis				
pH	NaCl[a]	sample volume[b]	extraction time[c]	adsorbent[d]	desorption time[c]	eluent volume[b]	R^2	adj R^2	SD	CV	lack of fit
4.96	17.23	7.82	4.99	25	4.99	6.91	0.88	0.77	539.52	19.27	0.26

[a]%. [b]mL. [c]min. [d]mg.

As shown in Figure 6, the extraction recovery of cyclophosphamide increases by an increase in the volume of the sample and eluent solvent of up to 8.0 and 7.0 mL, respectively. The highest recovery percentage of cyclophosphamide did not change much at higher volumes. This phenomenon can be justified by the increased interface of the liquid sample and dispersive solid sorbent. On the other hand, due to the limitation of active adsorbent receptors (limitation of sorbent capacity), from one point onward, due to the saturation of adsorbent capacity for bonding with the analyte, increasing the sample volume will not have much effect on the extraction efficiency.[24,31,32]

The applied amount of solid sorbent in the SPE, MEPS, and Dμ-SPE methods is a critical factor that can be effective in the extraction recovery of the target analyte from the matrix.[29,30] According to the experiment, the extraction recovery was increased along with the increase of the sorbent of up to 25.0 mg. At higher amounts, the extraction efficiency did not change because of the absorbent capacity completion. Therefore, the optimal amount of the adsorbent was

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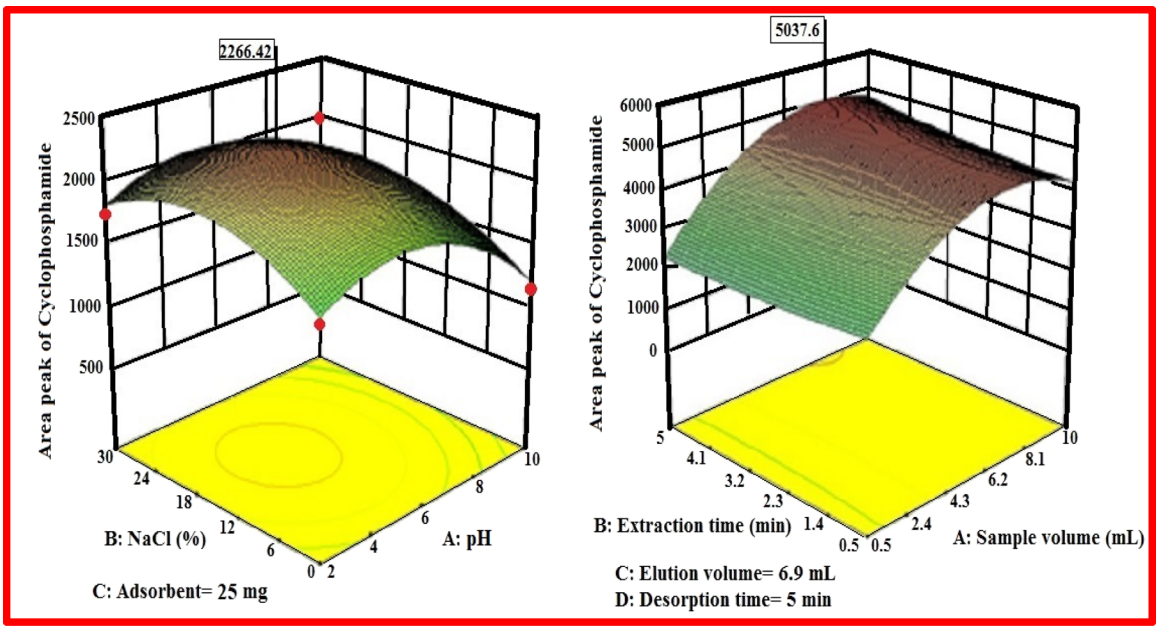

Figure 6. Effective extraction parameters on the efficiency of Dμ-SPE;HPLC analysis of urinary cyclophosphamide.

considered to be 25.0 mg for the highest extraction recovery percentage.

So, the highest extraction efficiency of urinary cyclophosphamide by the proposed method was observed in the following optimized conditions: pH, 5.0; sample volume, 8.0 mL; NaCl concentration, 17.2% (w/v); eluent volume, 7.0 mL; extraction and desorption time, 5 min; reconstitution volume, 200.0 μL; amount of sorbent, 25.0 mg.

Material constituents of MIL-53(Al) (benzene dicarboxylic acid (BDC) and aluminum chloride) are capable of providing a strong $\pi-\pi$ interaction between benzene dicarboxylic acid molecules and cyclophosphamide and also the separation of cyclophosphamide from the primary matrix.[34,43] It has been reported that $AlCl_3$ plays an important role in the MIL-53 (Al) structure to separate small molecules by MIL-53(Al).[34] In addition, carboxylic groups of BDC on the surface of the MIL-53 (Al) structure increase the capability of this sorbent to separate polar compounds.[36,43] The amino group in the MIL-53(Al)-NH_2 structure can lead to the separation of the organic analyte (such as cyclophosphamide) from the sample through covalent bonding and hydrogen interaction, which increases the efficiency of MIL-53(Al) to extract organic compounds from the complex matrix.[7,12]

3.3. Analytical Performance with Different Eluent Solvents. In the following, the performance of different eluent solvents (7.0 mL) including methanol, acetonitrile, acetone, methanol:acetonitrile (1:1 v/v), methanol:acetone (1:1 v/v), and acetone:acetonitrile (1:1 v/v) was investigated to minimize the carryover effect of the current procedure on the MIL-53(Al)-NH_2 adsorbent after each sampling at a concentration of 50.0 μg mL^{-1} (spiked sample). The results of this analysis illustrated that the application of methanol:acetone solution (1:1 v/v) can have the highest efficiency in adsorbent cleaning (carryover = 2.8%), and therefore, this solvent was selected as the optimal adsorbent cleaning solvent.

The sensitivity of the designed analytical method was determined based on the correlation of the standard solution concentration injected into the analyzer apparatus and the received response (area peak of cyclophosphamide). The calibration curve of the analyzer apparatus (HPLC/UV−Vis) was plotted by using several spiked cyclophosphamide concentrations in the urine samples of healthy and nonexposed humans. The results exhibited that the proposed method has a linear calibration curve throughout the concentration range of 0.14−120.0 μg mL^{-1}, with a favorable correlation coefficient (R^2 = 0.998).

In the next step, the precision of the procedure as a new analytical method was determined in terms of relative standard deviation (RSD %) for six replicate measurements of three standard solutions of cyclophosphamide (0.5, 50.0, and 100.0 μg mL^{-1}) at the intraday and interday, which were obtained under optimized conditions. The results of the intra- and interday experiments are mentioned in Table 2, and as can be seen, the precision values were calculated to be 2.7−3.9%.

Table 2. Accuracy and Precision of the MIL-53(Al)-NH_2@ Dμ-SPE;HPLC Method to Determine Urinary Cyclophosphamide[a]

QC concentration (μg mL^{-1})	accuracy (%)	intraday RSD (%)	interday RSD (%)	EF
0.5	−7.1	2.7	3.5	18.2
50.0	−6.4	3.1	3.9	18.8
100.0	−5.9	2.9	3.7	20.9

[a]QC, quality control; RSD, relative standard deviation; EF, enrichment factor.

In this study, due to the lack of a standard method for determining cyclophosphamide from urine samples, the accuracy was determined in three different concentrations (0.5, 50.0, and 100.0 μg mL^{-1}) based on the measurement of sample recovery and spike. In this way, different concentrations of cyclophosphamide were added to the blank samples and then the recovery percentage was calculated as follows:[44]

$$R = \frac{T_a T_b}{C} \times 100$$

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Table 3. Comparison of the Published Methods with the Proposed Method in This Study[a]

technique	accuracy (%)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	precision (%)	references
MEPS-LC−MS/MS	91.0−106.0	5.0	50.0	5−9	45
MEPS-LC−MS/MS	107.0−110.0		0.1	4−6	12
RP-HPLC-UV	99.8	0.2	1.0	0.27	46
HPLC-UV	99.0−99.7	0.123	0.15	1.5−1.7	47
SPE-GC−MS	83.0−116.0	0.004	0.001	0.5−10	48
D-SPE-UPLC−MS/MS	98.2	0.003	0.001	8.22	5
NTD-Carboxen- GC-ECD	95.2−108.5	100*	191*	4.8−8.9	7
SPE-HPLC-UV	94.0−115.0		540**	0.5−7.8	49
LC−MS/MS	97.0	0.005	3.0−175.0	0.8−1	50
Dμ-SPE@MIL-53(Al)-NH$_2$;HPLC	94.0−96.0	0.05	0.14	2.7−3.9	current study

[a]MEPS, microextraction by packed sorbent; SPE, solid-phase extraction; DSPE: dispersive solid-phase extraction; NTD, needle trap device; Dμ-SPE, dispersive microsolid-phase extraction; LC, liquid chromatography; GC, gas chromatography; HPLC, high-performance liquid chromatography; UPLC, ultraperformance liquid chromatography; MS, mass spectrometry; RP, reverse phase. *, μg m^{-3}; **, μMol.

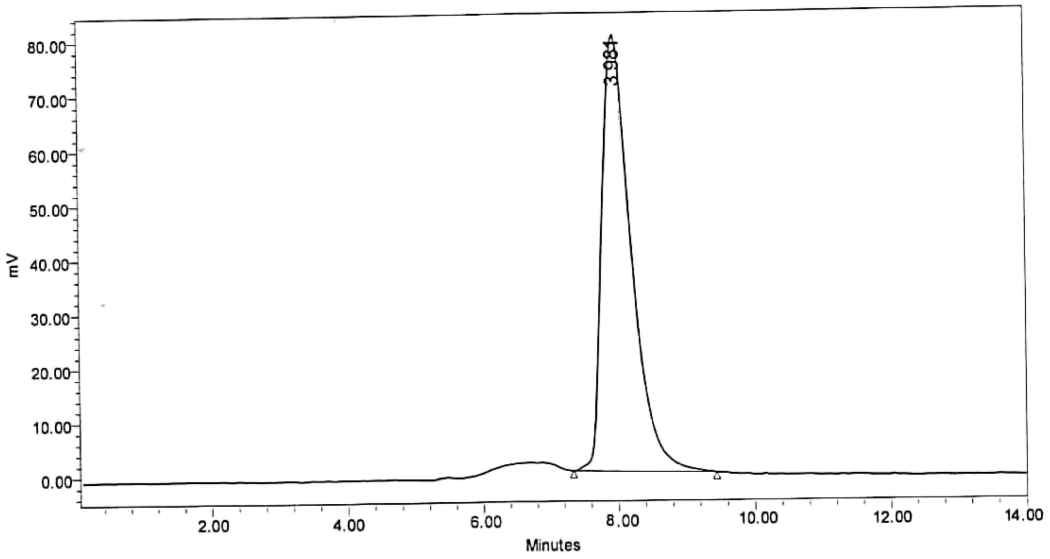

Figure 7. HPLC/UV−Vis chromatogram of cyclophosphamide in the real urine samples by the proposed MIL-53(Al)-NH$_2$@Dμ-SPE method.

where R is the recovery rate (percentage), T_a is the extracted compound (mg mL^{-1}), T_b is the desired compound in the blank sample (mg mL^{-1}), and C is the concentration of the compound added to the solution (mg mL^{-1}).

Finally, to express accuracy, the obtained numbers were subtracted from 100 and reported as negative.

The extraction efficiency of cyclophosphamide by the MIL-53(Al)-NH$_2$@Dμ-SPE method was estimated using direct injection of spiked urine samples (0.5, 50.0, and 100.0 μg mL^{-1}) into the HPLC system. The obtained results indicated that the average extraction efficiency)in the three mentioned concentrations (of urinary cyclophosphamide using the developed method under optimized conditions is in the range of 92.0−96.0%, which is an acceptable result compared to the previous studies.[6,12,13]

In this study, to estimate the LOD and LOQ values, the analyte concentration was reduced consecutively to reach a concentration corresponding to a peak with signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively (with five repetitions).

The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed method were calculated to be 0.05 and 0.14 μg mL^{-1}, respectively.

From a comparative perspective, the achieved results of the present study were compared with other similar research to the assessment of MIL-53(Al)-NH$_2$@Dμ-SPE performance (Table 3). As can be seen, the analytical performance of the proposed method in the present study is similar to and sometimes better than the results of similar previous studies. Therefore, the proposed MIL-53(Al)-NH$_2$@Dμ-SPE;HPLC technique can be used as a fast, simple, sensitive, user-friendly, and promising method for the extraction and analysis of cyclophosphamide from urine samples.

3.4. Analysis of Real Urine Samples. Finally, the designed procedure (MIL-53(Al)-NH$_2$@Dμ-SPE) was successfully implemented in real conditions (chemotherapy center of the hospital) for the determination of cyclophosphamide under optimized factors. The 24 h urine samples were taken from five staff of the chemotherapy center in one of the Iranian hospitals and were triplicate-analyzed under the optimized conditions of the proposed method. The mean concentration of cyclophosphamide in urine samples was 0.18 μg/24 h in the range of 0.08−0.22 μg/24 h with RSD of less than 4.1%. Also, Figure 7 shows a chromatogram obtained from cyclophosphamide extracted in a real urine sample using the MIL-53(Al)-NH$_2$@Dμ-SPE;HPLC method.

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4. CONCLUSIONS

In this paper, a simple, fast, inexpensive, and reliable analytical method was introduced for extraction and determination of cyclophosphamide in the urine sample by the MIL-53(Al)-NH_2 sorbent integrated with the dispersive microsolid-phase extraction method prior to the high-performance liquid chromatography technique. The influential factors on the extraction and preconcentration of urinary cyclophosphamide were optimized by the response surface methodology and central composite design. The performance of the proposed method was evaluated in terms of accuracy, precision, LOD, LOQ, sensitivity, carryover, and linearity.

The obtained results exhibited that the optimal extraction time, sample volume, adsorbent amount, and NaCl concentration of the sample were 4.99 min, 7.82 mL, 25 mg, and 17.23%, respectively. Acetone was also chosen as the most suitable elution solution. Moreover, the optimal desorption time, eluent volume, and pH were determined to be 4.99 °C, 6.91 mL, and 4.96, respectively. The percentage recovery of desired analytes was obtained in the range of 94−96%. The LOD and LOQ of the proposed method were calculated in 0.05 and 0.14 $\mu g\ mL^{-1}$, respectively. The results of intraday and interday were calculated in the range of 2.7 to 3.9%, which shows the high precision of the proposed method.

It should be noted the designed procedure was implemented in real conditions with satisfactory achievements. The utilized sorbent integrated with the dispersive microsolid extraction method has provided sufficient advantages for determining cyclophosphamide from the urine sample compared with a previous similar method.

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Notes

The authors declare no competing financial interest.

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