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Solid Phase Extraction of Letrozole by a Metal Organic Framework and its HPLC-UV Characterization

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We successfully synthesized TMU-24 as metal-organic framework (MOF) and employed as an effective sorbent for preconcentration of letrozole (LTZ) from biological samples. Using high-performance liquid chromatography with UV detector (HPLC-UV) coupling with solid-phase extraction (SPE), a sensitive, fast, and simple method for detection of LTZ has been developed. The optimal conditions, such as pH, type of eluent solvents, contact time, amount of adsorbent, and adsorption capacity are discussed. Under the optimized conditions, relative recoveries (RR) and preconcentration factor (PF) of the LTZ was obtained in the range of 92.4-99.6% and 33, respectively. Under the optimal conditions, LTZ can be determined with a limit of detection (LODs) $0.5 \mu\text{g l}^{-1}$ (based on $S/N = 3$), a linearity in the range of $1\text{-}250 \mu\text{g l}^{-1}$, and a reasonable linearity of 0.995. Also, the experimental adsorption isotherms models were investigated. The developed method was successfully used to the analysis of LTZ in urine samples.

Keywords: TMU-24, Metal-organic framework, Solid-phase extraction, Letrozole, HPLC-UV

INTRODUCTION

Letrozole (LTZ) can prevent excess estrogen of biosynthesis and is known as an estrogen receptor-positive breast cancer drug [1,2]. The terminal elimination half-life of LTZ is nearly 48 h. In addition, due to its low therapeutic dose and wide distribution in tissues, the plasma concentration of LTZ is rather low. Therefore, it is essential to establish an accurate and sensitive analytical methodology for the determination of LTZ [3-5].

Some techniques have been proposed to determine LTZ, including capillary electrophoresis, gas chromatography-mass spectrometry (GC-MS), and HPLC-MS. Instrumental analysis is disabled to quantify ultra-trace amounts of LTZ in real samples [6-11]. Thus, preconcentration and extraction of LTZ to overcome matrix interferences or to improve the detectability of the analytical technique are required for analysis of trace amounts of LTZ in real

samples. Solid-phase extraction (SPE) has been widely used for this purpose [12-18]. Different types of sorbents including activated carbon, peat, zeolites, silica, chitin or polymers, and inorganic mesoporous silica are applied for SPE [19-22]. Conventional sorbents exhibit numerous limitations such as low surface area, and long extraction times [23]. Due to the above-mentioned drawbacks of conventional sorbents, we have chosen a metal-organic framework (MOF). The MOFs are 2D or 3D crystalline hybrid porous solid structures comprising of metal ions joined to organic linkers acting as a bridge between metal centers [24-27]. MOFs have attractive structural properties such as high surface area, good chemical resistance, the crystal morphology, high void volume, pore shape and tunable pore sizes, low density, features of easy to be functionalized, water solubility and uniform morphology of structured nanoscale cavities that make MOFs attractive materials for the SPE. [24,28,29].

The study aims to fabricate a TMU-24 by using a solvothermal synthesis method. The applicability of MOF

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for the extraction of LTZ from biological is investigated, and they are also characterized by high-performance liquid chromatography.

EXPERIMENTAL

Materials

The cadmium nitrate tetrahydrate, N,N'-bis(4-pyridylformamide)-1,5-naphthalenediamine (bpfm) and 4,4'-oxybisbenzoic acid (H₂oba) methanol and sodium chloride were purchased from Merck. LTZ was purchased from Tofigh Daru Company, Tehran, Iran. Some physical properties and chemical structure of LTZ are shown in Table 1.

Apparatus

The FT-IR spectroscopy was performed by a Thermo Nicolet Avatar 360, USA. The geomorphology of nanostructures was characterized by FESEM (Tescan Mira3 FEG). The TGA was done with a Bahr STA-503 (Germany). Chromatography separation of analytes was performed using a Varian HPLC system (Walnut Creek, CA, USA).

Separation was done on ODS-3 column of Teknochroma (Barcelona, Spain) using a mobile phase consisting of acetonitrile and ultra-pure water (40:60) with a flow rate of 1 ml min⁻¹, and UV detection was set at 240 nm.

Synthesis of MOF

A mixture of H₂oba (0.318 g, 1 mmol), Zn (NO₃)₂.6H₂O (0.297 g, 1 mmol), bpfm (0.181 g, 1 mmol), and DMF (50 ml) was sonicated for 15 min to disperse, the vials were heat at 120 °C for 3 days, used a DMF wash, and dried at 25 °C [28].

Extraction Procedure

10 ml sample solution containing LTZ at 0.1 mg l⁻¹ adjusted at pH = 7 was transferred to a glass beaker. Then, 4 mg of TMU-24 as adsorbent was added. The sample solution was stirred for 5 min to extract the target LTZ. The sample solution was centrifuged and the supernatant was removed. 80 µl of acetonitrile with an ultrasonic elution time of 3 min was added. Finally, the samples were separated by centrifugation, and extraction solution was

analyzed by HPLC (Fig. 1).

Real Samples

The urine samples were obtained from healthy volunteers and then samples were stored in a polyethylene tube at 4 °C.

RESULTS AND DISCUSSION

Characterization of the Synthesis TMU-24

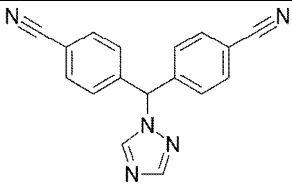
FT-IR spectrum of MOF is depicted in Fig. 2A. The characteristic peaks at 2742 cm⁻¹ and 2846 cm⁻¹ are assigned to C-H bending of benzene ring, the peak at 1646 cm⁻¹ is related to C=O of carboxylic acid group, and the peaks at 1377 cm⁻¹ and 1423 cm⁻¹ belong to the vibrations of the benzene framework [30]. TGA curve of TMU-24 is shown in Fig. 2B. As shown in Fig 2B, a weight loss of 78.6% is observed that 8.8% of which was related to the expulsion of desorption of water molecules and physically-bound organic solvent. The remaining 69.4% weight loss, represented destruction of organic moiety. The fabricated TMU-24 crystal structure and phase purity were characterized by PXRD pattern (Fig. 3A). Figure 3B is FE-SEM image of TMU-24. However, a cube like morphology crystals of fabricated TMU-24 were obtained.

Optimization of Parameters

The sorbent was used for SPE of the LTZ mainly based on π - π stacking interactions, hydrophobic and hydrogen-bond interactions between the MOF and the LTZ that play an important role in the adsorption process. It has been certified that the carboxyl groups in the MOF can interact with N of LTZ to form hydrogen-bonds.

The pH of sample solution plays a critical role in adsorption process. The adsorptions of LTZ (0.1 mg l⁻¹; 10 ml; 3 min) onto MOF as adsorbent (2 mg) were conducted at different pHs (from pH 5 to 9). Above pH 7, the adsorption efficiency was decreased with the increase of pH values. Therefore, pH = 7 was selected for further study. To choose a simple eluting solvent for SPE to obtain maximum extraction, a series of eluting solvents, including acetonitrile, acetone, methanol, and propanol, were investigated. The results showed that the acetonitrile exhibits the best recovery. Therefore, acetonitrile was

Table 1. 2 D Structure and Chemical Properties of LTZ

Chemical structure	
Molecular formula	C ₁₇ H ₁₁ N ₅
Molecular weight	285.303 g mol ⁻¹
λ_{\max}	241 nm

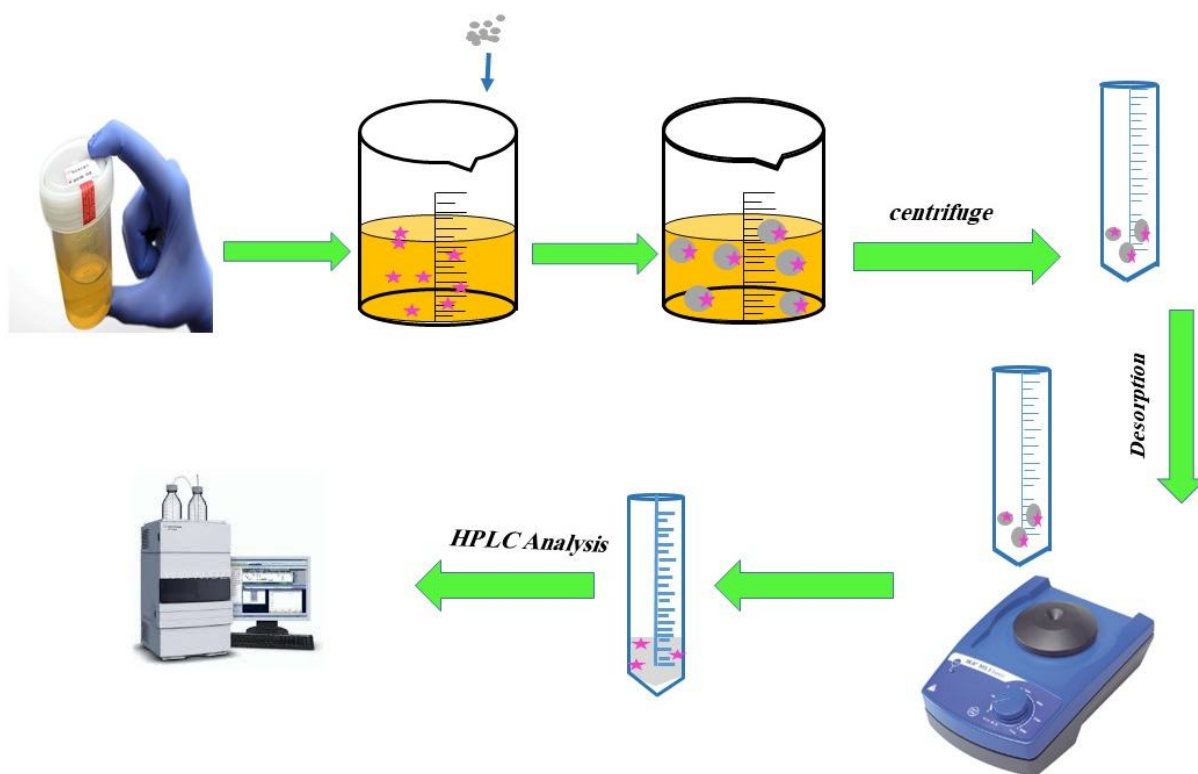


Fig. 1. Schematic illustration of the experimental setup for the solid phase extraction procedure.

employed as an elution solvent (Fig. 4A).

The effect of the amount of TMU-24 on the SPE of LTZ is significant. In order to save material and to achieve sufficient adsorption, the amount of TMU-24 was studied. This experiment studied the TMU-24 adsorbent when the

dosage is 1 mg, 2 mg, 4 mg, 6 mg and 8 mg (Fig. 4B). The extraction efficiency increased rapidly with the increase of TMU-24 from 1 to 4 mg. The recovery rate of LTZ was almost constant when the amount of adsorbent increased from 4 mg to 8 mg. Thus, 4 mg of TMU-24 was employed

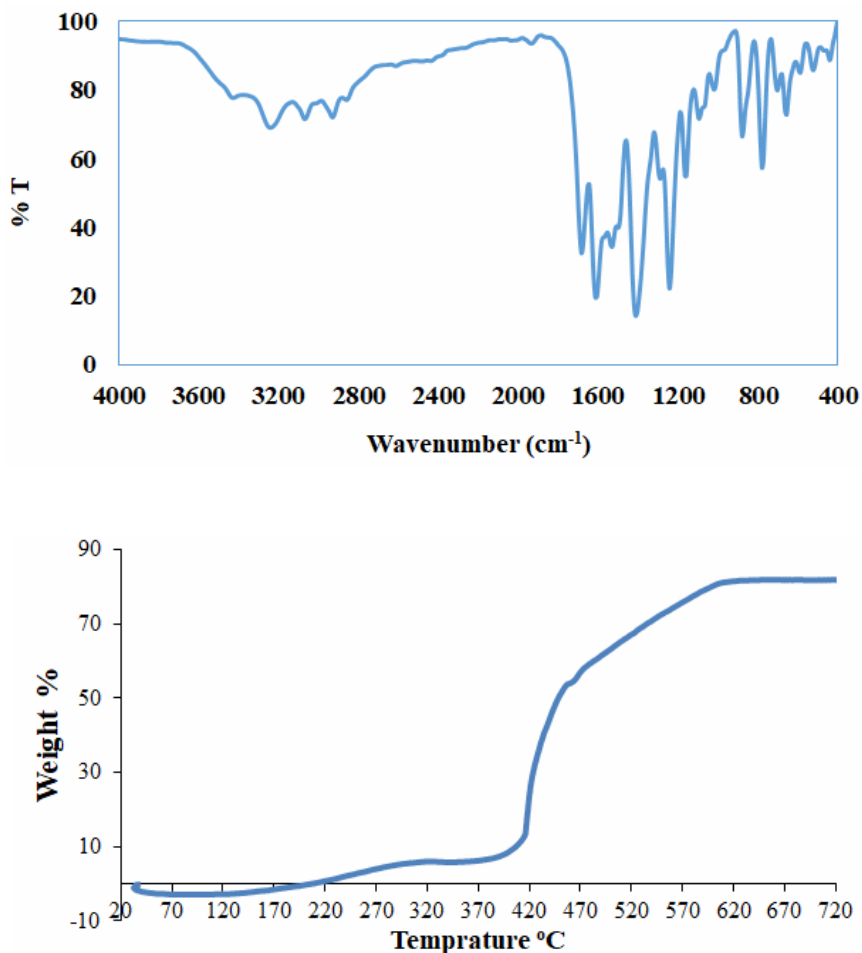


Fig. 2. A) FT-IR spectra of TMU-24. B) TGA thermograms of TMU-24.

for the following experiments.

Five extraction times of 1 min, 3 min, 5 min, 7 min and 9 min were studied. The result showed that the recovery for the analytes was greatly increased from 1 to 5 min and it did not change significantly after that. So, 5 min of adsorption time was chosen (Fig. 4C). The effect of solvent volume on desorption efficiency of LTZ was also investigated. Various volumes of desorption solution were investigated by varying it from 40 to 120 μ l. As the volume of acetonitrile increased from 40 to 80 μ l, the recovery of LTZ increased; when the volume of acetonitrile continued to increase to 120 μ l, the recovery of LTZ decreased because of dilution effect [31]. Thus, 80 μ l of acetonitrile was chosen to ensure complete elution of target analytes for further experiments

(Fig. 4D).

The reusability of TMU-24 adsorbent for the extraction of LTZ was also studied. After each SPE process, the sorbent was washed two times with acetonitrile and then dried at 70 °C before the next use. It was found that TMU-24 could be reused at least six times and it loses 5% of its initial extraction efficiency.

Adsorption Isotherm Study

The adsorption isotherm is used to predict the adsorption capacity of adsorbents. The Freundlich (Eq. (1)) and Langmuir (Eq. (2)) were the adsorption isotherm models employed here to describe letrozole adsorption equilibrium:

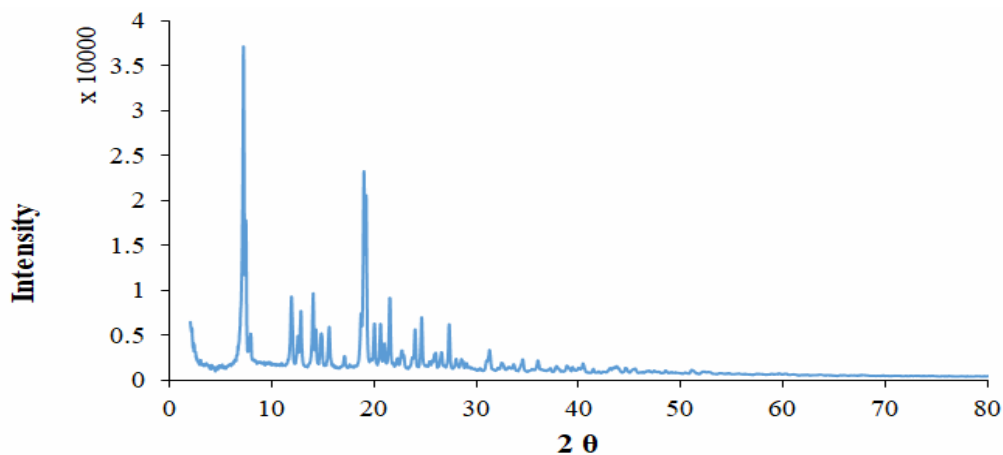


Fig. 3. A) X-ray diffraction pattern of TMU-24. B) SEM images of TMU-24.

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{C_e}{q_{max}} \quad (1)$$

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (2)$$

where q_e represents the adsorption amount at equilibrium, C_e is the letrozole concentration in sample solution at equilibrium, q_{max} is maximum adsorption capacity, $1/n$,

K_F and K_L are the Freundlich and Langmuir constant respectively [32,33].

According to the results, Langmuir model was adopted to analyse the adsorption isotherms of TMU-24 for LTZ. And the R^2 of linear regression between C_e/q_e and C_e was 0.98, suggesting that the uptake of LTZ on MOFs fits Langmuir's adsorption model well.

The obtained results show that the LTZ adsorption process by the synthesized TMU-24 as adsorbent is a

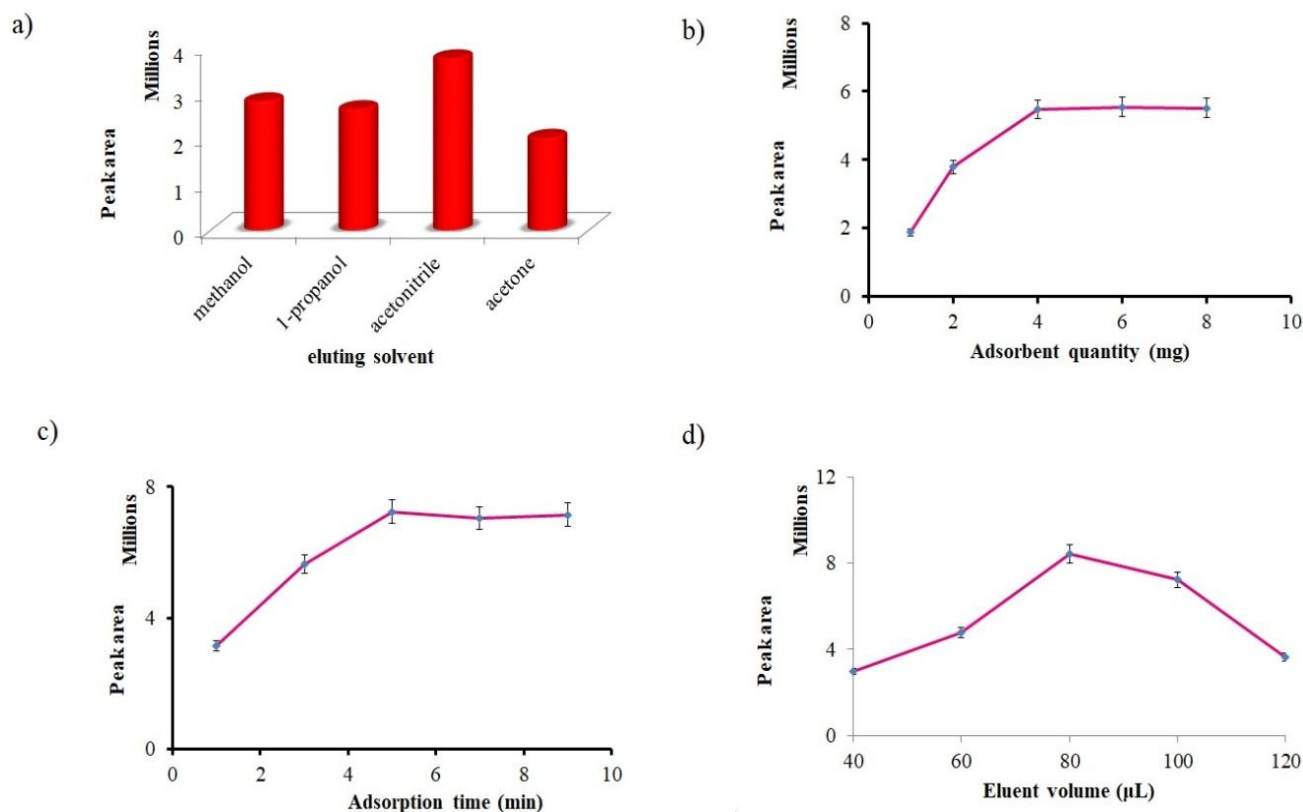


Fig. 4. Effects of the type of desorption solvent (a), amount of sorbent (b) equilibrium time (c) and eluent volume (d) on the proposed extraction procedure of LTZ using TMU-24 as the sorbent. Conditions: sample volume, 10 ml. Analyte concentration, $100 \mu\text{g l}^{-1}$, pH = 7.

Table 2. SPE Performance and Validation Data

Analyte	LOD ($\mu\text{g l}^{-1}$)	LDR ($\mu\text{g l}^{-1}$)	R^2	ER (%)	PF ^a	Precision (n = 3)			
						Intra-day		Inter-day	
						50 ($\mu\text{g l}^{-1}$)	100 ($\mu\text{g l}^{-1}$)	50 ($\mu\text{g l}^{-1}$)	100 ($\mu\text{g l}^{-1}$)
LTZ	0.5	1.0-250	0.995	26.5	33	3.3	2.8	4.7	4.1

^aPF was obtained for $50 \mu\text{g l}^{-1}$ of LTZ.

monolayer, reversible, and favorable chemisorption process.

Method Validation

A series of experiments was performed to evaluate

the developed SPE with TMU-24 as the sorbent for the HPLC-UV determination of LTZ under the optimized conditions. Parameters for analytical performance were investigated, including preconcentration factor (PF), limit of

Table 3. Comparative Summary of Various Methods Developed for Letrozole

Method		Linear range ($\mu\text{g l}^{-1}$)	LOD ($\mu\text{g l}^{-1}$)	RSD (%)	Sample	Ref.
MNP-PEGylated ^a (HPLC-UV)	dendrimer	0.1-20	0.92	< 2.2	Plasma-urine- drugmatrix (Tablet)	[34]
DLLME ^b (HPLC-UV)		5-500	0.7-1.5	<9.7	Plasma/urine	[35]
MWCNT ^c -resorcin (HPLC-UV)		200-2000	130	0.97	Plasma	[36]
Fused silica (MEKC ^d)		125-1500	37.5	3.76	Urine	[37]
HF-LPME ^f (HPLC-UV)		0.9-200	0.3	< 5.6	Urine	[38]
C8 (HPLC-UV)		500-5000	82.1	< 1.34	Water	[39]
TMU-24 (HPLC-UV)		1-250	0.5	<4.7	Urine	This study

^aMagnetic nanoparticles -polyethylene glycol. ^bDispersive liquid-liquid microextraction. ^cMulti-walled carbon nanotubes. ^dMicellar electrokinetic chromatography. ^fHollow fiber liquid-phase microextraction.

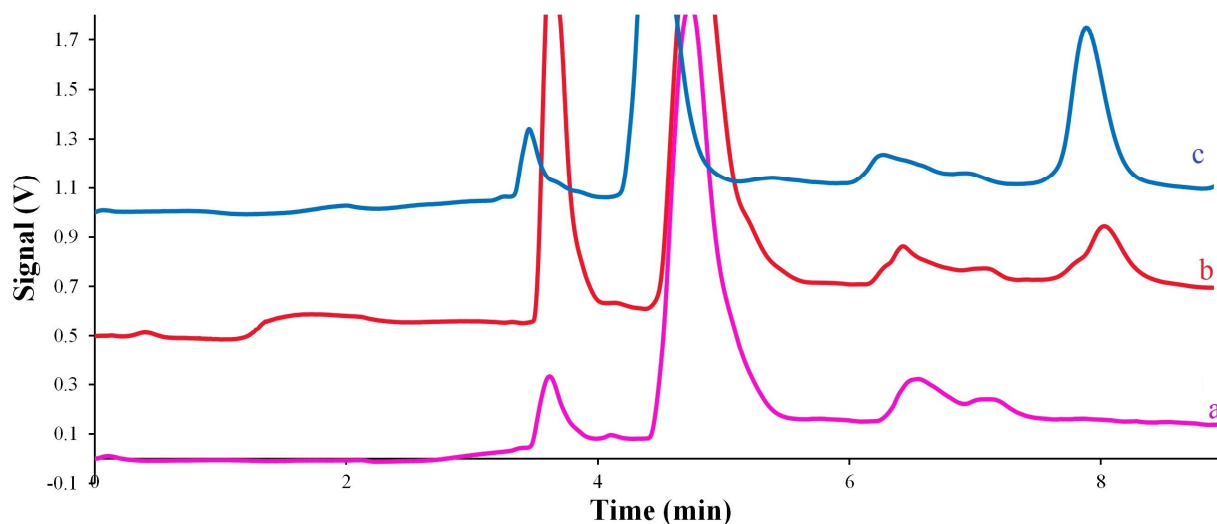


Fig. 5. The HPLC chromatograms obtained after the proposed extraction procedure from a urine sample (a) blank urine and (b) blank urine samples spiked with $20 \mu\text{g l}^{-1}$ (c) and $50 \mu\text{g l}^{-1}$. Conditions: sample volume, 10 ml. pH = 7; extraction time, 4 min; 80 μl of eluent; and desorption time, 3 min.

detection (LOD), intra-day and inter-day precisions, linearity, and extraction recovery (ER) (Table 2). Excellent linearity was obtained within $1\text{-}250 \mu\text{g l}^{-1}$ for LTZ with

correlation coefficients (R^2) 0.995. The LOD was attained $0.5 \mu\text{g l}^{-1}$. The obtained intra-day RSD% was 3.3% and for inter-day RSD% was 4.7%. The ER value was found to be

26.5 % since the PF value was achieved 33.

The results of a comparison made between the figures of merit of the proposed method and some of the published methods for extraction and determination of letrozole in literature are provided in Table 3 [34-39]. The results indicated that the proposed method has a comparable sensitivity and precision regarding the other reported techniques for determination of letrozole.

Analysis of Real Samples

To evaluate the applicability and accuracy of the MOF based SPE method for biological fluids, the extraction and preconcentration of LTZ was performed at trace levels in urine samples. Three replicate determinations of LTZ in the selected samples were performed under the optimal conditions. The accuracy and precision measurements were done with urine samples by spiking with two levels of 20, and 50 $\mu\text{g l}^{-1}$. The relative recoveries (RR) of LTZ at two spiked levels were between 92.4 and 99.6% (Table 3). The chromatograms are shown in Fig. 5. These results demonstrated the applicability of the applied procedure for determination of LTZ in urine samples.

CONCLUSIONS

TMU-24 as sorbents have been successfully prepared by solvothermal method. The introduced method demonstrated great potentials of MOF (TMU-24) as an appropriate adsorbent in SPE for the extraction of LTZ in urine samples. The developed method offered ease of operation, short analysis time, excellent sensitivity, good linearity and satisfactory recovery.

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