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ORIGINAL PAPER

Sensitive determination of Anastrozole and Letrozole in urine samples by novel magnetic nanoparticles containing tetraethylenepentamine (TEPA) prior to analysis by high-performance liquid chromatography-diode array detection

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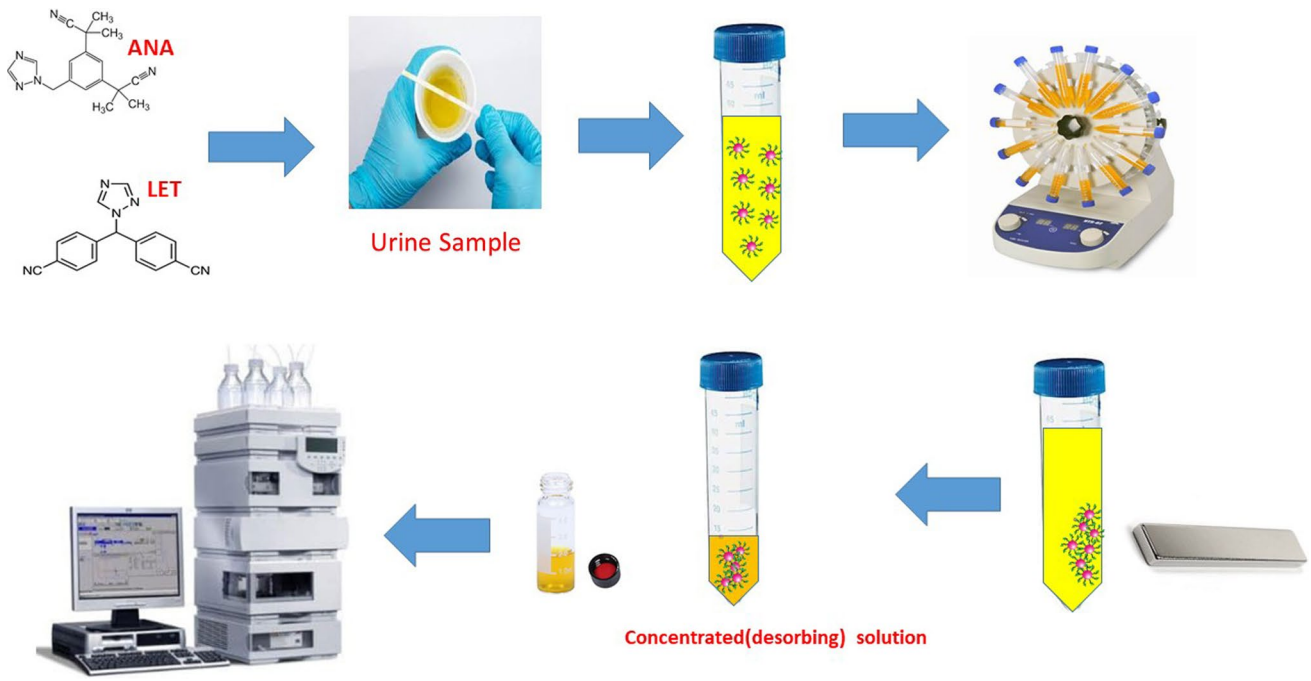
Abstract

Magnetic solid-phase extraction coupled to high-performance liquid chromatography (HPLC)-diode array detection method has been developed for the sensitive analysis of Anastrozole (ANA) and Letrozole (LET) in human urine samples. Characterization studies of the newly synthesized material (Fe_3O_4@TEPA) were carried out by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction, Raman spectroscopy, and scanning electron microscope techniques. The method has provided linearity for both the analytes ($r^2 > 0.9900$) in the range of 4.00–160.00 ng mL^{-1}. The limit of detections for ANA and LET was found to be 1.14 and 1.26 ng mL^{-1}, respectively. The limit of quantification for ANA and LET was obtained 3.78 and 3.86 ng mL^{-1}, respectively. The new method was simple, convenient, fast, and suitable for the direct analysis of ANA and LET in urine samples. Finally, the developed method was applied to the spiked human urine samples to test accuracy and repeatability. Recovery values for each of the molecules were calculated in the range of 93.4–108.0% (n = 3) at two concentration levels, while % RSDs were lower than 6.2%.

Graphical abstract



Extended author information available on the last page of the article

Keywords Anastrozole · Letrozole · RP–HPLC · Magnetic solid-phase extraction · Urine samples

Introduction

Breast cancer is one of the most common types of cancer in women, whereas the incidence of breast cancer in men is lower. The main reason for this difference is estrogen, which is known to have an important role in the development and proliferation of breast cancer (Wu and Butler 2011). The effect of estrogen can be prevented either by blocking the estrogen synthesis via antiestrogens or inhibiting by blocking enzymes involved in the biosynthesis process, such as aromatase. There are several agents to block this aromatization. The new generation aromatase inhibitors such as Anastrozole and Letrozole have a very strong effect in inhibiting/blocking the estrogen synthesis. Anastrozole and Letrozole are both non-steroidal aromatase inhibitors used in breast cancer treatment. Side effects of Anastrozole and Letrozole include nausea, vomiting, and headache (Olin and St. Pierre 2014).

Anastrozole (ANA) that selectively inhibits aromatase is a powerful non-steroidal aromatase inhibitor. It is chemically described as 1,3-benzene diacetonitrile, a, a, a', a'- tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl). It is the first aromatase inhibitor approved by the US Food and Drug Administration (US FDA) as adjuvant hormonal therapy in early stage breast cancer. Letrozole (LET) is used as a third-generation drug with highly selective as non-steroidal aromatase enzyme inhibitor that plays a key enzyme role in estrogen biosynthesis. LET is chemically known as 4-[(4-cyanophenyl)-(1,2,4-triazol-1-yl)methyl]benzonitrile. Aromatase is an enzyme involved in the production of estrogen by catalyzing the conversion of testosterone to estradiol. Aromatase is found in the adrenal glands, ovaries, placenta, testes, adipose (fat) tissue, and estrogen-producing cells in the brain. Aromatase is a specific component of the cytochrome P-450 enzyme system. Aromatase inhibitors are a class of drugs that work by blocking the enzyme aromatase, the enzyme that converts androgens to estrogen (Vanol et al. 2016).

There are several methods available in the literature for the determination of ANA and LET such as liquid chromatography-tandem mass spectrometry (HPLC–MS-MS) (Beer et al. 2010), reverse-phase high-performance liquid chromatography (RP–HPLC) (Acharjya et al. 2012), HPLC–DAD determination after extraction using fabric phase sorptive extraction (FPSE) (Locatelli et al. 2018), capillary gas chromatography with flame ionization detection (FID) (Berzas et al. 2003), spectrophotometric approaches (Mondal et al. 2007), potentiometric determination (Skawky and El-Tohamy 2021), and capillary electrophoreses (Rusu et al. 2017). In the another study, ANA and LET molecules were simultaneously determined by HPLC–MS/MS system without applying any pre-concentration step because this technique is very sensitive (van Nuland et al. 2019). When high concentrations of drug molecules are present in the samples, such as in pharmaceutical tablets, the determination could be also carried out directly (Galla et al. 2020). But, it is of great importance to apply pre-separation and pre-concentration techniques before the analysis of these antineoplastic drugs, due to the trace presence of ANA and LET in biological matrices and the degrading effects of the interfering species that exist in the dense matrix environment (Andreu et al. 2009).

Solid-phase extraction (SPE) is an extraction method generally used to separate a sample from other interfering species before using any analytical method (chromatographic, electroanalytical, etc.) to measure the amount of analyte in the sample. (Vuran et al. 2021). The principle of SPE is similar to that of liquid–liquid extraction (LLE), but SPE offers an excellent alternative to the conventional liquid–liquid extraction (LLE) methods in terms of high analyte recovery, highly purified extracts, the ability to simultaneously extract a wide range of analytes possessing different polarity and reduce organic solvent consumption (Shaikh and Kumar 2017; Demir et al. 2020). Magnetic solid-phase extraction (MSPE) is carried out based on its ability to adsorb and desorb analytes on a magnetic adsorbent ranging from mg or µg using an external magnetic field without further tedious steps (centrifugation or filtration) (Kabir et al. 2017; Yilmaz et al. 2018). The used solid-phase material can be easily isolated and collected after adsorption and desorption, making the sample pre-treatment procedure more convenient, time-saving, and cost-effective (Andreu et al. 2009; Zhang et al. 2016, 2019; Hu et al. 2021).

In this study, newly synthesized magnetic nanoparticles (Fe_3O_4@TEPA) were used for the first time as sorbent material in a magnetic solid-phase extraction of ANA and LET at trace levels. Surface modification of magnetic particles was carried out by TEPA which has hydrophobic properties (Su et al. 2017). Since the current research involves the development and evaluation of magnetic particles highly affinitive toward Anastrozole and Letrozole drugs, the objective of the present study was to develop a simple, precise, inexpensive, accurate, and validated RP-HPLC–DAD method for the estimation of these drugs using magnetic particles containing TEPA that can be easily used for routine analysis of human urine samples. The method was validated according to International Guidelines, and the recovery experiments were also carried out. The magnetic sorbent material has demonstrated satisfactory performance as a promising sorbent in the development of MSPE technique with high analytical performance.

Materials and methods

Instrumentation

The characterization processes of the newly synthesized magnetic nanomaterial were carried out using scanning electron microscope (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy. SEM and SEM–Mapping were performed using a Zeiss Gemini 500 Field Emission Scanning Electron Microscope to elucidate the morphological structures, while X-ray diffraction spectra were performed with a Bruker AXS D8 brand X-ray diffraction meter. Also, the Raman spectra of nanomaterials were obtained using a Raman Spectrophotometer (WITec alpha 300 M + micro-Raman system, Germany) with a 532 nm laser source. A Shimadzu (Prominence) HPLC (Kyoto, Japan) system was used for determination of drug molecules.

Chemicals and reagents

In the present study, all chemicals used were of analytical purity (99.5%). A double-distilled water system delivering water of 18.2 MΩ cm resistivity was used to obtain ultrapure water (MES, MP Minipure Dest Up, Turkey). The HPLC grade acetonitrile and methanol were used for HPLC–DAD analysis (Sigma-Aldrich, St. Louis, MO, the USA). A stock solution of Anastrozole and Letrozole, 100 µg mL^{-1} was prepared in the analytical grade methanol (Sigma-Aldrich, St. Louis, MO, the USA). In the magnetic nanoparticle synthesis step, $FeCl_3$, $FeCl_2$, trimethoxyphenylsilane (TMPS), and tetraethylenepentamine (TEPA) were obtained from Sigma-Aldrich (St. Louis, the USA).

Chromatographic analysis

The mobile-phase composition was AcN: MeOH: PBS (pH 3.0) (30:10:60, *v: v: v*) in isocratic elution mode. The flow rate was set at 1.0 mL min^{-1} performed using a Phenyl-Hexyl column (100 Å, LC Column 250 × 4.6 mm). The absorbance of target molecules was followed by PDA detector at 210 and 240 nm, respectively. The column temperature was maintained at 30 °C, and injection volume in the autosampler was 10 µL for all determinations. The obtained chromatogram is given in Fig. 1. HPLC system was flushed with 50% AcN to remove the residues of surfactants and the other organic compounds for 30 min after each day's work. The peak area of target molecules was calculated by LC Solution software provided by Shimadzu.

Synthesis of magnetic nanoparticles

A well-known procedure was applied in the first step of magnetic nanoparticle synthesis. Briefly, a mixture of $FeCl_3$ and $FeCl_2$ salts were dissolved by considering molar ratio (2:1) in 50 mL of 0.01 M HCl solution (Laurent et al. 2008). This solution was kept on a magnetic stirrer at 600 rpm and 65 °C. Then, 100 mL of 50% ethanol was added to the solution, and the temperature was maintained at 85 °C on a magnetic stirrer. The synthesis reaction was initiated by dropping 20 mL of 9 M NH_3*H_2O for 10 min in the presence of inert nitrogen gas. The resulting black magnetite Fe_3O_4 particles were collected using an external magnet, washed three times with 50% ethanol, and then dried in an oven at 60 °C for 4 h.

In the next step, 2 g of the previously synthesized Fe_3O_4 particles were dispersed in 50 mL of 50% ethanol (mixture of ethanol: water 1:1) and 3 mL of 8 M ammonia solution was added, while the mixture was stirring at 600 rpm and 80 °C on a magnetic stirrer. After 30 min, 2 mL of trimethoxyphenylsilane (TMPS) was added to the solution. Then, 2 mL of tetramethylenepentamine (TEPA) was dissolved in 2 mL of ethanol, and this dissolution was carried out using

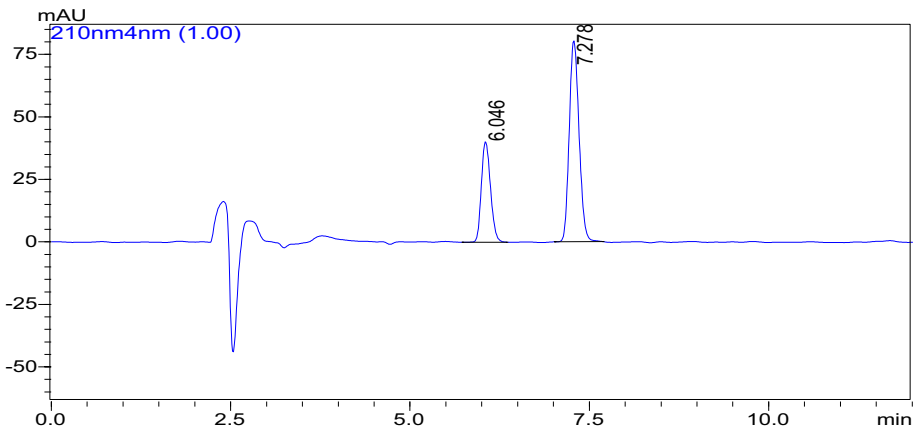


Fig. 1 Chromatogram of ANA and LET molecules before MSPE

a magnetic stirrer. Stirring was done for 6 h. The resulting magnetic particles were removed from the solution with an external magnet, washed five times with 50% ethanol, and then left to dry in an oven at 45° C.

Magnetic solid-phase extraction

The current method involves sample pre-treatment by magnetic solid-phase extraction (MSPE) and analytical determination by HPLC coupled with a photodiode array detector (PDA).

There are a few steps requiring optimization for sensitive and correct analysis. The analytical signal was increased by optimizing every relevant factor, and finally, the conditions for a sensitive analysis were obtained at the end of this study using the one-variable-at-time (OVAT) optimization procedure. The accuracy (precision and trueness) and recovery of the developed method with different experimental variables were evaluated and optimized. All optimization procedures were carried out using model solutions including Anastrozole and Letrozole at ppb levels.

50 mg of the newly synthesized magnetic material was weighted into a falcon tube and washed with 2 mL of ultrapure water for two times. After 40 mL of sample solution including anticancer drug molecules (4.0–160.0 ng mL^{-1}) was added, the pH of solution was set by using 2 mL of pH 10.0 Britton Robinson buffer solution (0.01 M), and final volume was completed to 50 mL. For the adsorption of target molecules on surface of magnetic particles, the tubes were placed on an orbital shaker at 80 rpm for 20 min, and then, the magnetic solid particles were carefully separated using a Neodymium magnet. After the separation of the aqueous phase, 400 µL AcN: MeOH (1:1, *v: v*) were added to tubes and vortexed for 30 s to easily desorb the molecules. Then, the samples were filtered via a 0.45 µm syringe-type filter and transferred to HPLC microvials. After every use, the magnetic particles were washed with 1 mL acetonitrile and 1 mL methanol two times. The solid phase was ready for re-use after drying at the end of this procedure.

Urine samples pre-treatment

Urine samples were prepared by little modification of a published method presented in the literature (Lu et al. 2020). The drug-free urine samples were collected from healthy donors with informed consent. All experiments were performed in compliance with the relevant laws and institutional guidelines. The samples were stored at − 4 °C. Before usage, the samples were kept at room temperature and diluted with ultrapure water (1:3, *v:v*). The diluted blank samples were then directly subjected to the developed MSPE procedure. The fortified samples were spiked with drug molecules at ng mL^{-1} level concentrations and then subjected to the MSPE procedure.

Results and discussion

Characterization of the magnetic nanoparticles

The FTIR spectra obtained for the newly synthesized nanomaterial are in the wavelength range of 4000–500 cm^{-1} as can be seen in Fig. 2. It is thought that the spectrum seen at 2329 cm^{-1} may belong to atmospheric C-N stretching vibrations in the reaction medium. The spectrum obtained at a wavelength of 2015 cm^{-1} originates from C–C=–C–C=–CH stretching vibrations. The spectrum observed at a wavelength

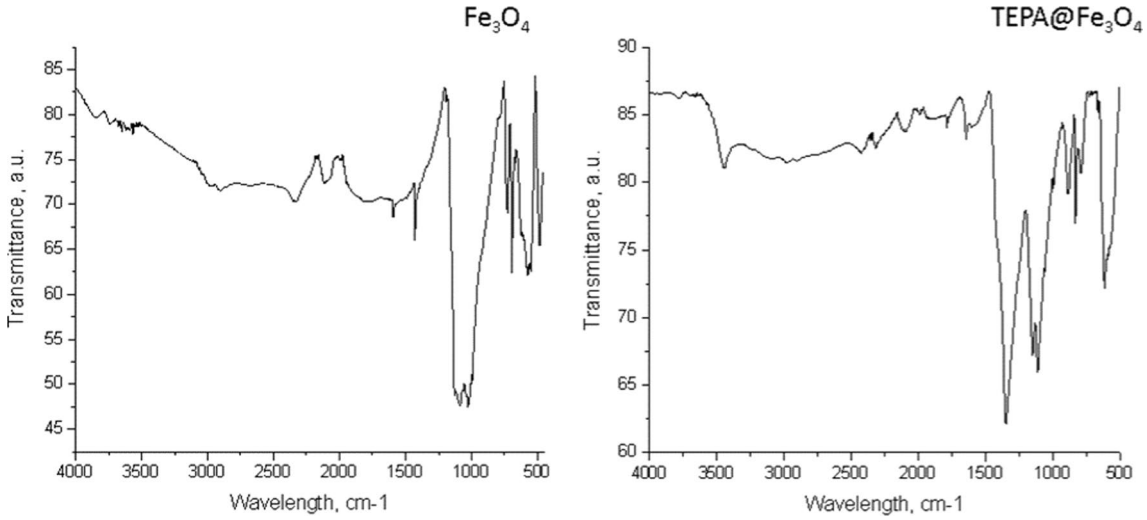


Fig. 2 FTIR spectrum of magnetic nanoparticles

of 1592 cm^{-1} characterizes the presence of conjugated species originating from C = O (Priya and Asharani 2018). $–CH_2$ bending vibrations are observed in the wavelength range of 1405–1465 cm^{-1}. It has been confirmed by the literature studies that it belongs to C–O and C–OH bending vibrations at wavelengths of 1125, 1086, 1026, 996 cm^{-1} (Zhong and Yun 2018). The FTIR spectrum seen at a wavelength of 727 cm^{-1} is the characteristic peak of –CH = CH- (cis) bending vibrations. The FTIR spectrum with a wavelength of 694 cm^{-1} was observed as the absorption peak of deformation bending vibrations in the benzene ring and was confirmed by the literature studies (Yu et al. 2018).

It was observed that the nanomaterial, which was analyzed using 532 nm laser in Raman spectrophotometer, underwent chemiluminescence over 1600 cm^{-1} wavelength and no significant peaks could be obtained as shown in Fig. 3a. Therefore, the measurements were taken on the same sample in the range of 200–1600 and 1600–4000 cm^{-1}, by changing the wavelength scale. The main peaks obtained are spectra with wavelengths of 289, 404, 610, and 1309 cm^{-1}. Raman spectrum seen at 289 cm^{-1} wavelengths characterizes metal–metal vibrational energy bands, and 404 cm^{-1} wavelength characterizes Si-metal vibrational bands (Wang et al. 2017). The spectrum taken at the 1309 cm^{-1} peak, which has the most intense peak area, characterizes the vibrational energies originating from $-CH_3$ and $-CH_2$ and is also the characteristic D band in Raman spectroscopy (Su and Chuang 2012).

X-ray diffraction (XRD) was also taken for magnetic nanomaterial as can be seen in Fig. 3b. X-ray diffractometry is a technique used to determine the interatomic distances and orientations of crystalline materials. Depending on its crystal structure, the X-ray is refracted at different angles and intensities, thus revealing the diffraction pattern of the material. The diffraction peaks obtained for the nanomaterial were 21.51°, 30.29°, 35.68°, 43.19°, 57.29°, and 62.94° (2theta). 21.51° and 30.29°; XRD diffraction peaks of 35.68°, 57.29°, and 62.94° are characteristic for Fe_3O_4 particles (Sarp and Yilmaz 2019). Another specific peak of the obtained nanomaterial is the 43.19° diffraction peak.

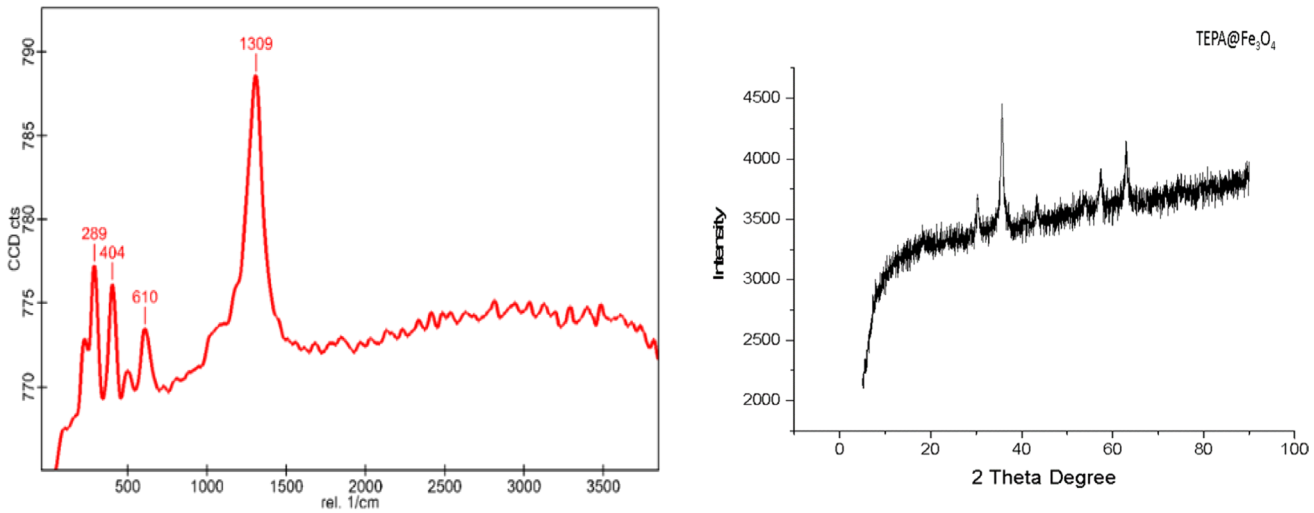
The novel nanomaterial was also characterized by scanning electron microscope (SEM), and the image is shown in Fig. 4. When the surface morphology of magnetic particles nanomaterial is examined, a mixed arrangement of nanomagnetite structures with magnetite structure is observed on the surface. It is seen that the surface morphology of magnetic nanomaterial has changed and the magnetite structure on the surface is surrounded by the binding species used in the synthesis phase.

Optimization of extraction factors

Effect of pH

The new magnetic material was synthesized for the trace analysis of ANA and LET, which are used as anticancer drugs, and then, all the necessary experimental parameters of the MSPE procedure were optimized step by step. The first optimization step was to examine the effect of sample pH.

Experimental variables for MSPE were optimized sequentially. First, the effect of pH on the amount of extracted



(a) Raman spectrum of magnetic nanoparticles nanoparticles

(b) XRD results of magnetic

Fig. 3 **a** Raman spectrum of magnetic nanoparticles. **b** XRD results of magnetic nanoparticles

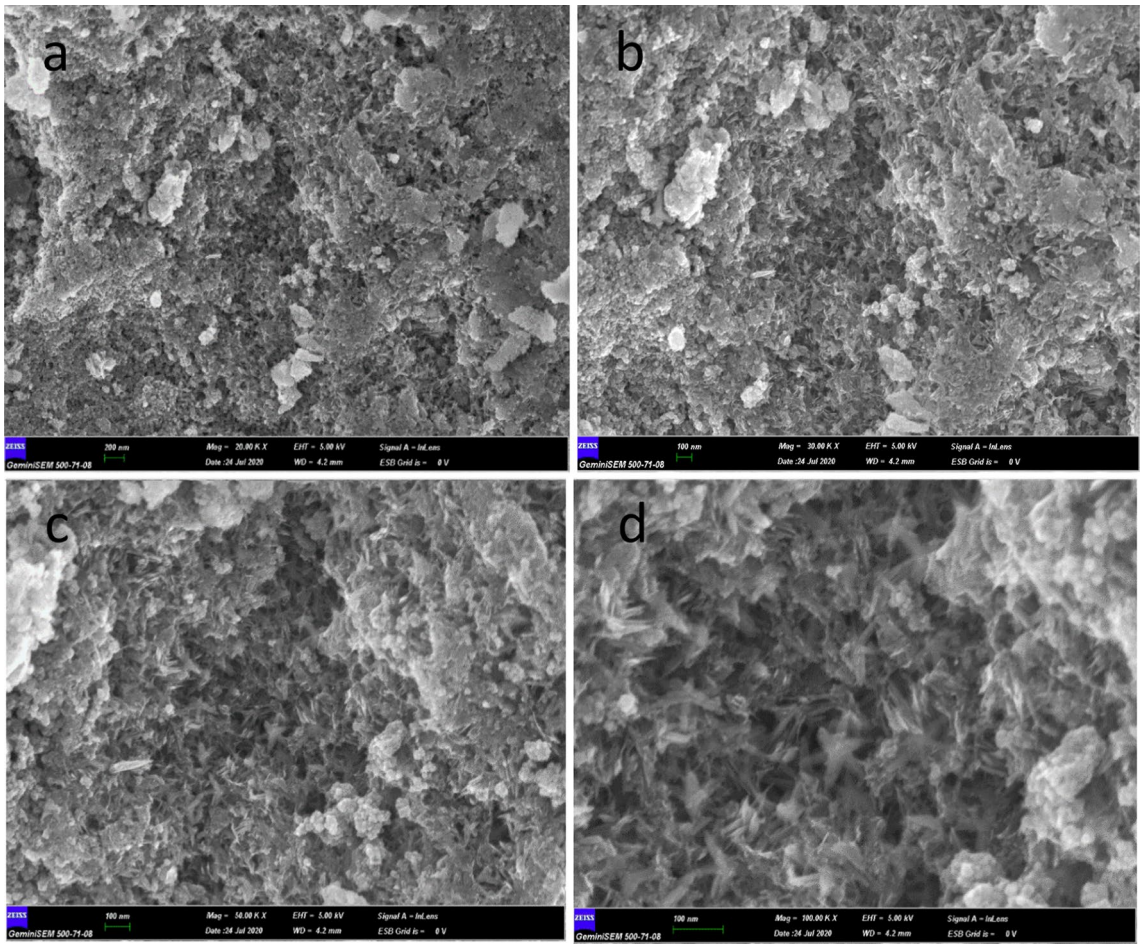


Fig. 4 SEM images of magnetic nanoparticles at **a** (20 k), **b** (30 k), **c** (50 k) and **d** (100 k) magnifications

drugs was investigated using a model solution containing 100 ng mL^{-1} of drugs in the pH range of 2.0–11.0. pH is one of the main parameters since the adsorption of analytes in the aqueous sample solution onto the adsorbent is the basis of solid-phase extraction applications. It creates an optimal environment for both analyte and adsorbent to achieve the best interactions and to accomplish higher analyte adsorption. Therefore, it is one of the first parameters to be optimized by adding Britton Robinson Buffer (BR) series changing from 2 to 11. As shown in Fig. 5, it was observed that high analyte absorption was achieved by shifting the solution pH from the acidic medium to the basic medium, and the extraction efficiency reached its utmost at pH 10. Since a decrease was observed after this pH, pH 10 was determined as the ideal operating condition. pKa values of ANA are 2.01 and 4.78, while pKa values of LET are 3.63, 5.40, and 4.40 (Rusu et al. 2017). If pKa values are considered together with the experimental data, it can be assumed that the deprotonated forms of both molecules are transferred easily. Thus, 0.01 M Britton Robinson's pH 10 buffer was used for the next enrichment procedure.

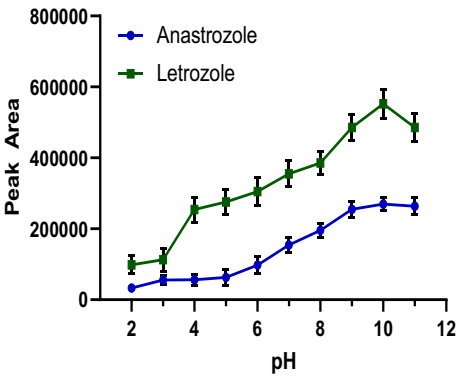


Fig. 5 pH Effect of MSPE

Optimization of adsorption and desorption time

In this optimization step, the adsorption equilibrium must be reached to obtain the best enrichment factor and extraction efficiency of the MSPE procedure. This balance is usually closely related to the adsorption time. Drugs retention

on magnetic particles surfaces is need enough duration of exposure. Considering other studies, it was set the speed of the orbital shaker to 80 rpm. Adsorption time was optimized in the range of 0–90 min for the model solutions including Anastrozole and Letrozole. Regarding the adsorption time, there was a significant increase in extraction efficiency in the range of 5–20 min, and then, no significant increase when the adsorption time exceeded 20 min. As highlighted in Fig. 6a, 20 min as interaction time is enough for analytes because the profiles reach the plateau at this specific time. For this reason, adsorption time was determined as 20 min for maximum extraction efficiency in subsequent MSPE studies.

The next optimization step is the desorption time, that is, the vortex time. The purpose of this process is to provide sufficient desorption of analytes from the adsorbent. For this purpose, the desorption (vortex) time for ANA and LET was studied in the range of 0–90 s. As displayed in Fig. 6b, it was observed that 30 s is a suitable time for quantitative desorption of target molecules. As such, in the subsequent studies, vortex time was maintained at 30 s.

Eluent type and volume

It is of great importance in terms of extraction efficiency to provide the stripping of the analytes from the magnetic particles in the most ideal conditions and to analyze them chromatographically. Different extraction yields were obtained with different solvents such as methanol, ethanol, acetonitrile, acetone to perform the elution process effectively. Figure 7a showed that AcN: MeOH (1:1, *v:v*) exhibited higher extraction recovery than other solvents. Therefore, this mixture was used as an eluent in subsequent studies.

Extraction efficiency and pre-concentration factor are directly related to the final desorption solvent volume. That is why over the range of 200–1500 µL of AcN: MeOH (1:1, *v: v*) were used to clarify the effect of the eluent volume. Moreover, the final desorption solvent volume should be

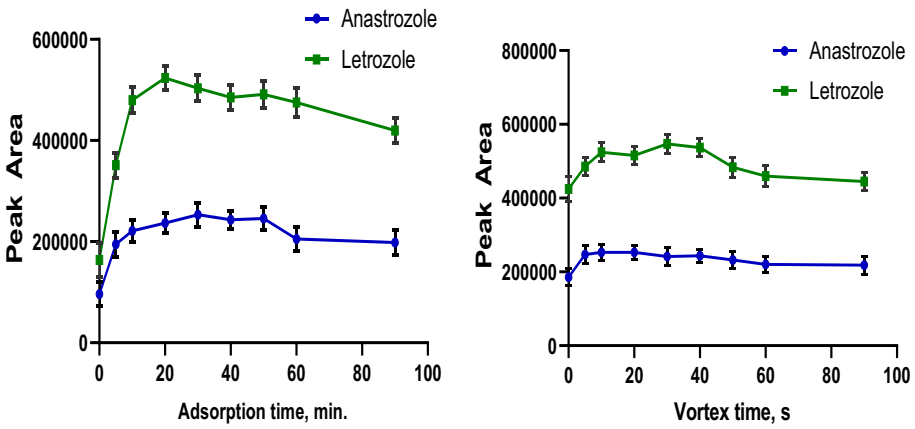


Fig. 6 **a** Optimization of adsorption time. **b** Impact of vortex time on analyte desorption

(a) Optimization of adsorption time desorption

(b) Impact of vortex time on analyte

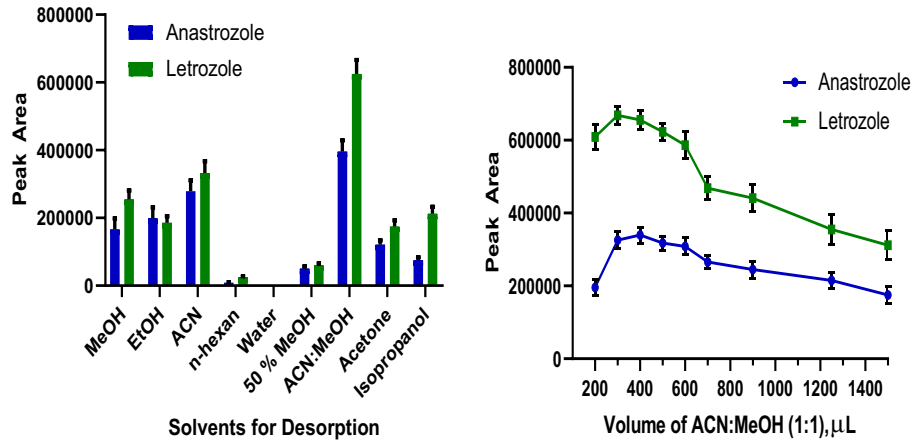


Fig. 7 **a** The effect of solvent on the desorption of target molecules. **b** Optimization of the volume of desorption solvent

(a) The effect of solvent on the desorption of target molecules desorption solvent

(b) Optimization of the volume of

enough for the filtration procedure. The results shown in Fig. 7b revealed that 400 µL of AcN: MeOH (1:1, *v:v*) was adequate to desorb the adsorbed analytes. As expected, the signals were increased by volume in the first points of optimization and decreasing again due to the dilution effect. For this reason, 400 µL of solvent volume was used in subsequent experimental studies.

Reusability and stability of magnetic sorbent

The developed magnetic material can be used in solid-phase extraction of the studied anticancer drug molecules. Separation of magnetic nanoparticles (MNPs) after every use was carried out by an external magnet easily. The reusability of sorbent materials is important parameter in batch-type SPE experiments. This directly effects both analysis cost and repeatability. The reusability tests of the synthesized magnetic materials were performed to observe the stability of the adsorbent to be reused after extraction process. The evaluation was carried out by comparing peak areas of model samples including 100 ng mL^{-1} of both anticancer drugs. After 15 cycle use, the change of peak area for ANA and LET molecules was lower than 10% of RSD. Magnetic material after every use was washed 2 mL of ACN:MeOH (1:1, *v:v*) and 2 mL of ultrapure water. Thus, the synthesized magnetic material was proven to be an effective solid-phase material.

Analytical performance criteria of the method

Under optimized conditions, the developed analytical method was investigated in terms of extraction efficiency, linearity, stability, accuracy, and selectivity. The linearity of this study was measured at 9 concentrations level. The calibration curve for ANA and LET was drawn by peak areas as the y-axis versus concentration as the x-axis. Also, the linear regression equations were calculated simultaneously. By using this equation was determined the slopes, intercepts, and correlation coefficients. According to ICH guidelines (ICH 2005), the calculations for LOD and LOQ were based on the SD of y-intercepts of regression analysis (r) and the slope (S), using the equations LOD = 3.3 r/S, LOQ = 10 r/S, where r is SD of the intercept, and S is the slope. Trueness, expressed in terms of recovery as allowed by method validation guidelines (Sultan 2014; Ostertag et al. 2021), was calculated using the measured and spiked concentration of the target molecules. All analytical figures of the merits method are given in Table 1.

After the ideal optimization processes for the MSPE procedure were completed, all the validation parameters of the newly developed MSPE-HPLC–DAD method were calculated by experimental data obtained using blank human urine samples fortified with different analytes concentration levels. These parameters are LOD, LOQ, the pre-concentration factor (PF), enhancement factor (EF), relative standard deviation (%RSD). Because the MSPE experiments were started with 50 mL of sample solution and the final volume of desorption solution was 0.4 mL after MSPE, the pre-concentration factor (PF) was determined as 125 at the optimization conditions. And also, the enhancement factor (EF) was calculated via calibration slopes of ANA and LET after and before MSPE. The relative standard deviations (RSD) of ANA and LET at a certain concentration (100 ng mL^{-1}) were reckoned by applying the MSPE method with five replications.

Table 2 compares a few analytical parameters of the developed method with published articles. Analysis of these drug molecules mainly focuses on urine and blood samples. As can be seen in Table 2, MS-based methods have better LOD and linearity merits as expected. The developed method has good and comparable results and easily applicable sides. The main aim of separation and pre-concentration methods is to submit an alternative way to an expensive hybrid system for sensitive analysis of target molecules.

Table 1 Analytical merits of the developed method

Parameter	Before MSPE		After MSPE	
	Anastrozole	Letrozole	Anastrozole	Letrozole
Linear range	1.0–20.0 µg mL^{-1}	2.0–20.0 µg mL^{-1}	4.0–160.0 ng mL^{-1}	4.0–160.0 ng mL^{-1}
LOD	0.23 µg mL^{-1}	0.57 µg mL^{-1}	1.14 ng mL^{-1}	1.26 ng mL^{-1}
LOQ	0.66 µg mL^{-1}	1.88 µg mL^{-1}	3.78 ng mL^{-1}	3.86 ng mL^{-1}
RSD % (50 ng mL^{-1})	3.40	3.75	3.25	2.85
Calibration Sensitivity	57.890	81.061	3194	6413
Determination Coefficient (R^2)	0.9985	0.9977	0.9907	0.9916
Pre-concentration factor	–	–	125	125
Enhancement factor	–	–	55.2	79.1

Table 2 Comparison of the new method with other reported methods

Analyte	Pre-treatment Procedure	Determination Method	Limit of Quantification (LOQ)	Linearity	Samples	References
ANA LET	SPE	LC–MS/MS		5–200 ng mL^{-1} 10–300 ng mL^{-1}	Human Plasma	Beer et al. (2010)
LET	SPE	LC–MS/MS	0.5 ng mL^{-1}	0.5–200 ng mL^{-1}	Human Plasma	Gomes et al. (2013)
LET	Conventional	HPLC	0.15 µg mL^{-1}	0.15–100 µg mL^{-1}	Rat Serum	Acharjya et al. (2012)
ANA LET	Conventional	Ultra-fast LC	100 ng mL^{-1}	25–75 µg mL^{-1} 25–75 µg mL^{-1}	Tablet Drugs	Galla et al. (2020)
LET	SPE	UPLC–MS/MS		0.1–100 ng mL^{-1}	Human Plasma	Vanol et al(2016)
ANA LET	FPSE	HPLC–DAD	0.05 µg mL^{-1} 0.25 µg mL^{-1} 0.10 µg mL^{-1}	0.025–10.0 µg mL^{-1}	Human whole blood plasma urine	Locatelli et al. (2018)
ANA LET	MSPE	HPLC- DAD	3.78 ng mL^{-1} 3.86 ng mL^{-1}	4.0–160.0 ng mL^{-1}	Urine samples	This method

Application of the developed method on spiked urine samples

The developed MSPE method was applied to real urine samples after adding two different concentrations of drug to check the accuracy of the method through recovery values. Samples were prepared as explained in Sect. 2.5. Results are given in Table 3. As the analytical data presented in the table, quantitative recovery values were obtained for all samples. The obtained chromatograms obtained from urine samples are presented in Fig. 8.

Conclusions

The novelty of this study demonstrated a practical application of Fe_3O_4@TEPA as an effective adsorbent in MSPE together with HPLC–DAD for the detection of anticancer drugs, such as ANA and LET in urine samples. Therefore, the proposed method was shown to provide simple, fast, well sensitive, accurate, and excellent recovery of enrichment and separation of ANA and LET. This proposed approach was successfully employed for analyzing simulated synthetic urine and real urine samples. The proposed procedure and the newly synthesized material have shown convincing analytical performances to predict the future usage of the proposed methodology in routine analyses in clinical laboratory.

Table 3 Results of simulated urine and real human spiked urine samples analyses

Sample	Added ng mL^{-1}	Found[a] ng mL^{-1}		RSD %		Recovery %	
		ANA	LET	ANA	LET	ANA	LET
Simulated Urine	0.0	< LOD	< LOD	–	–	–	–
	25.0	26.7 ± 1.5	23.4 ± 1.3	4.3	5.5	106.8	93.6
	50.0	48.1 ± 2.4	47.3 ± 2.0	4.9	4.2	96.2	94.6
Urine 1	0.0	< LOD	< LOD	–	–	–	–
	25.0	26.1 ± 1.6	24.8 ± 1.4	6.1	5.6	104.4	99.2
	50.0	49.2 ± 2.3	50.3 ± 2.2	4.7	4.4	98.4	100.6
Urine 2	0.0	< LOD	< LOD	–	-	–	-
	25.0	26.2 ± 1.2	27.1 ± 1.7	4.6	6.2	104.8	108.4
	50.0	51.8 ± 2.0	52.5 ± 1.9	3.8	3.6	103.6	105.0
Urine 3	0.0	< LOD	< LOD	–	–	–	–
	25.0	27.0 ± 1.3	25.4 ± 1.1	4.8	4.3	108.0	101.6
	50.0	53.0 ± 2.2	50.7 ± 2.9	4.2	5.7	106.0	101.4

[a]N:5, Mean ± SD

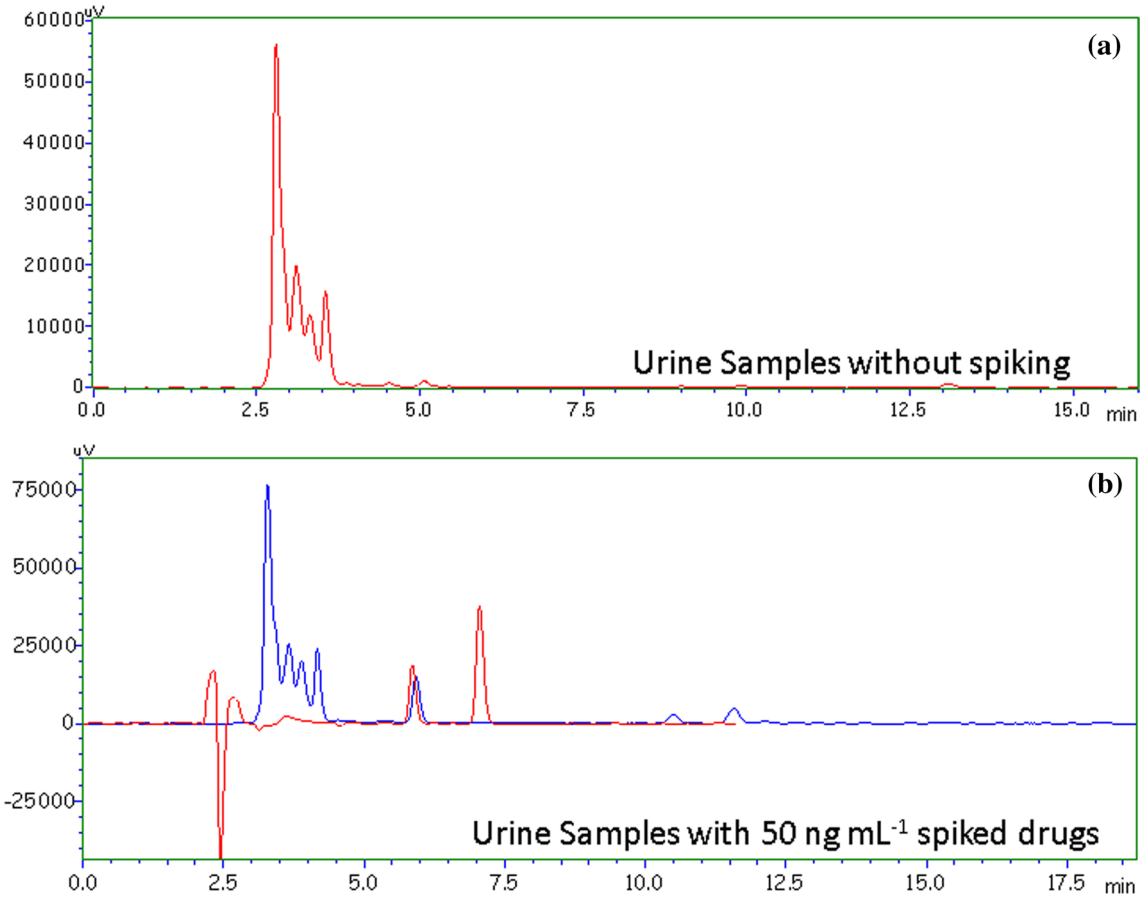


Fig. 8 Chromatograms obtained from urine samples A: without and B: with spiked ANA and LET Molecules

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Declarations

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

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