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Sensitive determination of Fluoxetine and Citalopram antidepressants in urine and wastewater samples by liquid chromatography coupled with photodiode array detector

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ABSTRACT

A new analyte separation and preconcentration method for the trace determination of antidepressant drugs, Fluoxetine (FLU) and Citalopram (CIT) in urine and wastewaters, was developed based on HPLC-DAD analysis after magnetic solid phase extraction (MSPE). In the proposed method, FLU and CIT were retained on the newly synthetized magnetic sorbent (Fe_3O_4@PPy-GO) in the presence of buffer (pH 10.0) and then were desorbed into a lower volume of acetonitrile prior to the chromatographic determinations. Before HPLC analysis, all samples were filtered through a 0.45 µm PTFE filter. Experimental parameters such as interaction time, desorption solvent and volume, and pH were studied and optimized in order to establish the detection limit, linearity, enrichment factor and other analytical figures of merit under optimum operation conditions. In the developed method, FLU and CIT were analyzed by diode array detector at the corresponding maximum wavelengths of 227 and 238 nm, respectively, by using an isocratic elution of 60% pH 3.0 buffer, 30% acetonitrile, and 10% methanol. By using the optimum conditions, limit of detections for FLU and CIT were 1.58 and 1.43 ng mL^{-1}, respectively, while the limit of quantifications was 4.82 and 4.71 ng mL^{-1}, respectively. Relative standard deviations (RSD%) for triplicate analyses of model solutions containing 100 ng mL^{-1} target molecules were found to be less than 5.0 %. Finally, the method was successfully applied to urine (both simulated and real healthy human) and wastewater samples, and quantitative results were obtained in recovery experiments.

1. Introduction

Therapeutic drug monitoring (TDM) is one of the most important research areas in drug discovery and development process used in pharmaceutical research. a TDM of antidepressants is necessary for an optimal supervision of patient drug regimen to avoid medical non-responsiveness, intoxication, complications or non-compliance [1,2]. Antidepressants have seen exponential growth in their use during the last couple of decades. Many antidepressants act by blocking the reuptake of norepinephrine and serotine substances in the brain [3]. When their structures are examined, it is seen that most of them have tricyclic or tetracyclic nuclei. These drugs are generally used for the treatment of mental depression which has become a health problem in many parts of the society today, causing loss of productivity and workforce in many areas of life. In some cases, it imposes substantial economic losses with the treatment process. If both drugs are used before the recommended time, it causes muscle stiffness, heart rhythm, sudden changes in blood pressure, fainting, and clouding of the mind called serotonin syndrome. If it is used under the clinical supervision, the liver interacts less with the toxin enzyme [4].

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Fluoxetine {*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propane-1-amine} was the first selective serotonin reuptake inhibitor (SSRI), synthesized and marketed under the name Prozac®, produced by the pharmaceutical company Eli Lilly [5,6]. Its pharmacologically active metabolite, norfluoxetine, derived from the biological N-demethylation of fluoxetine. This active metabolite has prolonged action with clinical activity of inhibition of the reuptake of 5-HT and inhibition of cytochrome P450 isoenzymes in the liver. Fluoxetine is metabolized by the CYP2D6 enzyme, such as neuroleptics and tricyclic antidepressants [7]. The drug is generally used in the treatment of diseases with similar effects belong to the antidepressant drugs group known as SSRIs.

Citalopram is a bicyclic phthalate and belongs to the SSRI family. It is a racemic drug used for the treatment of depression with the *S*-enantiomer being the pharmacologically active compound [8]. Citalopram (1-[3-(*dimethyl amino*) *propyl*]−1-(4-fluorophenyl)−1,3-dihydro-5-isobenzofurancarbonitrile is a "second generation" antidepressant drug, whose pharmacological activity is based on the selective serotonin reuptake inhibition. Its efficacy is comparable to tricyclic antidepressants, but it is better tolerated and is characterized by a lower risk of adverse effects [9].

In many drug formulations, active principle is one of the components, and in this scenario, after the drug consumption it is important to analyze in complex matrices (biological and/or environmental) the concentrations in terms of both monitoring the therapeutic dose and monitoring the excretion products after use. Two main problems encountered in these analyses are, in most cases, the complexity of the sample matrix and the concentration of the target molecules below the detection limits of the chromatographic system. In order to overcome these problems, it is preferred to use separation and enrichment methods with a suitable carrier system.

Several methods have been published for the determination of one or more antidepressants in complex matrices (biological fluids and environmental samples) for therapeutic drug monitoring, for toxicological purposes, or for environmental pollution evaluation. The methods available in the literature for trace determination of Fluoxetine and Citalopram are based on ion transfer stripping voltammetry [10], liquid chromatography–mass spectrometry LC–MS methods [11,12] and gas chromatography–mass spectrometry (GC–MS) [13], spectrofluorometric determination [14,15], liquid chromatography techniques [16–18], adsorptive square wave voltammetry (ASWV) [19]. However, due to the trace levels of antidepressants in complex matrices and the disruptive effects of the matrix components, clean up and preconcentration techniques have become an inevitable stage prior to the analysis of these drugs [20].

Magnetic solid phase extraction (MSPE), as a versatile approach of SPE, is carried out based on adsorption and desorption of the target molecules on a magnetic material. The used external magnetic field without some tedious steps (centrifugation or filtration) facilitates extraction steps. Sorbent particles can be easily isolated and collected during adsorption and desorption, making the sample pretreatment procedure more convenient, time-saving and cost-effective [21,22]. When magnetic solid phase extraction methods in the literature are examined, it is seen that carbon-based nanoparticles such as carbon nanotubes (CNTs), graphene oxide grafted nanostructures, nano diamond and carbon nanofibers (CNFs), which have high surface area and adsorption capacity and can be used repeatedly due to their inertness in the working solution environments, are frequently preferred as adsorbents [22–27].

In this study, Fe_3O_4 nanoparticles (NPs) were coated with graphene oxide-polypyrrole polymer (PPy-GO) and characterized by instrumental methods. The graphene oxide was preferred as a supporting material in order to provide multi imprinting sites, large surface area, and easy separation of magnetic nanocomposites. Then, the capability of these new sorbent (Fe_3O_4@PPy-GO) for simultaneous preconcentration and determination of two widely used antidepressant drugs (Fluoxetine and Citalopram) as model compounds were studied and examined by using magnetic solid phase extraction and HPLC-DAD system. Finally, the applicability of the proposed method was successively investigated for the extraction and determination of CIT and FLU in simulated urine, urine from healthy volunteers, and wastewater samples.

2. Materials and methods

2.1. Instrumentation

Characterization of synthesized magnetic nanoparticles was carried out using Raman spectroscopy, X-ray diffraction spectroscopy and scanning electron microscopy techniques. The Raman spectra of the nanomaterials were obtained using a Raman Spectrophotometer (WITEC alpha 300 M + micro-Raman system, Germany) with a 532 nm laser source. X-ray diffraction spectrum of magnetic nanoparticles was taken with a Bruker AXS D8 brand X-ray diffractometer. Scanning electron microscopes (SEM) and SEM Mapping analyses were performed using scanning electron microscopy (Zeiss Gemini 500 Field Emission Scanning Electron Microscope) to elucidate the morphological structures of magnetic nanoparticles. FT-IR spectra for the materials were obtained through the Perkin-Elmer Spectrum 400 FT-IR spectrometer (Waltham, MA). Chromatographic analysis of Fluoxetine and Citalopram were performed by the Shimadzu (Prominence) HPLC (Kyoto, Japan) system. All separations and determinations were performed on a phenyl hexyl column (Luna® 5 µm Phenyl-Hexyl 100 Å, 250 mm × 4.6 mm) under isocratic conditions.

2.2. Chemicals and reagents

In this study, all chemicals used were out or higher than 99.5% purity. Deionized water system with 18.2 MΩ cm resistivity was used to obtain deionized water (MES, MP Minipure Dest Up, Turkey). The HPLC grade acetonitrile (ACN) and methanol were used for HPLC-DAD analysis (Sigma Aldrich, St. Louis, MO, USA) without further purification steps. For HPLC analysis, were used a mixture of phosphate buffer solution (pH 3.0, 50 mM), methanol and acetonitrile (60:10:30) as mobile phase under isocratic elution conditions. Stock solutions of Fluoxetine (FLU) and Citalopram (CIT) (Sigma Aldrich, St. Louis, MO, USA) were prepared in methanol and calibration mix standards were prepared by serial dilutions. FLU was racemic standard, while CIT is a pure standard.

2.3. HPLC determination conditions

A phenyl-hexyl column was used as the most suitable stationary phase in this analysis. In order to determine the better mobile phase compositions, the mobile phases containing buffers at different pH values and various organic phase compositions were tested in order to obtain the most suitable conditions in terms of peak resolutions and symmetry. Optimized HPLC conditions in this work were given in Table S1, while in Fig. 1 was reported the chromatogram showing the peaks profile gained by increasing calibration standards concentration under the described conditions. All quantitative determinations were performed at 238 nm for CIT and 227 nm for FLU.

2.4. Synthesis of magnetic nanoparticles

2.4.1. Synthesis of graphene oxide by the Hummer method

3.0 g of graphite powder was added to the flask, which was cooled to 0 °C in an ice bath, and 70 mL of concentrated sulfuric

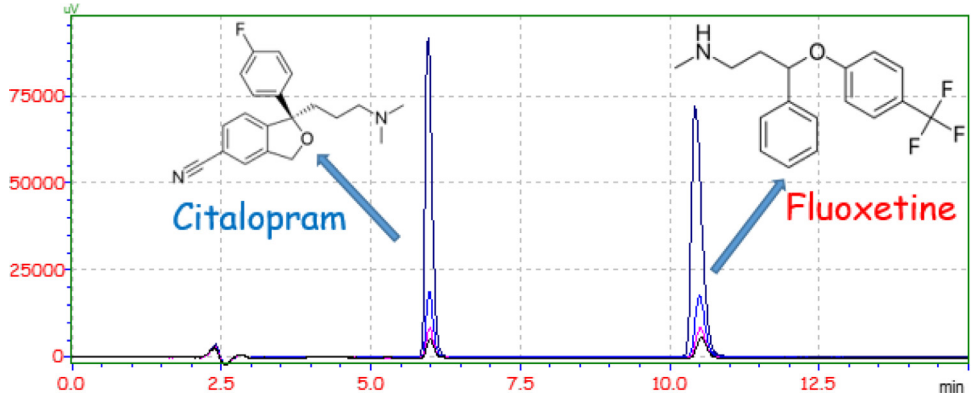

Fig. 1. Chromatogram of antidepressant drugs reported at 227 nm (wavelength where both the two molecules can be observed).

acid (≥ 99.9%) was slowly transferred onto graphite. Under vigorous stirring, 9.0 g of potassium permanganate, $KMnO_4$, was added to this reaction mixture and the reaction temperature was kept at around 20 °C for 30 min. Then, the reaction mixture was stirred at 40 °C for 30 min more. 150 mL of deionized water was added to the mixture and the reaction temperature was raised to 95 °C on a magnetic stirrer. After refluxing the reaction mixture at 95 °C for 15 min, 500 mL of deionized water and 15 mL of 30% (*w/v*) H_2O_2, hydrogen peroxide, were added to the reaction mixture, and the reaction was allowed to continue for 10 min. After this step, the reaction mass was cooled to room temperature. The brown-yellow reaction mixture was filtered and washed with 10% (*w/v*) hydrochloride acid, to remove unreacted reagents. The product obtained was then dried in an oven at 50 °C for 24 h.

2.4.2. Synthesis of magnetite graphene oxide

0.5 g of graphene oxide, which was synthesized in the previous step, was pulverized and weighed carefully. A mixture of 0.5 g of Iron (III) chloride and 2.0 g of sodium acetate, previously homogenized in 20 mL ethylene glycol, was added to the graphene oxide particles. After being kept in an ultrasonic bath for 10 min, it was transferred to an autoclave for hydrothermal synthesis. The hydrothermal synthesis unit was allowed to react at 180 °C for 12 h. After the reaction, the product was washed twice with ethanol and once with deionized water and allowed to dry in an oven at 70 °C.

2.4.3. Synthesis of magnetite graphene oxide-poly pyrrole (PPy) nano composite material (Magnetic PPy/GO)

0.5 g of the synthesized magnetite graphene oxide was weighed and dispersed in 200 mL of deionized water. Later, 500 µL of pyrrole was added to the mixture in an ice bath and 1.6 g of ammonium persulfate solution dissolved in 10 mL of water. This solution was added dropwise to the reaction medium. Stirring was continued until the reaction was complete. The synthesis product formed on the surface of the solution was separated from the mixture by filtration. It was washed 2 times with deionized water during the filtration process. It was left to dry in an oven at 70 °C.

2.5. Magnetic solid phase extraction

50 mg of Fe_3O_4@PPy-GO was weighed and transferred to 50 mL of falcon tubes. Then, 20 mL of sample solution including FLU and CIT in the range of 5.0–500.0 ng mL^{-1} and the volume of the tube was filled to 50 mL with distilled water. Falcon tubes were tightly closed and placed in orbital shaker device by setting 100 rpm for 20 min. After the time was over, magnetic particles were separated by using an external magnet, then 800 μL of acetonitrile was added and the tubes were vortexed for 40 s for the target molecules desorption. The ACN phase was taken into an injector, was passed through the 0.45 μm injector tip filter and transferred to the vials and placed in the HPLC device. The contents of samples for Fluoxetine and Citalopram were determined by HPLC-DAD system.

2.6. Preparation of simulated urine samples and wastewater samples

The application of proposed method was carried out by simulated urine, healthy human urine, and wastewater samples. Content of simulated urine samples was prepared as mentioned in literature [28–30]. 25.00 g of urea, 1.08 g of calcium chloride, 1.00 g of ammonium chloride, 1.60 g of potassium chloride, 1.40 g of sodium sulfate, 1.40 g of potassium dihydrogen phosphate, and 2.92 g of sodium chloride were dissolved in 1 L of ultra-pure water. The pH of simulated urine solution was adjusted to 6.0 using sodium hydroxide (0.1 M) or hydrochloride acid (0.1 M). The mixture was stirred on a magnetic stirrer for 15 min and kept in an ultrasonic water-bath. T The obtained solutions were stored in amber glass bottles until analysis.

The healthy human urine samples were collected in a capped sterile test tube from 2 of volunteers free from any kind of medication who had been informed about the experimental procedure and the nature of the study. All samples were left at room temperature for 20 min and then centrifuged for 10 min at 4000 rpm [31].

Wastewater sample was obtained from main wastewater discharge line of Cumhuriyet University in Sivas, Turkey. Wastewater samples were collected in amber glass bottles and immediately filtered through 0.45 µm cellulose nitrate membrane. Subsequently, pH of samples were adjusted to 3 to reduce biological activity [32] and were stored in the dark at +4 °C until analysis.

2.7. Method validation

The method validation was carried out according to International Conference on Harmonization guidelines [33–34]. Analytical figures of merit such as enhancement factor (EF), preconcentration factor (PF), relative standard deviation (RSD), limit of detection (LOD), limit of quantification (LOQ), linear range and correlation coefficient were calculated by considering correctness and sensitivity of the method.

Preconcentration factors (PF) were calculated by using the ratio of the initial solution volume (50 mL) to the last elution solvent volume (0.8 mL). The enhancement factors (EF) were obtained from the ratio of the slope of calibration curve of the analytes after MSPE application to that of prior MSPE application. The relative standard deviations (RSD%) were found by applying the MSPE method for seven repetition analysis, which includes 100 ng mL^{-1} of CIT and FLU. The LODs and LOQs values herein reported were

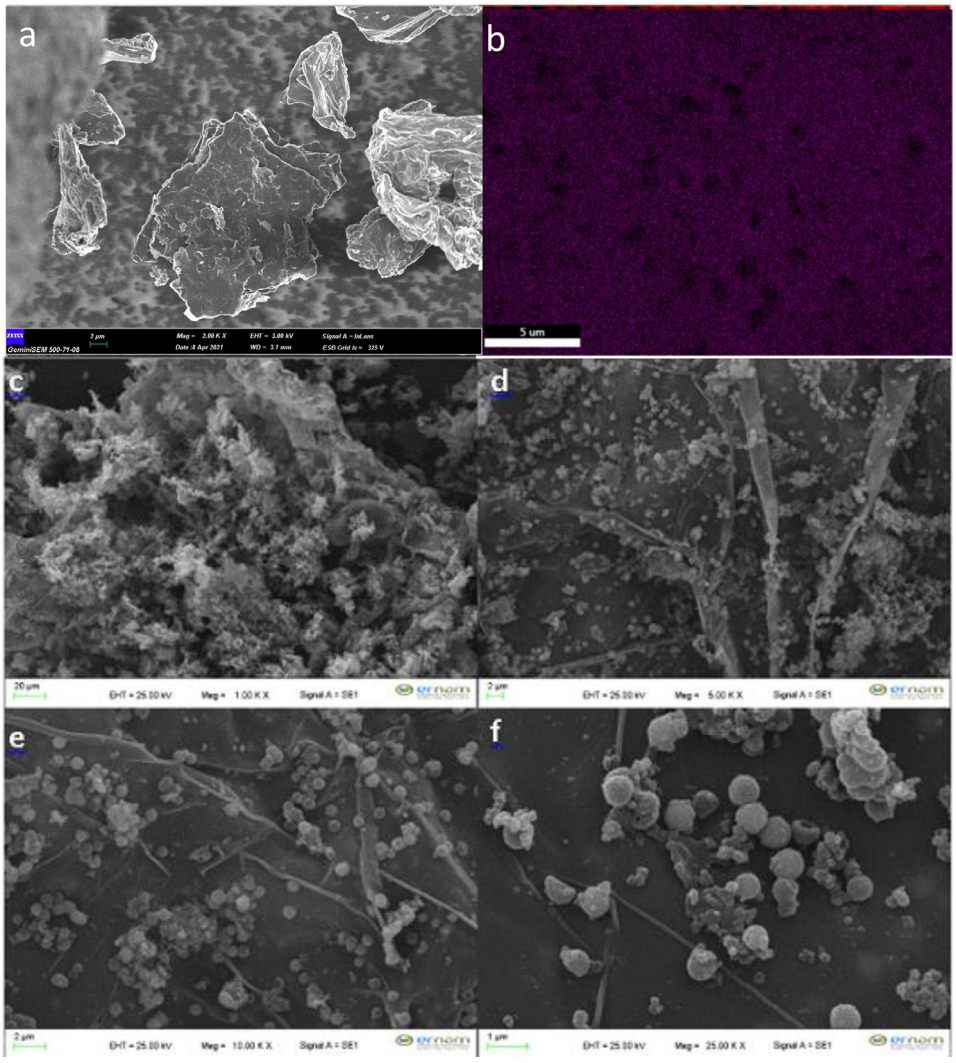

Fig. 2. SEM images of the developed magnetic material (a) Low-layer graphene oxide; (b) Formation of Fe_3O_4 magnetic particles by SEM-Mapping analysis; (c–f) The modification of magnetic graphene oxide with polypyrrole and the formation of polypyrrole particles.

obtained by means of the signal-to-noise ratio. Specifically, LOD was defined by a signal-to-noise ratio of 3:1, while the LOQ was defined by a signal-to-noise ratio of 10:1, accordingly to International Guidelines [34].

3. Results and discussions

3.1. Characterization of the magnetic nanoparticles

The results of FTIR analysis for the graphene oxide, magnetic graphene oxide and polypyrrole magnetic graphene oxide components used in the synthesis of the magnetite graphene oxide-polypyrrole nanomaterial material were given in the Fig. S1. The FTIR spectrum of graphene oxide is compatible with other studies currently available in the literature. Characteristic peaks of graphene oxide were detected: (C-O-C) (1230–1320 cm^{-1}), sp^2-hybrid C = C (1500–1600 cm^{-1}, in-plane vibrations), (COOH) (1650–1750 cm^{-1}, 3530 cm^{-1} carboxyl vibration modes.

Magnetic graphene oxide peaks are 588 cm^{-1}, known as the Fe-O characteristic peak, 1651 cm^{-1} (C = O) symmetrical stretching vibration peaks at 1085 cm^{-1} (C-O). In FTIR analysis of magnetic graphene oxide-polypyrrole nanomaterial as the end product of the synthesis, NH symmetric stretching vibration at 3271 cm^{-1} wavelengths, 3123 cm^{-1} (OH), 1714 cm^{-1}, 1614 cm^{-1}C=O stretching peaks with bending vibration of 1219 cm^{-1} (C-N)and 978 cm^{-1} (C-N) can be attributed to the presence of polypyrrole in the composite material.

In the SEM images given in Fig. 2 of the magnetic graphene oxide-polypyrrole nanomaterial, the layer in the form of a web cover, which is seen to corroborate with the studies in the literature, is known as "graphene oxide". It can be seen from the transparent SEM images that low-layer graphene oxide is successfully produced from graphite (Fig. 2a). The formation of Fe_3O_4 magnetic particles has been proven by SEM-Mapping analysis of Fe (Fig. 2b). As a result of the modification of magnetic graphene oxide with polypyrrole, the formation of polypyrrole particles is observed (Fig. 2c–f).

Fig. S2a shows the characteristic peaks of the synthesized graphene oxide (GO) in the D and G bands, which are compatible with the literature. It is easily understood from the fact that the D band of GO is more dominant than the G band where graphene oxide is successfully synthesized from graphite. Fig. S2b contains the Raman spectrum of magnetic graphene oxide. In the spectrum, it was observed that magnetite graphene oxide was synthesized from graphene oxide, and the suppression in the D and G bands can be clearly seen from the change in the ratio of the peaks to each

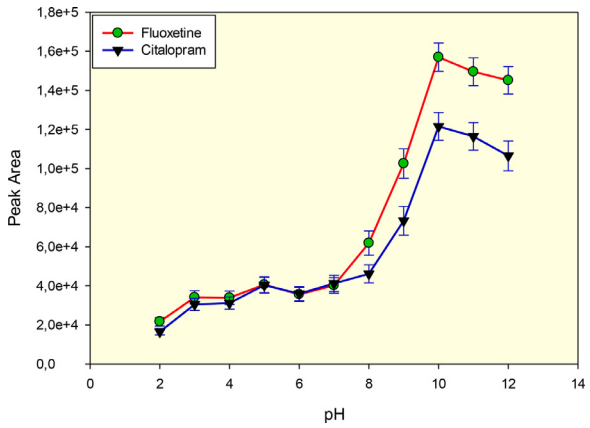

Fig. 3. pH effect on the developed method on analytes peak area (200 ng/mL).

other. Fig. S2c shows the Raman spectrum of the synthesis of pyrrole and magnetite graphene oxide. It was observed that the peaks at wavelengths of 978 cm^{-1} and 1047 cm^{-1} belong N-H bonds in pyrrole in the literature and that these peaks originated from pyrrole were formed in this spectrum and the desired structure was obtained as a result of the synthesis reaction. In Fig. S2d, it is seen that the spectra of magnetite graphene oxide and magnetite graphene oxide-pyrrole synthesis are overlapped, and the Raman shifts and the change in D and G band ratios can be easily noticed.

3.2. Extraction optimization experiments

The objective is to keep the analyte type in the solid phase at the highest possible level and to separate it from other substances in the environment, and after the separation process is achieved, all of the analytes in the solid phase pass into the solvent. Preliminary trials were made to determine the necessary parameters to achieve this. It was aimed to obtain a fast and easy separation process and to obtain the highest concentration of analyte by using as little amount of organic solvent as possible. Accordingly, a chromatographic method before MSPE experiments was developed for simultaneously determination fo CIT and FLU molecules by optimizing all parameters.

3.3. pH effect

Ambient pH is an important factor as it affects the adhesion of the analyte to the solid phase and the reactions between species. Model solutions containing both antidepressants respectively were interacted with a series solution in the range of 2.0–12.0. All experiments were studied with 50 mg of Fe_3O_4@PPy-GO. Following these processes, FLU and CIT molecules were retained on solid phase and separated with an external magnet. After desorption of target molecules, eluent solvent phase was transferred by a syringe and filtered through a 0.45 μm PTFE membrane filter, transferred into HPLC vials and subsequently injected into the HPLC system.

As can be illustrated in Fig. 3, the optimum pH value for the enrichment steps was observed at a pH value of 10.0. A literature review revealed that, the pKa value for Fluoxetine is 8.70, while the pKa value for citalopram is 9.50 [35]. As can be seen in the Fig. 3, the efficiency of extraction reaches to maximum beyond these values. Therefore, pH 10.0 was selected as the optimum for subsequent experiments.

3.4. Effect of Adsorption and Desorption time of extraction process

Adsorption and desorption processes of drug molecules on the surface of magnetic nanoparticles are carried out by means of an equilibrium process, guided by the partition coefficient of the analyte between the aqueous sample matrix and the magnetic nanoparticle-based sorbent. Generally, binding of the target molecules to solid sorbent needs longer time due to the slow diffusion of the analytes into the solid sorbent and the sluggish rate of the mass transfer of analytes from the bulk to the sorbent in absence of any external effect. To increase the mass transfer kinetics, this process was facilitated with an orbital shaker or a rotator by means of increasing interactions between sorbent and molecules. Desorption process is faster than adsorption because a pure and clean solvent is used to remove molecules directly from the sorbent surface. Rate and efficiency of desorption are increased by using vortex at high speed (up to 100 rpm). Time for both the processes should be optimized in order to find optimal extraction conditions. The first step of extraction (adsorption) was carried out by using an orbital shaker with 100 rpm. Model solutions including both molecules and adsorption time was studied in the range of 0–90 min. As can be seen in Fig. 4a, 20 min is enough for high recovery. Desorption time on the vortex was also studied in the range of 0–90 s as can be seen in Fig. 4b. The results show that 20 min for adsorption and 40 s for desorption are acceptable for the optimal extraction and desorption, respectively.

3.5. Eluent type and volume

After liquid sample matrix was seperated by using a syringe, retained target molecules on sorbent are removed by using an suitable solvent. The ideal solvent must be suitable with the chromatographic system and will not decompose molecular structure of the drugs. Various solvents were tried to find out the best solvent for desorption of both molecules. Experimental procedure with all steps was optimized by using 1 mL of various solvent in the last step. Methanol, ethanol, acetonitrile, water, isopropanol, acetone, 50% MeOH, *n*-hexane, and mixture of acetonitrile: methanol (1:1, *v:v*) were used as desorption solvent. As can be seen in Fig. 5a, the best signals were achieved with acetonitrile for both molecules. After the ideal solvent was determined as acetonitrile, the next optimization procedure was volume of the solvent used for desorption. The applied magnetic solid phase extraction procedure is based on the preconcentration of target molecules by means of decreasing volume of solution with extraction. The final volume of desorption solvent directly effects on the success of extraction procedure. In an ideal situation, the volume of desorption solvent should be at a minimum level for the maximum preconcentration factor which is evaluated by the first and last volume of solution, even if the recovery of drug molecules from the sorbent surface will be low due to weak interactions between solid and liquid phases. Moreover, the filtration process is not easy with volumes lower than 200 uL. Consequently, this optimization is also important for an ideal method. Volume of acetonitrile was studied in the range of 200–1500 µL. As can be seen in Fig. 5b, the highest signals were obtained with 800 µL of acetonitrile and this volume was selected for desorption process.

3.6. Reusability of magnetic nanoparticles

As known, development of new sorbents for drug residues in various medium is challenging for new studies. The developed sorbents can be used in many different areas such as drug delivery systems, adsorption studies, and solid phase material for commercial columns, etc. One of the most important indicators for a new sorbent is its robustness and reusability. As explained in the synthesis of magnetic particles section, the used NPs was prepared step by step in order to increase its durability. All experiments were carried out by using 50 mg of sorbent. In order to test reusability of the magnetic particles, all experimental steps

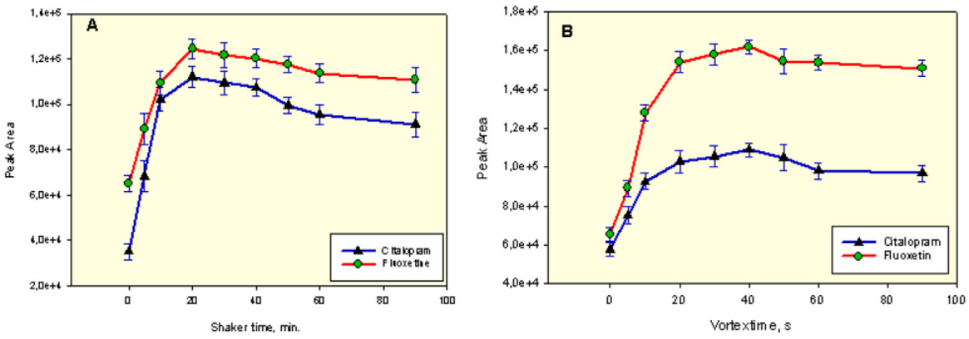

Fig. 4. Optimization of adsorption (A) and desorption (B) time on analytes peak area (200 ng/mL).

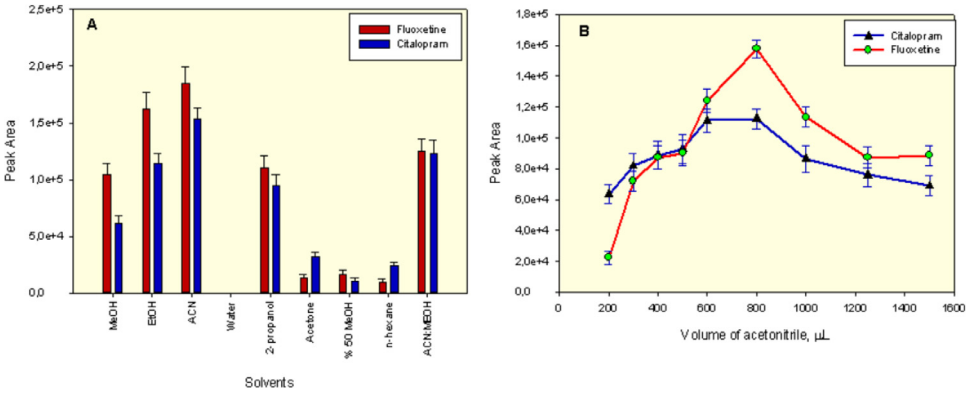

Fig. 5. Optimization of desorption solvent (A) and its volume (B) on analytes peak area (200 ng/mL).

in the optimized conditions were repeated by using model solutions including 100 ng mL^{-1} of both drug molecules. After every use, the NPs was washed 2 mL of acetonitrile: methanol mix and 1 mL of water. 50 mg of Fe_3O_4@PPy-GO was weighed again after it was dried in 40 °C. The evaluation of reusability was carried out by comparing peak areas after every use. After 20-cycles, the change of peak area for FLU and CIT molecules was lower than 10% of RSD%.

3.7. Analytical performances

Analytical validation of new MSPE-HPLC-DAD method was carried out after the developed magnetic solid phase extraction procedure was optimized systematically. The developed MSPE based methodology was applied to model solutions containing increasing concentrations of CIT and FLU antidepressants to determine the linear working range. The linear calibration curves for both molecules were found in the range of 5.0–500.0 ng mL^{-1}. Linearity of method describes the direct proportionality between the concentration of CIT and FLU molecules in model solutions and peak areas. 10 of calibration standards in the linear range were tested for 3 replicate analysis. All the analytical figures of merit (LOD, LOQ, RSD (%), Slope of Calibration, R^2, Preconcentration Factor, and Enhancement Factor) were reported in Table 1. In Table 2 were also reported the values of precision and trueness (recovery) observed for the real sample analyses, as reported in the next paragraph.

3.8. Analysis of real samples

Simulated urine, healthy human urine, and wastewater samples were analyzed in order to investigate the applicability of the proposed method by means of recovery tests. CIT and FLU contents of the studied samples were shown in Table 2. In none of the samples were detected both drug molecules. The recoveries of target molecules in the spiked samples were in the range of 96.2–104.8. Recovery values were calculated by using the ratio of the found amount of drugs to their spiked concentrations. These satisfactory results demonstrate that the prosed MSPE based HPLC-DAD method is suitable for trace determination of both drug molecules in the real samples.

3.9. Comparison of analytical figures of merit with the methods published in the literature

A comparison table with existing literature was given Table 3. The developed method has comparable merits with more complex approaches mass spectrometer (MS) based methods. As known, analysis cost is higher in MS based methods. The applicable linear range and convenience of simultaneous analysis of two antidepressants with the developed method are among the major advantageous features of this study. The reproducibility of the procedure highlights how this method can be effectively applied in different types of samples, both biological and environmental.

4. Conclusions

In the present work, the combined magnetic solid phase extraction procedure and HPLC-DAD method was examined as the extraction sorbent for the MSPE for CIT and FLU molecules. The proposed approach showed good sensitivity, wide linearity, simple operation, and excellent recovery for the selected antidepressant molecules. This proposed approach was successfully employed for analyzing simulated urine, healthy human urine, and wastewater samples. Briefly, polypyrrole coated NPs (Fe_3O_4@PPy-GO) was synthesized as a magnetic sorbent in SPE experiments for CIT and FLU in real samples before HPLC-DAD analysis, and several parameters were optimized to achieve optimum extraction conditions.

Table 1
Analytical figures of merit of the new method.

Parameter	Before MSPE Fluoxetine	Citalopram	After MSPE Fluoxetine	Citalopram
Linearity	1.0–20.0 μg mL^{-1}	1.0–20.0 μg mL^{-1}	5.0–500.0 ng mL^{-1}	5.0–500.0 ng mL^{-1}
LOD	0.38 μg mL^{-1}	0.32 μg mL^{-1}	1.58 ng mL^{-1}	1.43 ng mL^{-1}
LOQ	1.88 μg mL^{-1}	1.90 μg mL^{-1}	4.82 ng mL^{-1}	4.71 ng mL^{-1}
RSD (%)	4.7	3.8	3.2	3.5
Slope of Calibration	18.27	15.27	1425.14	1266.99
(R^2)	0.9975	0.9986	0.9954	0.9873
Preconcentration Factor	–	–	62.5	62.5
Enhancement Factor	–	–	78	83

Table 2
Analytical results obtained from real sample analyses using the developed method.

Samples	Added ng mL^{-1}	Found[a] ng mL^{-1} Fluoxetine	Citalopram	RSD% Fluoxetine	Citalopram	Recovery% Fluoxetine	Citalopram
Simulated	0.0	<LOD	<LOD	–	–	–	–
Urine	100.0	98.7 ± 4.1	104.8 ± 4.5	4.2	4.3	98.7	104.8
	250.0	255.1 ± 12.5	240.5 ± 11.5	4.9	4.8	102.0	96.2
Urine	0.0	<LOD	<LOD	–	–	–	–
1	100.0	95.4 ± 3.8	98.7 ± 3.5	3.9	3.5	95.4	98.7
	250.0	242.7 ± 10.5	246.8 ± 9.7	4.3	4.8	97.1	98.7
Urine	0.0	<LOD	<LOD	–	–	–	–
2	100.0	106.7 ± 5.0	95.8 ± 3.7	4.2	3.9	106.7	95.8
	250.0	239.4 ± 11.1	253.2 ± 10.7	4.6	4.2	95.7	101.3
Wastewater	0.0	<LOD	<LOD	–	–	–	–
1	100.0	99.8 ± 4.8	98.8 ± 3.5	4.8	3.5	99.8	98.8
	250.0	242.5 ± 11.2	255.9 ± 12.8	4.6	5.0	97.0	102.4
Wastewater	0.0	<LOD	<LOD	–	–	–	–
2	100.0	103.5 ± 3.6	104.5 ± 3.7	3.5	3.5	103.5	104.5
	250.0	259.5 ± 12.7	260.1 ± 12.5	4.9	4.8	103.8	104.0

[a] The average value of five replicates ± standard deviation ($N = 5$).

Table 3
Comparison of the analytical figures of merit of the new method with other methods published in the literature.

Target Molecules	Pre-treatment Procedure	Determination Method	LOD	Linearity	Samples	References
FLU	SPE	HPLC	25 ng mL^{-1}	25–500 ng mL^{-1}	Human Plasma	[36]
CIT	SPE	Capillary	8.1 ng L^{-1}	48.6–243.0 ng mL^{-1}	Human Plasma	[37]
FLU		Chromatography	9.3 ng L^{-1}	49.4–463.5 ng mL^{-1}		
CIT	SPE	LC–MS/MS	25 ng L^{-1}	2 – 346 ng L^{-1}	Spring and waste	[33]
FLU					water	
FLU	Liquid-liquid microextraction	GC–MS	3 ng mL^{-1}	10 - 500 ng mL^{-1}	Plasma	[38]
FLU	Stir Bar Sorptive Extraction	LC-MS	3 ng mL^{-1}	10–500 ng mL^{-1}	Human Plasma	[39]
CIT	Micro Solid phase extraction	HPLC-UV	0.2–1.0 ng mL^{-1}	2–800 ng mL^{-1}	Biologic samples	[40]
CIT	SPE	Chiral HPLC Method	10 ng mL^{-1}	100–500 ng mL^{-1}	Human Plasma	[41]
CIT	Molecular imprinting polymer/SPE	HPLC	0.5 ng L^{-1}	2–120 μg L^{-1}	Human Plasma and urine	[42]
CIT	Micro solid phase extraction	HPLC	10 ng mL^{-1}	50–2000 ng mL^{-1}	Human urine	[43]
FLU						
CIT	MSPE	HPLC	1.58 ng mL^{-1}	5–500 ng mL^{-1}	Urine and waste	This
FLU					water	method
			1.43 ng mL^{-1}	5–500 ng mL^{-1}		

In an analysis, it is aimed to use less amount of organic solvent, to be fast and economical, to prepare samples easily and to take less time, to obtain efficient results, and to be environmentally friendly. The main purpose of the solid phase extraction method in accordance with these parameters is to selectively extract the components that are dissolved in the solvent medium and desired to be analyzed into a solid phase and to enrich by transferring to a lower volume of solvent phase. Thus, components that are lower than the level that the devices can determine are concentrated to the measurable levels.

Declaration of Competing 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.

CRediT authorship contribution statement

Merve Sarıkaya: Conceptualization, Methodology, Investigation. **Halil Ibrahim Ulusoy:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project ad-

ministration. **Ummugulsum Morgul:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. **Songül Ulusoy:** Methodology, Writing – original draft, Writing – review & editing. **Angela Tartaglia:** Formal analysis, Visualization. **Erkan Yılmaz:** Data curtion, Writing – review & editing. **Mustafa Soylak:** Data curtion, Supervision, Project administration, Funding acquisition. **Marcello Locatelli:** Project administration. **Abuzar Kabir:** Writing – review & editing, Supervision.

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