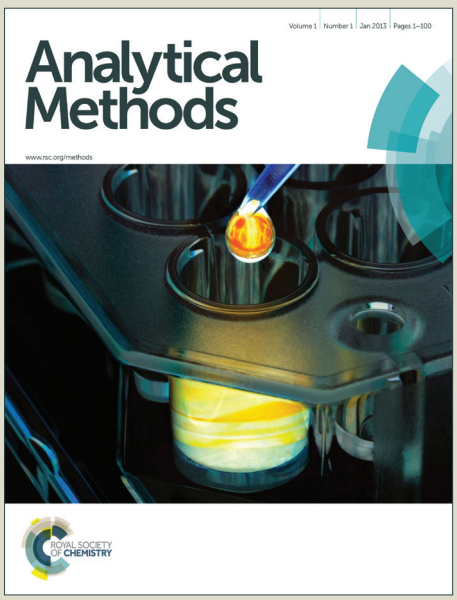
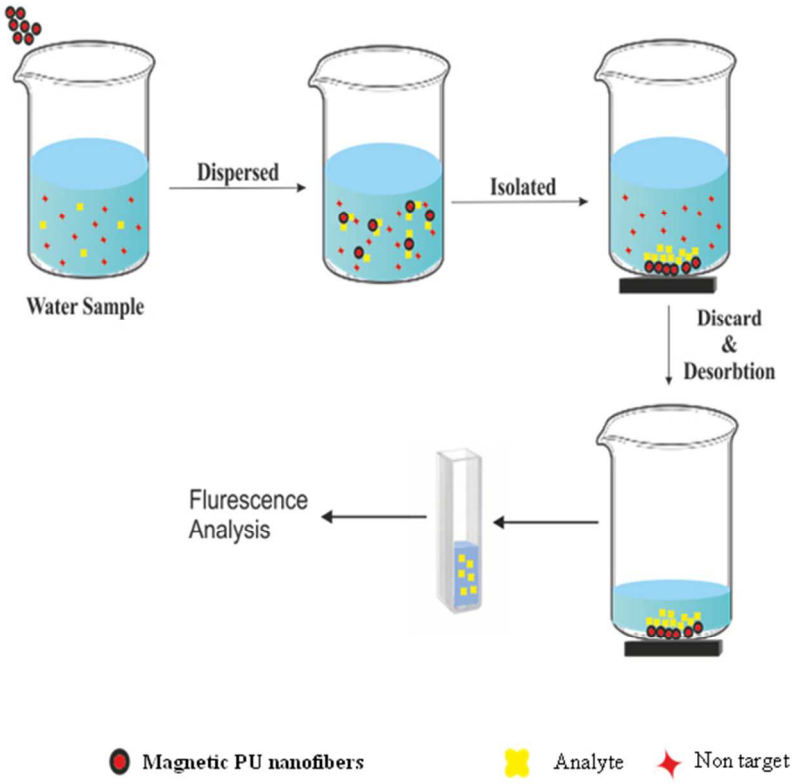
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods



The extraction/desorption setup for isolation of fluoxetine.
187x158mm (96 x 96 DPI)

An electrospun magnetic nanocomposite for a facile approach in micro-scaled analysis

Habib Bagheri[1], Ali Roostaie, Rasoul Daliri

Environmental and Bio-Analytical Laboratories, Department of Chemistry,

Sharif University of Technology, P.O. Box 11365-9516, Tehran-Iran

Abstract

A magnetic polyurethane (PU) nanocomposite was synthesized by electrospinning technique and applied for isolation and preconcentration of fluoxetine form aquatic and biological samples. The nanocomposite was electrospun using PU polymer solution containing the dispersed magnetic nanoparticles. The magnetic property of iron nanoparticles along with the use of electrospun technique led to the formation of a suitable sorbent toward isolation of fluoxetine. The magnetic PU nanofibers could be subsequently removed from the sample solution by applying a permanent magnet. The scanning electron microscopy (SEM) image of the magnetic PU nanofiber confirms that their diameters are in the range of 68–113 nm. The major parameters influencing the morphology of the magnetic PU nanofibers comprising the weight ratio of iron and PU components, the applied voltage and the coating time were optimized. Moreover, parameters including the eluting solvent, amount of sorbent, extraction time, pH and salinity of aqueous samples were considered for optimization. The detection limit of the developed method under optimized conditions and the use of a fluorescence spectrometry was 1 μgL^{-1}. The relative standard deviation (n = 5) at a concentration level of 150 μgL^{-1} was 2 %. The method was linear in the range of 50-5000 μgL^{-1} with a correlation coefficient of 0.9997. The whole procedure showed to be conveniently rapid, efficient and economical to extract fluoxetine from environmental and biological samples. Eventually, the developed method was applied to the analysis of water, urine, milk and plasma samples and relative recoveries of 76 to 99% were achieved.

Keywords: Magnetic polyurethane nanofibers; Electrospun; Fluoxetine; Biological samples

[1] Corresponding author. Tel.: +98-21-66005718; Fax: +98-21-66012983
E-mail address: bagheri@sharif.edu (H. Bagheri)

1. Introduction

Due to the low concentration of desired analytes and the complexity of biological and environmental samples, an efficient preconcentration step is usually necessary prior to the determination process. Classical liquid–liquid extraction (LLE) and solid phase extraction (SPE) are the most common techniques, but they are rather time-consuming and quite labour intensive. Moreover, the use of highly-pure organic solvents and their subsequent disposal are other major threats toward the environment and humans [1,2].

Minimizing sample preparation steps is quite effective to reduce possible sources of errors and expenses. The reduced number of steps in sample preparation has particular advantageous when the trace and ultra-trace determinations of the desired analytes in complex media is concerned.

Micro-solid phase extraction (μ-SPE) is an interesting alternative to the multistep SPE method for trace analysis of complex samples. Although, this μ-oriented setup is regarded as a total extraction method, but reduced amounts of sample and sorbent are needed. The key advantage of this technique lies on its minimized usage of solvent, its simplicity and being low cost [3].

More recently, some novel functional materials such as biomaterials [4], ion-imprinted materials [5], C_{60}–C_{70} and their derivatives [6, 7], mesoporous materials [8], magnetic materials [9], nanometer-sized materials [10, 11] and nanocomposites [12] have been extensively used in SPE. Nanocomposites often demonstrate unusual and beneficial properties. The improvement in properties of polymer-based nanocomposites compared to the pristine polymers has been reported in various scientific and technical literatures [13-15]. The combination of mechanical, magnetic, thermal and electrical properties of a material and low concentration of the nanoparticles in the polymer matrix has generated great deals of interest in the field of nanocomposites.

Among the applications of nanocomposites in various fields, magnetic nanocomposites are rather popular which is due to the incorporation of both magnetism and polymeric features. Up to now, various magnetic nanocomposites have been synthesized by modification of polymers with Ni, Co, CoO, Fe_3O_4 and Fe nanoparticles [16]. Among them, magnetic iron oxide (e.g., maghemite γ-Fe_2O_3 or magnetite Fe_3O_4) nanoparticles have attracted tremendous attentions due to their low toxicity, stability, and biocompatibility in the physiological environments [17–19].

Fluoxetine hydrochloride, (±) N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propylamine hydrochloride, is a selective serotonin reuptake inhibitor in presynaptic neurons. Since its introduction in 1980s, it is the most prescribed antidepressant drug worldwide. Lately, fluoxetine was used in the treatment of obsessive-compulsive and eating disorders, including anorexia and bulimia nervosa [20–22]. The development of a simple and robust method for the determination of fluoxetine in urine could be very useful for toxicological and therapeutic purposes. In the meanwhile, due to high consumption of this drug, there is a risk of spreading the residuals traces into the environmental waters. The determination of fluoxetine in biofluids is essential in therapeutic drug monitoring, pharmacokinetic analysis, and bioequivalence studies, while its determination in environmental water is also quite important [20–24].

The electrospinning is a simple and convenient method for producing nanofibers with adjustable diameters, polarities and porosities. They have malleability to conform to a wide variety of sizes and shapes. It is possible to control the nanofibers composition to achieve the desired properties and functionalities. The electrospinning process operates based on similar principle of electrospray ionization mass spectrometry [25].

Following our research interests, for first time, Fe_3O_4 manoparticles were doped into the macromolecular chains of PU network by electrospining technique. Although, the

employment of high performance liquid chromatography with fluorescence detection provides more selectivity but it requires much longer analysis time. Fluorescence spectroscopy, in spite of lack of sufficient selectivity, was therefore used for rapid determination of fluoxetine after extraction. The preliminary results revealed that the prepared magnetic PU nanocomposite was quite suitable toward the isolation of fluoxetine from different sample media. Various parameters affecting the synthesized polymer morphology and also the extraction/desorption steps were optimized and applied for the determination of fluoxetine in different aqueous and biological samples.

2. Experimental

2.1. Chemicals

Chemicals including $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, HCl, NaOH, N,N-dimethylformamide (DMF), acetone, methanol, acetonitrile, chloroform, hexane and ethanol were analytical grades and purchased from Merck (Darmstadt, Germany). Fluoxetine hydrochloride (99.37%) was prepared from Dr. Reddy Company (India). The stock solution (1000 mg L^{-1}) of fluoxetine was prepared in HPLC-grade methanol and stored in the refrigerator. The working standard solutions were prepared weekly by diluting the standard solution with methanol, and more diluted working solutions were prepared daily by diluting this solution with double distilled water (DDW). Sodium chloride was purchased from Merck. Diphenylmethane diisocyanate based PU was purchased from Bayer Material Science (Germany). The nitrogen gas was used for providing the inert atmosphere necessary for synthesis of NPs.

2.2. Incured samples

Human urine samples were collected from a healthy volunteer from our research group. Any precipitated material was fully removed using centrifugation at 10000 rpm for 10 min, and

then the supernatant of the urine was considered for analysis. Each urine sample was diluted (1:4) with water to minimize the matrix effects. The milk sample was purchased from a local supermarket, which was checked to be free of target analyte. The sample was centrifuged for 15 min at 10000 rpm. The supernatant of the milk sample was considered for analysis. Each milk sample was diluted (1:4) with water to minimize the matrix effects. Blood samples were obtained from a healthy human subject and centrifuged at 10000 rpm for 10 min. supernatant of the blood sample was stored at - 4 $^{\circ}$C until analysis. A plasma standard was prepared by adding amount of aqueous working standard to 49.9 ml of drug-free plasma eluted solution to yield final desired concentrations.

2.3. Apparatus

The fluorescence spectra were recorded using a Varian Cary Eclipse fluorescence spectrophotometer (Springvale, Victoria, Australia) equipped with 1 × 1 cm quartz cell and a xenon lamp. Spectra recording were carried out in fluorescence scan mode with the slit widths of 5 nm and the excitation and emission wave lengths of 246 and 310 nm, respectively. The PMT detector was used for recording the emission lines and set on 600 V. The pH of solutions was measured by Metrohm Herisan (Switzerland). A permanent magnet of NdFeB (100 ×50 ×40 mm) model N48 with magnetic field of 1.4 T was purchased from Ningbo strong magnet (Ningbo, China). The Scanning electron microscopy (SEM) images were obtained by aTSCAN VEGA II XMU (Brno, Czech Republic) and Fourier transform infrared spectroscopy (FTIR) spectra were recorded by a ABB Bomem MB100 (Quebec, Canada).

2.4. Preparation of magnetic nanoparticles

Magnetic nanoparticles were synthesized according to the procedure described previously [26]. Briefly, 5.2 g of $FeCl_3 \cdot 6H_2O$ and 2 g $FeCl_2 \cdot 4H_2O$ and 0.85 mL concentrated HCl were dissolved in 25 mL water under N_2 gas. This solution was added drop wise into 250 mL of sodium hydroxide solution (1.5M) under the N_2 atmosphere while it was vigorously stirred for 30 min (1000 rpm). The resulted black precipitates were separated using a 1.4 T magnet and washed several times with degassed water. Finally MNPs were dried in vacuum oven at 60 ºC for 5 hours.

2.5. Magnetic nanocomposite preparation by electrospinning

The first step in the preparation of nanocomposite by electrospinning was to dissolve the polymers in a suitable solvent. In the electrospinning process, a solution of high molecular weight polymer with high viscosity is necessary. It was found that PU is quite stable in many solvents such as methanol, ethanol, acetonitrile, chloroform, dichloromethane, tetrachloromethane, diethyl ether, ethyl acetate, hexane and toluene while it could be dissolved in DMF [27]. In all experiments, a solution of 15.8% (w/w) of polymers was dissolved in 1 mL of solvent [27]. After complete dissolving of polymer and obtaining a homogeneous solution, various amounts of MNPs were added to the polymer solution and under vigorous stirring for 20 min. Then 0.5 mL of produced solution was withdrawn into a 2.5 mL syringe which was eventually located in a syringe pump. For electrospinning, a syringe pump was used to deliver the polymer solution containing the nanoparticles with a flow rate of 0.5 mL min-1 under a voltage of 16 kV. A piece of aluminum foil (7 × 7 cm) was employed as a collector electrode. The collector and the polymer containing syringe needle were connected to the high voltage power supply terminals. All the electrospinning processes were performed under ventilation.

2.6. Extraction procedure

Primarily, an electrospun magnetic nanocomposite sheet of 1 mg was inserted in acetone to convert them into the separated magnetic PU nanofibers. After evaporation of acetone, 50 mL of water sample spiked with fluoxetine to a final concentration of 1 mg L^{-1} was added to a vial containing the magnetic PU nanofibers and then the sample vial was sealed and the solution was stirred at the maximum stirring rate (1000 rpm). Under the agitation, the magnetic PU nanofibres tumbled freely in the sample solution during the extraction to facilitate mass transfer. After 10 min at room temperature and 10% salt, the magnetic PU nanofibers were taken out using an external magnet and supernatant solution was decanted. Finally, magnetic PU nanofibers were immersed in a small amount of acetonitrile (1.5 mL) for complete desorption during 2 min and fluorescence spectrum of the desorbed solution was recorded by the spectrofluorimeter.

3. Results and discussion

According to our previous work, PU with acceptable flexibility and mechanical stability could be used as a medium for isolation of organic analytes from various samples. However, the PU-based nanofibers showed some degree of low thermal stability [27]. Therefore, it was necessary to use an organic solvent for the desorption process. By embedding MNPs into PU nanofibres, they can be easily collected by application of an external magnetic field with no need to perform any additional centrifugation or filtration of the sample, making the extraction and desorption processes more convenient and faster.

Parameters affecting the quality of the synthesized PU including the polymer concentration and electrospinning time, distance between electrodes were already optimized [27]. After successful preliminary experiments, it was necessary to optimize the synthesizing conditions in order to achieve the high quality fibers as far as the high extraction efficiency is concerned.

One way to enhance the efficiency of the electrospun nanofibers is to dope nanoparticles throughout the polymeric network. Addition of iron magnetic nanoparticles not only could provide this enhancement but they could induce magnetic property to the fabricated nanofibers. To do so, the effect of the Fe_3O_4 nanoparticles amounts, 0.005–0.09 g in 1 mL of PU solution, was also investigated.

3.1. Characterization of the magnetic PU nanofibres

Fourier transform infrared spectroscopy was also employed to examine the modification process and recognition of any changes on the PU film after nano Fe_3O_4 particles was embedded throughout the polymer by electrospinning process. According to Fig. 1, the FTIR spectrum of PU exhibited the typical bands which are: -NH, (free and bonded) at 3300-3400 cm^{-1}, CH_2 at 2850-2970 cm^{-1}, C=O in bonded urethane group at 1680-1720 cm^{-1} and -C-O-C- in ester group at 1053 cm^{-1}. However, the spectrum of magnetic PU nanofibres exhibits a new shoulder in the absorption peak appeared at 587cm^{-1} which is attributed to the Fe-O vibration in the composite.

The SEM images along with backscattered electrons (BSE) signals obtained for magnetic PU nanofibers mat are presented in Fig. 2, respectively. The SEM image of the dispersed iron oxide nanoparticles in water shows that the synthesized MNPs have rather high surface area (Fig. 2a) [26]. The SEM image of the magnetic PU nanofibres mat (Fig. 2c) confirms that the nanofibers diameters are in the range of 68–113 nm, although interestingly a layer of much narrower fibers is observable in compared with PU nanofibres (Fig 2b). Certainly these features make the magnetic PU nanofibres mat very porous which favours the increased surface area, sorption sites availability and higher mass transfer for analytes during the sorption/desorption steps.

BSE are beam electrons that are reflected from the surface by elastic scattering depending on the average atomic number of the material. Heavy elements strongly backscatter electrons, hence those areas appear brighter. Lighter elements tend to absorb electrons and thus appear darker. The BSE image is therefore called material contrast image and allows drawing conclusions about the distribution of chemical materials in the sample. Considering the surface micrograph obtained from BSE of the magnetic PU nanofibers mat (Fig. 2d), small white spots inside major nanofibres can mainly be distinguished. The grey phase is attributed to PU nanofibres and the white spots can be associated to the isolated Fe_3O_4 nanoparticles embedded into the nanofibers.

The Energy Dispersive X-Ray Analysis (EDX) spectra also confirm the results obtained by BSE (Fig. 3). The spectrum of the pristine PU nanofibres exhibits the following atomic composition (Fig. 3a): ~ 59% C, ~ 6% O and ~ 0.00% Fe. While the EDX chemical analysis of the magnetic PU nanofibres gives approximately the following atomic composition (Fig. 3b): ~ 64% C, ~ 21% O and 1% Fe. The iron content of the white region could be attributed to the Fe_3O_4 nanoparticles.

3.2. Optimization of μ-SPE procedure

In this μ-SPE procedure, several factors influencing the extraction/desorption process including the components ratio, type of organic solvents, sample pH, extraction time, desorption time, and salting-out effect were optimized.

The influence of desorbing solvent was investigated, to ensure effective elution of the trapped analyte from the sorbent. The elution solvent should be able to displace the target analyte from the sorbent at the lowest possible volume. Selecting the most appropriate solvent and time is quite essential for optimization of the desorption process. As mentioned, the magnetic PU nanofibres sheet exhibits enhanced stability in different solvents which

could be used for the analyte desorption. Five water-immiscible solvents with different polarities and water solubilities including chloroform, n-hexane, methanol, acetonitrile and ethanol were examined in order to find the most appropriate solvent for extraction. The results show that acetonitrile exhibited a superior role and it was therefore selected as desorbing solvent for further experiments (Fig. 4a).

The extraction efficiency of fluoxetine from aqueous solution containing different amounts of sodium chloride (0–30%, w/w) was investigated (Fig. 4b). Higher extraction efficiency for fluoxetine was obtained at lower NaCl concentration, while a slow decrease of extraction efficiency was found when the NaCl concentration was increased. The salting out effect has been commonly used in SPE and liquid–liquid extraction methods. Generally, salt addition can decrease the solubility of analytes in the aqueous phase while enhancing their partitioning into the organic phase. However, salt addition had an adverse effect on the overall efficiency of the method and was therefore abandoned. There are several reasons for this phenomenon: i) The addition of NaCl could suppress the thickness of electrical sorption layer at the sorbent solution interface, which leads to the decrease of layers formed on the sorbent surface[28]. ii) The presence of salt may increase the viscosity of solutions and reduce the sorption ability of sorbent. The amount of time required to attain equilibrium increased due to the rate of mass transfer of the analyte from the aqueous phase to the solid sorbent. Thus, the amount of time required to attain equilibrium decreased were performed without adding NaCl [29]. iii) The reduction of extraction efficiency may be due to the reduction of active sites of sorbent [30].

The magnitude of the distribution constant of analyte between sample solution and sorbent can be influenced by the sample pH. Therefore, the sample pH is a significant factor, which could affect the analyte extraction recovery from aquatic samples. The μ-SPE experiments were carried out with the pH range of 1.0–11.0 and the maximum response of

fluoxetine was obtained around pH 7 (Fig. 4c). The extraction response increased sharply until pH 7 and then gradually decreased. This result can be easily described considering the acid–base equilibrium of flouxetene and magnetic PU nanofibres in the solutions having different pH values. Since both fluoxetine and magnetic PU nanofibres-based sorbent contain –NH groups in their structures, both have the same charges and the electrostatic repulsion forces under acidic and basic conditions. Theses repulsion forces between ammonium groups in the analyte and nanofibres structure could overcome the π-π interactions, leading to subsequent low extraction efficiency. The reduction of signals are more pronounced at pHs below 2 and pHs above 9, while at pH 7 the synthesized magnetic PU nanofibres structure is rather neutral and sorption of non-charged compounds are expected to be more pronounced.

The amount of analyte extracted in μ-SPE is dependent on the rate of its mass transfer from the aqueous sample to the solid sorbent phase. The extraction time is a vital issue in whole procedure. In short extraction time, low enrichment factors are mostly expected and there would be insufficient time for the active sites to entrap the desired analyte while the use of longer time should be prevented. The effect of extraction time in the range of 2–20 min was considered and the relevant results proved that an extraction time of 15 min is a suitable value for further experiments (Fig. 4d).

The effect of Fe_3O_4 concentration on the extraction efficiency was investigated considering a concentration range of 0.5–9% (w/w). According to Fig. 5a, the rise of Fe_3O_4 nanoparticles concentration up to 2% led to higher extraction efficiencies and after that a decline is observed. When the amount of nanoparticles is increased, more probably, the sorbent capacity is enhanced due to the higher nanocomposite surface area. At higher iron concentration, the performance of electrospining process becomes rather difficult while lower

proportion of polymer content throughout the sorbent network might have a reduced effect in extraction efficiency.

The amount of magnetic PU nanofibres sorbent in the range of 0.5-3.5 mg was also optimized. According to Fig. 5b an amount of 2.0 mg of magnetic PU nanofibers provided the highest analytical response for fluoxetine. The small amount of magnetic PU nanofibers justifies the minimal elution volumes for efficient release of analyte from the sorbent. The effect of eluting solvent volume ranging from 0.5 to 3 mL was also studied (Fig. 5c). When solvent volumes were increased up to 2 mL, the extraction efficiency was increased. This trend is in fact due to the enhancement of analyte enrichment as the desorbing solvent volume is increased. But higher volumes of acetonitrile caused a decrease in the peak area which might be due to the dilution phenomenon.

The effect of desorption time over the range of 1–10 min was investigated using acetonitrile solvent. It was found out that desorption time of 2 min was quite sufficient for a suitable performance (Fig. 5d). The desorption process was incomplete when shorter periods of time were employed while after 2 min, there was a slight decrease in the desorption which might be probably due to the analytes being re-adsorbed by the magnetic nanofibers. No carryover effect was observed after repeating the desorption process.

3.3. Analytical results

The optimized μ-SPE method using the fabricated magnetic PU nanofibres was evaluated by quantitative analysis of the spiked DDW samples. Limits of detection and quantification, based on a signal-to noise ratio of 3 and 10, were 1 and 50 μg L^{-1}, respectively. The calibration curve of analyte was investigated in the range of 50–5000 μg L^{-1} and R^2 value was 0.9997. The precision of the method was determined by performing five consecutive

extractions from the aqueous solution. The relative standard deviation (RSD) was 2% at the concentration level of 150 μg mL^{-1}.

To study the matrix effect, the developed method was applied to tap water, Calan dam (Malayer, Iran) water, human urine, milk and human plasma samples. For extraction purposes, 50 mL from each prepared sample was extracted and the relative recoveries were obtained. Real samples were spiked at a concentration level of 150 μg L^{-1} and the analysis was carried out under the optimized conditions. Acceptable relative recoveries in the range of 76–99% for the selected analyte were achieved (Table 1). An absolute recovery of 96% was achieved by extracting 50 mL of DDW sample spiked at 150 μg L^{-1}. This indicates that the present method is quite suitable for the determination of fluoxetine in various real samples. Fig. 6 shows typical fluorescence spectra obtained after extraction and desorption of analyte from spiked real samples at a level of 150 μg L^{-1}.

The comparison study concerning the determination of fluoxetine (Table 2) [31-35] reveals the pronounced advantages of current method. Consuming little amounts of organic solvent and sorbent amount along with the short analysis time are clear advantages associated with the developed method.

4. Conclusions

The electrospun magnetic PU nanofibers mat was found to be porous and subsequently suitable to be used as novel extracting phase. By embedding MNPs into PU nanofibres, they can be easily separated from the sample solution by application of an external magnetic field with no need to perform any additional centrifugation or filtration of the sample, making the extraction/desorption processes more convenient and faster. Relative recoveries along with other analytical data confirm that the magnetic PU nanofibres sorbent could be an appropriate candidate as a suitable medium for μ-SPE of fluoxetine. Moreover, the developed method is

inexpensive, reproducible and efficient for extraction of fluoxetine in a variety of samples. The chemical structure of magnetic PU nanofibres contributes to hydrophobic, hydrogen bonding and π-π interaction between analyte and the magnetic PU nanofibres, making this nanocomposite a sensitive probe for extracting organic compounds.

Acknowledgements

The Research Council and Graduates School of Sharif University of Technology (SUT) are thanked for supporting the project.

References

[1] E. Baltussen, C.A. Cramers, P.J.F. Sandra, Sorptive sample preparation, Anal. Bioanal. Chem. 373 (2002) 3–22.

[2] E.K. Paleologos, D.L. Giokas, M.I. Karayannis, Micelle-mediated separation and cloud-point extraction, Trends Anal. Chem. 24 (2005) 426–436.

[3] S.Kanimozhi, C.Basheer, K. Narasimhan, L. Liu, S. Koh, F.Xue, M. Choolani, H.K. Lee, Application of porous membrane protected micro-solid-phase-extraction combined with gas chromatography-mass spectrometry for the determination of estrogens in ovarian cyst fluid samples, Anal. Chim. Acta 687 (2011) 56-60.

[4] A.A. Menegario, P. Smichowski, G. Polla, On-line preconcentration and immobilized on controlled pore glass, Anal. Chim. Acta 546 (2005) 244–250.

[5] G.Z. Fang, J. Tan, X.P. Yan, Synthesis and evaluation of an ion-imprinted functionalized sorbent for selective separation of cadmium ion, Sep. Sci.Technol. 40 (2005) 1597–1608.

[6] M.G. Pereira, E.R. Pereira-Filho, H. Berndt, M.A.Z. Arruda, Determination of cadmium and lead at low levels by using preconcentration at fullerene coupled to thermospray flame furnace atomic absorption spectrometry, Spectrochim. Acta B: Atomic Spectroscopy 59 (2004) 515–521.

[7] B.A. Lesniewska, I. Godlewska, Z.B. Godlewska, The study of applicability of dithiocarbamate-coated fullerene C60 for preconcentration of palladium for graphite furnace atomic absorption spectrometric determination in environmental samples Spectrochim. Acta B: Atomic Spectroscopy 60 (2005) 377–384.

[8] D. Perez-Quintanilla, I. Hierro, M. Fajardo, I. Sierra, Preparation of 2-mercaptobenzothiazole derivated from aqueous media onto a mesoporous silica chemically modified with 2-mercaptopyrimidineal, J. Mater. Chem. 16 (2006) 1757–1764.

[9] C.Z. Huang, B. Hu, Silica-coated magnetic nanoparticles modified with gamma-mercaptopropyltrime-thoxysilane for fast and selective solid phase extraction of trace amounts of Cd, Cu, Hg, and Pb in environmental and biological samples prior to their determination by inductively coupled plasma mass spectrometry, Spectrochim. Acta B: Atomic Spectroscopy 63 (2008) 437–444.

[10] P. Liang, Y. Liu, L. Guo, Determination of trace rare earth elements by inductively coupled plasma atomic emission spectrometry after preconcentration with multiwalled carbon nanotubes, Spectrochim. Acta B: Atomic Spectroscopy 60 (2005) 125–129.

[11] X.L. Pu, Z.C. Jiang, B. Hu, H.B. Wang, γ-MPTMS modified nanometer-sized alumina micro-column separation and preconcentration of trace amounts of Hg, Cu, Au and Pd inbiological, environmental and geological samples and their determination by inductively coupled plasma mass spectrometry, J. Anal. Atom. Spectrom. 19 (2004) 984–989.

[12] N.A. Booker, D. Keir, A. Priestley, C.D. Rithchie, D.L. Sudarmana, M.A. Woods, Sewage clarification with magnetite particles, Water Sci. Technol. 23 (1991) 1703–1712.

[13] T. J. Pinnavaia, G. W. Beall, Polymer-clay nanocomposites, Wiley, New York, NY (2000).

[14] M. Rosoff, nano-surface chemistry. Marcel Dekker, New York, NY (2000).

[15] D. R. Paul, L. M. Robeson, Polymer nanotechnology: nanocomposites, Polymer, 49 (2008) 3187-3204.

[16] O. Philippova, A. Barabanova, V. Molchanov, A. Khokhlov, Magnetic polymer beads: Recent trends and developments in synthetic design and applications, European Polymer Journal, 47 (2011) 542-559.

[17] L. C. A.Oliveira, , R. V. R. A. Rios, J. D. Fabris, R. M. Lago, A simple preparation of magnetic composites for the adsorption of water contaminants, J.Chem. Educ. 81 (2004) 248-250.

[18] I. Safarik, M. Safarikova, V. Buricova, Study of sorption of triphenylmethane dyes on a magnetic carrier bearing an immobilized copper phthalocyanine dye**,** Collect. Czech. Chem. Commun. 60 (1995) 1448–1456.

[19] J.D. Orbell, L. Godhino, S.W. Bigger, T.M. Nguyen, N. Lawrence, Oil spill remediation using magnetic particles an experiment in environmental technology J. Chem. Educ. 74 (1997) 1446–1450.

[20] Fluoxetine Hydrochloride Capsules and Tablets, Eli Lilly and Company Material Safety Data Sheet, 2004, Indiana, USA.

[21] M.A. Raggi, F. Bugamelli, G. Casamenti, R. Mandrioli, D. De Ronchi, V. Volterra, Analytical methods for the quality control of prozac capsules. J. Pharm. Biomed. Anal. 18 (1998) 699–706.

[22] R.F. Suckow, M.F. Zhang, T.B. Cooper, Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, Clin. Chem. 38 (1992) 1756–1761.

[23] N. Unceta, A. Gómez-Caballero, A. Sánchez, S. Millán, M.C. Sampedro, M.A. Goicolea, J. Sallés, R.J. Barri, Simultaneous determination of citalopram, fluoxetine and their main metabolites in human urine samples by solid-phase microextraction coupled with high performance liquid chromatography, J. Pharm. Biomed. Anal. 46 (2008) 763–770.

[24] M.A. Raggi, R. Mandrioli, G. Casamenti, V. Volterra, C. Desiderio, S. Fanali, Improved HPLC determination of fluoxetine and norfluoxetine in human plasma, Chromatographia 50 (1999) 423–427.

[25] H. Bagheri, A. Aghakhani, Polyaniline-nylon-6 electrospun nanofibers for headspace adsorptive microextraction, Anal. Chim. Acta 713 (2012) 63– 69.

[26] Z. Xiaoli, S. Yali, C. Yaqi, M. Shifen, Preparation of alkanethiolates functionalized core/shell Fe3O4@Au nanoparticles and its interaction with several typical target molecules, Environ. Sci. Technol. 42 (2008) 1201-1206.

[27] H. Bagheri, A. Aghakhani, Novel nanofiber coatings prepared by electrospinning technique for headspace solid-phase microextraction of chlorobenzenes from environmental samples, Anal. Methods 3 (2011) 1284-1289.

[28] Y. Huang, Q. Zhou, and G. Xie, Development of micro-solid phase extraction with titanate nanotube array modified by cetyltrimethylammonium bromide for sensitive determination of polycyclic aromatic hydrocarbons from environmental water samples, J. Hazard. Mater. 193 (2011) 82–89.

[29] T. P. Lee, B. Saad, W. S. Khayoona, B. Salleh, Molecularly imprinted polymer as sorbent in micro-solid phase extraction of ochratoxin in coffee, grape juice and urine, Talanta 88 (2012) 129-135.

[30] F. Ahmadi, H. Asgharloo, S. Sadeghi, V. Gharehbagh-Aghababa, H. Adibi, Post-derivatization procedure for determination of hippuric acid after extraction by an automated micro solid phase extraction system and monitoring by gas chromatography, J. Chromatogr. B 877 (2009) 2945–2951.

[31] J.R. Flores, J.J.B. Nevado, G.C. Penalvo, N.M. Diez, Development and validation method for determination of fluoxetineand its main metabolite norfluoxetine by nonaqueous capillary electrophoresis in human urine Talanta 65 (2005) 163–171.

[32] R. Mandrioli, V. Pucci, D. Visini, G. Varani, M.A. Raggi, Separation of five recently commercialized selective serotonin reuptake inhibitor antidepressants by capillary electrophoresis J. Pharm. Biomed. Anal. 29 (2002) 1127–1134.

[33] A.H. Prabhakar, V.B. Patel, R. Giridhar, Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. J. Pharm. Biomed. Anal. 20 (1999) 427–432.

[34] C. Fernandes, P. Jiayu, P. Sandra, F.M. Lancas, Stir Bar Sorptive Extraction-LC-MS for the Analysis of Fluoxetine in Plasma, Chromatographia 64 (2006) 517–521.

[35] H. Bagheri, O. Zandi, A. Aghakhani, Extraction of fluoxetine from aquatic and urine samples using sodium dodecyl sulfate-coated iron oxide magnetic nanoparticles followed by spectrofluorimetric determinationanal. Chem. Acta 692 (2011) 80-84.

Figure captions

Fig. 1. FTIR spectra of PU (a), magnetic PU nanofibers mat (b) and Fe_3O_4 nanoparticles (c).

Fig. 2. SEM images of magnetic nanoparticles (a), PU nanofibres (b), magnetic PU nanofibres (c) and BSE image of magnetic PU nanofibres (d)

Fig. 3. The EDX of PU nanofibre (a) and magnetic PU manofibres (b).

Fig. 4. Effects of desorption solvent (a), ionic strength (b), sample pH (c) and extraction time (d) on the extraction efficiency.

Fig. 5. Effect of nanocomposite components ratio (a), sorbent amount (b), desorption time (c) and solvent volume on the extraction efficiency.

Fig. 6. Fluorescence spectra of fluoxetine extracted from Calan Dam water (a) human plasma sample (b) human urine sample (c) and blank water sample (d) under optimized condition.

Fig. 1

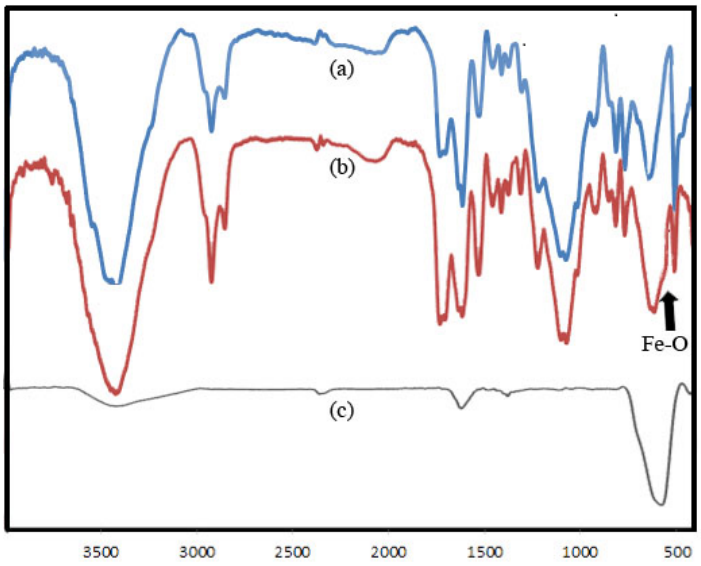


Fig. 2

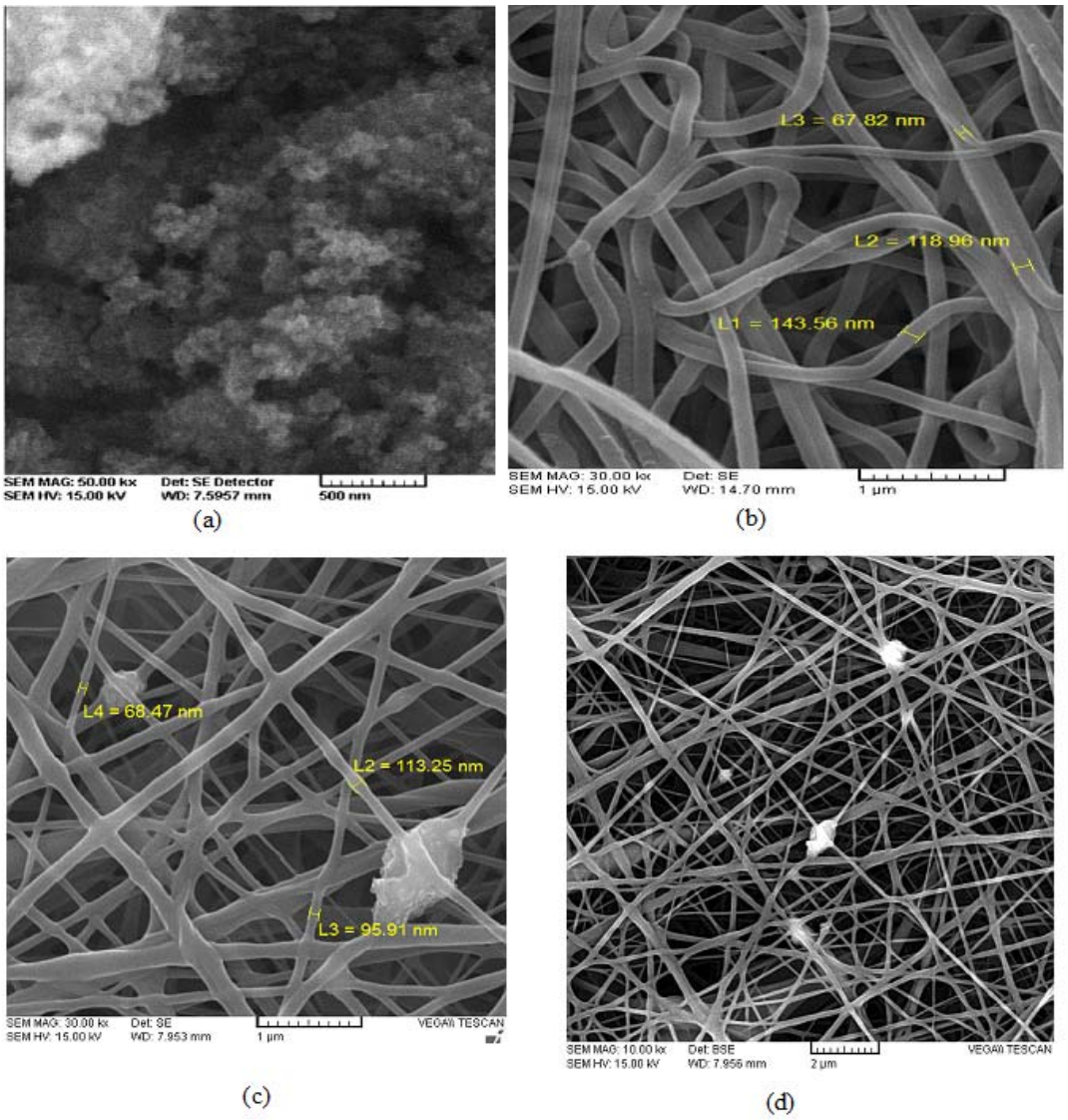
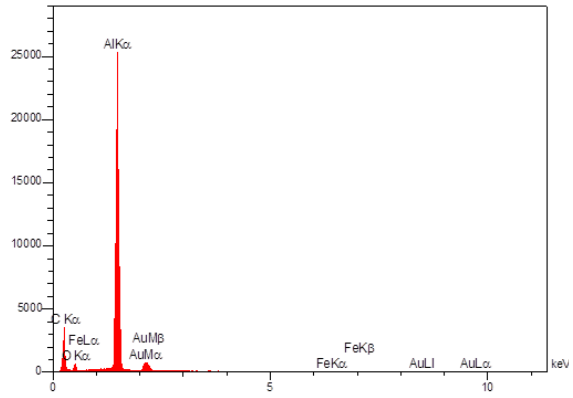
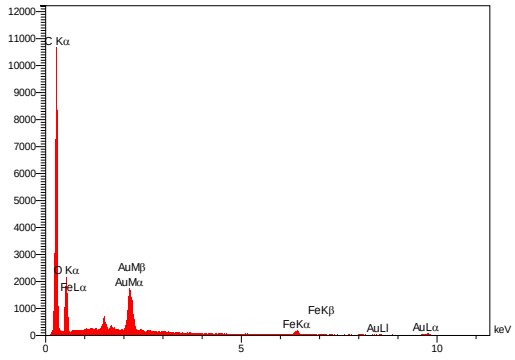


Fig. 3



Elt	Line	Int	Error	K	Kr	W%	A%
C	Ka	351.6	178.4626	0.3230	0.1331	59.49	76.71
O	Ka	61.9	31.3836	0.0283	0.0117	6.13	5.93
Al	Ka	3664.0	12.9206	0.5876	0.2420	29.57	16.97
Fe	Ka	0.0	0.0000	0.0000	0.0000	0.00	0.00
Au	La	3.4	0.3700	0.0611	0.0252	4.82	0.38

(a)



Elt	Line	Int	Error	K	Kr	W%	A%
C	Ka	1003.3	171.9524	0.7513	0.3929	63.56	79.07
O	Ka	228.5	26.2434	0.0852	0.0446	20.92	19.54
Fe	Ka	26.3	0.5471	0.0171	0.0089	1.11	0.30
Au	La	10.1	0.6388	0.1464	0.0765	14.40	1.09

(b)

Fig. 4

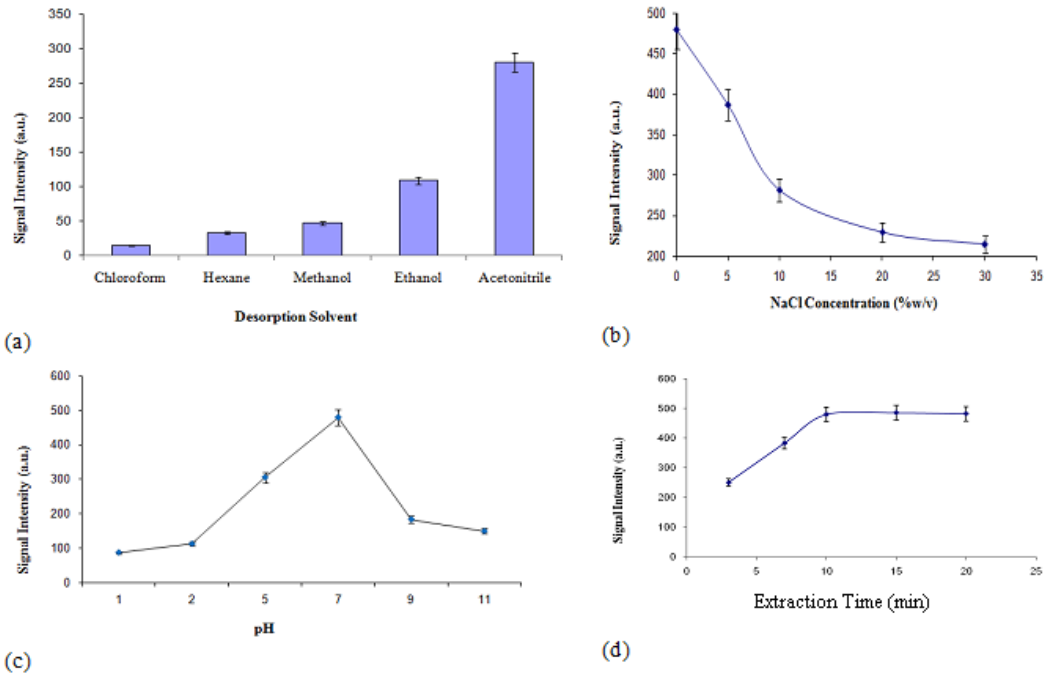


Fig. 5

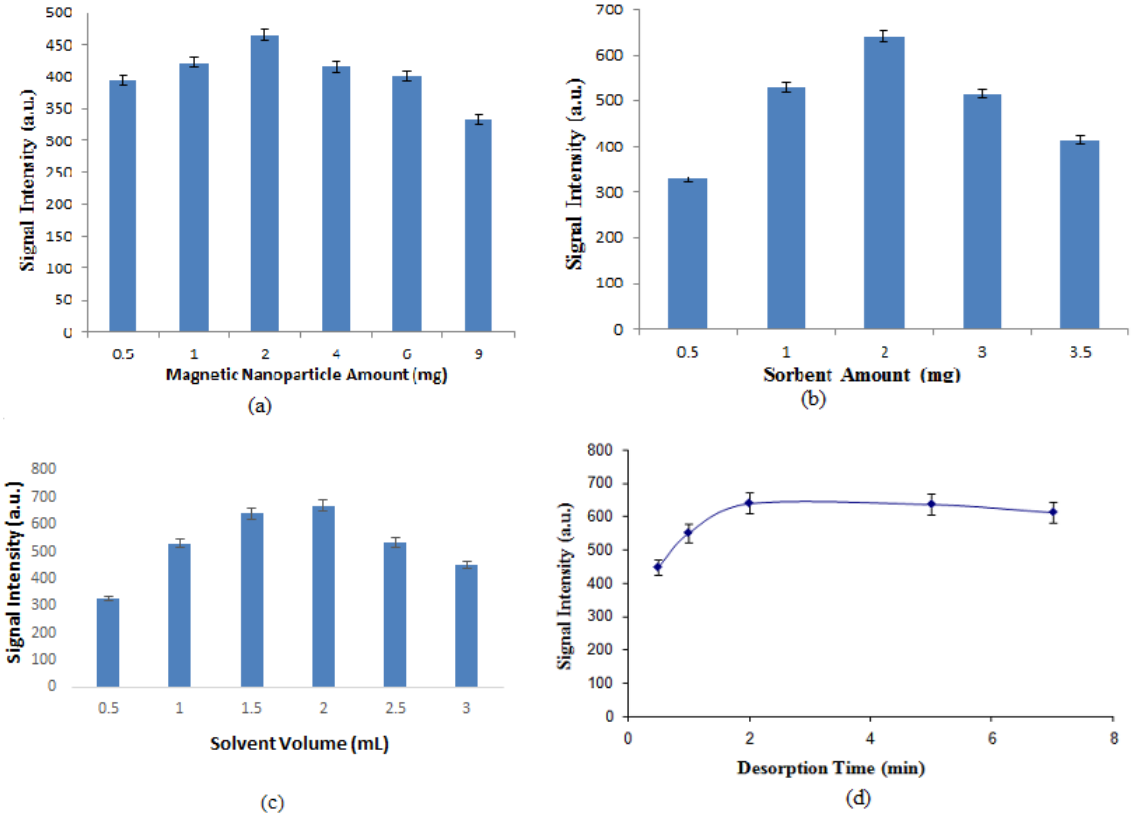


Fig. 6

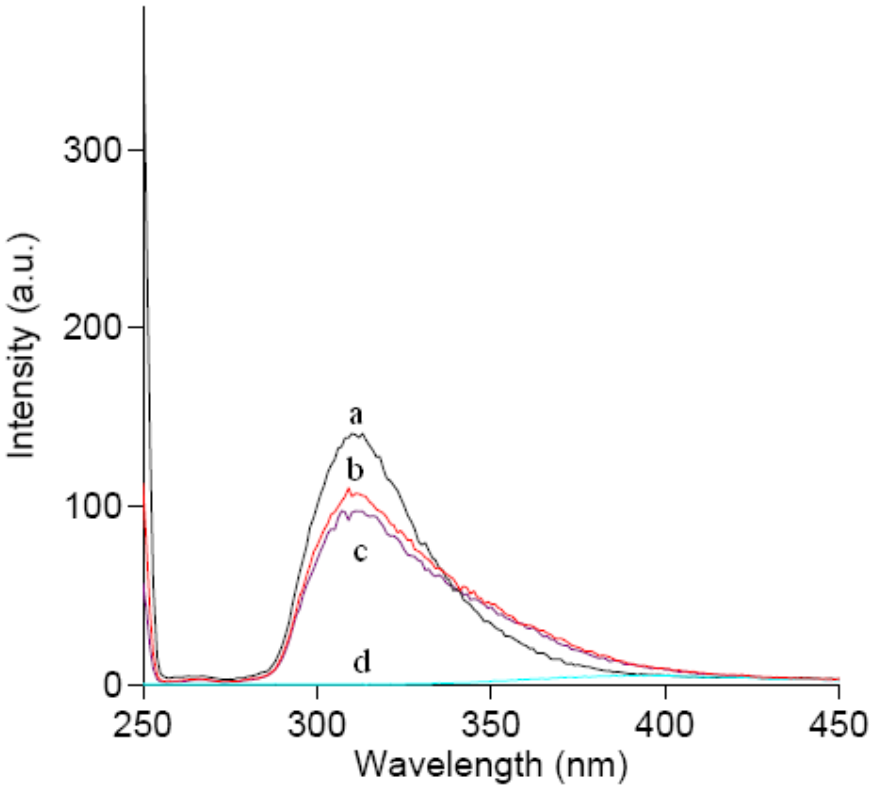


Table 1: Relative recoveries obtained for fluoxetine in different real samples.

Sample	Fluoxetine added ($\mu g\ L^{-1}$)	Fluoxetine found ($\mu g\ L^{-1}$)	Recovery (%)	RSD% (n=3)
Tap water	0	0	-	-
	150	147	98	2
Kalan dam	0	0	-	-
	150	148.5	99	3
Urine	0	0	-	-
	150	114	76	5
Milk	0	0	-	-
	150	135	90	4
Blood	0	0	-	-
	150	123	82	3

Table 2**:** Comparing the current work with some other methods used for the determination of fluoxetine.

Method	Recovery (%)	LOD[a] (μgL^{-1})	LDR[b] (μgL^{-1})	RSD[c] (%)	Ref.
Spectrofluorimetry	97	70	250-5000	2.2	[32]
LLE-CPE-FL	102	100	5000-50000	3.5	[32]
Spectrophotometry	19	-	1000-2000	1.0	[33]
SBSE-LC-MS	52-63	3	10-500	5.0	[34]
LLE-HPLC-FL	97-99	-	25-1000	1.0	[21]
SPE-CZE	89	10	100-200	3.0	[31]
Magnetic-SPE- spectrofluorimetry	80-104	20	50-1000	1.4	[35]
Developed method	76-99	1	50-5000	2	-

[a] Limits of detection
[b] Linear dynamic range
[c] Relative standard deviation