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Original Research Article

The Dispersive Solid-Phase Extraction of Fluoxetine Drug from Biological Samples by the Amine-Functionalized Carbon Nanotubes with HPLC Method

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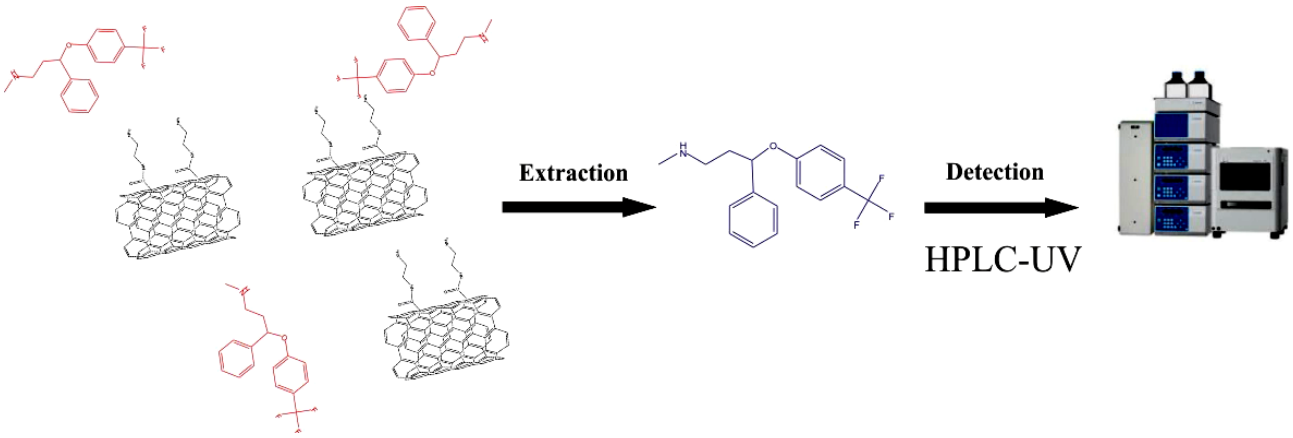
ABSTRACT

A rapid and selective dispersive solid-phase extraction technique, by the combination of Fe_3O_4@MWCNT-amine nanoparticles with HPLC (high-performance liquid chromatography), was expanded to detect the fluoxetine trace amounts in biological samples. The effective parameters on fluoxetine extraction were investigated by Fe_3O_4@MWCNT-amine, and the optimum conditions for fluoxetine extraction were sample pH 10.0, adsorption time of 28 min, and eluent (acidic methanol). Under the optimum extraction conditions, the limit of quantification (LOQ) and detection of limit (LOD) were found to be 18 and 6 μg L^{-1}, respectively. Likewise, a linear range method with the concentration of fluoxetine in the range of 40–800 μg L^{-1} was applied. The analysis of several biological samples such as human plasma, urine, and tap water samples was successfully performed by applying the SPE method, which is an easy and sensitive method and an appropriate alternative for the analysis of fluoxetine.

GRAPHICAL ABSTRACT



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Introduction

Fluoxetine is a known drug capable of treating major depressive disorder, and disorder of obsessive-compulsive with inhibitors of selective serotonin reuptake. Fluoxetine works by preventing the uptake of 5-HT (5-hydroxytryptamine) by the brain neurons and increase 5-HT neurotransmission via action on 5HT2c receptors [1]. Fluoxetine has the chemical formula of $C_{17}H_{18}F_3NO$ and is soluble in water [2]. This hydrophobic molecule is absorbed from the gastrointestinal tract via oral administration, but its adsorption from digestion depends on the pH value of the stomach. After oral administration, fluoxetine is metabolized by cytochrome P450 (CYP2D6) typically to N-desmethyl derivative norfluoxetine [3]. Fluoxetine molecules are excreted principally in urine, about 20% as norfluoxetine and its glucuronide, and just 10% as fluoxetine and fluoxetine-glucuronide.

The biomedical path through human effluent is perhaps the most consistent route that pharmaceuticals follow for entering the environment. Therefore, the determination of fluoxetine in the biological samples needs an efficient analytical method. Ingested drug molecules are excreted as biologically active shape, either as the original substance or as an energetic metabolite [4,5]. Many investigators have detected fluoxetine in municipal sewages [6] and also in freshwaters [7].

High-performance liquid chromatography (HPLC) was introduced as a suitable method for the convenient and rapid detection of analytes owing to its selectivity, simplicity, sensitivity, universality, and precision. However, before HPLC analysis, due to the little levels and intricate matrix interferences from real samples, utilization of a pre-enrichment and purification technique is necessary. It is noteworthy, before chromatographic analysis, implementing an appropriate pretreatment procedure for the pre-concentration of purpose analytes and interferences reduction of sample matrix is of high importance. Appropriate techniques of sample preparation contribute to effectively clean up, separating, and enrich an analyte before analysis. In recent methods of extraction, the attention has mostly been given to miniaturize the matrix interferences sample by the concept of Green Chemistry and automation, and to minimize harmful solvent utilization and human errors, respectively [8].

Solid-phase extraction (SPE) is an efficient and extremely sensitive sample pretreatment technique for extraction, sampling process, and concentration. This technique with its many applications in the separation and pre-concentration of most heavy metals, pharmaceutical molecules in environmental samples has an important place in sample preparation [9-12].

Nanomaterials derived from carbon nanotubes have attracted much attention in important scientific and industrial sectors due to their special properties that have been identified in recent years, including their large area. Carbon nanomaterials were applied to pollutants adsorption from water sources [13-16], construction of biosensors[17,18], storage systems of energy [19], batteries, super-capacitors, and systems of drug delivery [20-22].

The objective of this research was to obtain nano sorbent for the pre-concentration of fluoxetine. A method, namely dispersive SPE, was developed using the nano sorbent for the fluoxetine pre-concentration and enrichment from human plasma, human urine, and water samples. The efficient factors on the SPE extraction process were optimized for fluoxetine sorption, and under the optimum conditions, the analytical parameters as LOD and LOQ of this technique were calculated and compared with other adsorbents.

Material and methods

Chemicals, reagents, devices, and HPLC conditions

Fluoxetine and ciprofloxacin with 98% of purity were obtained from Darou Pakhsh Holding Company (Iran). Acetonitrile (C_2H_3N, 99%), sodium hydroxide (NaOH, 98%), $FeCl_2$, and $FeCl_3$ with 98% of purity were purchased from Merck

(Darmstadt, Germany). Ethylenediamine ($C_2H_8N_2$, 99%) was obtained from Sigma-Aldrich (USA). The nanotubes of multi-walled carbon were purchased from carbonstructures Co., (Iran).

The fluoxetine stock solution (500 mg L^{-1}) was prepared by dissolving 50 mg of fluoxetine in 0.1 L volumetric flasks with ultrapure water. The fluoxetine working solutions were created from a stock solution with distilled water. Different concentration of fluoxetine solutions (1-10 mg L^{-1}) was used for drawing calibration curves.

Infrared spectra were recorded in FT-IR of Perkin Elmer RX1 spectrometer. A spectrometer UV–Visible UV1700 was employed to evaluate the fluoxetine. XRD analysis was done by the Philips apparatus with the PW1730 model. The surface morphology was characterized using scanning electron microscopy (Tescan, Mira III).

HPLC separation is equipped with a UV-VIS detector. The C_{18} column was used for the separation operation (150×3.9 mm, 5 µm). The acetonitrile (55:45, v/v) and buffer of sodium phosphate (0.05 mol L^{-1}, 3.07 of pH) were applied for the mobile phase.

Then, the flow rate was set at 1.0 mL min^{-1}, a wavelength of 230 nm, and the column temperature was set at 30 ºC. The methanol and acetonitrile solvents were provided in HPLC grade.

Sampling and preparation of biological samples

Human sample blood collected in the morning was stored in an anticoagulant glass tube and then centrifuged at 3000 rpm at 20 min. Afterward, the supernatant plasma was kept at -20 °C. The proteins and other substances were removed by adding a methanol-containing aqueous solution (8 mL) to a 10 mL glass vial containing 2 mL of plasma following vortexing for 1 min and then centrifuged in 10000 rpm at 20 min. Next, the supernatants were gathered. Besides, the sample of urine was collected of healthy volunteers and then frozen and thawed at room temperature. Before the extraction procedure, the urine samples were centrifuged in 12000 rpm at 10 min and then filtrated *via* a filter of 0.45-µm Millipore. Tap water was obtained from the tap at the Varamin city in a suitable bottle.

Synthesis of magnetite nano-sorbent (Fe_3O_4@MWCNT)

The following procedure was done for the preparation of magnetite nano-sorbent. At first, the 0.08 g ($FeCl_2$) and 0.21 g ($FeCl_3$) was dissolved into deionized water (20 mL). Then 0.04 g of MWCNT (Multi-walled carbon nanotubes) was admixed and heated for 20 min at 50 ºC. Then, the resultant mixture was put into an ultrasonic bath for 20 min. Afterward, this solution was heated for 40 min by adding 1 mL of NaOH. After collecting, nano-sorbents were separated with magnetic separation and washed with water and ethanol, respectively, to remove extra reagents and solvent [23].

Preparation of amine-functionalized carbon nanotubes (Fe_3O_4@MWCNT-amine)

The collected nano-sorbents (MWCNT-COOH) were added to an ethylenediamine solution (20 mL) and then this mixture was put in the ultrasonic bath at 5 h. Next, the mixture was stirred for 24 h at 60 ºC, and finally, the product was collected by vacuum filtration and then washed with methanol and finally dried in an oven vacuum [24,25].

Batch method of fluoxetine adsorption/desorption

A set of fluoxetine solutions (1 mL) with 1–40 µg mL^{-1} concentrations were taken in a test micro-tube and their pH was adjusted to 10 with buffer solution. The 0.02 g of Fe_3O_4@MWCNT-amine was added to all tubes and these mixtures were shaken for 5 min. The adsorbent has been centrifuged and the sorbed fluoxetine was eluted with acidic methanol. The fluoxetine concentrations in the eluate were assessed with HPLC [26,27].

Study of isotherm

The study of isotherm was performed with adding 0.02 g of adsorbent to a series of a beaker filled with 10 mL of fluoxetine diluted solutions (1–10 mg mL^{-1}). The beakers were shaken for 28 min in pH 10 at 25 °C. The final fluoxetine concentration in solution was determined by the HPLC system. The fluoxetine amount in equilibrium qe (mg g^{-1}) Fe_3O_4@MWCNT-amine was obtained with the following equation:

$$q_e = \frac{C_0 - C_e}{m} V \qquad (1)$$

where C_0 and C_e are fluoxetine concentrations of initial and equilibrium, respectively (mg L^{-1}); V (L) is the solution volume, and m is the Fe_3O_4@MWCNT-amine mass used (g) [28-30].

Result and Dissection

Characterization of Fe_3O_4@MWCNT-amine adsorbent

In Figure 1a, the peaks at 2850 and 2960 cm^{-1} confirmed the C-H bonds of the nanotube [31]. The characteristic absorption bands of MWCNT seen in 1622 cm^{-1} and 1430 cm^{-1} were attributed to the C=O and C=C stretching bonds of the graphite structure [32,33]. The distinguished bands at 1032 and 1115 cm^{-1} were corresponding to the C-O bond. Also, the wide band at 3453 cm^{-1} was due to the N-H bending. Besides, the absorption band at 1620 cm^{-1} belonged to the plane bend of N-H in amine connected to MWCNT.

Figure 1b represents the FTIR of Fe_3O_4@MWCNT-amine after fluoxetine adsorption. As it can be observed, the band at 3434 cm^{-1} relates to the N-H functional group of adsorbed fluoxetine on the Fe_3O_4@MWCNT-amine. Besides, the peak at 1455 cm^{-1} is indexed to the vibrations of the aromatic ring (C-C) from fluoxetine.

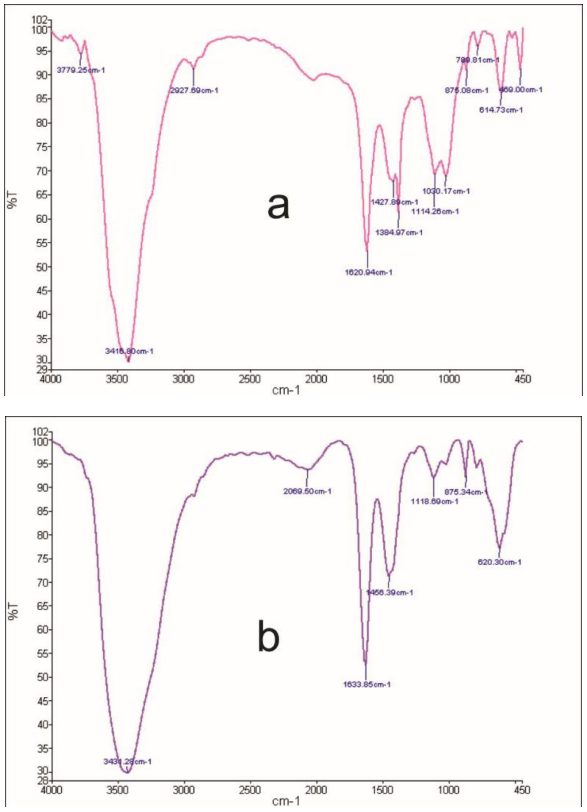

Figure 1: FTIR spectra of Fe_3O_4@MWCNT-amine before (a) and after (b) fluoxetine adsorption

FESEM analysis

Figure 2(a-b) shows FESEM images of Fe_3O_4@MWCNT-amine before and after fluoxetine adsorption. Fe_3O_4@MWCNT-amine is aligned with a thickness of about 10–15 nm and a length of multiple micrometers Figure 2(a). Brighter spots indicate the amine group on the Fe_3O_4@MWCNT (Figure 2a). After fluoxetine adsorption by Fe_3O_4@MWCNT-amine, the width of the adsorbent increased to 29 nm (Figure 2b).

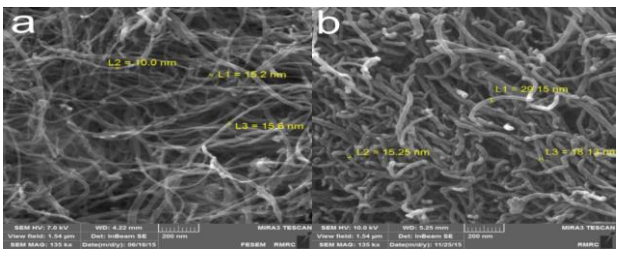

Figure 2: FESEM images of Fe_3O_4@MWCNT-amine before (a) and after (b) fluoxetine adsorption

XRD analysis

Figure 3 displays XRD patterns of Fe_3O_4@MWCNT-amine before and after fluoxetine adsorption. In Figure 3a, the broad peak at 2θ = 26° is related to the graphite structure reflection of MWCNT [33]. The peaks at 18.5°, 30.3°, 35.3°, 43°, 56.5°, and 65° can be indexed to magnetite of Fe_3O_4 [34]. After fluoxetine adsorption on Fe_3O_4@MWCNT-amine, the changing was observed in the intensity of 2θ = 26° and the location of other peaks (Figure 3b).

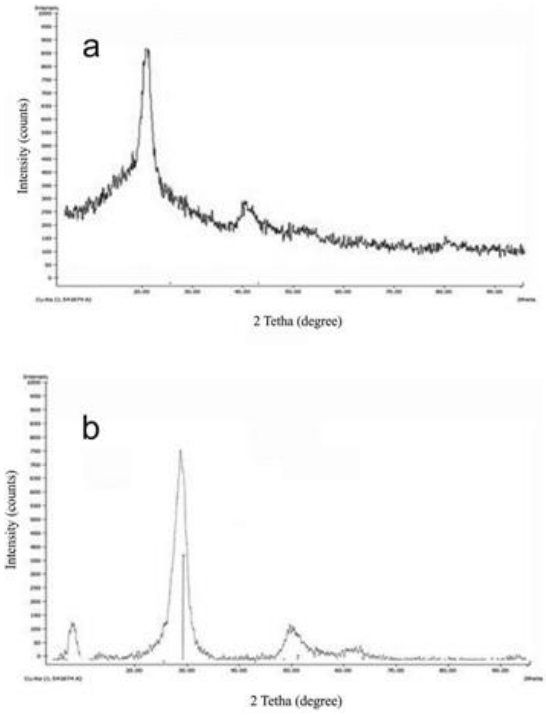

Figure 3: XRD patterns of Fe_3O_4@MWCNT-amine before (a) and after (b) fluoxetine adsorption

Factors influencing the measurement of fluoxetine extraction

To evaluate the pH effect on fluoxetine extraction, the pH was set in the 2-10 ranges. The results reported in Figure 4 demonstrate that by adsorption at pH equal to 10 for fluoxetine, suitable conditions for MWCNT protonation are observed. Also, the highest uptake of fluoxetine on the Fe_3O_4@MWCNT-amine was operated by amine group in the best electrostatically conditions at pH=10.

Time is a significant factor in fluoxetine adsorption. For optimal fluoxetine adsorption, the reaction time of 28 min (at pH=10) was achieved to have the most appropriate drug adsorption. The results in Figure 5 shows that the profile of fluoxetine uptake by Fe_3O_4@MWCNT-amine reflects the proper accessibility of the active sites in the Fe_3O_4@MWCNT-amine.

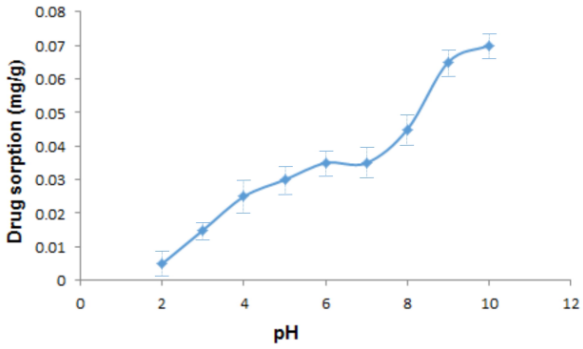

Figure 4: The fluoxetine adsorption in terms of pH

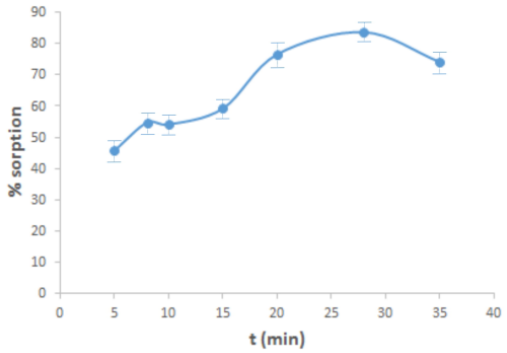

Figure 5: Investigating the time effect on drug adsorption rate at pH=10

Type of elution solvent

The type of elution solvent for drug adsorption is one of the main factors that have a great impact on the drug extraction system. In this research, elution solvents (neutral methanol, acidic methanol (75 methanol/25 HCl), basic methanol (75 methanol/25 NaOH), neutral ethanol, acidic ethanol (75 ethanol/25 HCl), basic ethanol (75 ethanol/25 NaOH), and acetonitrile) were applied and the optimal elution solvent for the fluoxetine adsorption was selected (Figure 6). Considering the maximum drug adsorption with acidic methanol, this elution solvent was selected

for the extraction of fluoxetine with the best balance created between the Fe_3O_4@MWCNT-amine and the elution solvent.

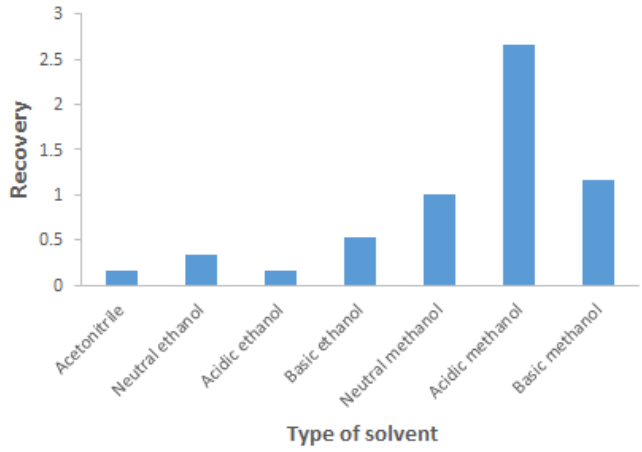

Figure 6: Effect of elution solvent on drug adsorption

Study of isotherm

Langmuir isotherm is based on the assumption that maximal adsorption occurs when a saturated layer of fluoxetine molecules is present on the surface of Fe_3O_4@MWCNT-amine, the adsorption energy is constant, and there is no transfer of drug molecules to the surface. This model is expressed as follows [35]:

$$\frac{c_e}{q_e} = \frac{c_e}{q_{max}} + \frac{1}{q_{max} K_L} \quad (2)$$

where q_{max} is the highest fluoxetine sorption capacity relates to perfect monolayer coating at Fe_3O_4@MWCNT-amine (mg g^{-1}) and K_L is constant of Langmuir model (L mg^{-1}), qe is the fluoxetine adsorbed on Fe_3O_4@MWCNT-amine surface (mg g^{-1}), Ce is the fluoxetine concentration at equilibrium (mg L^{-1}). The result fitted fine in the model of Langmuir as shown by the value of the coefficient (R^2 = 0.9955). The K_L and q_{max} values were achieved by 1.02 L mg^{-1} and 3.85 mg g^{-1}, respectively. The experimental data well fitted by this model and demonstrated the fluoxetine-binding sites homogeneous nature on Fe_3O_4@MWCNT-amine. The essential characteristics of the model can also be represented in terms of R_L (separation factor), expressed with an equation.

$$R_L = \frac{1}{1 + (K_L c_0)} \quad (3)$$

Where C_0 is the concentration of initial fluoxetine (mg L^{-1}). For desirable sorption, the R_L (separation factor) value lies in the 0-1 range. Thus, the R_L with a value of 0.65 calculated at pH=10 lies between 0 and 1 confirming favourable fluoxetine adsorption.

Analytical performance

Calibration curve of fluoxetine extraction method

After optimizing all the factors affecting the adsorption of fluoxetine, the calibration curve of the extraction method by Fe_3O_4@MWCNT-amine was drawn. For this aim, different concentrations of fluoxetine were added to the balloons (10 ml), and the sorbent was added to the balloons in optimum conditions. Then the extraction procedures of fluoxetine were performed. This technique gives an excellent linear range of 1-10 mg L^{-1} for fluoxetine with a coefficient of correlation (R^2) of 0.9958. The extraction recovery was determined as the percentage of the total fluoxetine which was extracted from the solid phase and subsequently into the desired eluent.

The limits of detection (LODs) for the presented method were obtained according to the slope of the fluoxetine concentration curve employing a signal-to-noise ratio of 3 (LOD = 6 µg L^{-1}). The quantification limit (LOQ) was calculated from 18 µg L^{-1} [36].

Precisions of method

To evaluate the precisions of the procedure (according to the relative standard deviation), analysing the extraction amounts of 4 solutions of fluoxetine in intra-day and inter-day were studied. To do this goal, 4 standard fluoxetine solutions with optimum concentrations in balloons of 10 ml have been prepared in entirely similar conditions according to the presented method. By achieving the recovery of fluoxetine,

the relative standard deviation (RSD) for intra- and inter days was achieved by 2.03 % and 2.40 % (n=3), respectively

Influence of interference specie

The interference effect of the species on the measurement of fluoxetine was investigated under optimum conditions considering the biological matrices. For this aim, the dye samples were mixed with various concentrations of ciprofloxacin (the time of analysis is one hour after the addition of ciprofloxacin) and the absorption intensities have been compared with a sample of fluoxetine in the absence of ciprofloxacin. The interference species (ciprofloxacin) was added with concentrations of 5-15 mg L^{-1}. An interference species of fluoxetine is shown in Table 1. As can be observed, in higher concentrations, the more ciprofloxacin effect is. (The dilution decreases the absolute amount of ciprofloxacin).

Table 1: Investigating the ciprofloxacin effect on fluoxetine extraction

Interference drug (mg L^{-1})	Fluoxetine extraction (%)
5	3.2
10	2.5
15	1.73

Method application

The introduced method was used for the detection of the fluoxetine of real samples. Human biological fluids and water samples were collected for the extraction of the fluoxetine. The concentration of fluoxetine in the human sample blood, urine, and water samples were determined (Table 2). These samples were spiked with fluoxetine standards to determine matrix effects. The powerful recovery in plasma demonstrates a strong competition of plasma matrices for fluoxetine binding or occlusion of binding sites on the Fe_3O_4@MWCNT-amine by plasma components. Besides, a good recovery for urine and water samples demonstrate the reasonability and reliability of the introduced method procedure.

Table 2: Determination of fluoxetine in human sample blood, urine, and water samples

Samples	Fluoxetine adding (µg L^{-1})	Found[a] (µg L^{-1})	Recovery (%)	RSD (%)
Plasma	0	7.4	95	3.5
	50	57.5	98	4.6
	150	156.54	98.36	4.8
Urine	0	N.D[b]	-	-
	50	49.4	98	5.9
	150	148.9	98	5.7
Tap water	0	N.D[b]	-	-
	50	47.25	94.5	4.5
	150	145.40	93.6	4.3

[a] For three determinations
[b] Not Detection

Conclusion

This research aimed at developing an efficient, easy, inexpensive, and suitable method for evaluating the drug value of fluoxetine in real samples. It was found out that batch adsorption of fluoxetine on Fe_3O_4@MWCNT-amine adsorbent depends on factors such as primary fluoxetine concentration, time, pH. The optimum dose of Fe_3O_4@MWCNT-amine was determined to be 0.02 g. The results indicated that the reaction time at optimizing pH=10, between the Fe_3O_4@MWCNT-amine and the fluoxetine for getting equilibrium is 28 min. Among the factors affecting the extraction of fluoxetine, the acidic methanol demonstrates the best recovery from the Fe_3O_4@MWCNT-amine. The Langmuir

adsorption isotherm for fluoxetine was investigated in the interaction with Fe_3O_4@MWCNT-amine, and the results indicated the adsorption capacity of 3.85 mg g^{-1}. In this study, the LOQ and LOD were observed to be 18 and 6 µg L^{-1}, respectively. A linear range for the fluoxetine concentration in dispersive solid-phase extraction method was found in the range of 40−800 µg L^{-1} Finally, the resulting dispersive solid-phase extraction based on amine-functionalized carbon nanotubes presented a high extraction efficiency of fluoxetine.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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