**Facile Preparation of Graphene-Modified Magnetic Nanoparticles and Their Application in the Analysis of Four Anti-depressant Drugs in Plasma and Urine**

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**Abstract**

**Background**: Anti-depressants are used to treat depression and some anxiety and personality disorders. In this study, a magnetic sorbent was prepared for the extraction of some antidepressant drugs from plasma and urinary samples. In order to extract the target compounds from the samples, magnetic Fe3O4 nanoparticles coated with graphene were used as the sorbent.

**Methods**: Vibrating sample magnetometry, scanning electron microscopy, energy dispersive X-ray spectroscopy, Brunauer–Emmett–Teller nitrogen sorption/desorption analysis, Fourier transform infrared spectroscopy, and X-ray diffraction were applied to investigate the synthesized sorbent. First, an ammoniacal solution of the target compounds was exposed to the sorbent for the adsorption purpose. It was facilitated by vortexing. Then, by using an external magnetic field, the sorbent particles containing the adsorbed analytes were separated from the solution. Acetonitrile was used to desorb the analytes from the sorbent. Then, the eluate containing the analytes was separated from the sorbent in the presence of an external magnetic field, mixed with 1,1,2-trichloroethane (at µL-level), and quickly injected into the ammoniacal solution containing dissolved KCl. After centrifuging the formed cloudy solution, an aliquot of the settled organic phase was injected into a gas chromatograph equipped with a mass spectrometer.

**Results**: Low limits of detection (LODs) and quantification were obtained in the ranges of 0.66-1.03 and 2.1-3.4 ng/mL, respectively. The method led to acceptable extraction recoveries (55-66%), high enrichment factors (214-275), good repeatability (relative standard deviation ≤ 5.7% for intra- and inter-day precisions), and good linearity of the calibration curves (r2 ≥ 0.996).

**Conclusion**: The proposed method can be applied for the successful extraction of anti-depressant drugs from plasma and urine samples.

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**Introduction**

Depression describes a mood disorder that causes a strong sense of sadness and lack of interest. Various factors are contributing to the increase of antidepressant drugs consumption today including economic stress and emotional problems. Fluoxetine belongs to the selective serotonin reuptake inhibitor class of anti-depressants. Amitriptyline, imipramine, and clomipramine are drugs of the tricycle anti-depressants (TCAs) class. Therapeutic drug monitoring program is important in clinical pharmacology and forensic sciences since it can aid the effective control of pharmacotherapy and drug poisoning. This point can be more important in the case of TCAs because of their narrow therapeutic range. Different analytical methods have been used to determine anti-depressant drugs in various samples including high performance liquid chromatography and gas chromatography (GC). The above-mentioned methods have made considerable progress in recent years; however, matrix effect and low concentration of analytes make it difficult in directly using of them in the analysis of the target compounds. Consequently, sample preparation techniques need to be used on real samples before their introduction to analytical devices leading to cleaning, enrichment, and improved sensitivity. Traditional sample preparation procedures include liquid-liquid extraction (LLE) and solid phase extraction (SPE). There are certain problems associated with both methods. SPE involves the adsorption of analytes onto a solid sorbent located in a cartridge. Afterwards, the adsorbed analytes...
are desorbed with a small volume of an organic solvent. Lower organic solvent usage and less matrix effect are the advantages of SPE over LLE. The main disadvantages of SPE are cartridge obstruction, high back pressure, the need for suction, and the use of organic solvents in conditioning the sorbents and also elution of the analytes. Dispersive solid phase extraction (DSPE) was developed to solve these problems by dispersing sorbent particles directly into the sample solution instead of being located into cartridge. In DSPE, first, the sorbent is added into a sample solution containing the analytes and vortexed. Then, the sorbent particles are separated from the sample solution by centrifugation or filtration. In the following, the sorbent containing the analytes is contacted with an appropriate organic desorption solvent. DSPE has been studied with various sorbents including metal organic frameworks, molecularly imprinted polymers, carbon nanotubes, etc. The above-listed sorbents require a centrifugation or filtering step prior to being used in another step. In order to speed up the above method, in recent years, magnetic sorbents have been used instead of non-magnetic sorbents, which do not require centrifugation or filtration step in the sample preparation procedure. The method in which magnetic sorbents are used is called magnetic dispersive solid phase extraction (MDSPE). In MDSPE, a magnetic sorbent is rapidly separated from the solution by applying an external magnetic field. The widespread use of magnetic nanomaterials in research and industrial works is due to their unique properties. Iron, nickel, cobalt, and their oxides are used as the magnetic part of the sorbent to synthesize magnetic compounds. Various reagents are used to cover the magnetic part of the sorbent to prevent its oxidation. Different methods have been applied for the synthesis of magnetic nanoparticles (MNPs) such as co-precipitation, microwave exposure, thermal decomposition, sonochemistry, microemulsion, pulsed laser decomposition, and chemical vapor deposition. Graphene is one of the materials used to cover the magnetic part of the sorbent which is due to its mechanical and thermal stability and high surface area. Recently, magnetic nanocomposites based on graphene have been prepared by various methods. DSPE and MDSPE are often followed by a dispersive liquid-liquid microextraction (DLLME) method. The purpose of combining MDSPE and DLLME is reaching to low limits of detection (LODs), high selectivity, and high enrichment factors (EFs).

In this study, first, in order to prepare the magnetic sorbent, Fe$_3$O$_4$ MNPs were synthesized and then modified with graphene by co-precipitation method. The prepared sorbent was used as an efficient and inexpensive sorbent in MDSPE integrated with DLLME to extract four anti-depressant drugs containing fluoxetine, amitriptyline, imipramine, and clomipramine from plasma and urine samples prior to being analyzed by GC-mass spectrometry (MS).

Materials and Methods

**Chemicals and solutions**

Fluoxetine was supplied from Abidi Pharmaceutical Company (Tehran, Iran). Amitriptyline was provided from Darupakhsh Company (Tehran, Iran). Imipramine and clomipramine were bought from Soha Pharmaceutical Company (Karaj, Iran). 1,1,1-Trichloroethane (1,1,1-TCE), 1,2-dibromoethane (1,2-DBE), and carbon tetrachloride were supplied from Janssen (Beerse, Belgium). Acetonitrile (ACN), acetone, methanol, chloroform, sodium sulfate, and sodium chloride (all analytical grade) were from Merck (Darmstadt, Germany). 2-Propanol was from Caledon (Georgetown, Canada). Ammonia solution (25%, w/w), graphene, ethanol, FeCl$_3$$\cdot$6H$_2$O, and FeSO$_4$$\cdot$7H$_2$O used in the synthesis of the sorbent were bought from Merck. Deionized water was provided from Ghazi Pharmaceutical Company (Tabriz, Iran). A stock solution of the anti-depressants (1000 μg/mL of each) was prepared in methanol. Daily used standard solutions were prepared by dilution of the stock solution with deionized water.

**Samples**

A plasma sample of a healthy individual was prepared from the Iranian Blood Transfusion Organization (Tabriz, Iran). Also, a blank urine sample was obtained from a healthy person who has not received the studied drugs. Two plasma samples and two urine samples from the patients who used the studied anti-depressant drugs were obtained (Tabriz University of Medical Sciences (IR. TBZMED.VCR.REC.1400.463), Iran). In order to remove plasma proteins, 1 mL ACN (in two parts, each part 0.5 mL) was added to 1 mL plasma, vortexed for 2 minutes, and centrifuged for 5 minutes at 7000 rpm. The obtained supernatant was diluted with 0.05 mol/L ammonium solution at a ratio of 1:3. Urine was diluted with 0.05 mol/L ammonium solution at a ratio of 1:1 before using in the proposed method.

**Instrumentation**

Separation and determination of the anti-depressant drugs was done by an Agilent 7890A-5975C GC-MS (Agilent Technologies, CA, USA) system equipped with a split/splitless injection port. Temperature programing of column oven was as follows: initially the temperature was set at 80°C for 5 minutes and then raised to 300°C at a rate of 12°C min$^{-1}$ and held for 8 minutes. Injection port temperature was set at 300°C and worked in a pulsed split mode. The carrier gas was helium with purity of 99.9999% (Gulf Cryo, Dubai, UAE) at 1.2 mL/min flow rate. An HP–5MS capillary column (30 m × 0.25 mm i.d., with a film thickness of 0.25 μm) (Hewlett-Packard, Santa Clara, USA) was used in the separation of anti-depressants. MS operation conditions were as follows: ion source temperature: 280°C; transfer line temperature: 300°C; voltage of detector: –1700 V; ionization of the analytes at 70 eV; and acquisition rate: 20 Hz. A Hettich centrifuge
model D-7200 (Kirchlegation, Germany) was used for centrifugation of the samples. An L46 vortex (Labinco, Breda, the Netherlands) was used in sample preparation step. A Metrohm pH meter model 654 (Herisau, Switzerland) was utilized to adjust pH. For the synthesis of the sorbent, a magnetic heater-stirrer (Heidolph MR 3001K, Germany) was used. A zero dead volume 1-µL microsyringe (Hamilton, Switzerland) was used for injection of the analytes into the separation system. An ultrasonic water bath (Branson 3510, Danbury, CT, USA) was used in synthesis of the sorbent. Also, a Siemens D500 diffractometer (Siemens AG, Karlsruhe, Germany) at the scan range and rate of 4-73° and 1° min⁻¹, respectively, was used for the X-ray diffraction (XRD) analysis. A Fourier transform infrared (FTIR) spectrophotometer (Bruker, Billerica, USA) was used to record FTIR spectrum of the sorbent in the range of 400-4000 cm⁻¹. Brunauer-Element-Teller (BET) analysis based on nitrogen adsorption/desorption was performed using a BELSORP mini II analyzer (BEL, Japan). A Lake Shore 7304 vibrating sample magnetometer (VSM) (Lakeshore, USA) was employed to evaluate the magnetic property of the prepared sorbent. In order to obtain information about surface morphology and elemental analysis of the sorbent, a Mira 3 microscope (Tescan, Czech Republic) was used for scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analyses.

**Sorbent synthesis**

MNPs were synthesized by co-precipitation method. Initially, 0.174 g graphene was added to 40 mL deionized water and put in a sonication bath for 30 minutes. Then 0.530 g FeCl₂·6H₂O and 0.360 g FeSO₄·7H₂O were added to the solution. The mixture was transferred into a water bath thermostated at 80°C. Then 12 mL concentrated ammonia solution was added dropwisely to the above-mentioned solution and stirred for 2 hours at 300 rpm. The produced MNPs were separated from the solution using a magnet. The obtained MNPs were washed three times (each time with 20 mL) with a mixture of ethanol-water (50:50, v/v) to reach neutral pH. Finally, they were dried at room temperature for 24 hours. The synthesized sorbent was analyzed using FTIR, VSM, EDX, SEM, BET, and XRD methods before being used in the extraction process.

**Extraction procedure**

**MDSPe**

Five milliliters of ammonia solution (0.05 mol L⁻¹) spiked with 25 ng/mL of each drug or real sample (see section samples) was transferred in a 10-µL conical bottom glass test tube. After that, 20 mg of the sorbent was added and the mixture was vortexed for 3 minutes. Then the sorbent containing the adsorbed analytes was collected by an external magnetic field. ACN (1.5 mL) was added onto the sorbent and vortexed for 3 minutes. The solution was separated from the sorbent in the presence of external magnetic field and used in the next process.

**DLLME**

To the eluate obtained from the previous step, 31 µL of 1,1,2-TCE was added as an extraction solvent and then the resulting solution was injected into 5 mL ammonia solution (0.1 mol/L) containing KCl (1.0 mol/L) placed in a 10-mL conical bottom glass test tube with a 5-µL glass syringe. The obtained cloudy solution was centrifuged at 8000 rpm for 5 minutes. One microliter of the sedimented phase (10 ± 0.5 µL) was injected into GC-MS.

**EF and extraction recovery (ER) calculation**

The ratio of analyte concentration in the sedimented phase (C_{sed}) to its initial concentration in the sample (C₀) is called EF. Eq. (1) shows it.

\[
EF = \frac{C_{\text{sed}}}{C_0}
\]  

(1)

Also, ER is the percentage of the total analyte amount (n₁) extracted into the sedimented phase (n_{sed}). Eq. (2) shows it.

\[
ER = \frac{n_{\text{sed}}}{n_1} \times 100 = \frac{C_{\text{sed}} \times V_{\text{aq}}}{C_0 \times V_{\text{aq}} \times 100} = EF \times \frac{V_{\text{sed}}}{V_{\text{aq}}} \times 100
\]  

(2)

Here, V_{sed} and V_{aq} are the volumes of the sedimented phase and aqueous phase, respectively.

**Results and Discussion**

**Sorbent characterization**

Several techniques were used to evaluate the properties of the prepared nanocomposite including FTIR, VSM, EDX, XRD, SEM, and BET. The crystalline structures of graphene, Fe₃O₄, and the synthesized nanocomposite were studied using XRD patterns. Fe₃O₄ has six relevant peaks in XRD pattern (30.1°, 35.7°, 43.3°, 53.9°, 57.5°, and 63.0°). The peak around 2θ angle of 35.73° in Figure 1a is related to the diffraction pattern of Fe₃O₄ particles. Also, the sharp peak around 20 angle of 26.36° in Figure 1b is related to the diffraction pattern of graphene. The diffraction pattern of nanocomposite in Figure 1c has the peaks of graphene and Fe₃O₄ (26.60, and 35.66°) and it confirms the presence of graphene and Fe₃O₄ in the structure of the sorbent. It proves the successful formation of the composite.

In order to identify the functional groups, Fe₃O₄, graphene, and the sorbent were analyzed by FTIR. According to Figure 1d, the two intense peaks observed at 569 and 636 cm⁻¹ are related to the stretching vibrations associated to the Fe-O bond in Fe₃O₄. Figure 1e shows FTIR spectrum of graphene. It has strong and broad O-H stretching vibration band at 3433 cm⁻¹, carboxyl (C = O) stretching bond at 1727 cm⁻¹, and C-O stretching vibration at 1076 cm⁻¹. The peaks at 1727 and 1076 cm⁻¹ in Figure 1f are related to C=O and C-O stretching vibration bands, respectively, confirming the successful modification of Fe₃O₄ with graphene.

SEM analysis was performed to evaluate the surface morphology of the sorbent particles. The obtained results
in Figure 1g demonstrate the aggregation of spherically-shaped particles of the nanocomposite. The mean diameter of the nanoparticles is about 31 nm (Figure 1h).

So, the obtained morphology shows a perfect surface for the adsorption of the analytes.

VSM is one of the techniques used for measuring magnetic property of MNPs as a function of an applied external magnetic field between 3 and -3 Tesla. Considering the results in Figure 1i, saturated magnetization of the nanocomposite (32 emu/g, curve B) is lower than pure Fe$_3$O$_4$ (51 emu/g, curve A) which is because of its graphene coating, but it is suitable for collecting the sorbent particles by an external magnetic field from the solution.

In order to obtain the type and percentage of constituents in the sorbent structure, EDX analysis was performed. According to Figure 1j, the presence of three elements including C, Fe, and O with the percentages of 31.11, 35.37, and 33.52 %, respectively, in the sorbent structure was assigned.

Additionally, BET analysis was performed on the sorbent. The specific surface area and average diameter of the sorbent pores were obtained as 48.58 m$^2$/g and 6.95 nm, respectively.

**Optimization of MDSPE parameters**

**Ammonia solution concentration**

Considering the pK$_b$ values of the studied anti-depressants (pK$_b$ values of amitriptyline 4.3, clomipramine 4.8, imipramine 4.6, and fluoxetine 4.2), at pHs higher than 10, the neutral forms of the analytes are present which are adsorbed from the aqueous solution onto the sorbent surface. In the pHs lower than 10, the analytes are in their ionic forms and have tendency to stay in the aqueous phase. From another point of view, the surveyed drugs are in their ammonium forms having a positively charged nitrogen on their structures. So, in order to neutralize the analytes charge and facilitate their extraction, the pH of solution was enhanced using ammonia solution to convert the analytes into their neutral forms. Therefore, in order to increase the extraction efficiency of the analytes, different concentrations of ammonia solution were tested. As a result, 0.01, 0.05, 0.10, 0.20, and 0.40 mol/L ammonia solutions were examined and compared to the case in which analytes were dissolved in deionized water. According to the results in Figure 2, 0.05 mol/L ammonia solution was selected for the next experiments due to resulting higher ER values.

**Graphene percentage in the sorbent and weight of nanocomposite**

Due to oxidation probability and also low adsorptive capability, Fe$_3$O$_4$ MNPs should be coated with some chemical compounds. Graphene was used for this purpose in the present study. The percentage of graphene...
in the nanocomposite should be optimized in order to achieve high adsorption efficiency. For the optimization of graphene content in the sorbent, different percentages of graphene including 25%, 50%, and 75% with respect to the initially produced bare Fe₃O₄ amount were examined. According to Figure 3, 75% graphene was chosen as the optimum percentage because the resulted sorbent in this case shows higher ERs. It is noted that the percentage of 90% was also tested in the preparation of the nanocomposite. However, the obtained sorbent particles were not collected completely in the presence of an external magnetic field. So, 75% of graphene was opted for the synthesis of the nanocomposite.

The weight of the sorbent is also a very important parameter in MDSPE process and it can affect the ER values. For this purpose, different weights of the synthesized nanocomposite including 3.0, 5.0, 10, 15, 20, and 25 mg were selected. Figure 4 shows that 20 mg has the highest efficiency among the studied weights. Therefore, 20 mg was selected as the optimum sorbent weight for the subsequent analyses.

**Study of ionic strength**

In order to enhance ionic strength of the solution and reduce solubility of the analytes in the ammonia solution, two salts were tested. For this purpose, NaCl and Na₂SO₄ (1.0 mol/L of each salt) were evaluated and the obtained results were compared with those of the saltless conditions. According to Figure 5, with salt addition, the ERs of the analytes are reduced. This observation can be due to the increased viscosity of the solution that reduces adsorption of the analytes onto the sorbent surface. Consequently, the MDSPE process involved no addition of salt to the basic solution.

**Optimization of vortexing time in adsorption step**

During the adsorption step, vortexing time is vital to ensure that the nanocomposite particles contact adequately with the analytes. The adsorption of the analytes was facilitated with vortexing for 1, 3, 5, 7, and 9 minutes. The results (data not shown here) showed that the appropriate vortexing time was 3 minutes and the ER values were reduced after 3 minutes. Therefore, the subsequent experiments were conducted by 3 minutes vortexing.

**Desorption/disperser solvent type and volume**

A desorption/disperser solvent was used for desorbing the anti-depressants from the sorbent particles surface and dispersing the extraction solvent used in DLLME step into an aqueous phase. It should be an organic solvent which is miscible in both aqueous and organic solvents (the organic solvent refers to the extraction solvent in DLLME). Thus, 1.0 mL of acetone, methanol, ACN, and 2-propanol were used for this purpose. In order to obtain 10 ± 0.5 µL of the sedimented phase volume in DLLME using 1,1,2-TCE as the extraction solvent, volumes of 27, 27, 27, and 34 µL of 1,1,2-TCE were used for each of the mentioned desorption/disperser solvents, respectively. The results in Figure 6 show that ACN is the suitable desorption/disperser solvent due to its resulted high ERs for all of the studied anti-depressant drugs. For the evaluation of ACN volume, different volumes of 0.5, 1.0, 1.5, and 2.0 mL were tested. In this stage, 22, 27, 31, and 33 µL of 1,1,2-TCE were used to reach 10 ± 0.5 µL sedimented phase volume, respectively. The results (data not reported here) showed that 1.5 mL of ACN was the optimum volume for desorbing the anti-depressants from the sorbent surface. So, 1.5 mL of ACN was utilized for the desorption of the analytes from the sorbent surface in the further studied.

**Optimization of vortexing time in desorption step**

The period of time in which anti-depressant drugs are desorbed from the sorbent surface by vortexing should also be optimized. For this purpose, vortexing times of 1, 3, 5, and 7 minutes were evaluated. According
to the results obtained, 3.0 minutes was sufficient for the efficient removal of the analytes from the sorbent surface. As a consequence, 3.0 minutes was selected as the optimum vortexing time for desorption in the subsequent experiments.

**Optimization of DLLME parameters**

**Ammonia concentration**

In this study, in order to reach high ERs, different concentrations of ammonia solution (0.01, 0.05, 0.10, 0.20, and 0.40 mol/L) were tested and compared to that of the deionized water. According to results (data not shown here) 0.10 mol/L ammonia solution resulted in highest ERs and it was selected to be applied in the next experiments.

**Type and volume of the extraction solvent**

The extraction solvent should have some properties such as being miscible with the disperser solvent (the eluate obtained from the MDSPE step) and having little solubility in water. Also, in this method it has to have a higher density than water to be placed at the bottom of the conical glass test tube after centrifuging. Thus, 1,2-DBE, 1,1,1-TCE, 1,1,2-TCE, and carbon tetrachloride were selected according to the mentioned characteristics. Volumes of 31, 33, 31, and 27 µL of the above-mentioned extractants, respectively, along with 1.5 mL of ACN were used to reach the sedimented phase volume of 10 ± 0.5 µL. According to Figure 7, 1,1,2-TCE was selected as the extraction solvent. Then volume of 1,1,2-TCE was evaluated to investigate its effect on concentrating the analytes and its consequent effect on EFs. For this purpose, volumes of 31, 36, 41, and 46 µL of 1,1,2-TCE were used. According to the obtained results (data not shown here) the EFs decreased with increasing the volume of 1,1,2-TCE due to dilution effect. It is noted that volume of the sedimented phase increased to 10, 12, 21, and 25 µL using 1,1,2-TCE in the mentioned volumes, respectively. So, 31 µL of 1,1,2-TCE was selected as the optimum volume of the extractant in this study.

**Ionic strength optimization**

In order to evaluate the ionic strength of the aqueous phase used in the DLLME process, Na$_2$SO$_4$, KCl, and NaCl (0.5 mol/L of each) were used as salting-out agents. According to the results in Figure 8, KCl increases the ER values of all analytes more than the other tested salts. So, it was selected as the salting-out agent in this step. Then, KCl concentration was evaluated. Concentrations of 0.5, 1.0, 1.5, and 2.0 mol/L of KCl were tested. According to the results (data not shown here) up to 1.0 mol/L due to increasing ionic strength of the solution, ERs of the analytes increased. In the concentrations higher than 1.0 mol/L, the ERs decreased due to increasing viscosity of the solution. So, 1.0 mol/L KCl was selected as the appropriate salt concentration in this section of the experiments.

**Method validation**

After optimization of the parameters of the developed method, some quantitative parameters such as LOD, limit of quantification (LOQ), relative standard deviation (RSD), ER, EF, linear range (LR) of the calibration curves, and coefficient of determination ($r^2$) were examined to validate the method. The validation results are listed in Table 1. The ERs and EFs were in the ranges of 55–66% and 214–275, respectively. The LODs and LOQs (calculated as the signal-to-noise ratios of 3 and 10, respectively) were obtained 0.66-1.03 and 2.1-3.4 ng/mL, respectively. This method indicated wide LRs with $r^2$ values between 0.996 and 0.999. Repeatability of the method as RSD was assessed by determining intra- (n = 6) and inter-day (n = 4) precisions at a concentration of 20 ng/mL of each anti-depressant. They were in the ranges of 2.6-5.4 and

![Figure 6. Selection of desorption/disperser solvent type. Extraction conditions: are the same as those used in Figure 5, without salt addition.](image)

![Figure 7. Selection of extraction solvent in DLLME. Extraction conditions: are the same as those used in Figure 6, except 1,1,2-TCE was used as the extractant.](image)

![Figure 8. Study of ionic strength effect in DLLME. Extraction conditions: are the same as those used in Figure 7, except 1,1,2-TCE was used as the extractant.](image)
Table 1. Quantitative features of the developed analytical method for the analysis of anti-depressant drugs

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD a</th>
<th>LOQ b</th>
<th>LR c</th>
<th>r² d</th>
<th>RSD % e</th>
<th>EF ± SD f</th>
<th>ER ± SD g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intra-day</td>
<td>Inter-day</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.72</td>
<td>2.3</td>
<td>2.3-250</td>
<td>0.998</td>
<td>5.4</td>
<td>5.7</td>
<td>275 ± 5</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.66</td>
<td>2.1</td>
<td>2.1-250</td>
<td>0.996</td>
<td>2.6</td>
<td>3.6</td>
<td>253 ± 12</td>
</tr>
<tr>
<td>Imipramine</td>
<td>0.92</td>
<td>3.0</td>
<td>3.0-250</td>
<td>0.999</td>
<td>4.9</td>
<td>5.3</td>
<td>214 ± 21</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>1.03</td>
<td>3.4</td>
<td>3.4-250</td>
<td>0.999</td>
<td>4.1</td>
<td>4.4</td>
<td>271 ± 25</td>
</tr>
</tbody>
</table>

a Limit of detection (S/N = 3) (ng/mL).
b Limit of quantification (S/N = 10) (ng/mL).
c Linear range (ng/mL).
d Coefficient of determination.
e Relative standard deviation at a concentration of 20 ng/mL of each analyte for intra- (n = 6) and inter-day (n = 4) precisions.
f Enrichment factor ± standard deviation (n = 3).
g Extraction recovery ± standard deviation (n = 3).

3.6-5.7%, respectively.

Application on real samples analysis

Applicability of the developed approach was evaluated in human plasma and urine samples. Figure 9 shows GC-selected ions monitoring (SIM)-MS chromatograms of standard solution (25 µg/mL of each anti-depressant in methanol), the extracted plasma and urine samples from the patient who used fluoxetine and the extracted plasma and urine samples from the patient who used imipramine. The following ions were used in obtaining chromatograms: m/z 119, 104, and 211 for fluoxetine, m/z 58, 202, and 215 for amitriptyline, m/z 58, 85, and 269 for clomipramine, and m/z 58, 193, and 234 for imipramine. According to the results, fluoxetine with the concentrations of 86 ± 9 and 19 ± 0.06 ng/mL (n = 3) were found in the plasma and urine samples of the patient who used fluoxetine, respectively. Also, imipramine with the concentrations of 186 ± 16 and 96 ± 5 ng/mL (n = 3) were found in the plasma and urine samples of the patient who used imipramine, respectively. To get information about the matrix effect in the used samples, added-found method was carried out. Thus, the samples and deionized water were spiked with the anti-depressants at two concentration levels (20 and 40 ng/mL of each anti-depressant) and the suggested method was performed on them. Table 2 listed the relative recovery data for the samples compared to deionized water. Considering these results, matrices of the studied samples have no significant effect on performance of the developed method and the method is appropriate for analysis of the studied anti-depressants in these samples.

Comparison of the method with other approaches

The ER, EF, LOD, LOQ, RSD, r², and LR values of some previously reported analytical methods and the developed method are summarized in Table 3 for the analyzing the selected anti-depressants. The LODs and LOQs of the method presented in this study are better than or comparable with the mentioned methods. Repeatability of the studied method is acceptable and its RSDs lower than or are comparable with those of the other approaches. The approach has comparable LRs compared to the other mentioned methods. The advantages of the developed method were compared with other methods.
analytical method are wide LRs, low LODs and LOQs, reasonable ERs, high EFs, and low RSD values for analyzing the intended anti-depressants.

Conclusion
This study demonstrated the successful usage of graphene/Fe₃O₄ nanocomposite in an MDSPE-DLLME method followed by GC-MS for analyzing four anti-depressants in plasma and urine. The synthesized sorbent was characterized by FTIR, XRD, VSM, BET, SEM, and EDX.

Wide LRs (3.4-250 ng/mL), acceptable ERs (55-66%), high EF values (214-275), and low LODs (0.66-1.03 ng/mL) and LOQs (2.1-3.4 ng/mL) were obtained. Also, easy preparation of the sorbent and low amount consumption of the sorbent (20 mg) and organic solvents were the superiorities of the approach. The method was applied for analyzing the studied anti-depressants in plasma and urine samples at ng/mL concentration level.

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Conceptualization: Mir Ali Farajzadeh.
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Formal analysis: Sanaz Barazandeh.
Funding acquisition: Mir Ali Farajzadeh.
Investigation: Sakha Pezhhanfar.
Methodology: Sakha Pezhhanfar.
Project administration: Mir Ali Farajzadeh.
Resources: Sanaz Barazandeh.
Supervision: Sakha Pezhhanfar.
Validation: Sanaz Barazandeh.
Visualization: Sanaz Barazandeh.
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The authors declared that they have no conflict of interest.

Data Availability Statement
All data generated during the study are included in the article.

Ethical Approval
This study was approved by the ethical committee of the Tabriz University of Medical Sciences (Ethics No. IR.TBZMED.VCR.REC.1400.463).

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