BMC Chemistry

RESEARCH

Open Access

Extraction of methamphetamine and pseudoephedrine by modified graphene oxide solid phase extraction method coupled to HPLC-UV in urine sample

Nasim Nourani[1,2,3], Yousef Javadzadeh[2,4], Ali Shayanfar[5], Arezou Taghvimi[2,6], Ahad Bavili-Tabrizi[3] and Siavoush Dastmalchi[2,3,7*]

Abstract

Methamphetamine, pseudoephedrine, and related drugs are among the first drugs used for the stimulation of the central nervous system. In this study, two adsorbents based on graphene oxide (GO) were synthesized and used for the analysis of methamphetamine and pseudoephedrine. The prepared nano-adsorbents based on GO in this study were coated by polyaniline (PANI) and Fe_3O_4/C-nanodot/GO (magnetic adsorbent). The average size of nanoparticles (GO/PANI) was 18.43 nm. The specific surface area and pore size diameter of Fe_3O_4/C-nanodot/GO were 22.71 m^2 g^{-1} and 15.23 nm, respectively. Experimental variables affecting the extraction efficiency of the analytes such as pH of the sample solution, amount of adsorbent, extraction time, and type of eluents were investigated and optimized by response surface methodology. Under optimum conditions, GO/PANI and Fe_3O_4/C-nanodot/GO were considered appropriate solid phase extraction adsorbents for HPLC-based analyses of the studied drugs in human urine samples. However, GO/Fe_3O_4 nano adsorbent (Fe_3O_4/C-nanodot/GO) showed superior working condition than GO/PANI. The validated proposed analytical methods can be used for the quantitative determination of methamphetamine and pseudoephedrine in actual samples.

Keywords Methamphetamine, Pseudoephedrine, Solid phase extraction, Graphene oxide, Urine

*Correspondence:
Siavoush Dastmalchi
dastmalchi.s@tbzmed.ac.ir; siavoush11@yahoo.com
[1]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Medicinal Chemistry, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[6]Qualty Control Department, Zahravi Pharmaceutical Co., km 19 of Tabriz-Tehran road, Serm Daroo street, Tabriz, Iran
[7]Faculty of Pharmacy, Near East University, POBOX:99138, Nicosia, North Cyprus, Mersin 10, Turkey

Introduction

Methamphetamine and related drugs were first introduced as therapeutic agents for the treatment of various diseases by stimulating the central nervous system. Today these agents are being abused [1] and have become a global public health concern. Continuous and long-term use of these drugs causes complications such as psychosis and violent behaviors in regular consumers, which leads to many challenges for medical professionals and healthcare systems [2]. Therefore, it is essential to determine the amount of such compounds in different samples taken from people consuming these derivatives [3]. These

drugs can be found in various types of biological samples. Hair, plasma, and urine are the most common samples used to determine the amount of these drugs [4]. Urine is the most common biological matrix for determination of therapeutic agents due to its ease of collection, availability, and non-invasive sampling [5, 6]. Sample preparation is a crucial step in analytical procedures. It mainly helps quantitative analysis of analytes by preconcentrating them to traceable levels in matrices [7, 8]. Furthermore, the sample preparation step is used to clean up complex matrices from interfering components present in the original samples. One of the commonly used methods for sample preparation is solid phase extraction (SPE). In this method, briefly, the extraction is done in such a way that the analyte(s) in an aqueous sample is/are extracted by a stationary phase and then eluted by a suitable organic solvent [9, 10].

SPE has various types, among which dispersive solid phase extraction (DSPE) and magnetic solid phase extraction (MSPE) were used in this study. DSPE has a shorter extraction time than SPE and it is also considered a green extraction method due to the low consumption of organic solvent [11, 12].

In MSPE-based methods, a magnet can be used to quickly separate the analyte adsorbed on the magnetic solid phase from the sample without the need for filtration and centrifugation steps [13, 14]. In addition, the functionalization of magnetic nanoparticles may allow for the preparation of adsorbents with a variety of properties suitable for use in complex matrices [15, 16]. Carbon-based structures present more advantages than silica-based materials rationalizing their frequent application in solid phase extraction (SPE). Biocompatibility, thermal and chemical stability, better surface modification and, feasible pore creation are some superior aspects of carbonic structures, which make them more appropriate for use in SPE [15, 17]. Carbon nanotubes (CNTs), graphene, graphene oxide, and black graphite powder are examples of carbon-based materials, which show excellent extraction properties in SPE due to their high adsorption affinity toward most of the organic compounds. Another appropriate feature of such adsorbents is their effectiveness in mediums with a wide range of acidic and basic pHs [18]. For example, covalent organic framework (COF) and many nanocomposite compounds are used in biosensors field [19–21]. These materials have high speed and sensitivity in identifying analytes, most of these compounds have a high surface-to-volume ratio, and this leads to the detection of biomarkers [22]. Numerous studies are showing the applications of carbon-based adsorbents in the extraction of methamphetamine and other pharmaceuticals from biological mediums [17, 23, 24]. To extract methamphetamine and pseudoephedrine from urine specimens, adsorbents based on graphene oxide (GO) were used. GO, the oxidized form of graphene, has hydrophilic functional groups, including hydroxyl, epoxy, carbonyl, aldehyde, and carboxyl [25], which makes the attachment and release of biomolecules and drugs much easier [26]. Density functional theory (DFT) calculations showed that GO has a high chemical potential for reactivity, which is caused by the transfer of hydrogen atoms from organic materials to the surface of GO, while the calculations performed for carbon materials without functional groups such as graphite have reaction barriers [27]. In another computational study that used GO as an adsorbent in aqueous environments and human urine, the adsorption capacity (1253.17 mg/g) at pH 5.0 was obtained using an adsorbent dose of 0.125 g/L at 298 K, and the measured adsorption performed in simulated environment and human urine showed excellent performance of adsorbents for drug removal at real concentrations excreted by kidneys (removal values higher than 60%) [28]. In this study, we have used two types of GO-based adsorbents called GO/polyaniline and Fe_3O_4/C-nanodot/GO. These types of adsorbents can alter the optical and electrical conductivity of GO-based nanomaterials [29]. The chemical and physical properties of GO-based nanocomposites allow for structural modifications according to the required properties for a given separation problem [30].

Optimization is a critical factor in developing an analytical method [10], usually performed by experimental design (or design of experiments, DOE) method. The interaction between factors cannot be identified using a one-factor-at-a-time method, but it allows mixing different parameters with a minimum number of experiments. One of these designs is the Behnken box design, in which optimal conditions can be reached with fewer experiments [11].

The aims of this study were to develop two SPE methods for the extraction of methamphetamines and pseudoephedrine from urine, followed by quantitative analysis by the high-performance liquid chromatography (HPLC) method.

Methods and materials

Reagents

Methamphetamine and pseudoephedrine were supplied from Bahar-Afshan Company (Tehran, Iran) and Jalinous Pharmaceutical Company (Tehran, Iran), respectively. Potassium permanganate ($KMnO_4$), sulfuric acid (H_2SO_4), ortho-phosphoric acid (H_3PO_4), hydrogen peroxide (H_2O_2), aniline ($C_6H_5NH_2$), hydrochloric acid (HCl, 37%), acetonitrile (CH_3CN), methanol (CH_3OH), $FeCl_3.6H_2O$ were procured from Merck (Germany). Ammonium persulfate ($(NH_4)_2S_2O_8$, APS) provided from Amresco (USA), purified graphite powder (99.5%), and ethylene glycol were purchased from Titrachem

(Tehran, Iran). Sodium dihydrogen monosodium phosphate (H_2NaPO_4), sodium hydroxide (NaOH), potassium hydroxide (KOH) were provided from Ghatran Shimi (Tehran, Iran), and pasteurized cow's milk with 1.5% fat obtained from Pegah Dairy company (Tabriz, Iran). All solutions were prepared using ultrapure water obtained from the Milli-Q® Gradient water purification system (Millipore Corporation, Bradford, MA, USA). All the reagents were of analytical grade and used as received without further purification.

Instruments

HPLC analyses were performed on a Knauer (Germany) system equipped with a UV–VIS detector (K-2500, Knauer, Germany), pump (K-1001, Knauer, Germany), and an injector consisting of a 20 µL loop. A Fourier transform infrared (FT-IR) spectrometer (Tensor 27, Bruker, Germany) was used to record IR spectra in the range of 400–4000 cm^{-1}. Probesonication (U 200 H, Heielscher, Germany) was used for the dispersion of GO. Scanning electron microscopy (SEM) MIRA3 FEG–SEM (Tescan, The Czech Republic) was utilized for the morphologic analysis. Zeta potential measurements were performed using a zetasizer (Nano ZS ZEN 3600, England), and pH was measured using a Metrohm 827 pH-meter (Switzerland). A vortex (STA 001, Farzaneh Arman Co, Iran) was used to improve the extraction efficiency. Specific surface areas and micropore volumes (Vmicro) were determined using Brunauer-Emmett-Teller surface area and porosity analyzer (Micromeritics TriStar II Plus, USA).

Chromatography condition

HPLC analyses of methamphetamine and pseudoephedrine extracted from urine samples were performed using a C18 chromatography column (250×4.6 mm, 5 µm, precolumn, Berlin) by applying a mobile phase composed of acetonitrile-phosphate buffer (pH=2.8, 10 mM), (15:85, v/v). The sample injection volume was 20 µL and the analytical wavelength was 210 nm.

Adsorbents synthesis

Synthesis of graphene oxide (GO)

GO was prepared by Hummer's green method described in the literature [31–33]. Briefly, 300 mg of graphite powder was stirred in the presence of 12 mL of sulfuric acid on ice. Then, 1.5 g of potassium permanganate was gradually added to the medium while stirring. The reaction mixture was transferred to an oil bath (silicon oil) adjusted at 35 °C and stirred for 24 h. The product of this step was a brown pasty mass to which 12 mL of distilled water was added and the reaction was continued as the temperature was increased to 75 °C. After one hour, the reaction was stopped by adding a few drops of hydrogen peroxide and the obtained product was subjected to Buchner vacuum filtration setup using Whatman paper, and then washed with water. The resulting product was dried in the oven at 50 °C (Figure S1).

Synthesis of GO/ Polyaniline (PANI) nanocomposites

An appropriate amount (116 µL) of the aniline was added to 0.1 M hydrochloric acid solution (200 mL) to obtain a pale green solution. To the solution was added ammonium persulfate (0.07 g) and 40 mL of the GO suspension (3 g/L) [34, 35]. Then, 100 mL of sodium hydroxide alkaline solution (0.04 g/L) was added to the reaction mixture, and the reaction was continued for 24 h. The development of an emerald green color indicated the formation of polyaniline on the surface of GO. The produced GO/PANI nanocomposites were separated from the mixture by centrifugation at 6000 rpm, washed with water, and finally dried at 50 °C. A schematic illustration of the synthesis steps of the GO (a) and PANI (b) was demonstrated in Figure S1.

Synthesis of Fe3O4/C-nanodot/GO hybrid material

This absorbent was prepared in three steps similar to the reported method in the reference [36]. In the first step, GO was prepared using the method described above. Then carbon nanodots were made by mixing 25 mL of cow's milk with 20 mL of water and transferring the mixture to a hydrothermal container and incubating at 180 °C for 8 h. The obtained product was centrifuged and washed with water and ethanol and dried at 50 °C overnight. In the last step, 1 g of $FeCl_3.6H_2O$ and 4 g of sodium acetate were wholly dissolved in 50 mL of ethylene glycol with constant stirring. To the obtained solution was added 0.5 g of GO and 0.25 g of prepared carbon nanodots. To homogenize the obtained mixture, it was subjected to ultrasonic vibration for 15 min. Finally, it was transferred to the hydrothermal container and heated at 180 °C for 8 h. After the completion of the reaction, the product was separated from the aqueous phase using a magnet, washed with water and acetone, and dried in an oven at 50 °C. A schematic illustration of the synthesis steps of the Fe_3O_4/C-nanodot/GO is shown in Figure S2.

Pre-treatment of urine samples

All fasting urine samples were prepared daily. A 10 mL urine sample was transferred into a clean Falcon tube and its pH was adjusted to 10 using potassium hydroxide solution. The sample was centrifuged at 6,000 rpm for 10 min to remove the produced precipitates and then, the supernatant was diluted (1 mL urine supernatant plus 6 mL water) and used for extracting the drugs by two methods outlined below.

Dispersive solid phase extraction (DSPE) procedure using GO/PANI

An appropriate amount of GO/PANI (50 mg) was weighed and added to 7 mL of the diluted urine sample and vortexed for 4.5 min. The solid phase was collected using a centrifuge and the supernatant was removed. To extract the analytes from the adsorbent, 400 µL of extraction solvent (acetone) were added to the solid phase and the mixture was sonicated for 5 min using a sonicator. Then the solid phase was separated by centrifugation and 20 µL of the eluant was injected into the HPLC system.

Magnetic dispersive solid phase extraction (MDSPE) procedure using Fe3O4/C-nanodot/GO

The appropriate amount of Fe_3O_4/C-nanodot/GO (20 mg) was weighed and added to 7 mL of a diluted urine sample and shaken for 20 s. The solid phase was collected using a magnet and the supernatant was removed. To extract the analytes (methamphetamine and pseudoephedrine) from the adsorbent, 400 µL of extraction solvent (acetone) was added to the solid phase and sonicated for 5 min using a sonicator. Then, the solid phase was easily separated with a magnet, and the eluant was injected into the HPLC system equipped with a 20 µL loop.

Figure S3 represents a schematic illustration of the solid phase extraction which applied in this study.

Table 1 Experiments designed by Box–Behnken method for optimization of methamphetamine (concentration) extraction by GO/PANI

Run	pH	Adsorbent	Time	Solvent	AUC of methamphetamine
1	4	50	7	Acetone	7927
2	4	50	3	Methanol	14026.5
3	8	50	5	Methanol	11,403
4	4	50	3	Acetone	16245.5
5	8	80	3	Methanol	9662
6	4	80	5	Methanol	-[a]
7	4	20	5	Methanol	4273.5
8	4	20	5	Acetone	5511.5
9	12	50	7	Methanol	7360.5
10	12	80	5	Acetone	11,848
11	8	20	7	Methanol	10151.5
12	4	80	5	Acetone	23,855
13	12	80	5	Methanol	7119
14	4	50	7	Methanol	7283
15	8	20	3	Acetone	7979
16	12	20	5	Acetone	4004
17	8	50	5	Acetone	24,705
18	8	20	7	Acetone	13169.5
19	8	50	5	Acetone	25,471
20	12	50	3	Acetone	-[a]
21	12	50	7	Acetone	11,848
22	8	80	7	Methanol	4123.5
23	8	80	3	Acetone	13514.5
24	12	20	5	Methanol	1949
25	12	50	3	Methanol	1451
26	8	80	7	Acetone	7348.5
27	8	50	5	Acetone	-[a]
28	8	50	5	Methanol	13,160
29	8	50	5	Methanol	13,694
30	8	20	3	Methanol	1909

[a]Outlier data

Optimization of extraction parameters using response surface methodology

The response surface method has been used to optimize factors influencing the extraction performance in this study. It determines the main effects and interactive effects of the factors and gives the possibility to obtain the most information according to the minimum number of experiments [37]. In this study, Box-Behnken, a fractional factorial design, was employed to determine of the optimal condition [38]. The type of the extraction solvent, pH, time, and the amount of adsorbent were selected as independent variables of the extraction. The optimization design was accomplished with MINITAB (Minitab Inc., release 17). According to the initial study, two solvents for elution after SPE (acetone and methanol) and the low (pH=4, amount of absorbent=20 mg and time for DSPE=3 min and MDSPE=7 min), and high levels (pH=12, amount of absorbent=80 mg and time for DSPE=3 min) of each variable that affect the experiment were selected. The experimental matrix and the value of the area under the peak of the analyte for each experiment are shown in Tables 1 and 2.

Method development

The optimized DSPE and MSPE methods for the extraction were used to quantify the analytes. The standard solutions prepared by adding the standard analytes to the blank matrix and were used for constructing the calibration curves. For method validation, FDA guidelines were used. Linear range, sensitivity, accuracy, precision, and specificity were investigated in the urine matrix.

Analysis of real urine samples

First, permission was obtained from the Ethics Committee of Tabriz University of Medical Sciences with an approval code of 1400.083 to collect the real samples from healthy volunteers who were exposed to NPO (nil per os) for 8 h. No age or sex restrictions were imposed on individuals. Then, real samples prepared from individuals were treated and analyzed using the developed method.

Table 2 Experiments designed by Box–Behnken method for optimization of methamphetamine (concentration) and pseudoephedrine (concentration) for extraction by Fe_3O_4/C-nanodot

Run	pH	Adsorbent mass (mg)	Time (min)	Solvent	AUC of pseudoephedrine	AUC of methamphetamine
1	8	80	0.1	Methanol	1213	-[a]
2	8	50	1.05	Methanol	1624.5	1270
3	4	80	1.05	Methanol	1732.5	1856
4	8	20	2	Methanol	1350	1083
5	4	50	2	Methanol	1671	-[a]
6	8	50	1.05	Acetone	1077	1149.5
7	12	20	1.05	Methanol	198.5	1005
8	8	50	1.05	Methanol	721	1414.5
9	12	20	1.05	Acetone	0	980
10	12	80	1.05	Methanol	235.5	2049.5
11	12	50	0.1	Acetone	251.5	1244.5
12	4	50	0.1	Acetone	2407.5	1815.5
13	8	80	2	Methanol	1802	1619.5
14	12	80	1.05	Acetone	298	1642
15	8	50	1.05	Acetone	1077	1149.5
16	4	50	0.1	Methanol	2300.5	1612
17	8	80	2	Acetone	1158.5	1391.5
18	8	20	0.1	Methanol	1000	1691.5
19	4	20	1.05	Acetone	1504.5	-[a]
20	8	50	1.05	Methanol	1429.5	1703.5
21	8	80	0.1	Acetone	1213	1073.5
22	4	20	1.05	Methanol	-[a]	-[a]
23	12	50	2	Acetone	360	-[a]
24	8	20	2	Acetone	1885.5	-[a]
25	12	50	0.1	Methanol	102	-[a]
26	4	80	1.05	Acetone	1176.5	1988
27	4	50	2	Acetone	1193	2458
28	8	50	1.05	Acetone	1077	1149.5
29	8	20	0.1	Acetone	1020.5	1103.5
30	12	50	2	Methanol	196	955.5

[a]Outlier data

Results and discussion

The adsorption mechanism

GO and its modified forms are among the most commonly used materials in the advanced technologies, such as electronic devices, energy storage devices, (bio) sensors, biomedical applications, supercapacitors, membranes, catalysts, and water purification. Its many advantages have increased its use, but one of the drawbacks that may limit working with it is the restacking and aggregation of the layers as a result of interaction forces between carbon layers through π-π bonds. The re-accumulation of carbon layers leads to aggregation, minimizing the available surface area and blocking the active adsorption sites in GO. This problem can be solved by inserting nanoscale particles between these carbon layers and creating a space between these layers. In the current work and line with the aforementioned strategy, GO has been altered by dispersing C nanodots between carbon layers to resolve its re-accumulation and aggregation problem. The materials were then modified with Fe_3O_4 nanoparticles to separate C-nanodot/GO hybrid material from the solution medium using a magnetic field during analyte adsorption and desorption processes [36]. In a different approach, the growth of polyaniline chains was used to prevent the re-stacking of GO sheets as described in detail by Mitra et al. [35]. The prepared GO-based adsorbents are able to interact with drug molecules through π-π, H-bond, electrostatic and charge transfer interactions provided by aromatic, unsaturated double bonds, hydroxy, carboxylate/carboxyl, and epoxy groups as illustrated schematically in Figures S4 and S5.

Characterization of GO/Polyaniline (GO/PANI) nanocomposites

FT-IR spectrum of GO is shown in Figure S6. For GO, the specific absorption peaks were obtained at around 3400 cm^{-1}, 2931 and 2852 cm^{-1},1714 cm^{-1}, 1621 cm^{-1}, and 1173 to 1056 cm^{-1} belong to the O–H, C–H, C=O, C=C, and different C-O-C vibrations, respectively [36]. The infrared spectrum of GO/PANI shown in Figure

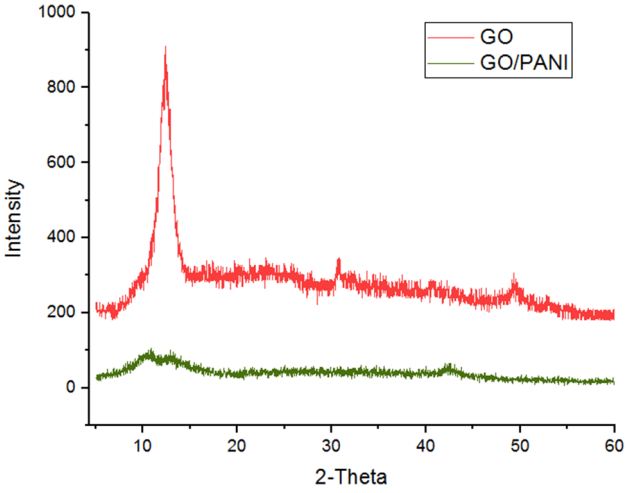

Fig. 1 XRD spectra of GO and GO/PANI

S6 indicates peaks at 1087 cm^{-1} and 1226 cm^{-1} correspond to the absorption of the aromatic out of plane C-H deformation and C-N stretching vibrations, 1404 cm^{-1} are related to the absorption of benzoide C=C group. The specific absorption peak of C=C related to N=Q=N (with Q representing the quinoid ring) is shown at 1556 cm^{-1}. Also, peaks at 1720 cm^{-1}, 2923 and 2852 cm^{-1}, and 3116 cm^{-1} are respectively for C=O stretching, asymmetric and symmetric C-H stretching, and =C–H stretching vibrations. N-H stretching vibrations gave rise to peaks from 3370 to 3517 cm^{-1}, overlapping with O-H vibration.

Zeta potential values of 1 $mg.mL^{-1}$ of GO and GO/PANI dispersions were −21.5 and −13.39 mV, respectively (Figures S7 and S8 in supplementary data). The results indicate that the negative Zeta potential of GO helps aniline monomers adsorption on nanocomposite via the electrostatic interaction between the amino group and carboxyl group and π-π conjugation interactions between benzene rings [39]. When the XRD spectrum of GO is examined, the presence of peaks confirms GO formation [35, 40]. Moreover, it is worth noting that the peak at 2θ:11.78 becomes almost invisible upon the formation of GO/PANI nanocomposite, reflecting an exfoliation of the GO sheets in this product [41] (Fig. 1).

The results of SEM analysis (Fig. 2) show that the average particle size diameter of the produced GO/PANI particles is in the range of 18.43 nm (Fig. 3). The polymer structure can be seen in different areas without altering the GO layered structure.

Characterization of the magnetic adsorbent

FT-IR spectra of C-nanodot and Fe_3O_4/C-nanodot/GO materials are shown in Figure S9. The characteristic peak for Fe-O stretching vibrations is observed at around 563 cm^{-1}.

The XRD spectrum for GO has shown two peaks at angles of 2θ:11.7 and 42.3 which confirms the formation of GO according to the references [40]. When the same test was done for the magnetic absorber, peaks were shown at 2θ:28.4, 36.8, and 57.2 angles which confirmed the magnetization of the prepared adsorbent [4]. From

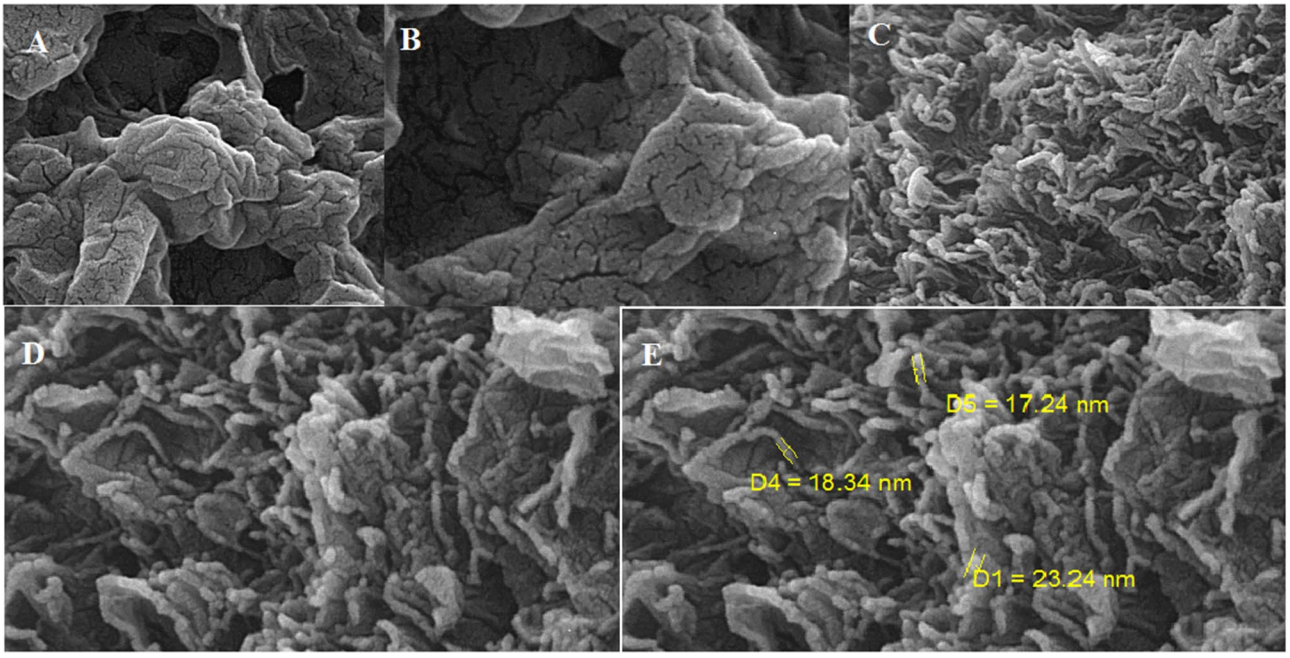

Fig. 2 Scanning electron microscopy (SEM) of: (**A**) GO at 500 nm scale, (**B**) GO at 200 nm scale, (**C**) GO-polyaniline composite at 500 nm scale, (**D**) GO-polyaniline composite at 200 nm scale, (**E**) GO-polyaniline composite at 200 nm scale by indicating density

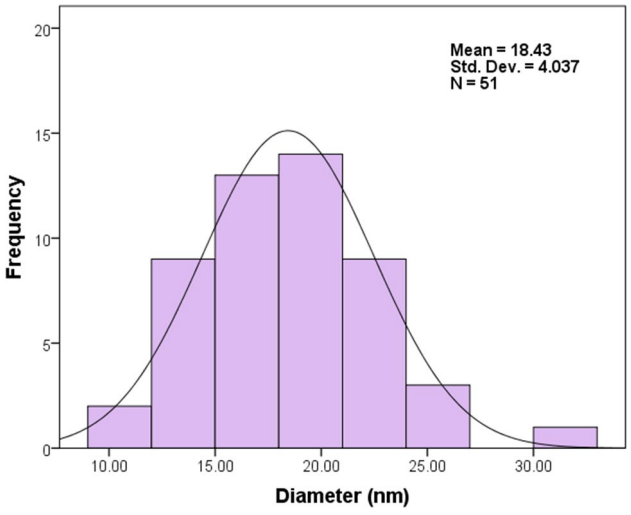

Fig. 3 The average particle size of GO/PANI absorbent was obtained using ImageJ software

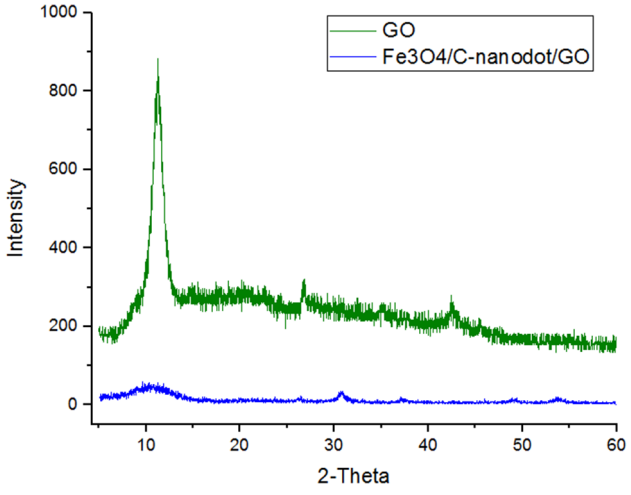

Fig. 4 XRD spectra of GO, Fe_3O_4/C-nanodot/GO

the comparison of the spectra of these two materials, we conclude that GO has decreased at 2θ:11.7 and peak 42.3 has also disappeared, which indicates that the accumulation of GO sheets has decreased [36](Fig. 4).

VSM characterization of Fe_3O_4/C-nanodot/GO, was carried out at room temperature. The S-shaped diagram shows the formation of the absorbent magnetic property. The maximum saturation of the Fe_3O_4/C-nanodot/GO was found to be 30 emu g^{-1} (Fig. 5). Based on a previous study, VSM for Fe_3O_4 is equal to 54 and in our study, the prepared magnetic absorber is equal to 30. Reduced saturation magnetization in Fe_3O_4/C-nanodot/GO is attributed to non-magnetic materials which quench the surface moment, which in turn decreases saturation magnetization [42].

Specific surface area, an important parameter for nanoparticle adsorbents, can be determined by using the

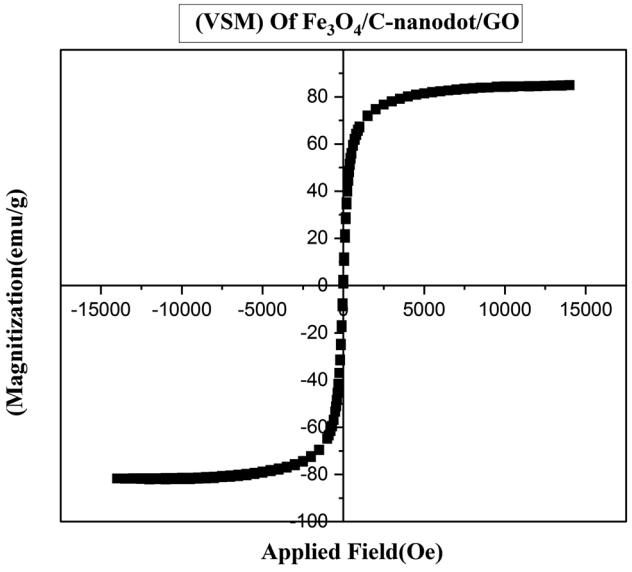

Fig. 5 Vibrating sample magnetometer (VSM) Of Fe_3O_4/C-nanodot/GO

Table 3 BET surface properties of adsorbents

	Pore size diameter (nm)	Pore volume (cm^3g^{-1})	Specific surface area ($m^2 g^{-1}$)
GO/Fe_3O_4/C-nanodot	[1]15.23695	[1]0.161430	22.7133
	[2]15.92494	[2]0.157950	

1. Adsorption

2. Desorption

Brunauer–Emmett–Teller (BET) method. The method works based on the adsorption isotherm of a non-reactive gas, such as nitrogen, carbon dioxide or, argon, with the surface area of the solid or porous materials. To obtain BET isotherms, the sample was subjected to the adsorption isotherm of N2 at 77 °K, and the BET equation was applied to the data in the P/Po range of 0.01 to 0.90 (49-point BET). The sample was degassed at 423.15 °K (150 ℃) for 4 h before surface area measurement. The collected data for Fe_3O_4/C-nanodot/GO is displayed as the BET isotherm, which plots the amount of gas adsorbed as a function of the relative pressure. The results of the BET analysis of surface properties of Fe_3O_4/C-nanodot/GO magnetic adsorbent are summarized in Table 3. The specific surface area of the adsorbent was assessed with the help of N2 adsorption–desorption isotherms shown in Fig. 6. The results were of high quality indicated by an excellent correlation coefficient (R^2=0.997) as illustrated in Fig. 7. It can be deduced from the shape of the plot in Fig. 6 that nitrogen gas follows a Type-IV adsorption–desorption isotherm on the surface of Fe_3O_4/C-nanodot/GO adsorbent. The Barrett–Joyner–Halenda (BJH) desorption isotherm has been used for the pore size distribution study. The mesoporous nature of the adsorbent

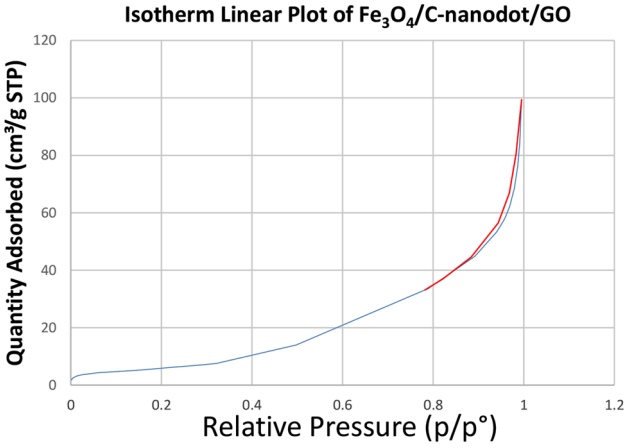

Fig. 6 A BET N2 adsorption/desorption isotherms of Fe_3O_4/C-nanodot/GO

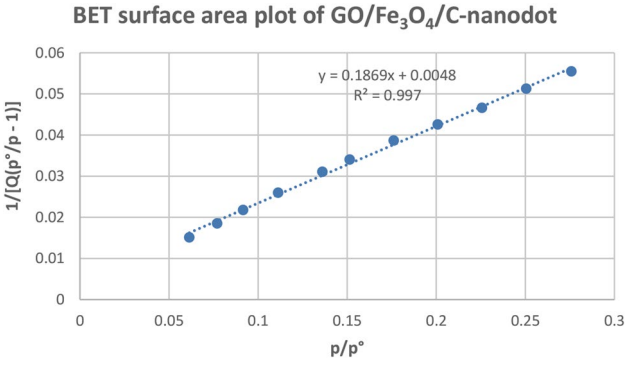

Fig. 7 BET surface area plot of GO/Fe_3O_4/C-nanodot

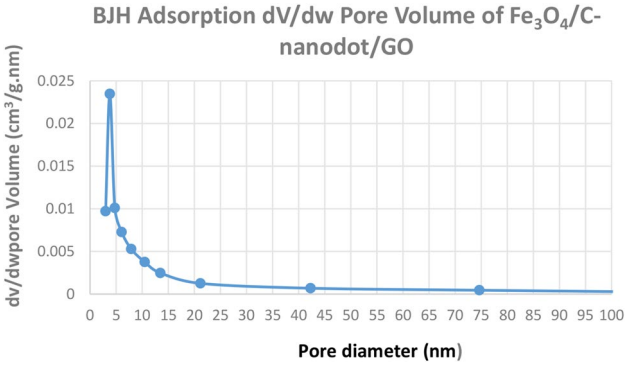

Fig. 8 BJH pore size distribution of GO/Fe_3O_4/C-nanodot

can also be confirmed from the distribution of pore size measured based on the desorption (equilibrium) branch of the isotherm by the BJH method (Fig. 8). The presence of a sharp peak at the pore radius of 3.8 nm is indicative of a mesopores architecture for Fe_3O_4/C-nanodot/GO magnetic adsorbent.

Optimization of methamphetamine extraction using GO/PANI

GO/PANI could only extract methamphetamine from the urine samples. Therefore, to optimize the developed method for the quantification of methamphetamine, the affecting parameters in the extraction process were assigned as the independent variables in the experimental design procedure using Minitab software. Different experimental conditions (runs) suggested by the software are shown in Table 1. Under different experimental conditions, the peak area of the sample (area under the analyte peak in the chromatogram) was taken into account as the response value (Table 1). Based on the results, the solvent of choice for obtaining the best results was acetone. From the analysis of the results with the step-by-step multiple linear regression equation, the response value (AUC for analyte).

was correlated to the remaining factors according to the following equation:

$$\begin{aligned} AUC = &-35914 + 1829\,pH + 906\,adsorbent \\ &+ 10956\,Time - 281.6\,pH \times pH - 5.63\,adsorbent \\ &\times adsorbent - 1172\,Time \times Time \\ &+ 416\,pH \times Time - 52.4\,adsorbent \times Time \end{aligned}$$

The Correlation coefficient (R^2), adjusted R^2, and predicted R^2 are 0.79, 0.68, and 0.51, respectively. P-value of all parameters was <0.15 except for time. This shows that the parameters and their mutual effects have a statistically significant effect on the area under the curve (AUC). The data confirm that the applied parameters have a significant effect on AUC, except for time, which has no significant effect (p-value=0.91). The surface plots for pH, time, and the amount of adsorbent are shown in Fig. 9.

The analysis of the results shows that factors with P-values less than 0.05 had the greatest effect on the extraction. The findings show that low pH and time and medium levels of adsorbent can increase the absorption efficiency of the analyte. The optimum values for the tested parameters are as follows: pH=4, solvent: acetone, adsorbent: 50 mg, and time: 4.5 min (Fig. 9). The studied drugs are weak bases and convert to ionized form in acidic pH [43, 44].

Optimization of studied amphetamines extraction using magnetic adsorbent

In this part of the study, fractional factorial design was used to determine the optimal conditions for the extraction of methamphetamine and pseudoephedrine from urine samples. The response surface method was used for the optimization process. The main parameters affecting the extraction efficiency, including pH, time, adsorbent, and solvent were selected as the independent variables. The peak area values were considered as the response

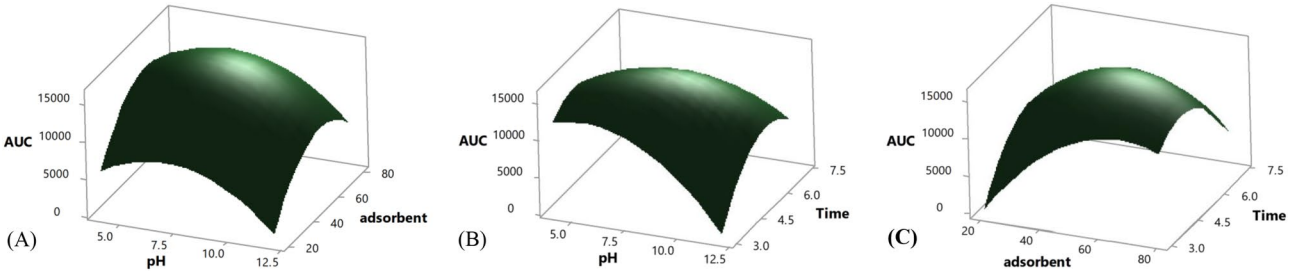

Fig. 9 Surface plot of absorption of methamphetamine (**a**) pH vs. adsorbent and (**b**) adsorbent vs. time (**c**) pH vs. time for GO/PANI composite

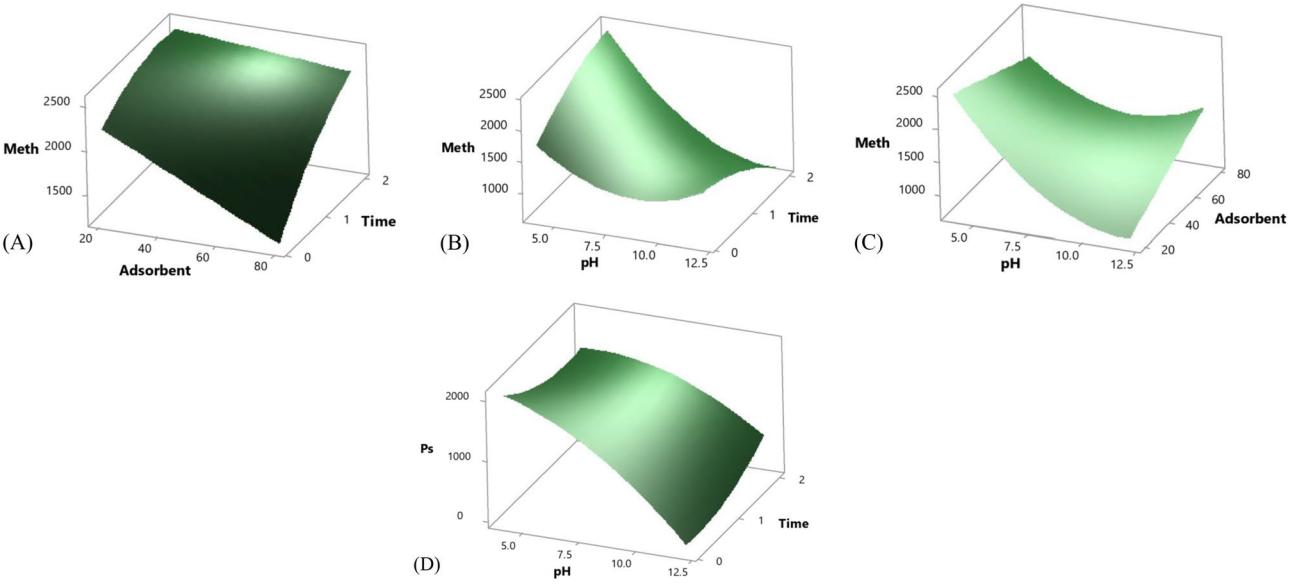

Fig. 10 Surface plot of AUC of methamphetamine and pseudoephedrine (**A**) pH vs. adsorbent and (**B**) adsorbent vs. time (**C**) pH vs. time and (**D**) pH vs. time for Fe_3O_4/C-nanodot/GO composite

for choosing the best condition for the extraction. The obtained values have been reported in Table 2. The optimized solvent for the elution was acetone and the stepwise analysis of quantitative parameters indicated that pH has the most statistically significant effect ($p<0.05$) on the extraction. The following equations were obtained from the quantitative analyses relating the peak area to the evaluated parameters:

Methamphetamine:

$$Peak\ area = 4538 - 616\,pH\ - 28.91\,Adsorbent + 705\,Time + 27.18\,pH \times\ pH\ - 144.1\,Time \times\ Time + 2.974\,pH \times\ Adsorbent - 95.5\,pH \times\ Time + 7.10\,Adsorbent \times\ Time$$

Pseudoephedrine:

$$Ps = 2256 + 38\,pH\ - 978\,Time\ - 18.44\,pH \times\ pH + 212\,Time \times\ Time + 67.3\,pH \times\ Time$$

Correlation coefficient (R^2), adjusted-R^2, predicted R^2 values for methamphetamine were 0.89, 0.81 and 0.57. The same statistical parameters for pseudoephedrine were 0.82, 0.89 and 0.71, respectively. These data confirm a good correlation between the peak area and the parameters under study. In addition, adjusted R^2 values and predicted R^2 confirm the correctness and adequacy of the proposed models with Box-Behnken design to optimize the parameters.

The analysis of the results shows that the most important factor in the extraction is the pH due to its prominent effects on the surface charge of adsorbent binding sites and the degrees of ionization of the studied analytes. The optimal values of each parameter are as follows: pH=4, solvent: acetone, amount of absorbent: 20 mg, and time: 20 s. (Fig. 10).

Method validation

Linearity and calibration curves

The calibration curve for the quantification of methamphetamine in the urine matrix was constructed using five calibration points at the concentration range of 0.25–1.20 mg/L for GO/PANI adsorbent. The obtained determination coefficient (R^2) was 0.984.

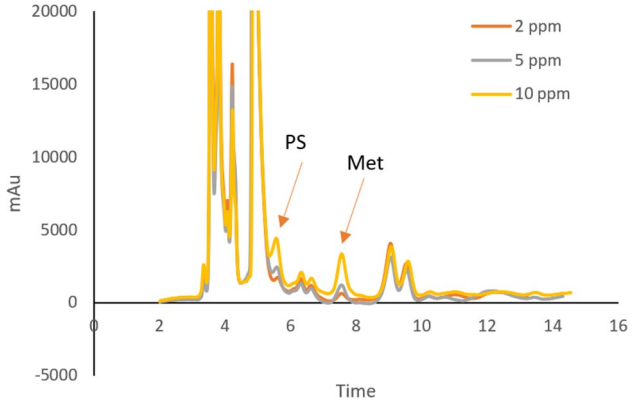

Fig. 11 Chromatogram of different concentrations of methamphetamine and pseudoephedrine in urine samples

Table 4 Comparison of the proposed methods with other techniques for analysis of Met and PS in urine

Method	Analyte	Concentration range (µg/L)	LOD/LLOQ (µg/L)
EM-SPME[3]-GC-FID [45]	Methamphetamine	5-500	2.0
MIP-SPME[4]-CE [46]	Pseudoephedrine	5-500	1.1
DLLME-SFOP[1]-HPLC-UV [48]	Methamphetamine	10-3000	2
Head-space-SPME[2]-GC-MS [49]	Methamphetamine	0.5–1000	0.2
DSPE-HPLC-UV (this study)	Methamphetamine	250-12000	250
MSPE[5]-HPLC-UV (this study)	Methamphetamine and Pseudoephedrine	1000–15,000	1000

[1]Electromembrane solid-phase microextraction-GC-FID

[2]Molecularly imprinted polymer- solid-phase microextraction-CE

[3]Dispersive liquid–liquid microextraction - solidification of floating organic drop-HPLC-UV

[4]Headspace solid-phase microextraction- GC-MS

[5]Magnetic solid-phase extraction-HPLC-UV

The representative regression equation is as follows: $Y = 7870.9X - 266.55$.

For the magnetic adsorbent, the calibration curve was constructed with five calibration points at the concentration range of 1–15 mg/L for methamphetamine and pseudoephedrine in urine matrix. Figure 11 illustrates chromatograms for the urine samples spiked with different concentrations of methamphetamine and pseudoephedrine applied to the HPLC system. The determination coefficient (R^2) values obtained for methamphetamine and pseudoephedrine were 0.993 and 0.970, respectively. The representative regression equations respectively are as follows: $Y = 6460.5X + 941.61$ and $Y = 3409.2\,X - 1099.8$.

The sensitivity of the established method which named the lower limit of quantitation (LLOQ) was calculated based on FDA guidelines for bioanalytical validation of small molecules. It is the minimum value of calibration curve which RSD and the error of the back-calculated concentration were less than 20%. Therefore, LLOQ for methamphetamine extracted by GO/PANI adsorbent was 0.25 mg/L while for both methamphetamine and pseudoephedrine extracted by Fe_3O_4/C-nanodot/GO obtained at 1 mg/L.

Comparing various analytical methods (Table 4) by various instrumental and different extraction approaches [45–48] indicates that generally, GC gives better sensitivity than other methods. The established extraction method used in this work is just useful for the clean-up of urine samples and shows acceptable sensitivity for the detection of methamphetamine and pseudoephedrine in real urine samples. It has the ability to clean up and extract two drugs simultaneously, and our goal was to measure them in clinical samples by a fast and simple extraction methods.

Each of the proposed methods has advantages and disadvantages. The advantages of magnetic adsorbent (Fe_3O_4/C-nanodot/GO) include (a) convenient and fast collection of analyte from the adsorbent surface using a magnet, which avoids the time-consuming operation of passing the column or filtration, (b) relatively low-cost of magnetic adsorbents compared to polyaniline adsorbent, (c) much less extraction time compared to polyaniline adsorbent, (d) cleaner chromatogram, and (e) simultaneous extraction of two drugs. The following are among the disadvantages of Fe_3O_4/C-nanodot/GO: (a) longer synthesis time compared to polyaniline adsorbent, and (b) higher concentration analytical range of analyte compared to that of polyaniline adsorbent.

Precision and accuracy

Precision was utilized to show the method's repeatability and represented by the relative standard deviation (%RSD). Accuracy shows the closeness of the measured values to the actual (nominal) values and is indicated by relative recovery. Intra-day assays for the technique validation were determined using three concentrations (low, middle, and high concentration based on the range of the calibration curve) with three replicates to determine methamphetamine and pseudoephedrine in urine samples. Based on the reported value in Table 5, the RSD% and relative recovery values were in the range of ±17% and 83–115%, respectively. They are in acceptable range based on FDA guideline and indicative of acceptable accuracy and precision for the reported methods.

Specificity

The specificity of HPLC-UV was investigated to corroborate the quantitation of methamphetamine and pseudoephedrine in the presence of other drugs (2 mg/L) which could be co-administrated/abused (e.g., alprazolam,

Table 5 Accuracy and precision of the proposed methods to quantify methamphetamine and pseudoephedrine in urine samples in one day and with three replicates

Analyte	Adsorbent	Spiked Concentration(mg/L)	Found Concentration (mg/L)	Recovery %	RSD%
Methamphetamine	GO/PANI	0.30	0.34	114.9	4.01
		0.60	0.53	87.8	8.55
		0.90	0.81	89.6	3.88
Methamphetamine	Fe_3O_4/C-nanodot/GO	3.0	3.03	100.9	13.0
		6.0	5.00	83.3	2.4
		9.0	9.73	108.1	8.6
Pseudoephedrine	Fe_3O_4/C-nanodot/GO	3.0	3.29	109.6	11.8
		6.0	6.22	103.7	3.9
		9.0	9.70	107.8	5.0

chlordiazepoxide, codeine, caffeine, dextromethorphan, diazepam, morphine, oxazepam, oxycodone, buprenorphine, and tramadol) with the studied drugs. Except for the codeine in the presence of pseudoephedrine (Figure S10), no interfering peak was observed in the recorded chromatograms. This result shows that HPLC conditions are selective enough for methamphetamine and pseudoephedrine extraction and determination.

The extraction process of our methods takes a short time and acceptable extraction efficiency for the analysis of the studied drugs in urine samples.

Real samples analysis

The above-mentioned method described for the extract of methamphetamine from urine samples was used for the treatment of real urine samples collected from a patient. One mL of patient urine (interval between sampling and drug administration was 5 h) pre adjusted at pH 4 with H_3PO_4 solution (85%) was diluted with 6 ml of water and used for the extraction. Using the optimal extraction conditions by Fe_3O_4/C-nanodot/GO, the extraction was performed, and then the final eluted sample in acetone was injected into the HPLC. The peak area for methamphetamine was used to determine the concentration of methamphetamine based on the constructed calibration curve. The obtained methamphetamine concentration was 14.3 mg/L. In addition, two concentrations of methamphetamine were spiked to the real samples (i.e., standard addition calibration method). Under the latter condition, a good linearity was obtained between the peak area and concentration and the quantified methamphetamine concentration was 16.7 mg/L. The results indicated a good agreement between the determined concentrations (i.e., 14.3 and 16.7 mg/L) confirming the accuracy of the developed method.

Conclusion

In this study, the extraction of two psychostimulant drugs in laboratory urine samples using GO-based adsorbents was investigated. The experimental design was used to establish the optimal conditions for the extraction process with the least number of experiments. Parameters such as the extraction solvent, pH, time, and the amount of the used adsorbents were determined for the optimum extraction conditions. The results indicated that using GO/PANI adsorbent for the extraction can lead to the detection of as low as 0.25 mg/L methamphetamine from the urine matrix. On the other hand, the Fe_3O_4/C-nanodot/GO can extract methamphetamine and pseudoephedrine simultaneously at the minimum concentration of 1 mg/L in the test sample. Considering that the urine laboratory sample is a complex matrix, the magnetic adsorbent showed a better clean-up ability compared with GO/PANI. In addition, the extraction time by Fe_3O_4/C-nanodot/GO (20 s) is less than that of GO/PANI (5 min). Collectively, the introduced adsorbents can practically be applied for the sensitive determination of the tested psychostimulants in human urine.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01331-y.

Supplementary Material 1

Author contributions

Y.J., A.T., and S.D. designed the project, N.N. performed experiments, A.S. and S.D. analyzed the data, N.N. wrote the main manuscript text, A.S., A.B., and S.D. revised the manuscript. All authors reviewed the manuscript.

Funding

The current study results from NN's Ph.D. thesis. Financial support was received from Tabriz University of Medical Sciences under grant number (66097).

Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request. Figures S1-S10 are available as supplementary data.

Declarations

Human ethics and consent to participate

Ethical Statement All of the participants who donor urine sample signed an informed consent form. All methods in this project were carried out in accordance with relevant guidelines and regulations and approved by the

Ethics Committee of Tabriz University of Medical Sciences (Ethical code: IR.TBZMED.REC.1400.083).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 May 2024 / Accepted: 25 October 2024
Published online: 05 November 2024

References

1. Shokrollahi M, Seidi S, Fotouhi L. <ArticleTitle Language="En">In situ electrosynthesis of a copper-based metal–organic framework as nanosorbent for headspace solid-phase microextraction of methamphetamine in urine with GC-FID analysis. Microchim Acta. 2020;187(10):1–10.
2. Farrell M, Marsden J, Ali R, Ling W. Methamphetamine: drug use and psychoses becomes a major public health issue in the Asia Pacific region. Volume 97. Blackwell Science Ltd Oxford, UK; 2002. pp. 771–2.
3. Nourani N, Taghvimi A, Bavili-Tabrizi A, Javadzadeh Y, Dastmalchi S. Microextraction Techniques for Sample Preparation of Amphetamines in Urine: A Comprehensive Review. Crit Rev Anal Chem. 54(5):1304-1319.
4. Abdelrahman AA, Betiha MA, Rabie AM, Ahmed HS, Elshahat M. Removal of refractory Organo–sulfur compounds using an efficient and recyclable {Mo132} nanoball supported graphene oxide. J Mol Liq. 2018;252:121–32.
5. Longo V, Forleo A, Ferramosca A, Notari T, Pappalardo S, Siciliano P, Capone S, Montano L. Blood, urine and semen Volatile Organic Compound (VOC) pattern analysis for assessing health environmental impact in highly polluted areas in Italy. Environ Pollut. 2021;286:117410.
6. Cruz-Vera M, Lucena R, Cárdenas S, Valcárcel M. Sorptive microextraction for liquid-chromatographic determination of drugs in urine. TRAC Trends Anal Chem. 2009;28(10):1164–73.
7. Vogliardi S, Tucci M, Stocchero G, Ferrara SD, Favretto D. Sample preparation methods for determination of drugs of abuse in hair samples: a review. Anal Chim Acta. 2015;857:1–27.
8. Niu Z, Zhang W, Yu C, Zhang J, Wen Y. Recent advances in biological sample preparation methods coupled with chromatography, spectrometry and electrochemistry analysis techniques. TRAC Trends Anal Chem. 2018;102:123–46.
9. Buszewski B, Szultka M. Past, present, and future of solid phase extraction: a review. Crit Rev Anal Chem. 2012;42(3):198–213.
10. Kumazawa T, Hasegawa C, Lee X-P, Hara K, Seno H, Suzuki O, Sato K. Simultaneous determination of methamphetamine and amphetamine in human urine using pipette tip solid-phase extraction and gas chromatography–mass spectrometry. J Pharm Biomed Anal. 2007;44(2):602–7.
11. Rocío-Bautista P, González-Hernández P, Pino V, Pasán J, Afonso AM. Metal-organic frameworks as novel sorbents in dispersive-based microextraction approaches. TRAC Trends Anal Chem. 2017;90:114–34.
12. Saito K, Takase M, Yasumura Y, Ito R. Analysis of Methamphetamine in Urine by HPLC with Solid-Phase Dispersive Extraction and Solid-Phase Fluorescence Derivatization. Chem Pharm Bull. 2023;71(1):24–30.
13. Han X, Chen J, Li Z, Quan K, Qiu H. Magnetic solid-phase extraction of triazole fungicides based on magnetic porous carbon prepared by combustion combined with solvothermal method. Anal Chim Acta. 2020;1129:85–97.
14. Wang Y, Tong Y, Xu X, Zhang L. Developed magnetic multiporous 3D N-Co@C/HCF as efficient sorbent for the extraction of five trace phthalate esters. Anal Chim Acta. 2019;1054:176–83.
15. Xie L, Jiang R, Zhu F, Liu H, Ouyang G. Application of functionalized magnetic nanoparticles in sample preparation. Anal Bioanal Chem. 2014;406:377–99.
16. Yuan S, Xiang Y, Chen L, Xiang P, Li Y. Magnetic solid-phase extraction based on polydopamine-coated magnetic nanoparticles for rapid and sensitive analysis of eleven illicit drugs and metabolites in wastewater with the aid of UHPLC-MS/MS. J Chromatogr A. 2024;1718:464703.
17. Kala KL, Anbuchezhiyan G, Pingili K, Singh PK, Vel V, Yusuf K, Aljuwayid AM, Islam MA, Christopher D. Employing a Carbon-Based Nanocomposite as a Diffusive Solid-Phase Extraction Adsorbent for Methamphetamine for Therapeutic Purposes. *Adsorption Science & Technology* 2023, 2023:8650678.
18. Wang L, Liu J, Zhao P, Ning Z, Fan H. Novel adsorbent based on multi-walled carbon nanotubes bonding on the external surface of porous silica gel particulates for trapping volatile organic compounds. J Chromatogr A. 2010;1217(37):5741–5.
19. Singh AR, Mohan B, Raghav N, Sagar, Virender A, Pombeiro AJL. Understanding the mechanisms and applications of luminescent covalent organic frameworks (COFs) for multi-analyte sensing. J Mol Struct. 2025;1321:139945.
20. Mohan B, Gupta RK, Pombeiro AJ, Ren P. Advanced luminescent metal–organic framework (MOF) sensors engineered for urine analysis applications. Coord Chem Rev. 2024;519:216090.
21. Mohan B, Mehak, Virender, Kumar A, Modi K, Singh G, Garazade IM, Parikh J, Pombeiro AJ, Solovev AA. Detecting cancer biomarkers using electrochemical composite nanomaterials tools. Vietnam J Chem. (2024) doi.org/10.1002/vjch.202300399
22. Kumar A, Kumar N, Chauhan A, Mohan B. A reversible Schiff base chemosensor for the spectroscopic and colorimetric sensing of Eu3 + ions in solution and solid-state. Inorg Chim Acta. 2024;560:121833.
23. Han X, Chen J, Li Z, Qiu H. Combustion fabrication of magnetic porous carbon as a novel magnetic solid-phase extraction adsorbent for the determination of non-steroidal anti-inflammatory drugs. Anal Chim Acta. 2019;1078:78–89.
24. Suárez B, Simonet B, Cárdenas S, Valcárcel M. Determination of non-steroidal anti-inflammatory drugs in urine by combining an immobilized carboxylated carbon nanotubes minicolumn for solid-phase extraction with capillary electrophoresis-mass spectrometry. J Chromatogr A. 2007;1159(1–2):203–7.
25. Agarwal V, Zetterlund PB. Strategies for reduction of graphene oxide – A comprehensive review. Chem Eng J. 2021;405:127018.
26. Liu J, Cui L, Losic D. Graphene and graphene oxide as new nanocarriers for drug delivery applications. Acta Biomater. 2013;9(12):9243–57.
27. Boukhvalov DW, Dreyer DR, Bielawski CW, Son YW. A computational investigation of the catalytic properties of graphene oxide: Exploring mechanisms by using DFT methods. ChemCatChem. 2012;4(11):1844–9.
28. da Silva Bruckmann F, Foucaud Y, Pinheiro RF, Silva LFO, Oliveira MLS, Badawi M, Dotto GL. Removal of phenazopyridine from water, synthetic urine, and real sample by adsorption using graphene oxide: A DFT theoretical/experimental approach. Chemosphere. 2024;363:142738.
29. Yang N, Zhai J, Wan M, Wang D, Jiang L. Layered nanostructures of polyaniline with graphene oxide as the dopant and template. Synth Met. 2010;160(15–16):1617–22.
30. Narayanaswamy V, Obaidat IM, Kamzin AS, Latiyan S, Jain S, Kumar H, Srivastava C, Alaabed S, Issa B. Synthesis of graphene oxide-Fe3O4 based nanocomposites using the mechanochemical method and in vitro magnetic hyperthermia. Int J Mol Sci. 2019;20(13):3368.
31. Hummers WS Jr, Offeman RE. Preparation of graphitic oxide. J Am Chem Soc. 1958;80(6):1339–1339.
32. Mitra M, Chatterjee K, Kargupta K, Ganguly S, Banerjee D. Reduction of graphene oxide through a green and metal-free approach using formic acid. Diam Relat Mater. 2013;37:74–9.
33. Stankovich S, Dikin DA, Piner RD, Kohlhaas KA, Kleinhammes A, Jia Y, Wu Y, Nguyen ST, Ruoff RS. Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. Carbon. 2007;45(7):1558–65.
34. Mitra M, Kulsi C, Chatterjee K, Kargupta K, Ganguly S, Banerjee D, Goswami S. Reduced graphene oxide-polyaniline composites—synthesis, characterization and optimization for thermoelectric applications. RSC Adv. 2015;5(39):31039–48.
35. Huang Y, Lin C. Facile synthesis and morphology control of graphene oxide/polyaniline nanocomposites via in-situ polymerization process. Polymer. 2012;53(13):2574–82.
36. Yuvali D, Narin I, Soylak M, Yilmaz E. Green synthesis of magnetic carbon nanodot/graphene oxide hybrid material (Fe3O4@ C-nanodot@ GO) for magnetic solid phase extraction of ibuprofen in human blood samples prior to HPLC-DAD determination. J Pharm Biomed Anal. 2020;179:113001.
37. Ebrahimi-Najafabadi H, Leardi R, Jalali-Heravi M. Experimental design in analytical chemistry—part I: theory. J AOAC Int. 2014;97(1):3–11.
38. Ferreira SC, Bruns R, Ferreira HS, Matos GD, David J, Brandão G, da Silva EP, Portugal L, Dos Reis P, Souza A. Box-Behnken design: An alternative for the optimization of analytical methods. Anal Chim Acta. 2007;597(2):179–86.
39. Zhang W, Cao S, Wu Z, Zhang M, Cao Y, Guo J, Zhong F, Duan H, Jia D. High-performance gas sensor of polyaniline/carbon nanotube composites promoted by interface engineering. Sensors. 2019;20(1):149.
40. Han Q, Wang Z, Xia J, Chen S, Zhang X, Ding M. Facile and tunable fabrication of Fe3O4/graphene oxide nanocomposites and their application in the magnetic solid-phase extraction of polycyclic aromatic hydrocarbons from environmental water samples. Talanta. 2012;101:388–95.

41. Bissessur R, Liu PK, White W, Scully SF. Encapsulation of polyanilines into graphite oxide. Langmuir. 2006;22(4):1729–34.
42. Sharma S, Sharma S, Sharma N, Sharma S, Paul S. Waste Chicken Eggshell-Derived CaO Based Magnetic Solid Base Catalysts for the One-Pot Synthesis of Tetrahydro-4H-chromenes and Benzopyranopyrimidines. Catal Lett. 2024;154(2):532–52.
43. Beckett A, Rowland M. Urinary excretion kinetics of amphetamine in man. J Pharm Pharmacol. 1965;17(10):628–39.
44. Brunton L, Parker K. Pharmacokinetics and pharmacodynamics: the dynamics of drug absorption, pharmacogenetics, distribution, action, and elimination. Goodman Gilman's Man Pharmacol Ther. New York: McGraw-Hill 2008. pp. 1–25.
45. Rezazadeh M, Yamini Y, Seidi S. Application of a new nanocarbonaceous sorbent in electromembrane surrounded solid phase microextraction for analysis of amphetamine and methamphetamine in human urine and whole blood. J Chromatogr A. 2015;1396:1–6.
46. Deng D-L, Zhang J-Y, Chen C, Hou X-L, Su Y-Y, Wu L. Monolithic molecular imprinted polymer fiber for recognition and solid phase microextraction of ephedrine and pseudoephedrine in biological samples prior to capillary electrophoresis analysis. J Chromatogr A. 2012;1219:195–200.
47. Jabbari NR, Taghvimi A, Dastmalchi S, Javadzadeh Y. Dispersive solid-phase extraction adsorbent of methamphetamine using in-situ synthesized carbon-based conductive polypyrrole nanocomposite: focus on clinical applications in human urine. J Sep Sci. 2020;43(3):606–13.
48. Ahmadi-Jouibari T, Fattahi N, Shamsipur M. Rapid extraction and determination of amphetamines in human urine samples using dispersive liquid–liquid microextraction and solidification of floating organic drop followed by high performance liquid chromatography. J Pharm Biomed Anal. 2014;94:145–51.
49. Song A, Wang J, Lu G, Jia Z, Yang J, Shi E. Oxidized multiwalled carbon nanotubes coated fibers for headspace solid-phase microextraction of amphetamine-type stimulants in human urine. Forensic Sci Int. 2018;290:49–55.

Publisher's note