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Article

Mixed-Hemimicelle Solid Phase Extraction Followed by Dispersive Liquid-Liquid Microextraction of Amphetamines from Biological Samples

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In this work, the synthesized Fe_3O_4 nanoparticles was coated by sodium dodecyl sulfate and then it was used as a sorbent in mixed-hemimicelle solid phase extraction of some amphetamines, as psychoactive drugs, from biological samples. This extraction method was combined with dispersive liquid-liquid microextraction to enhance enrichment factors of targeted analytes. Effect of different parameters influencing the hybrid extraction performance, such as sodium dodecyl sulfate amount and sample pH, were investigated. The method showed linearity in the range of 1.0-250 and 2.0-250 μg L^{-1} for the most of analytes in urine and plasma samples, respectively. The limits of detection, based on signal to noise of 3, were found 0.1-0.2 and 0.3-0.5 μg L^{-1} in urine and plasma samples, respectively. The results of the intra-day and inter-day precision were less than 13.5% for all amphetamines. The amounts of relative recoveries in spiked urine and plasma samples were found in the range of 90-96 and 87-93%, respectively.

Keywords: iron oxide magnetic nanoparticle, mixed-hemimicelle solid phase extraction, dispersive liquid-liquid microextraction, amphetamine, biological sample

Introduction

Amphetamines and their related derivatives are powerful stimulants of the central nervous system. However, they are often misused by recreational users. A chronic abuse of amphetamines often results in hallucinations and psychosis, as well as dysphoria and depression upon withdrawal.[1] Hence, their dosage in biological matrices, especially in urine, remains a major challenge for analytical chemists. For years, immunoassays were mostly applied to determine the dosage of amphetamines and related drugs.[2] Nevertheless, such methods are not selective enough and positive results must be confirmed by a second more specific technique. Therefore, several methods, such as gas chromatography (GC),[3,4] high performance liquid chromatography (HPLC),[5] GC-mass spectrometry (MS),[6-12] HPLC-MS,[13,14] capillary electrophoresis (CE),[15] and CE-MS,[16] along with sample preparation methods have been developed for the determination of amphetamine and related compounds at low concentration in biological matrices. In order to eliminate interferences from the biological matrices, liquid-liquid extraction (LLE),[17-19] supercritical fluid extraction (SFE),[20] solid-phase extraction (SPE),[21,22] and solid-phase microextraction (SPME)[23-25] have been proposed.

However, there are several disadvantages for conventional extraction procedures such as LLE and SPE. These procedures are time-consuming and laborious, and the large amounts of organic solvents used in the extraction procedures cause problem regarding health and the environment. SPME also suffers from some drawbacks; the fibers are usually fragile and have limited lifetime in complex matrices such as biological fluids and the sample carry-over is also a problem. Also, SFE is inaccessible to the most of routine laboratories due to the high cost of equipment and the need for a skillful operator.

Application of iron oxide magnetic particles in sample preparation field has been reported in magnetic solid phase extraction (MSPE) methodology since 1999.[26] High sample capacity, high efficiency, as a result of high sorbent surface area, ease of sorbent separation, using an external magnetic field, and being rapid and inexpensive are the advantages of this methodology. Such benefits lead to the superiority of MSPE technique over other convectional SPE methods.[27] Both forms of naked and coated magnetic particles have been used for inorganic analytes,[28] while functionalized particles have been largely employed for organic pollutants.[29,30] Furthermore, several SPE methods based on

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surfactant-coated Fe_3O_4 particles have been reported.[31-33] In the SPE technique based on mixed-hemimicelles (MHSPE), sorbent is obtained by coating of the magnetic sorbents with ionic surfactants through electrostatic adsorption. Different structures, including hemimicelles and admicelles, could be formed on the surface of nanoparticle according to ionic surfactant amounts.[28] In a way that, hemimicelles consist of monolayers of electrostatically adsorbed surfactants and admicelles are bilayers of surfactant formed from electrostatic adsorption on the surface and interaction of hydrocarbon chains.

Dispersive liquid-liquid microextraction (DLLME) is a simple, fast and inexpensive sample preparation method in which consumption of toxic organic solvents is low. DLLME method usually provides high enrichment factors due to large surface area between extraction phase and sample solution. In this method, a cloudy solution is formed when an appropriate mixture of extraction and dispersing solvents is injected into an aqueous sample. Despite having several advantages, the matrix has significant impact on DLLME performance. Dilution of complex samples also causes detection problems at low concentration levels. So, this methodology is not frequently used for complex matrices alone. Recently, SPE techniques have been used in combination with DLLME method to take the advantages of both methods including simplicity, low solvent consumption, rapid extraction time along with high recovery and high enrichment factors. This combination could also provide applicability in complex matrices.[34-38]

In this work, we developed a MHSPE method coupled with DLLME for the isolation and determination of amphetamines from biological samples based on the use of sodium dodecyl sulfate (SDS)-coated Fe_3O_4 nanoparticles prior to the HPLC-UV analysis.

Experimental

Reagents and chemicals

HPLC grade acetonitrile and methanol, acetone, ethanol, carbon tetrachloride, carbon disulfide, triethylamine and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Iron(II) sulfate, iron(III) chloride, sodium dodecyl sulfate, sodium chloride, sodium hydroxide were also obtained from Merck. HPLC grade water was purchased from Caledon (Ontario, Canada).

Stock solutions of 3,4-methylenedioxy amphetamine (MDA), 3,4-methylenedioxyethyl amphetamine (MDEA), 3,4-methylenedioxymethyl amphetamine (MDMA), and 3,4-methylenedioxypropyl amphetamine (MDPA) (Salars, Como, Italy) were prepared at a concentration of 100 mg L^{-1} in methanol and they were stored at –18 ºC. Working solutions were prepared daily from the stock solution.

Apparatus

An Agilent 1200 series HPLC system including a quaternary pump and a UV detector were used for separation and determination of the analytes. The separation was performed on Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm internal diameter, 5 µm) column. An acetate buffer solution (0.05 mol L^{-1}) containing 0.1% triethylamine (pH = 3.9) and methanol (35:65) were used as mobile phase in isocratic elution mode. The chromatographic data were collected and recorded using ChemStation software. The direct sample introduction was carried out using a Rheodyne manual injector (Rohnert Park, CA, USA) with a 20 µL loop. Column temperature was kept constant at 25 °C using a thermostatted column compartment. The flow rate was 1 mL min^{-1} and detection was performed at 210 nm.[39]

The structural properties of the synthesized Fe_3O_4 were examined by X-ray diffraction (XRD) Siemens, D5000 X-ray diffractometer at room temperature. Infrared (IR) spectrum was recorded in the wave numbers ranging 2200-400 cm^{-1}, with a Fourier transform infrared (FTIR) spectrophotometer EQUINOX55-Bruker. The morphology of Fe_3O_4 was examined by a Hitachi S4160 field emission scanning electron microscopy (FE-SEM). Magnetic separation was performed using a 1.4 Tesla magnet (10 × 5 × 4 cm).

Synthesis of Fe_3O_4 magnetic nanoparticles

Iron oxide magnetic particles were synthesized according co-precipitation method.[40,41] In summary, an amount of 2.794 g $FeSO_4.7H_2O$, 3.110 g $FeCl_2$ and 0.85 mL HCl were dissolved in 25 mL degassed deionized water. This solution was added dropwise to 250 mL sodium hydroxide solution (1.5 mol L^{-1}) at 80 ºC under nitrogen atmosphere while stirring in three necked round bottom. The black colloidal product was collected using a 1.4 T magnet and it was washed several times by degassed deionized water. This product kept in degassed deionized water in a 250-mL volumetric flask. Iron oxide magnetic particles concentration estimated 10 mg mL^{-1}.

Extraction procedure of the proposed method

Firstly, a 2.5 mL urine sample was diluted four times, after pH adjustment (pH = 3). A volume of 10.0 mL of this solution containing 100 µg L^{-1} of amphetamines was transferred into a 25-mL Becker. Following 1.2 mL iron

oxide magnetic particles suspension and 1 mL of SDS solution (1 mg mL^{-1}) was added. The mixture was shook for 5 min at room temperature. Subsequently the sorbent was isolated from solution using a 1.4 T magnet. After decanting the solution, 1 mL methanol was used to do desorption process in 2 min using vortex mixer. The sorbent was again separated using magnet and the eluent was transferred to a vial to accomplish DLLME technique. The final solution was mixed with 55 µL carbon disulfide, as extracting solvent in DLLME process. The entire solution, which contains amphetamines, was rapidly injected into a conical test tube containing 5 mL double distilled water. After centrifugation of cloudy solution, the sedimented phase was withdrawn into a microsyringe and it was transferred into a vial. This solution was dried using a gentle flow of N_2 gas. The residue was dissolved in 30 µL methanol and then injected into the HPLC system.

In order to perform extraction from plasma samples, an amount of 1.0 mL human plasma sample was acidified with 50 µL hydrochloric acid (2 mol L^{-1}) and then 100 µL tirchloroacetic acid (4 g mL^{-1}) was added. These two compounds led to disturb drug protein binding and denature the proteins in order. Subsequently, the sample was centrifuged at 10000 rpm for 5 min to remove precipitated proteins. Eventually the supernatant was diluted in a 10 mL volumetric flask and the extraction was performed as the extraction procedure for urine sample.

The effect of influential parameters on MHSPE process including SDS amount, sample pH, extraction time, type and volume of desorption solvent, and desorption time were investigated. Type and volume of extracting solvent effect on DLLME was also studied. A univariate approach was employed to optimize effective factors. Moreover, the peak area was used to assess the extraction efficiency under investigated condition.

Results and Discussion

Characterization of Fe_3O_4 particles

X-Ray diffraction pattern for Fe_3O_4 (Figure S1) displayed sharp peaks at 2θ = 30.1, 35.5, 43.1, 53.4, 57, 62.6º which is in accordance with the literature.[42] These six characteristics peaks for Fe_3O_4 marked by their indices (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0) were observed. It reveals that the particles were pure Fe_3O_4 with an inverse spinal structure. According to the FE-SEM images of Fe_3O_4 (Figure S2), the diameter of Fe_3O_4 nanoparticles was in the range of 22-36 nm which obviously indicate formation of nanoparticles. Vibrating sample magnetometer (VSM) analysis was also performed. Magnetic saturation for Fe_3O_4 obtained was 50 emu g^{-1} (Figure S3), which enables simple isolation of Fe_3O_4 nanoparticles from solution by an external magnetic field.[43]

Optimization of MHSPE-DLLME process

SDS amount

The amount of ionic surfactant has a significant effect in MHSPE method. To investigate the effect of surfactant amount, various volumes of SDS solution (1 mg mL^{-1}) in the range of 0 to 2.5 mL (0 to 8.7 mmol L^{-1}) was added into acidic sample solution. It is worth mentioning that the critical micelle concentration (CMC) for SDS is about 7 mmol L^{-1} in acidic pH. As illustrated in Figure 1a, the extraction efficiencies were increased up to 1 mg (3.5 mmol L^{-1}) and slight declines were observed after addition of 2 mg (7 mmol L^{-1}) SDS. At adjusted solution pH level, the organic analyte molecules appear in cationic forms. In addition, gradual SDS addition leads to the formation of first hemimicelle, with hydrophobic interaction, and then mixed-hemimicelle, with potential for both hydrophobic and electrostatic interactions. This fact is responsible for the raise in extraction efficiency. Further increase in SDS amount caused formation of SDS micelles in sample solution and resulted in the loss of analytes in magnetic isolation step. So an amount of 1 mg SDS was used as optimum value and it was used in further experiments.

Sample pH

Amphetamine compounds are weak bases with a pKa of 9-10. It means that the amine group of amphetamines are protonated at pH < 9. In addition, the point of zero charge for Fe_3O_4 magnetic particles is obtained at pH 6.5. The surface charges below and above this pH are positive and negative, respectively.[44] Having effective interaction between SDS molecules and nanoparticles, the sample pH must be acidic. This pH could also provide suitable electrostatic interaction between sorbent and amphetamines.

The effect of sample pH was studied in the range of 2-10 and the results are illustrated in Figure 1b. As expected, better extraction efficiencies were obtained in acidic solutions for all analytes. Protonation of sulfate sites in SDS molecules, which leads to less coverage of Fe_3O_4 magnetic particles, could be responsible for low extraction efficiencies in strong acidic media. Consequently, the sample pH of 4 was chosen.

Extraction time

Extraction process was investigated in the range of 2-15 min. As demonstrated in Figure 1c, there has been a sharp rise for all amphetamines up to 5 min. After a decrease, a steady signal has been obtained. Hence, an extraction time

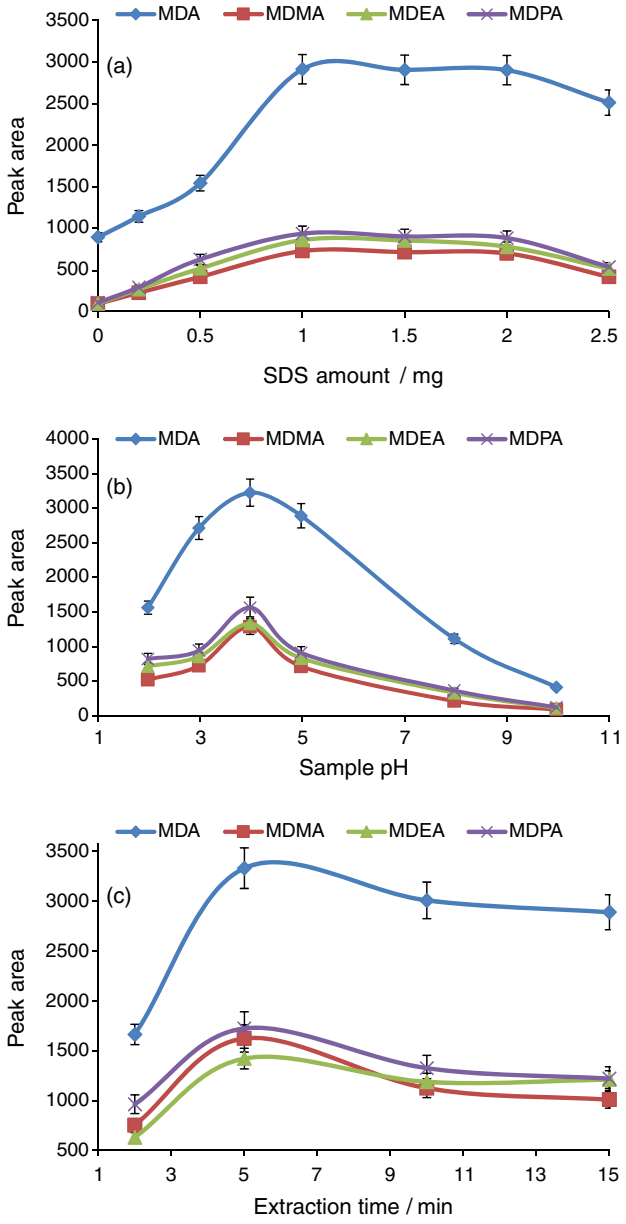

Figure 1. Effect of (a) SDS amount; (b) sample pH; (c) extraction time on mixed-hemimicelle solid phase extraction efficiency. Conditions: 10 mL sample solution containing 100 μg L^{-1} of amphetamines, 1.2 mL iron oxide (10 mg mL^{-1}), 1 mL methanol as elution solvent, 45 μL CS_2 as extraction solvent. MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA:3,4-methylenedioxypropyl amphetamine.

of 5 min was used as optimized time. Dispersion of high surface area SDS-coated magnetic sorbent in sample solution resulted in such a low extraction time.

Desorption conditions

In order to select the most suitable solvent for desorption process, acetonitrile, methanol, ethanol and acetone were utilized and the best results were obtained from methanol (Figure 2a). The volume of methanol was also studied. As depicted in Figure 2b, the best results were obtained from 1.5 mL methanol. Since desorption solvent in MHSPE step possess the role of dispersive solvent in DLLME process, further volume increase resulted in decreasing DLLME efficiency due to the raise in solubility of analytes. Desorption time was also investigated in the range of 1 to 8 min and the time of 2 min was quite suitable (Figure 2c).

Type and volume of extracting solvent in DLLME

Performance of DLLME is mainly determined by the type and volume of extractant. The extraction solvent must have higher density than water and possess high extraction capability for the analytes. In addition, it should be immiscible in water. Therefore, in this work, $CHCl_3$, CCl_4 and CS_2 were evaluated as potential extractants. The

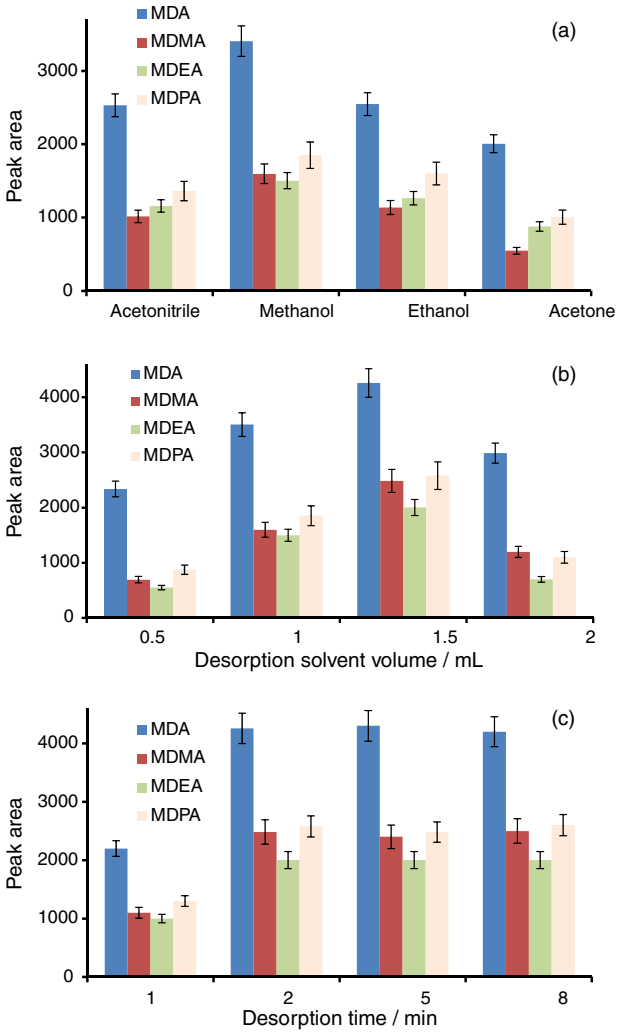

Figure 2. Optimization of desorption solvent (a) type; (b) volume; (c) time in mixed-hemimicelle solid phase extraction. Conditions: 10 mL sample solution containing 100 μg L^{-1} of amphetamines at pH = 4, 1.2 mL iron oxide (10 mg mL^{-1}), 1 mg SDS, 5 min extraction time. MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine.

results showed that, carbon disulfide was the best extracting solvent (Figure 3a). The study on the effect of extracting solvent volume was performed in the range of 45-85 µL of carbon disulfide and the best results were obtained in 55 µL for all amphetamines (Figure 3b). As carbon disulfide volume was increased, the volume of sedimented phase also grew which led to the dilution of final phase and the drop in signal values was observed.

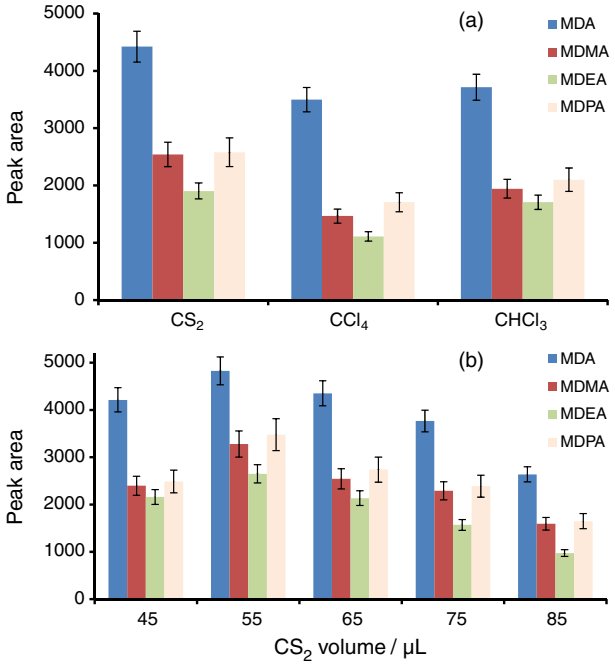

Figure 3. Optimization of extracting solvent (a) type; (b) volume in dispersive liquid-liquid microextraction process. Conditions: 10 mL sample solution containing 100 µg L^{-1} of amphetamines at pH = 4, 1.2 mL iron oxide (10 mg mL^{-1}), 1 mg SDS, 5 min extraction time, elution using 1.5 mL methanol in 2 min. MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine.

Method validation

In order to evaluate the proposed method, quantitative analysis was performed in urine and plasma samples, considering optimized conditions. As tabulated in Table 1, good correlation coefficients were found for MDA, MDMA, MDEA and MDPA in both urine and plasma media using external standard calibration curve. The linear dynamic range (LDR) for MDA was in the range of 0.5-250 µg L^{-1} in urine sample. The LDRs were ranged from 1-250 µg L^{-1} for the rest of amphetamines. In addition, the method showed linearity in the ranges of 1-250 µg L^{-1} for MDA, and 2-250 µg L^{-1} for the rest of analytes in plasma sample. The limit of detection (LOD), based on signal to noise ratio of 3 were obtained 0.1 and 0.3 µg L^{-1} for MDA in urine and plasma sample, respectively. These values were found 0.2 and 0.5 µg L^{-1} for the rest of studied amphetamines. In addition, the limits of quantification, based on a signal to noise ratio of 10, varied between 1.0-2.5 µg L^{-1} for four amphetamines. The results of the intra-day and inter-day precision at concentration level of 5 µg L^{-1} in urine sample and at 10 µg L^{-1} in plasma sample are presented in Table 2. As shown, acceptable relative standard deviation (RSD%) values (< 13.5%) were obtained for all compounds.

Accuracy and precision assays were done according to some validation guides as the ICH (International Conference on Harmonization-Validation of Analytical Procedures) and FDA. Stability of MDA, MDMA, MDEA and MDPA at three levels of concentration in plasma extracts (5, 10 and 200 µg L^{-1}) and in urine extracts (10, 150 and 700 µg L^{-1}) was evaluated. Samples were kept at 4 ºC and the same extract was injected just after being prepared, and 3 and 5 days after preparation. The stability of the extracts at three levels of concentration for the two matrices was evaluated during a week. Coefficients of variation (%) are low, even at the 5th day. However, at that time, several unknown peaks interfered in the base line of the chromatogram. It is, therefore, recommendable that the extracts are analyzed in a relatively short period after preparation.

To evaluate interference and method specificity, several blank (no analyte) urine and plasma samples were evaluated for co-eluting chromatographic peaks that might interfere with detection of the analytes of interest. Although mass spectrometry are often preferred for quantitative

Table 1. Some analytical data obtained for MHSPE-DLLME of amphetamines using SDS-coated Fe_3O_4 nanoparticles and HPLC-UV

Sample	Urine				Plasma			
Compound	MDA	MDMA	MDEA	MDPA	MDA	MDMA	MDEA	MDPA
LDR / (µg L^{-1})	0.5-250	1.0-250	1.0-250	1.0-250	1.0-250	2.0-250	2.0-250	2.0-250
R^2	0.998	0.995	0.993	0.997	0.996	0.993	0.991	0.996
LOD / (µg L^{-1})	0.1	0.2	0.2	0.2	0.3	0.5	0.5	0.5
LOQ / (µg L^{-1})	1.0	1.5	1.5	1.5	1.5	2.5	2.5	2.5

MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine; LDR: linear dynamic range; LOD: limit of detection; LOQ: limit of quantification.

Table 2. Intra-day and inter-day precision of the MHSPE-DLLME/HPLC-UV of amphetamines from biological samples

Compound	RSD (n = 5) / %			
	Urine (5 μg L^{-1})		Plasma (10 μg L^{-1})	
	Intra-day	Inter-day	Intra-day	Inter-day
MDA	6.1	8.8	8.6	10.8
MDMA	8.4	10.5	10.2	12.6
MDEA	7.3	9.8	9.4	12.4
MDPA	9.7	11.3	11.1	13.5

MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine; RSD: relative standard deviation.

determination of amphetamines compounds from biological samples, no interference peaks were detected, in the retention time of the analytes using UV detector.

The accuracy of the method (A%) was investigated by the standard additions of low, medium and high concentrations level (10, 50 and 100 μg L^{-1} for plasma, and 5, 50 and 100 μg L^{-1} for urine), and calculating the deviation percentage between the calculated and the nominal value [accuracy (%) = (mean calculated concentration/nominal concentration) × 100]. The accuracy, calculated as the percentage of target concentration, was 93.5-98.7% for urine and 90.4-94.6% for plasma.

Table 3 compares the proposed method with other extraction techniques in the determination of the targeted analytes in urine and plasma samples. The quantitative results of the proposed method were better than those of for molecularly imprinted-solid phase extraction with simultaneous derivatization and DLLME-GC-FID[45] in urine sample. The quantitative results of the proposed method are better than those of for solid-phase microextraction (SPME)[46] and microwave-assisted extraction (MAE)[47] techniques in urine sample. The LOD and RSD values of the proposed method are also better than those of for DLLME[48] method in urine sample. DLLME alone needs extra steps in sample preparation for the extraction of the analytes in plasma sample comparing with SPE-DLLME method[49] and MHSPE-DLLME method. Also, it needs more dilution in biological fluids in comparison with the SPE-DLLME and MHSPE-DLLME methods. This drawback causes problem in trace detection of amphetamines. The proposed method possesses comparable results with our previous SPE-DLLME-GC-FID study[49] using C18 sorbent, despite the flame ionization detector being more sensitive than UVD. The problem with cartridge blockage is removed using magnetic separation. Magnetic separation also eliminates the time consuming processes of loading sample in classical SPE and speeds up the sample preparation step. Moreover, easy elution of SDS molecules from magnetic nanoparticles, using organic solvent leads to facile desorption and no memory effect would happen.

Comparison of the proposed method with the SPE method without DLLME procedure also showed the superiority of the hyphenated technique. The calibration graphs were in the range of 20-500 μg L^{-1} in urine and 50-500 μg L^{-1} in plasma for most of selected analytes using the SPE method. It indicates that the preconcentration factor increased by using DLLME procedure because of the large surface area between the extraction solvent and the selected analytes.

Urine sample analysis

To evaluate the matrix effect, experiments were performed using human urine. Firstly, a urine sample was diluted four times. After pH adjustment (pH = 3), a volume of 10.0 mL of this solution containing 5 μg L^{-1} of amphetamines was transferred into a 25 mL Becker and extraction was done as similar to the MHSPE-DLLME procedure. Figures 4a and 4b show the chromatograms obtained from urine samples extracted before and after spiking at concentration level of 5 μg L^{-1} of the amphetamines. As illustrated, the chromatograms confirm

Table 3. Comparison of the proposed method with other extraction techniques for the determination of the targeted analytes in urine and plasma

Method	Sample	RSD / %	LDR / (μg L^{-1})	LOD / (μg L^{-1})	Reference
Moleculary imprinted-solid phase extraction with simultaneous derivatization and DLLME-GC-FID	urine	6.8	50-1500	18	45
Solid-phase microextraction-GC-MS	urine	≤ 14.3	100-10000	5.0-15.0	46
Microwave-assisted extraction-GC-FID	urine	5.5-6.9	50-15000	10-20	47
DLLME-GC-FID	urine	8.2-10.1	1.0-500	0.3-0.8	48
SPE-DLLME-GC-FID	urine	5.7-8.4	1.0-500	0.1-0.3	49
	plasma	6.4-9.7	2.5-500	0.2-0.7	
MHSPE-DLLME-HPLC-UV	urine	6.1-9.7	1.0-250	0.1-0.2	this work
	plasma	8.6-11.1	2.0-250	0.3-0.5	

RSD: relative standard deviation; LDR: linear dynamic range; LOD: limit of detection; DLLME-GC-FID: dispersive liquid-liquid microextraction-gas chromatography-flame ionization detector; GC-MS gas chromatography-mass spectrometry; SPE: solid phase extraction; MHSPE: mixed hemimicelles SPE.

Table 4. Determination of amphetamines in urine and plasma samples using developed method and the relative recoveries of spiked biological samples

Sample	Initial concentration / (μg L^{-1})				Added concentration / (μg L^{-1})				Found concentration ± SD (n = 3) / (μg L^{-1})				Relative recovery / %			
	MDA	MDMA	MDEA	MDPA	MDA	MDMA	MDEA	MDPA	MDA	MDMA	MDEA	MDPA	MDA	MDMA	MDEA	MDPA
Urine	n.d.	n.d.	n.d.	n.d.	5.0	5.0	5.0	5.0	4.8 ± 0.2	4.7 ± 0.3	4.5 ± 0.3	4.6 ± 0.4	96	94	90	92
Plasma	n.d.	n.d.	n.d.	n.d.	10.0	10.0	10.0	10.0	9.3 ± 0.7	9.0 ± 0.9	8.8 ± 0.8	8.7 ± 0.9	93	90	88	87

MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine; n.d.: not detected; SD: standard deviation.

the absence of amphetamines in non-spiked samples. According to Table 4, the amounts of relative recoveries were obtained in the range of 90-96% in urine sample. These satisfactory results indicate good sample clean-up along with negligible matrix effect.

Plasma sample analysis

In order to perform extraction from plasma samples, an amount of 1.0 mL human plasma sample was acidified with 50 μL hydrochloric acid and then 100 μL tirchloroacetic acid (4 g mL^{-1}) was added. Subsequently, the sample was centrifuged at 10000 rpm for 5 min to remove precipitated proteins. Eventually the supernatant was diluted and extraction was performed accordingly. Figure 5 shows chromatograms obtained from plasma samples extracted before and after spiking at concentration level of 10 μg L^{-1} of the amphetamines. As illustrated, the chromatograms confirm the absence of amphetamines in non-spiked samples. According to Table 3, the amounts of relative recoveries were obtained in the range of 87-93% in plasma sample. These satisfactory results indicate good sample clean-up along with negligible matrix effect.

Conclusions

Surface morphological studies and structural analysis results along with VSM analysis confirm successful

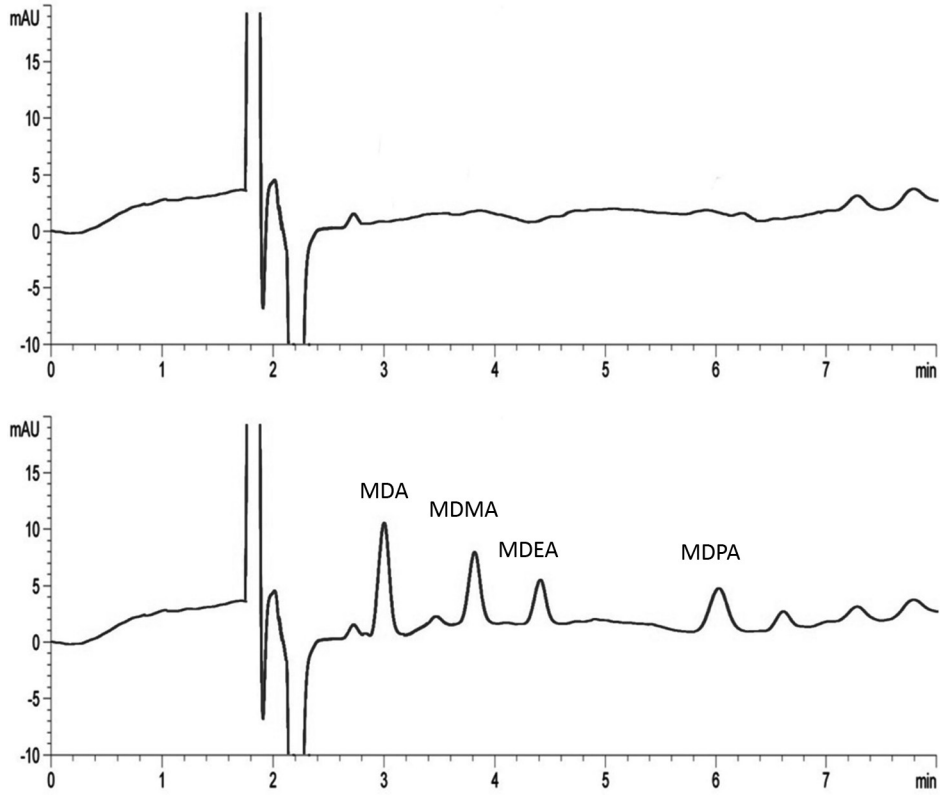

Figure 4. Chromatograms obtained from urine sample (a) before; (b) after being spiked with amphetamines at concentration level of 5 μg L^{-1}. MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine.

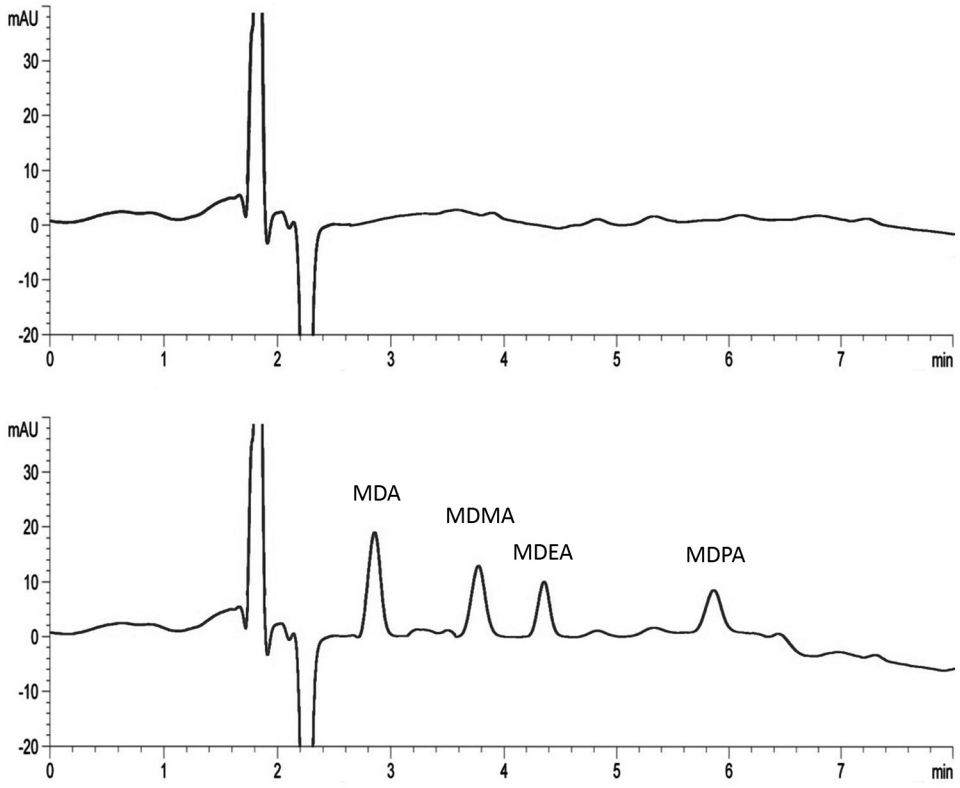

Figure 5. Chromatograms obtained from plasma sample (a) before; (b) after being spiked with amphetamines at concentration level of 10 μg L^{-1}. MDA: 3,4-methylenedioxy amphetamine; MDMA: 3,4-methylenedioxymethyl amphetamine; MDEA: 3,4-methylenedioxyethyl amphetamine; MDPA: 3,4-methylenedioxypropyl amphetamine.

synthesis of Fe_3O_4 magnetic nanoparticles. The proposed MHSPE-DLLME/HPLC-UV method, using SDS-coated Fe_3O_4 nanoparticles, demonstrates sufficient sensitivity and selectivity, good reproducibility, acceptable accuracy, applicable sample clean-up and insignificant matrix effect in extraction and determination of amphetamines from biological samples. However, stability tests proved that analysis should be performed shortly after sample preparation. Comparing the developed method to some other techniques of extraction and determination of amphetamines from urine and plasma samples corroborates reasonable superiority of the method using UV as a general detector.

Supplementary Information

Supplementary data (X-ray diffraction pattern of Fe_3O_4, FE-SEM images of Fe_3O_4, magnetization curve of Fe_3O_4 nanoparticles) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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