Journal of the Iranian Chemical Society
https://doi.org/10.1007/s13738-021-02339-0

ORIGINAL PAPER

Dispersive solid-phase extraction for simultaneous determination of four amphetamines drugs in urine using gas chromatography–mass spectrometry

Mahir Z. Elboraie[1] · Abdalla A. Elbashir[1,2] · Eltayeb E. M. Eid[3] · Hassan Y. Aboul-Enein[4]

Received: 24 May 2021 / Accepted: 5 July 2021

Abstract
A sample preparation method based on modified Quick, Easy, Cheap Effective, Rugged, and Safe (QuEChERS) and GC–MS method was developed and validated for identification and quantification of amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) in human urine. Several derivatization parameters such as derivatization temperature, time, and volume of derivatizing agent were optimized. dSPE parameters were optimized. Under the optimized conditions, the method was validated with respect to selectivity, linearity, limits of detection and quantification, precision, accuracy, and recoveries. Linearity was obtained in the concentration range of 50–1200 ng/mL for all the drugs. The intra- and inter-day precision and accuracy were acceptable. The RSD values ranging from 0.08 to 3.43% and 0.23% to 3.0% for intra- and inter-day precision were obtained, respectively. Accuracy was within the required limits (± 15% and ± 20% at LOQ) for all analytes at three concentrations studied. Analyte recoveries that ranged from 87.8 to 95% were obtained. These figures resulted within the best recovery percentages as comparing with the previous studies. The method was successfully applied to the routine work of the Sudanese Forensic laboratories to detect and quantify the AP, MA, MDA, and MDMA in urine samples.

Keywords Drug abuse · QuEChERS · GC–MS · MSTFA · Human urine · Dispersive solid-phase extraction

Introduction

The secondly most widely used drugs of abuse (DOA) are amphetamines, opiates, and cocaine; they are second to cannabis in most countries [1]. Amphetamine (AP) and its related compounds are powerful stimulants of the central nervous system, acting on neurons in the brain to create feelings of pleasure and well-being [2–4], they include a number of therapeutic agents and drug abused (illicit drugs) [2, 3, 5]. The abuse of amphetamines is continuing to be listed among the most commonly attending recreational drugs worldwide especially among adolescent and youths for performing hard activities like truck drivers [5–8], they are used most frequently for doping during competitions [2, 9]. A new generation of synthetic amphetamines with modified ring system, which derived from illegal origin have been consumed as a new drug of abused especially by young people [6, 10]. Since these drugs are synthesized in a clandestine laboratory, there would be no knowledge of actual composition of the product and no documentation of ensuring safety, so the abuse of these designer drugs poses some risk to the abusers [6, 10].

A number of analytical methods for the determination of amphetamines in biological samples have been reported in the literature. These include gas chromatography–mass spectrometry (GC–MS) [11–14], liquid chromatography–mass spectrometry (LC–MS) [14–16], and capillary

✉ Abdalla A. Elbashir
aaeahmed@kuf.edu.sa

✉ Hassan Y. Aboul-Enein
haboulenein@yahoo.com

[1] Department of Chemistry, Faculty of Science, University of Khartoum, 11115 Khartoum, Sudan

[2] Department of Chemistry College of Science, King Faisal University, P.O.Box 400, Al-Ahsa 31982, Saudi Arabia

[3] Department of Pharmaceutical Chemistry and Pharmacognosy, Unaizah College of Pharmacy, Qassim University, Buraydah, Saudi Arabia

[4] Division of Pharmaceutical and Drug Industries Research Division, Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

Published online: 28 July 2021

electrophoresis–mass spectrometry (CE-MS) [17]. Prior to the GC analysis, derivatization is necessary to improve the amphetamine analysis [18, 19], that is because the analysis of free amphetamines by GC–MS is associated with difficulties and results in poor peak resolution [18, 19].

For obtaining successfully analysis of large numbers of samples, forensic laboratories require rapid analysis methods, which cannot be achieved by using the conventional sample preparation technique that is generally employed in forensic laboratories for urine drug testing.

To perform a reliable and accurate chromatographic analysis, sample preparation is important step to isolate the target drug [20, 21]. Various sample preparation procedures have been employed for the extraction and pre-concentration of amphetamines from biological fluids, such as liquid–liquid extraction (LLE) [22–24], liquid-phase microextraction (LPME) [25, 26], dispersive liquid–liquid microextraction (DLLME) [27, 28], solid-phase extraction (SPE) [29–32], and solid-phase microextraction (SPME) [33, 34]. LLE and SPE are widely used; however, LLE needs large amounts of high-purity solvents that are expensive and toxic, whereas SPE is time consuming and expensive [35].

QuEChERS (quick, easy, cheep, effective, rugged, and safe) approach was commonly used for the analysis of pesticide residues in fruits and vegetables [36], and acrylamide in food [37]. This cleanup procedure has lately demonstrated its effectiveness for the determination of drugs or toxic compounds in human biofluids [1, 2] using LC–MS/MS and CE-MS. This technique is simple, rapid, and therefore compatible for forensic analysis requirements.

The present paper describes the development and validation of a GC–MS method based on QuEChERS extraction for the simultaneous determination of the (AP, MA, MDA, and MDMA) in human urine for forensic purposes. Basic QuEChERS was modified by optimizing the quantity of the salts in order to obtain the high recovery of studied drugs.

Experimental

Chemicals and materials

Analytical standard of amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) was purchased from Lipomed Serviced to Health, Lipomed AG Fabrikmattenweg 44,144 Arlesheim, Switzerland. The entire standards were 1.0 mg/mL solution in methanol. The silylating reagent N-methyl(trimethyl-silyl) triflouroacetamide [MSTFA] was obtained from Sigma-Aldrich, 3050 Spruce street, St. Louis MO 63,103, USA. The kits for screening immunoassay analysis (Rapid Response Multi-Drug Test Panel) were delivered from BTNX Inc. 570 Hood Road, unit 23, Markham ON L3R 4G7, Canada. The chemicals methanol (MeOH), acetonitrile (ACN), sodium hydroxide (NaOH), anhydrous magnesium sulfate ($MgSO_4$), and sodium chloride (NaCl) were bought from SDFCL, 315–317, TV Industrial Estate, 248, Worli Road, Mumbai 30, India; all chemicals were of analytical grade. Dispersive solid-phase extraction (dSPE) tubes part number CUMC182CT (2.0-mL dSPE tube) were supplied by UCT (Bristol, USA). The standard drugs were mixed and diluted in methanol to prepare stock solution at 10 µg/mL of (AP, MA, MDA, and MDMA) and stored at − 20 °C before use.

Samples

Urine samples received to the Forensic Laboratories for criminal analysis and blank urines collected from laboratory staff member were stored at − 20 °C before analysis. 2.0 mL of the blank urine was spiked to 200 µL of the calibration work standard drugs (each of the eight concentration levels) and placed in a 10-mL test tube.

Sample preparation

The extraction of the drugs was performed using acetonitrile organic solvent and purified using Q-sep dispersive solid-phase extraction (dSPE) tubes.

This method involves two steps—Step one (Extraction): 2.0 mL of acetonitrile (containing 200 mg of NaOH-apparent pH 12.4) was added to the 2.0 mL of blank urine sample that is placed in the 10-mL test tube and spiked with the 200 µL of the drugs. 400 mg of anhydrous $MgSO_4$ and 100 mg of anhydrous NaCl were added to the test tube. The test tube was vortexed and centrifuged at 2000 rpm for 10 min. Step two (cleanup): 1.0 mL of the supernatant was transferred into a dSPE tube containing 150 mg of $MgSO_4$, and 50 mg of C18, the mixture was vortexed and centrifuged at 3000 rpm for 5 min, the supernatant was filtered through 4.5-µm filter paper into a 5 mL vial, the vial content was totally dried under steam of nitrogen and derivatized using MSTFA reagent at optimum derivatization conditions (80 °C/25 min) that are obtained step-by-step in this work. Then, the extract and derivatized drugs underwent GC–MS analysis. The recovery test was carried out by spiking the drugs after the filtration step, at three concentration levels with the calibration curve.

Optimization of QuEChERS and dSPE process

In the QuEChERS and dSPE optimization process, the amount of NaCl and anhydrous $MgSO_4$ were studied. The amount of NaCl and anhydrous $MgSO_4$ was studied by first fixing the $MgSO_4$ at 400 mg and varying the amounts of NaCl added (50–200 mg). This method involves two

steps—Step one (Extraction): 2.0 mL of acetonitrile (containing 200 mg of NaOH-apparent pH 12.4) was added to the 2.0 mL of blank urine sample that is placed in the four 10-mL test tubes and spiked with the 200 vμL of the drugs followed by the addition of 400 mg of anhydrous $MgSO_4$. The first, second, third, and fourth tubes were added with 50,100, 150, and 200 mg of anhydrous NaCl, respectively, and the mixture was vortexed and centrifuged at 2000 rpm for 10 min. The effect of varying amounts of $MgSO_4$ (350–600 mg) was studied at 100 mg NaCl (best amount). Similar procedure as for NaCl was followed.

Optimization of the derivatization procedure

A systematic optimization of the derivatization experimental procedure was deemed necessary to achieve higher yields and enhance method sensitivity. Parameters including derivatization time, temperature, and volume of the derivatizing agent were step-by-step studied and optimized.

Method validation

The developed method was validated in terms of linearity, selectivity, accuracies, precisions, limits, and recovery. In order to obtain these validation data, calibration curves were constructed based on peak area obtained with the drugs at eight different concentration levels (50, 100, 200, 500, 800, 1000, and 1200 ng/mL) that spiked to blank urine samples and three analyses were made for each point level. The range of linearity was detected. Coefficient of determination (r2) was used to express the linearity of the regression line. Linearity was accepted if $r2 \geq 0.98$ [5]. Chromatographic selectivity was evaluated by the presence or absence of co-eluting peaks at the retention times of the analytes [38]. The accuracy of the method was evaluated by spiking blank matrix at three different concentration levels (low, 50; medium, 500; and high, 1200 ng/mL) and through the calculation of the percentage deviation between the calculated value and the nominal value [accuracy (%) = (experimental concentration/theoretical concentration) *100] [5, 38, 39]. Precision is defined as the degree of agreement between independent results obtained under specific circumstances [39]. Precision data were quantified by analyzing the peak areas of three replicates of three concentrations levels within the calibration curves and calculating the RSD. Within-day precision and time-different intermediate precision were calculated as the relative standard deviation (RSD) = $s/\times 100\%$ where RSD values below 15% and at LOQ below 20% were acceptable for quantitative analysis [5, 39, and 40]. The Limit of quantitation (LOQ) is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [39], where at this level, the analytes should have accuracy and precision values within ± 20% bias and below 20% relative standard deviation (RSD), respectively, where the limit of detection (LOD) is defined as the minimum detection ability for the system, or as the amount of analyte in the sample below which the spectrometer system cannot detect the analyte [22 and 41]. The LOD and LOQ were calculated based on the approach of "Standard Deviation of the Response and the Slope" whereby the LOD is expressed as LOD = 3.3 s/S and the LOQ is expressed as LOQ = 10 s/S, where s is the standard deviation of the linear regression line and S is the slope of the curve [38, 39]. The recovery was determined by comparing the response from the drug spiked to the blank urine samples and then extracted, with the response from already extracted blank urine samples and then spiked to the same analyte at the equivalent concentration level. The recovery was expressed as percentage. Analytical recovery between 80 and 120% was considered acceptable [4, 38].

GC–MS analysis

GC–MS analysis was performed using Shimadzu GC–MS-TQ8040, coupled to an AOC-5000 Autosampler. Analytic chromatographic separation was achieved using a capillary column (RTx-5MS, 30 m–0.25 mm i.d- and 0.25 um thickness). Carrier gas (helium) was maintained at a total flow rate of 50 mL/min, all injections were in splitless mode with a column flow rate of 1.4 mL/min, the injection port temperature was 250 °C. The ion source and interface temperature were 200, 250 °C, respectively, mass cut time was 3 min. The mass spectrometer was operated at 70 eV in the electron impact (EI) mode with the types of ionization mode (scan ionization mode and selected ion monitoring mode). In scan ionization mode, the m/z ions used were in the range of 35 m/z to 400 m/z, where in selected ion monitoring (SIM) mode, only specific ions were used, where one ion was used as a quantifier, while two other ions were used as qualifiers for the analysis of the target compounds.

Results and discussion

Optimization of amount of NaCl and anhydrous MgSO4 added in QuEChERS

It has been reported that the quantity of the salt added affects recovery of the dSPE extraction [1]. Therefore, the optimum amount of salt combination ($MgSO_4$ and NaCl) added was investigated by fixing the amount of anhydrous $MgSO_4$ (400 mg) and varying the amounts of NaCl added (50,100, 150, and 200 mg). The optimum peak areas of AP, MA, MDA, MDMA were obtained by adding 100 mg of NaCl and were used in subsequent experiments. The effect of varying the amount of anhydrous $MgSO_4$ salt (350–600 mg) at

100 mg of NaCl was then studied. The optimum peak areas of AP, MA, MDA, MDMA were obtained with 400 mg anhydrous $MgSO_4$. The role of anhydrous $MgSO_4$ is to enhance the extraction efficiency by drying the extraction solvent (acetonitrile) to achieve the maximum amount of analytes extracted. The role of anhydrous NaCl is to enhance the extraction efficiency by separating the two layers completely, the solvent layer and the aqueous layer (urine) with no emulsion in between [37].

Optimization of the derivatization procedure

The first parameter studied was the temperature of derivatizing reaction. 100 µL of 500 ng/mL amphetamine was totally evaporated and dried under steam of nitrogen, and then 100 µL of MSTFA was added in the residue and the time of heat was set at 20 min, the derivatization temperature was ranged from 60, 70, 80, and 90 °C. The best result was obtained at 80 °C and thus it was selected for the following experiment.

To study the effect of time on the derivatization efficiency, the temperature was set at 80 °C and different derivatization reaction times (15, 20, 25, and 30 min) were studied. The best results were obtained at the derivatization reaction time 25 min.

The volume of the derivatizing agent was also studied: 50, 100, 150, and 200 µL MSTFA were added in the residue. Best results were obtained with 50 µL, and thus, this volume was selected for further experiments. The finally selected derivatization parameters were as follows: 50 µL of MSTFA was added to the evaporated drug and derivatized for 25 min at 80 °C and then injected at the GC–MS system. The optimized derivatization parameters were used in all experimental work in this study.

Chromatograms for the simultaneous separation of the amphetamines drugs AP, MA, MDA, and MDEA with and without derivatization are shown in Fig. 1a and b, respectively. Without derivatization, the peak area values were very low, because the drug is volatile and some of the underivatized drug is lost by evaporation [13, 14].

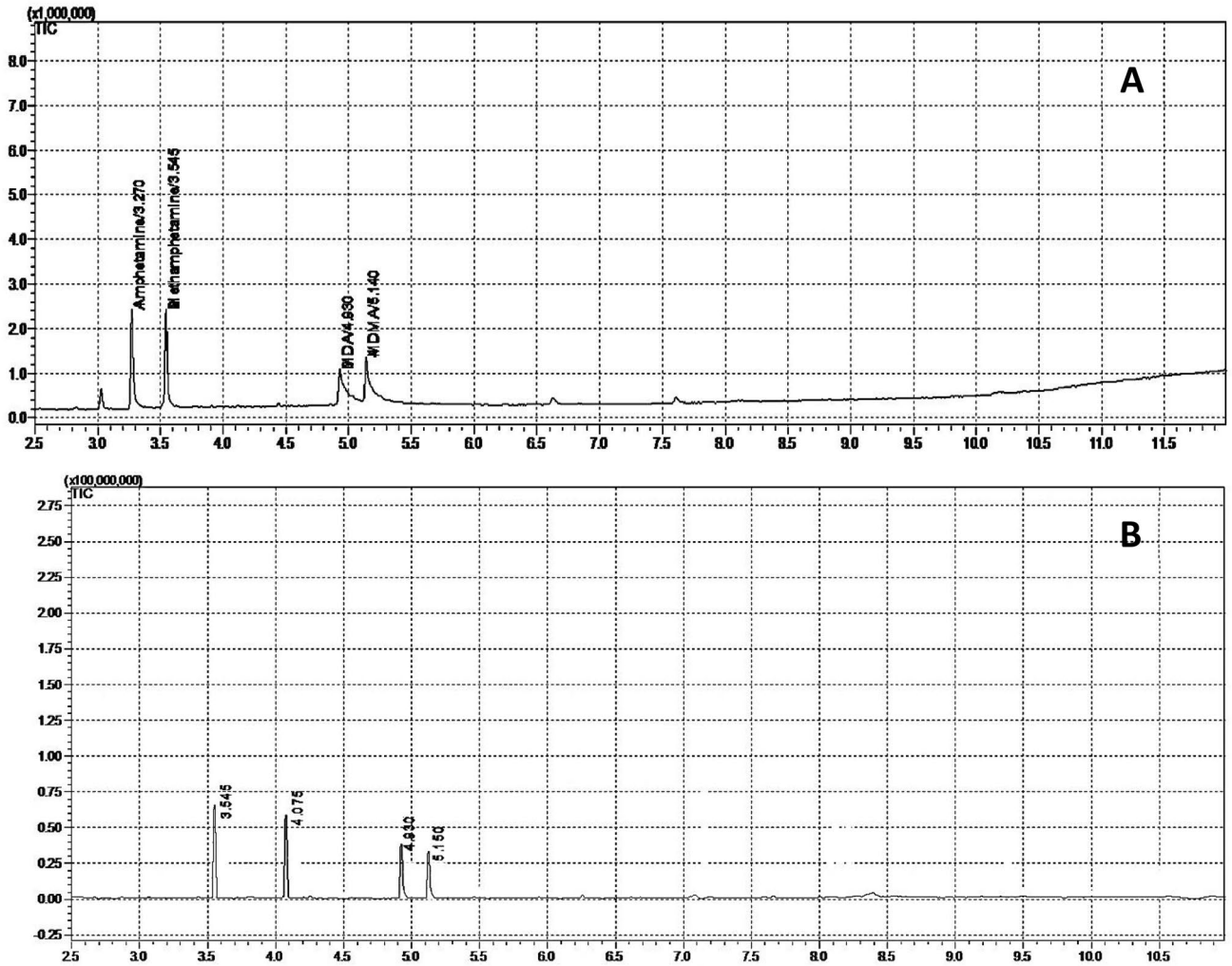

Fig. 1 The chromatograms of mixture of standard of AP, MA, MDA, and MDMA **a** underivatized **b** derivatized

Unlike the standard drugs, the extracted and derivatized urinal drugs were GC–MS identified and quantified using the selected ion monitoring (SIM), where one peak (the highest) was used for quantification for each extracted drug. Figures 2 shows the chromatogram of the urinal extracted derivatized amphetamines drugs using (SIM) mode.

The most relative abundance masses m/z and the retention times of the derivatized standard amphetamines drugs are shown in Table 1. Their masses and retention times were used to identify the corresponding ones that urinary extracted, using the methodology mentioned above then derivatized and GC–MS analyzed. The retention times reported here are shorter than those reported by Jurado et al., 10.9 min [4], Nishida et al., 11.3 min [3], and by Villamor et al., 12.8 [6].

Method validation

To validate the performance of the proposed modified QuEChERS with dSPE method for AP, AM, MDA, and MDEA, parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and reproducibility) (RSD%), and accuracy (recovery) were evaluated at optimum extraction conditions using 2.0 mL of acetonitrile (containing 200 mg of NaOH-apparent pH 12.4, 400 mg anhydrous MgSO4, 100 mg NaCl) and optimum derivatization condition using 50 µL of MSTFA reagent at 80 °C temperature for 25 min.

Selectivity

Eight different urine samples obtained from laboratory staff (drug non-abuser) were tested for selectivity test. There was no interfering peak at the retention times of the analytes. Representative chromatograms obtained from blank urine sample, spiked urine sample, and urine positive sample are shown in Fig. 3a, b, and c, respectively.

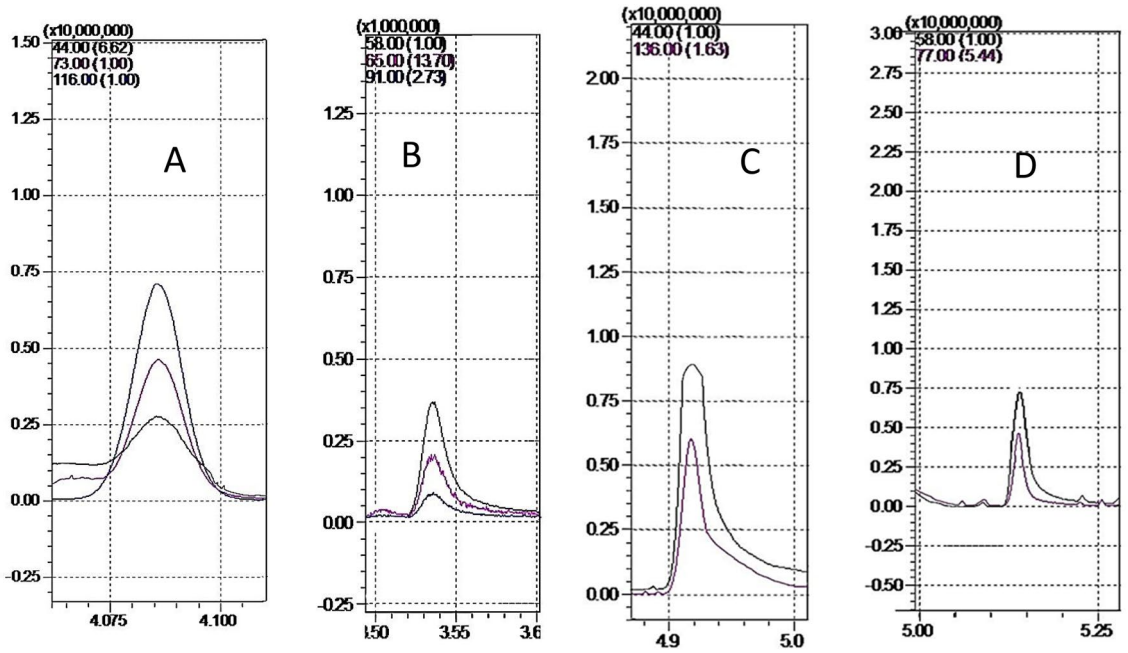

Fig. 2 Urinal extracted derivatized peaks using selective ion monitoring (SIM) mode. **a** AP **b** MA **c** MDA **d** MDMA

Table 1 Retention times and mass ions the simultaneous GC–MS analysis of the derivatized standard amphetamines drugs

Analyte	Retention time	Identified mass ions	Quantified mass ion
Derivatized methamphetamine	3.545	58, 65, 91	58
Amphetamine-TMS derivative	4.085	44,73,116	116
Derivatized MDA	4.930	44,136	44
Derivatized MDMA	5.140	58, 77	58

Linearity, limit of detections (LODs), and limit of quantifications (LOQs)

The linearity of the method was investigated at eight different concentration levels of AP, AM, MDA, and MDEA ranging from 50 to 1200 ng/mL. Good linearity was obtained for all analytes with coefficient of determination, (r2) in the range of 0.984–0.997.

The LOD and LOQ of this method were calculated using the calibration curves. The LOD was established using LOD = 3.3 x (s/S) and the LOQ = 10 x (s/S), where s is the standard deviation of the intercept and S is the slope of the curve. The LOQ and LOD were found to be 42 and 13 ng/mL, respectively, which was higher than those obtained by Jurado et al., using SPME coupled with GC–MS for analysis of the same drugs in urine samples [4]. However, the values obtained by our methods are lower than those obtained by Gentili et al., using HS-SPME [40]. The results of the linearity experiments and LODs and LOQs are shown in Table 2.

Precision and accuracy

The results for intraday and inter-day precision and accuracy are shown in Tables 3 and 4, respectively. The intra-precision and inter-precision (as RSD) were within the required limits for all analytes. The within-day precision was measured as percentage standard deviation at three replicate analyses at three concentrations level 50, 500,

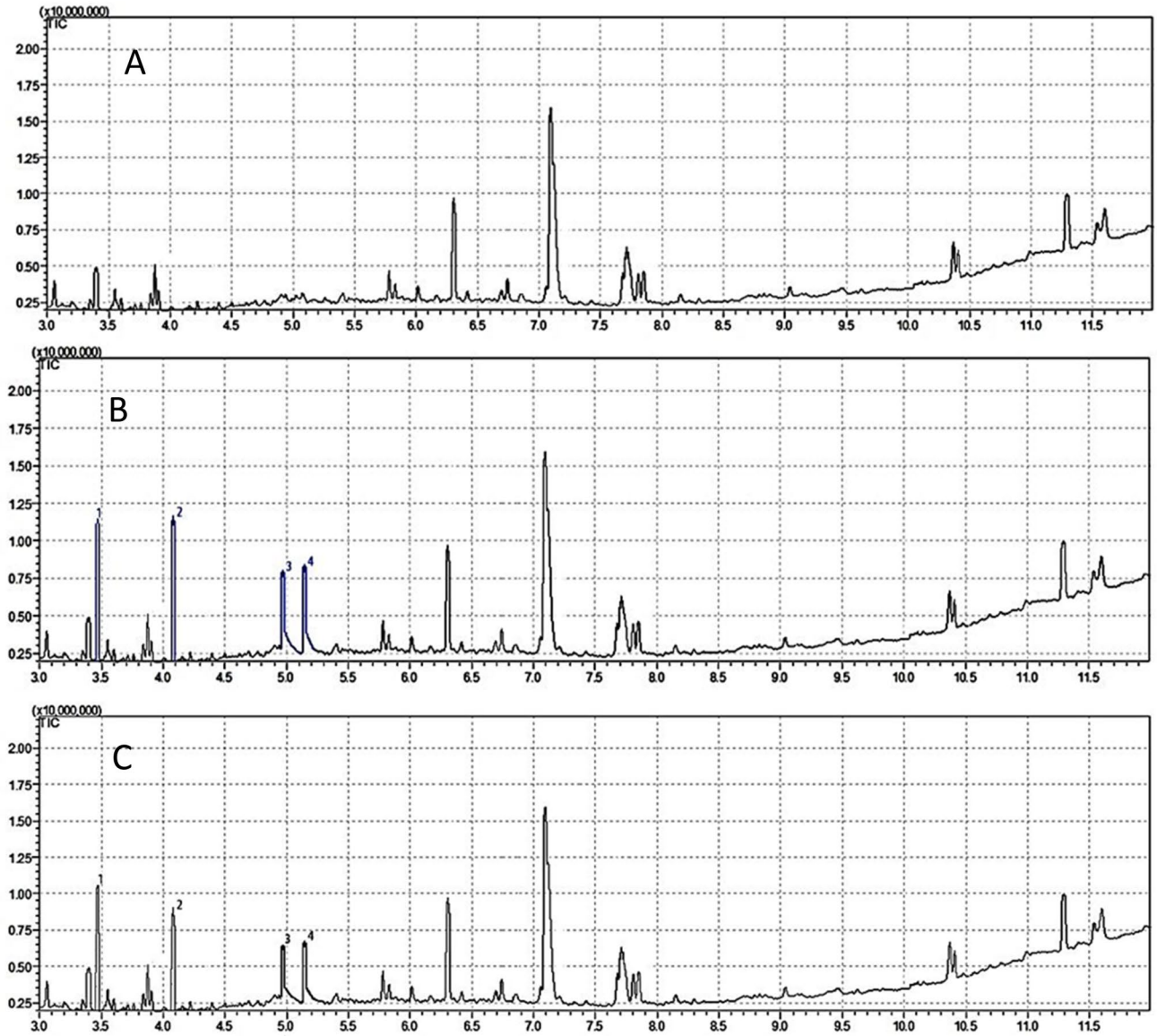

Fig. 3 Chromatograms of **a** blank urine sample, **b** spiked urine sample, and **c** positive urine sample

Table 2 Correlation coefficient, concentration linear range for the urine linear regression simultaneous analysis of urinal extracted amphetamines and drugs (in mixture) and their LOQ and LOD

Drug analyte	Determination coefficient (R^2)	LOQ (ng/mL)	LOD (ng/mL)
AP	0.984	43.17	12.95
MA	0.992	43.02	12.91
MDA	0.997	42.93	12.88
MDMA	0.993	43.00	12.90

and 1500 ng/mL. Based on the results obtained from Table 3, the method exhibits precision within % RSD values ranging from 0.08 to 3.43%. The intraday accuracy for AP, MA, MDA, and MDMA was found to be in the range of 92.2–106.1%, 85.2–95.6%, 92.6–116.6%, and 87.8–117.4%, respectively, at three concentration levels Table 3.

Similarly, the different-day precision was assessed, the method exhibits precision within % RSD values ranging from 0.20 to 3.0%, Table 4. Accuracy was within the required limits (± 15% and ± 20% at LOQ) for all analytes at three concentrations studied. The inter-day accuracy for AP, MA, MDA, and MDMA was found to be in range of 90.8–113.4%, 92.2–104%, 85.2–107.6%, and 87.6–114.8%, respectively, at three concentration levels Table 4.

Table 3 Intra-assay precision (a day precision) and accuracy at different concentration levels (low, intermediate, and high) for amphetamines drugs

Analyte	Theoretical concentration (ng/mL)	Mean peak area ($n=3$)	SD	%CV (RSD) (%)	Experimental concentration (ng/mL)	Accuracy %	[a]Accuracy bias (%)
AP	50	49,207	62.5	0.13	46.1	92.2	−7.8
	500	49,896	39	0.08	520	104%	4.0
	1200	50,992	106.8	0.21	1274	106.1%	6.16
MA	50	43,186	39	0.1	42.5	85.2%	−1.5
	500	43,677	77.1	0.2	469.6	93.9%	−6.08
	1200	44,456	57	0.13	1143	95.6%	−4.75
MDA	50	32,253	1105	3.43	46.3	92.6%	−7.4
	500	32,810	829	2.53	572	114.4	14.4
	1200	33,686	275	0.82	1399	116.6	16.58
MDMA	50	28,393	24.4	0.1	56	112%	12.0
	500	28,769	46.5	0.16%	439	87.8%	−12.2
	1200	29,722	338.3	1.14%	1409	117.4%	17.72

[a]Accuracy: [(found-added/added)] × 100

Table 4 Inter-assay precision and accuracy at different concentration levels (high, intermediate, and low) in five different days for amphetamines drugs

Analyte	Theoretical concentration (ng/mL)	Mean peak area ($n=15$)	SD	%CV (RSD) (%)	Experimental concentration (ng/mL)	Accuracy %	[a]Accuracy bias (%)
AP	50	49,215	380.3	0.77	51.6	103.2	3.2
	500	49,965	608	1.22	567	113.4	13.4
	1200	50,723	220	1.23	1089	90.8	−9.25
MA	50	43,190	101	0.23	46.1	92.2	−7.8
	500	43,735	126.6	0.29	520	104	4.0
	1200	44,462	89.1	0.20	1151	96	−4.1
MDA	50	32,251	953	3.0	44.4	88.8	−11.2
	500	32,774	873	2.7	538	107.6	7.6
	1200	33,286	667	2.0	1022	85.2	−14.8
MDMA	50	28,381	181	0.64	43.8	87.6	−12.4
	500	28,825	84.5	0.29	496	99	−0.8
	1200	29,691	615.4	2.1	1378	114.8	14.8

[a]Accuracy: [(found-added/added)] × 100

Table 5 Concentrations of amphetamine and related compounds in 17 immunoassay amphetamine positive out of 21 urine samples received to the forensic laboratories

Sample Number/ analyte	Concentration (ng/mL)			
	MDMA	MDA	MA	AP
1	1226	865	271	246
2	1103	904	258	253
3	1099	865	x	x
4	1080	423	262	241
5	1068	830	227	166
6	1056	569	255	188
7	969	546	x	136
8	760	537	189	x
9	753	865	126	x
10	720	482	174	154
11	538	530	189	x
12	517	448	x	x
13	517	482	x	x
14	513	318	x	x
15	499	320	x	x
16	491	x	x	x
17	456	x	x	x

x: absence of the target analytes

Application of the method

From 21 urine samples that received to the Sudanese Forensic Laboratories, 17 urine samples were found to be amphetamine screening positive. In overall, 17 urine samples were tested positive for amphetamine with an average concentration of 758.7 ng/mL (one sample was found to be over the linear concentration), 15 samples were tested positive for methamphetamine with an average concentration of 603 ng/mL, 9 samples were tested positive for MDA with average concentration of 216.8 ng/mL and 7 samples were tested positive for MDMA with average concentration of 197.7 ng/mL. The results are summarized in Table 5.

Conclusion

A fast GC–MS method based on QuEChERS extraction for the simultaneous determination of the AP, MA, MDA, and MDMA in human urine for forensic purposes was developed and validated. The study involves the optimization of derivatization procedure and the quantity of the partition salts in the QuEChERS cleanup procedure. The optimization of the quantity of the partition was found to enhance extraction recovery. The method is considered to be fast and needs about half an hour to complete the sample analysis, 20 min for sample preparation and cleanup, and 12 min for the GC–MS analysis. The method developed was successfully applied for the analysis of urine samples, and out of 21 samples, 17 were tested positive for amphetamines. This method has proven to be suitable for application in the analytical routine analysis of forensic laboratory.

Author's contributions All the authors contributed equality to this manuscript.

Funding Not applicable.

Data availability Available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval An ethical approval for using urine samples was received from Sudan Police Headquarters, General Administration of Forensic Evidence, Forensic Laboratories, Khartoum, Sudan.

Human and animal rights Not applicable.

Consent to participate Not applicable.

References

1. S. Dulaurent, S. El Balkhi, L. Poncelet, J.M. Gaulier, P. Marquet, F. Saint-Marcoux, QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood. Anal. Bioanal. Chem. **408**(5), 1467–1474 (2016)
2. B.D.S. Vagner, L.D.L. Claudimir, and D. Daniel; Determination of Amphetamine and Derivatives in Urine Using a Modified QuEChERS and Capillary Electrophoresis Tandem Mass Spectrometry Analysis, Agilent Technologies, Inc., (2016) 5991–7019.
3. M. Nishida, A. Namera, M. Yashiki, M. Kojima, Routine analysis of amphetamine and methamphetamine in biological materials by gas chromatography-mass spectrometry and on-column derivatization. J. Chromatogra. B **789**, 65–71 (2003)
4. C. Jurado, M.P. Giménez, T. Soriano, M. Menéndez, M. Repetto, Rapid analysis of amphetamine, methamphetamine, MDA, and MDMA in urine using solid-phase microextraction, direct on-fiber derivatization, and analysis by GC-MS. J. Anal. Toxicol. **24**, 11–16 (2000)
5. E. Miranda-G, M. Sorda, A.M. Salazar, C. Contras, L. Bautista, A.E. Rojas Gaecia, P. Ostrosky-Wegman, Determination of amphetamine, methamphetamine and hydroxyamphetamine derivatives in urine by gas chromatography-mass spectrometry and its relation to CYP2D6 phenotype drug users. J. Anal. Toxicol. **31**, 31–36 (2007)
6. J.L. Villamor, A.M. Bermejo, P. Fernández, M.J. Tabernero, A new GC-MS method for the determination of five amphetamines in human hair. J. Anal. Toxicol. **29**, 135–139 (2005)
7. B. Moslah, M. Araoud, M.A. Nouioui, S. Najjar, D. Amira, N. Ben Salah, A. Hedhili, Fast screening tests for the simultaneous detection of 11 drugs of abuse in urine specimens. A forensic

epidemiology study of 28,298 cases in Tunisia. Forensic Sci. Int. **283**, 35–40 (2018)
8. L. Guo, Z. Lin, Z. Huang, H. Liang, Y. Jiang, Y. Ye, Z. Wu, R. Zhang, Y. Zhang, Y. Rao, Simple and rapid analysis of four amphetamines in human whole blood and urine using liquid–liquid extraction without evaporation/derivatization and gas chromatography–mass spectrometry. Forensic Toxicol. **33**, 104–111 (2015)
9. M. Thevis, A. Thomas, W. Schanzer, Current role of LC-MS-(MS) in doping control". Anal. Bioanal. Chem. **401**, 405–420 (2011)
10. J.Y. Kim, S. Suh, J. Park, M.K. In, Simultaneous determination of amphetamine-related new psychoactive substances in urine by gas chromatography-mass spectrometry. Analytical Toxicol. **42**, 605–616 (2018)
11. T. Saito, H. Mase, S. Takeichi, S. Inokuchi, Rapid simultaneous determination of ephedrines, amphetamines, cocaine, cocaine metabolites, and opiates in human urine by GC-MS. J. Pharm. Biomed. Anal. **43**, 358–363 (2007)
12. G. Merola, S. Gentili, F. Tagliaro, T. Macchia, Determination of different recreational drugs in hair by HS-SPME and GC/MS. Anal. Bioanal. Chem. **397**, 2987–2995 (2010)
13. S. Mohr, J.A. Wei, J. Spreitz, M.G. Schmid, Chiral separation of new cathinone- and amphetamine-related designer drugs by gas chromatography-mass spectrometry using trifluoroacetyll-prolyl chloride as chiral derivatization reagent. J. Chromatogr. A **1269**, 352–359 (2012)
14. S. Malaca, T. Rosado, J. Restolho, J.M. Rodilla, P.M.M. Rochae, L.S.C. Margalho, M. Barroso, E. Gallardo, Determination of amphetamine-type stimulants in urine samples using microextraction by packed sorbent and gas chromatography-mass spectrometry". J. Chromatogr. B **1120**, 41–50 (2019)
15. R. Kronstrand, I. Nystrom, J. Strandberg, H. Druid, Screening for drugs of abuse in hair with ion spray LC-MS-MS. Forensic Sci. Int. **145**, 183–190 (2004)
16. J. Wang, Z. Yang, J. Lechago, Rapid and simultaneous determination of multiple classes of abused drugs and metabolites in human urine by a robust LC-MS/MS method-application to urine drug testing in pain clinics. Biomed. Chromatogr. **27**, 1463–1480 (2013)
17. P.M. O'Byrne, P.V. Kavanagh, S.M. Mcnamara, S.M. Stokes, Screening of stimulants including designer drugs in urine using a liquid chromatography tandem mass spectrometry system. J. Anal. Toxicol. **37**, 64–73 (2013)
18. I. Kohler, J. Schappler, S. Rudaz, Highly sensitive capillary electrophoresis-mass spectrometry for rapid screening and accurate quantitation of drugs of abuse in urine. Anal. Chim. Acta **780**, 101–109 (2013)
19. J.M. Holler, S.P. Vorce, T.Z. Bosy, A. Jacobs, Quantitative and isomeric determination of amphetamine and methamphetamine from urine using a nonprotic elution solvent and R-(-)-α-methoxy-α-triflouromethlphenylacetic acid chlouride derivatization. J. Anal. Toxicol. **29**(7), 652–657 (2005)
20. A. Orfanidis, O. Mastrogianni, A. Koukou, G. Psarros, H. Gika, G. Theodoridis, N. Raikos, A GC–MS method for the detection and quantitation of ten majordrugs of abuse in human hair samples. J. Chromatogr. B **1047**, 141–150 (2017)
21. N.H. Kwon, Y.R. Lee, H.S. Kim, J.C. Cheong, J.Y. Kim, Hybrid solid-phase extraction for selective determination of methamphetamine and amphetamine in dyed hair by using gas chromatography-mass spectrometry. Molecules **24**, 2501 (2019)
22. D.E. Raynie, Modern extraction techniques. Anal. Chem. **78**, 3997 (2006)
23. M. Pellegrini, F. Rosati, R. Pacifici, R. Zuccaro, F.S. Romolo, A. Lopez, Rapid screening method for determination of Ecstasy and amphetamines in urine samples using gas chromatography-chemical ionisation mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **769**, 243–251 (2002)
24. D. Peroni, W. Van Egmond, W.T. Kok, H.G. Janssen, Advancing liquid/liquid extraction through a novel microfluidic device: theory, instrumentation and applications in gas chromatography. J. Chromatogr. A **1226**, 77–86 (2012)
25. M.K. Woźniak, M. Wiergowski, J. Aszyk, P. Kubica, J. Namieśnik, M. Biziuk, Application of gas chromatography–tandem mass spectrometry for the determination of amphetamine-type stimulants in blood and urine. J. Pharm. Biomed. Anal. **148**, 58–64 (2018)
26. R. Wang, X. Qi, L. Zhao, S. Liu, S. Gao, X. Ma, Y. Deng, Ionic-liquid-based dispersive liquid–liquid microextraction coupled with high-performance liquid chromatography for the forensic determination of methamphetamine in human urine. J. Sep. Sci. **39**, 2444–2450 (2016)
27. S.C. Franco de Oliveira, M. Yonamine, Measurement uncertainty for the determination of amphetamines in urine by liquid-phase microextraction and gas chromatography-mass spectrometry. Forensic Sci. Int. **265**, 81–88 (2016)
28. D. Djozan, M.A. Farajzadeh, S.M. Sorouraddin, T. Baheri, Molecularly imprinted solid phase extraction combined with simultaneous derivatization and dispersive liquid-liquid microextraction for selective extraction and preconcentration of methamphetamine and ecstasy from urine samples followed by gas chromatography. J. Chromatogr. A **1248**, 24–31 (2012)
29. T. Ahmadi-Jouibari, N. Fattahi, M. Shamsipur, 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. **94**, 145–151 (2014)
30. L.W. Chung, G.J. Liu, Z.G. Li, Y.Z. Chang, M.R. Lee, Solvent-enhanced microwave assisted derivatization following solid-phase extraction combined with gas chromatography-mass spectrometry for determination of amphetamines in urine. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **874**, 115–118 (2008)
31. F. Wei, Y. Fan, M. Zhang, Y.Q. Feng, Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith in-tube solid-phase microextraction applied to simultaneous analysis of some amphetamine derivatives in urine by capillary zone electrophoresis. Electrophoresis **26**, 3141–3150 (2005)
32. A. Namera, A. Nakamoto, M. Nishida, T. Saito, I. Kishiyama, S. Miyazaki, M. Yahata, M. Yashiki, M. Nagao, Extraction of amphetamines and methylenedioxyamphetamines from urine using a monolithic silica disk-packed spin column and high-performance liquid chromatography-diode array detection. J. Chromatogr. A **1208**, 71–75 (2008)
33. S.A. Haeri, S. Abbasi, S. Sajjadifar, Bio-dispersive liquid liquid microextraction based on nano rhamino lipid aggregates combined with magnetic solid phase extraction using Fe3O4@PPy magnetic nanoparticles for the determination of methamphetamine in human urine, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1063 (2017) 101–106,
34. J. Zeng, J. Chen, M. Li, F. Subhan, F. Chong, C. Wen, J. Yu, B. Cui, X. Chen, Determination of amphetamines in biological samples using electro enhanced solid phase microextraction-gas chromatography. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **1000**, 169–175 (2015)
35. I. Racamonde, R. Rodil, J.B. Quintana, R. Cela, In-sample derivatization-solid-phase microextraction of amphetamines and ecstasy related stimulants from water and urine. Anal. Chim. Acta **770**, 75–84 (2013)
36. M. Barroso, I. Moreno, B. da Fonseca, J.A. Queiroz, E. Gallardo, Role of microextraction sampling procedures in forensic toxicology. Bioanalysis **4**, 1805–1826 (2012)

37. M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J. AOAC Int. **86**, 412–431 (2003)
38. M.M.A. Omar, W.A. Wan Ibrahim, A.A. Elbashir, Sol–gel hybrid methyltrimethoxysilane–tetraethoxysilane as a new dispersive solid-phase extraction material for acrylamide determination in food with direct gas chromatography–mass spectrometry analysis. Food Chem. **158**, 302–309 (2014)
39. T. Mills, W. Price, P. T. Price, and C. Roberson; "INSTROMENTAL DATA FOR DRUG ANALYSIS"; ELSEVIER Series in Forensic and Police Science; Barry A. J. Fisher, Editor; New York. Amsterdam. Oxford.
40. C.A. Gouveia, A. Oliveira, S. Pinho, C. Vasconcelos, F. Carvalho, R.F. Moreira, R.J. Dinis-Oliveira, "Simultaneous quantification of morphine and cocaine in hair samples from drug addicts by GC–EI/M. Biomed. Chromatogr. **26**, 1041 (2012)
41. S. Gentili, C. Mortali, L. Mastrobattista, P. Berretta, S. Zaami, Determination of different recreational drugs in sweat by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME GC/MS): Application to drugged drivers. J. Pharm. Biomed. Anal. **129**, 282–287 (2016)