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Application of gas chromatography–tandem mass spectrometry for the determination of amphetamine-type stimulants in blood and urine

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a b s t r a c t

Amphetamine, methamphetamine, phentermine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) are the most popular amphetamine-type stimulants. The use of these substances is a serious societal problem worldwide. In this study, a method based on gas chromatography–tandem mass spectrome-try (GC–MS/MS) with simple and rapid liquid-liquid extraction (LLE) and derivatization was developed and validated for the simultaneous determination of the six aforementioned amphetamine derivatives in blood and urine. The detection of all compounds was based on multiple reaction monitoring (MRM) tran-sitions. The most important advantage of the method is the minimal sample volume (as low as 200 μL) required for the extraction procedure. The validation parameters, i.e., the recovery (90.5–104%), inter-day accuracy (94.2–109.1%) and precision (0.5–5.8%), showed the repeatability and sensitivity of the method for both matrices and indicated that the proposed procedure fulfils internationally established acceptance criteria for bioanalytical methods The procedure was successfully applied to the analysis of real blood and urine samples examined in 22 forensic toxicological cases. To the best of our knowledge, this is the first work presenting the use of GC–MS/MS for the determination of amphetamine-type stimu-lants in blood and urine. In view of the low limits of detection (0.09–0.81 ng/mL), limits of quantification (0.26–2.4 ng/mL), and high selectivity, the procedure can be applied for drug monitoring in both fatal and non-fatal intoxication cases in routine toxicology analysis.

Keywords: Amphetamine-type stimulants (ATS), Drug analysis, Toxicological screening, GC–MS/MS, Biological specimens

1. Introduction

The use of drugs constitutes a serious worldwide societal and economic problem. The World Drug Report published in 2016 by the United Nations Office on Drugs and Crime (UNODC) stated that approximately 1 in 20 adults between the ages of 15 and 64 used at least one drug in 2014. Despite the many public campaigns against drug use, sexual assaults, car accidents, and instances of aggressive behaviour caused by doped people is still commonplace. Therefore, there is urgent need for the development of new, rapid, and sensitive methods for the determination of illicit drugs in various biological specimens for efficient confirmation of their use. Such results are also useful in routine toxicology analysis, can be provided as important evidence in courts of law and are helpful for the effective control of drug distribution or drug-associated crimes [1].

Amphetamine (AM), methamphetamine (MA), phentermine (PM), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-

Abbreviations: ATS, amphetamine-type stimulants; AM, amphetamine; CE, collision energies; DLLME, dispersive liquid-liquid extraction; GC, gas chromatography; HS-SPME, head-space microextraction; IT/MS, ion trap mass spectrometry; LC, liquid chromatography; LC-UV, liquid chromatography UV detection; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MA, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxy-*N*-ethylamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MRM, multiply reaction monitoring mode; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PEA, phenethylamine; PM, phentermine; Rs, peak resolution; SPE, solid phase extraction; SPME, solid-phase microextraction; UA-LDS-DLLME, ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction; UNODC, United Nations Office on Drugs and Crime; TFAA, trifluoroacetic anhydride; THC, tetrahydrocannabinol; QC, quality control samples.

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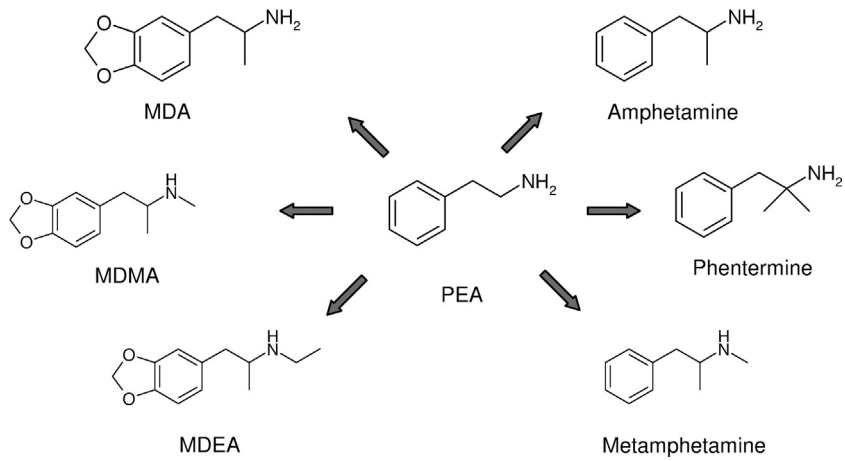

Fig. 1. Structures of six PEA derivatives included in the study.

methylenedioxy-*N*-ethylamphetamine (MDEA) are the most popular drugs from the class of amphetamine-type stimulants (ATS), also referred as amphetamine derivatives. These compounds are analogues of phenethylamine (PEA) (Fig. 1). PEA derivatives can be easily obtained by replacing or substituting one or more hydrogen atoms in the structure of phenethylamine via a substitution reaction [2]. These compounds remain the most commonly used drugs, followed by cannabis, with 13.9–54.8 million users around the world. Additionally, the global seizure of amphetamine derivatives between 2003 and 2012 was 144 tons. Both PEA and its derivatives tend to stimulate the central nervous system and offer psychedelic and hallucinogenic effects, which makes their use attractive [1,3].

Procedures based on chromatographic separation are the most powerful analytical tools for the identification and quantification of drugs in routine toxicological analysis [4]. Among them, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS) are the techniques of choice for the determination of ATS in biological materials [5,6]. However, these techniques have several disadvantages. The methods based GC–MS in many cases are not selective or sensitive enough for drug determination in biological matrices. Moreover, a large volume of sample for the extraction procedure (0.5–5 mL) is required, as well as the use of complicated and expensive consumables (e.g., solid-phase extraction cartridges) [5,7]. LC–MS/MS-based protocols demand large amounts of organic solvents for the separation and elution of analytes and therefore are expensive and environmentally unfriendly, with potential toxic and hazardous effects to health [5,8]. To overcome these drawbacks, many studies have paid greater attention to miniaturized sample preparation procedures, e.g., dispersive liquid-liquid microextraction (DLLME) or solid-phase microextraction (SPME). However, these methods also require large sample volumes, are time consuming, and in some cases are troublesome and inaccurate [6,9]. Therefore, there is a strong need for the development of new chromatographic techniques that achieve low limits of detection (LODs) with high selectivity using μL volumes of sample for analysis. Furthermore, according to the principles of "green analytical chemistry", gas chromatography should be the first choice whenever possible, because mobile phases in liquid chromatography may be a source of pollution [8].

Gas chromatography–tandem mass spectrometry (GC–MS/MS) is becoming increasingly popular in toxicology laboratories, because it is ideal for the analysis of complex matrices and the determination of trace analytes. Moreover, methods based on GC–MS/MS combine the "green character" of GC with the high sensitivity and selectivity of triple quadrupole detection and hence provide accurate and reliable results from the test samples. Up to now, this technique have been applied for several clinical and forensic purposes. Recently, the procedures for the determination of THC [10], cocaine [11] and their metabolites in hair samples were published. The GC–MS/MS technique is also considered a suitable tool for the quantification of THC [12], cocaine and metabolites [13] in human oral fluid, as well as for ketamines in human urine and plasma samples [14].

Despite the large number of published data describing procedures for ATS analysis, to the best of our knowledge, there is a lack of information concerning the application of GC–MS/MS technique for the determination of these drugs in biological specimens. Therefore, the aim of this study was to develop and validate a novel and rapid GC–MS/MS-based procedure for the determination of amphetamine derivatives in blood and urine for clinical, occupational and forensic purposes. The applicability of the presented method was evaluated by the quantification of amphetamine derivatives in 22 cases wherein the use of drugs was suspected. Blood and urine are the most preferred specimens for drug testing and were selected in this study due to the fact that the concentration of drugs can be closely correlated with the resulting impairment, pharmacological and toxic effects.

2. Materials and methods

2.1. Chemicals and standards

A multi-component certified standard methanolic solution (Amine Mixture-6) containing six amphetamine derivatives ((±)-amphetamine, (±)-MDEA, (±)-methamphetamine, (±)-MDMA, MDA, and phentermine) at concentrations of 250 μg/mL was purchased from Cerilliant Corporation (Round Rock, TX, USA). A solution of rac-metamphetamine-D5 (*rac*-mAMP-D5) in methanol at a concentration of 0.1 mg/mL was obtained from LGC Standards (Bury, UK) and was used as an internal standard (IS).

All solvents were of HPLC-grade purity and were supplied by Sigma-Aldrich (St. Louis, MO, USA), as well as trifluoroacetic anhydride (TFAA) *ReagentPlus*® (≥99% purity). Analytical-grade hydrochloric acid (HCl) at a concentration of 35–38% and sodium hydroxide (NaOH) powder were obtained from POCH (Gliwice, Poland). Ultrapure water was produced by a Millipore Milli-Q Gradient A10 water system (Billerica, MA, USA).

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2.2. Biological specimens

Blood and urine samples were collected in 2016 from subjects investigated by the police and suspected of taking drugs or obtained during autopsy (postmortem cases). All samples were stored at 4 °C prior to analysis. To validate the method, blood or urine was obtained from the local blood donation bank (Gdańsk, Poland) from subjects without a history of drug use or volunteers that declared no drugs use, respectively.

2.3. Preparation of stock solutions

Stock solutions of the analytes were prepared in methanol by diluting the certified standard solution to concentrations of 0.2, 1 and 10 μg/mL. A stock solution of the IS was prepared in methanol at a concentration of 1 μg/mL. These solutions were used for calibration and validation and were stored at −20 °C until analysis. Fresh stock solutions were made every two weeks.

2.4. Extraction-derivatization procedure

The six amphetamine derivatives were extracted by a modified method developed originally for the determination of synthetic cathinones, which are similar in structure to the compounds studied in this work [15]. The procedure is based on alkaline (pH of approximately 13) liquid-liquid extraction (LLE) with a nonpolar solvent (ethyl acetate) using only 200 μL of sample. A derivatization step was performed to acylate the amine groups via reaction with TFAA, according to the procedure developed in our previous study [16].

A 200 μL aliquot of the appropriate matrix, 5 μL of the IS stock solution, and 400 μL of 0.1 M NaOH were added to a screw-capped glass centrifuge tube. The tube was stirred for 1 min. After the addition of 2 mL of ethyl acetate, mechanical shaking was carried out for 2 min. Finally, the sample was centrifuged for 5 min at 5000 rpm, and the top layer was transferred to a new glass tube. The bottom layer was discarded. Subsequently, 100 μL of HCl solution in methanol (1:9, v/v) was added. The extract was evaporated to dryness under a gentle stream of inert gas (nitrogen) at 40 °C, and the dry residue was dissolved in 50 μL of ethyl acetate. Subsequently, 50 μL of TFAA was added to perform the derivatization (20 min, 55 °C). Finally, the solution was evaporated to dryness, and the residue was reconstituted in 50 μL of ethyl acetate. A 2 μL aliquot was injected into the GC–MS/MS system.

Importantly, to prevent possible loss of analytes during evaporation in the stream of nitrogen, compounds of interest were transformed into salts (hydrochlorides) by the addition of HCl solution in methanol due to the comparatively high volatility of the free bases. The concentration of analytes in a few samples were initially higher than range in the calibration curve. In these cases, the original samples were diluted with an appropriate drug-free matrix, the extraction procedure was performed again, and the extracts were injected into the GC–MS/MS system.

2.5. GC–MS/MS conditions

For chromatographic analysis, a Shimadzu GC-2010 PLUS system (Kyoto, Japan) equipped with an AOC-20i autoinjector, AOC-20s autosampler, and split/splitless injection port was used. The separation of analytes was performed on a Phenomenex ZB-5MSi capillary column (30 m × 0.25 mm id, and 0.25 μm film thickness; Zabelin, Poland) using helium (purity ≥99.999%) as the carrier gas. The flow rate of the carrier gas was initially set to 1 mL/min, and then, the constant gas linear velocity was maintained at 36.6 cm/s by the system. The splitless injection mode was used for 0.1 min. The oven temperature gradient program was as follows: from 100 °C to 200 °C at 15 °C/min and finally ramp to 300 °C at 25 °C/min. Post-run conditioning was set at 300 °C for 2 min to eliminate carry-over effects and all impurities co-extracted from the complex biological matrix. A solvent delay time of 3.7 min was used. The temperatures of the injection port and MS transfer line were set at 260 and 285 °C, respectively.

A Shimadzu TQ8050 triple quadrupole mass spectrometer equipped with a highly sensitive and stable ion source (Kyoto, Japan) was used in the study. Ionization was achieved in electron-impact ionization mode (70 eV), and the source temperature was set at 250 °C. The mass spectrometer was operated with a filament current of 60 μA. Argon (purity ≥99.999%) was used as the collision-induced dissociation (CID) gas. Quantification was performed using multiple reaction monitoring (MRM) transitions. All transitions were chosen based on selectivity and abundance. Two MRM transitions, a quantifier and a qualifier, were monitored for each compound, except for the IS (only one transition was selected). The specific transitions with optimum collision energies (CEs) for all analytes are listed in Table 1. Data handling and system operations were controlled by the GCMS Solution and Insight GCMS software (version 4.45, Shimadzu Corporation).

2.6. Method validation

Under optimized conditions, several parameters, such as: the selectivity, matrix effect, linearity, limit of detection (LOD), limit of quantification (LOQ), carry-over effect, recovery, and repeatability were evaluated to validate the developed method according to the principles for bioanalytical methods used in forensic toxicology [17,18].

2.6.1. Selectivity

Due to the potential application of the method for both clinical and forensic purposes, the selectivity experiment was performed for blank samples with both ante- and postmortem origins, because differences in the matrix composition in these two cases can be observed. In this study, control matrix samples obtained from 10 various subjects (ante- and postmortem origin) were analysed after the extraction-derivatization procedure.

2.6.2. Matrix effect

To verify the suppression/enhancement of the detector response for the extracted analytes compared to the signal of the same analytes injected in the solvent eight calibration solutions were prepared in triplicate in methanol as well as in extracts obtained from each drug-free matrix. Subsequently, both calibration curves were constructed (analyte peak areas to the IS peak areas versus the analyte concentrations). An assessment of the matrix effects (ME) was performed by comparing the slopes of both calibration curves.

2.6.3. Calibration, linearity, LOD and LOQ

The calibration solutions were prepared in triplicate by fortifying 200 μL drug-free blood or urine samples with an appropriate amount of the stock solution to obtain concentrations of 1, 5, 10, 25, 50, 100, 200, 500 ng/mL. The concentration of IS in each sample was maintained at 25 ng/mL by adding 5 μL of the IS stock solution. Finally, the extraction procedure was performed. Eight-point calibration curves were constructed using the peak area ratio (analytes vs IS) plotted against the corresponding concentration. Weighted least squares regression was applied to the calibration curves to improve the accuracy. Six weighting factors ($1/\sqrt{x}$, $1/x$, $1/x^2$, $1/\sqrt{y}$, $1/y$, $1/y^2$) were evaluated, according to the procedure described by Aszyk et al. [19]. The one with the lowest sum of relative errors and the highest accuracy was selected for each analyte and was considered for evaluation of the linearity as well as the intra- and inter-day repeatability. The linearity of the weighted calibration curves was

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Table 1
Retention times and parameters used in MRM mode for the studied analytes and IS.

Time segment	Compound[a]	RT[b]	Quantifier		Qualifier		RA[e]
			Transition[c]	CE[d]	Transition[c]	CE[d]	
1	AM	4.68	118.0 → 91.0	25	140 → 68.9	25	100/78
	PM	4.81	154.0 → 59.1	10	154 → 69.1	25	100/22
2	MA	5.61	154.0 → 110.0	10	154 → 69.0	25	100/47
3	MDA	7.32	135.0 → 77.1	15	135 → 51.1	30	100/87
4	MDMA	8.11	154.0 → 110.1	10	154 → 69.1	25	100/55
	MDEA	8.37	168.0 → 140.0	10	162 → 104.0	15	100/66
2	*rac*-mAMP-D5 (IS)	5.58	158.0 → 113.0	10	–	–	–

[a] Compounds as derivatives with TFAA.
[b] RT: retention time.
[c] Precursor ion → product ion (*m*/*z*).
[d] CE: collision energy (V).
[e] RA: relative abundance of quantifier/qualifier transitions.

investigated in the range of 1–500 ng/mL and was assessed as the correlation coefficient (r). The LOD was established based on following formula: $LOD = 3.3 \times S_b/a$, where *Sb* is the standard deviation of the intercept and *a* is the slope of the calibration curve. The LOQ was assessed as three times the LOD.

2.6.4. Carry-over effect, recovery, and repeatability

The potential for carry-over of analytes and the IS to the subsequent sample in the batch was investigated by injecting 2 μL of extracted blank sample spiked with analytes and the IS at the highest concentration level from the calibration curve followed by extraction solvent (ethyl acetate). The experiment was performed six times. The recovery and repeatability test was performed for three different concentration levels within the linear range of the assay (quality control samples, QC): low 5 ng/mL (LQC), medium 50 ng/mL (MQC), and high 500 ng/mL (HQC). The recoveries (in%) were evaluated by comparing the analyte-to-IS peak area ratios of the fortified and extracted drug-free blank blood and urine samples with the corresponding analyte-to-IS peak area ratios of the appropriate matrix extracts spiked with standards (n = 6). In this experiment, the IS was added post-extraction to avoid its loss during the extraction step. The repeatability was assessed as the intra- and inter-assay accuracy and precision (in terms of the coefficients of variation). Intra-day measurements were carried out by analysing (n = 6) both blood and urine blank samples spiked with analytes and the IS. The analyses were repeated over the next 3 consecutive days to estimate the inter-day assay repeatability as between-day averages. In both cases, the accuracy (A%) was determined as the ratio of the mean measured concentration and the nominal concentration.

3. Results and discussion

3.1. Separation and detection

To obtain high sensitivity and selectivity for the identification and quantification of all analytes, careful optimization of the MS/MS parameters was performed. Initially, the MRM transitions for the individual compounds were evaluated. For this purpose, 2 μL of the derivatized mixture of analytes and the IS at a concentration of 1 μg/mL was injected into the GC–MS/MS system in full scan mode (30–500 *m*/*z*). Subsequently, four of the most abundance ions were used for fragmentation with variable CEs in the range of 5–45 V (5 V step) during examination of the product ions. The results were collected and compared using the Shimadzu MRM Optimization Tool software, and the appropriate MRM transitions, based on the selectivity and abundance, were chosen for further analysis. The selection of the proper source temperature is also a key factor in GC–MS analysis to achieve a high sensitivity and S/N ratio. During this study, temperatures ranging from 200 to 260 °C at 10 °C steps were tested, and the results were compared in terms of peak intensity for all analytes and the IS. The best temperature (250 °C), i.e., that which provides the highest sensitivity, was chosen for quantification analyses.

The chromatographic conditions, such as the injector, initial and final column temperature, as well as the column temperature rate and carrier gas flow rate, were optimized. The use of splitless injection mode for 0.1 min provided a higher S/N ratio for the analytes and more symmetric peak shapes compared to longer splitless times and to split injection mode. The total method run-time was 12.7 min with a data acquisition time of 5 min. Four MRM time segments were created to increase the dwell time for each transition and to enhance the sensitivity. The corresponding retention times for all analysed compounds are presented in Table 1. The analyte peaks, obtained using the optimized oven program, were fully resolved (Rs of over 1.5), except for IS and MA (Rs = 1.0), as shown in Fig. 2a. This low Rs is due to the use of a deuterated form of MA as IS, hence achieving separation of these compounds is difficult. However, separation is not required in this case, since other specific transitions can be monitored independently.

3.2. Method validation

Full validation of the developed method was performed. The validation data for the method obtained by the aforementioned approach are summarized in Tables S1, S2, and S3 in the Electronic Supplementary Material (ESM).

No analytes, IS, or additional peaks that obstruct the identification and quantification of the compounds of interest were present in the six blank blood and urine samples at the retention times of the analytes (Fig. 2). Therefore, it can be concluded that neither endogenous matrix constituents nor any of the reagents added during the extraction-derivatization steps interfered with the studied analytes. According to these data, the developed method can be considered as selective and highly specific and can be applied for the quantification of the selected substances in both studied matrices.

The results obtained during the matrix effect experiments are listed in Table S1. Negative values of ME indicate the suppression, while positive values indicate the enhancement of the detector signal. Indeed, matrix effects in the range of −20% to 20% are considered as soft in GC–MS/MS-based methods and can be neglected, while when higher values are obtained, it is necessary to use certain methods to reduce the influence of the matrix on the detector response [20]. Based on the obtained results, significant matrix effects for amphetamine and phentermine were observed in both the analysed matrices. Therefore, to overcome these effects, matrix-match calibration, instead of external calibration, was used in

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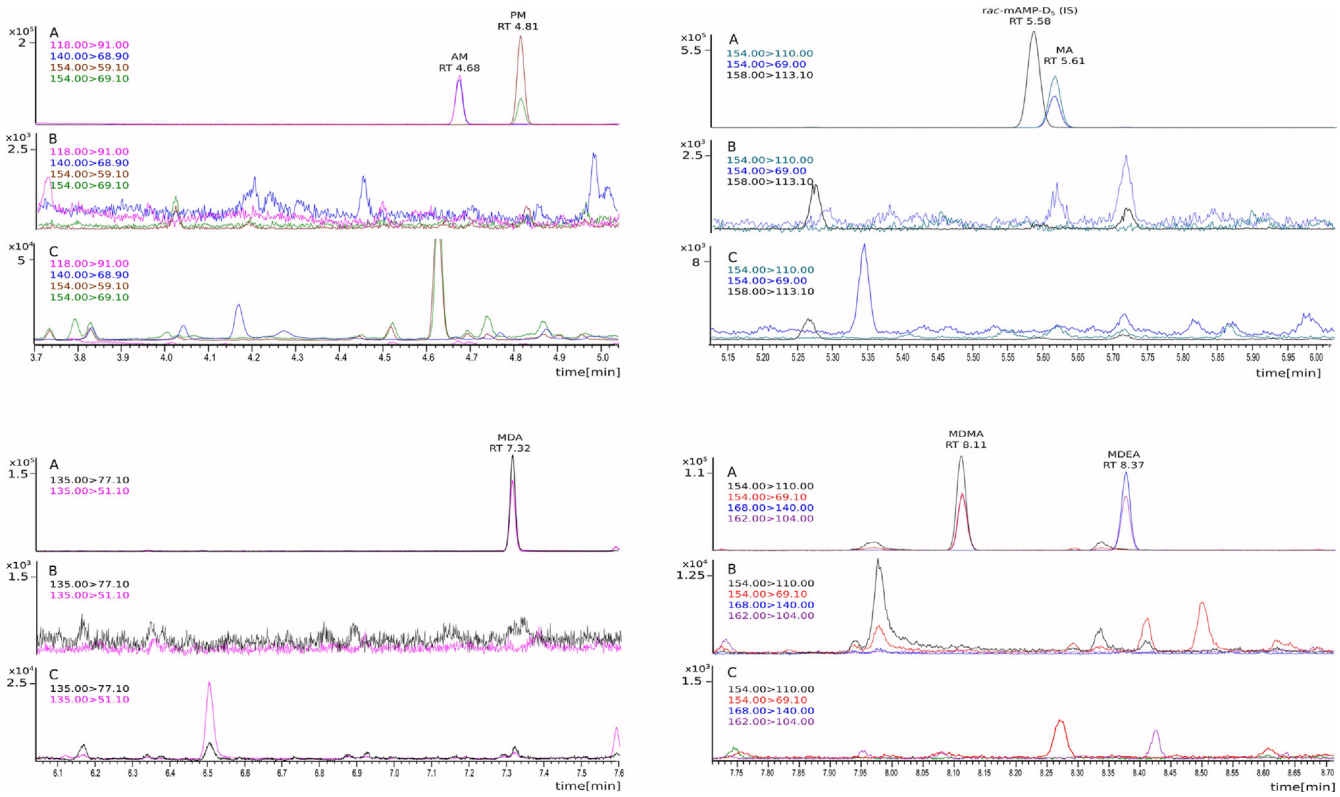

Fig. 2. GC-EI-MS/MS chromatograms (four time segments): a) mixture of standard solution of analytes (10 ng/mL) and IS; b) extracted blank blood sample; c) extracted blank urine sample.

further experiments. Moreover, to compensate for the instability of the MS signal during analysis and the loss of analytes in the extraction-derivatization procedure (correction of recoveries), internal standard calibration was performed.

The linearity was studied in the concentration range of 1–500 ng/mL for both matrices. The correlation coefficients (r) of the weighted calibration curves were greater than 0.99 and 0.98 for blood and urine, respectively. The LODs and LOQs were satisfactory and therefore, the developed method was determined to be sensitive and enables the quantification of all analytes in both matrices even at low concentration levels.

The recovery and repeatability data for the 3 concentration levels over the studied concentration range were summarized in Table S3 and showed that the investigated method can be considered accurate and precise. Additionally, the mean CVs were less than 20% at the low concentration level and less than 15% at other concentration levels, which meets the established international criteria for the validation of bioanalytical methods.

3.3. Comparison with other analytical procedures

Table 2 summarizes the results obtained using the method developed in this study along with selected results published in the literature. To the best of our knowledge, the presented work is the first attempt at applying the GC–MS/MS technique for the simultaneous determination of six ATS in blood and urine. Moreover, the proposed procedure offers several advantages, including enhancement of the sensitivity and selectivity. However, the most important benefit of the developed method is the minimal sample volume used for extraction, i.e., 200 μL, and lower LODs and LOQs were achieved compared to the literature data. Additionally, the volume of the extraction solvent was also reduced compared to other published data involving the LLE methods. Furthermore, the SPE methods demand large volume of solvents for conditioning of sorbents what is not required in LLE-based methods. The obtained LODs are comparable with those obtained using the LC–MS/MS technique, which is not possible when GC is coupled with other detection techniques. Based on the above, the proposed method can be used as an alternative to the LC–MS/MS technique in regard to sensitivity and selectivity; however, GC–MS/MS stands out in terms of "green chemistry".

3.4. Applicability of the method

The blood and/or urine samples collected from suspected abusers (n = 22) in 2016 were analysed for ATS content using the method developed in this study. The samples were referred to the Department of Forensic Medicine, Faculty of Medicine, of the Medical University of Gdańsk for routine toxicology analysis and analysed in the Department of Analytical Chemistry, Faculty of Chemistry, of the Gdańsk University of Technology in the framework of cooperation. Only in 11 cases were the urine samples accompanied by blood samples for analysis (3 urine samples in non-fatal cases and 8 in fatal cases). The data from the analysis of all samples are listed in Table 3. The presence of the investigated compounds, namely, AM (in 21 samples), MDA (4), MDMA (6), and MA (1), was confirmed. The results showed that men constitute approximately 59% of all investigated cases. The mean concentration of amphetamine in non-fatal cases was lower in blood and higher in urine compared to postmortem cases. Similar conclusions cannot be made for the other compounds due to the low number of cases investigated. In 6 cases, more than 1 compound was detected, which may suggest combined drug intoxication (polydrug abuse) or indicate impurities present in illegally available drugs. These situations constitute an additional hazard for human health because

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Table 2
Comparison of selected analytical procedures for the determination of amphetamines in blood and urine.

Compounds	Sample volume [mL]	Sample preparation	Extraction solvent volume [mL]	Detection method	LOD/LOQ [ng/mL]	Recovery [%]	Ref.
Blood							
AM, PM, MA, MDA, MDMA, MDEA	0.2	LLE	2	GC-MS/MS	0.22-0.81/0.65-2.4	91–104	This work
MDA, MDEA	0.5	SPE	–	GC-IT/MS	2/5	72–98	[21]
AM, MA, MDA, MDMA, MDEA	1	SPE	–	GC-MS	-/5	88–103	[22]
AM, MA, MDA, MDMA	0.2	UA-DLLME	0.2	GC-MS	10/40	77–92.4	[6]
AM, MA, MDA, MDMA	1	UA-LDS-DLLME	0.1	GC-MS	2-4/-	79.3–100.3	[23]
AM, MDA, MDMA, MDEA	0.2	LLE	0.6	LC-MS/MS	0.3/0.5	93–96	[24]
AM, MA, MDA, MDMA, MDEA	0.1	LLE	2.5	LC-MS/MS	0.25-1.25/2.5	94–109	[5]
Urine							
AM, PM, MA, MDA, MDMA, MDEA	0.2	LLE	2	GC-MS/MS	0.09-0.45/0.26-1.38	90.5–104	This work
AM, MA, MDA, MDMA, MDEA	4	HS-SPME	–	GC-FID	30-40/-	5.1–47	[25]
AM, MA, MDA, MDMA, MDEA	2	LLE	5	GC-MS	13-50/ 25-100	86–98	[7]
AM, MA, MDA, MDMA, MDEA	0.5	LLE	0.8	GC-MS	0.5-1.7/3.3-5.7	81.9–103.4	[26]
MDA, MDMA, MDEA	1	SPE	–	GC-MS	10/25	90–112	[27]
AM, MA, MDA, MDMA	1	UA-LDS-DLLME	0.1	GC-MS	1-4/-	80.6–99.9	[23]
MDA, MDMA	1	SPE	–	GC-IT/MS	3.5/10	84–94	[21]
AM, MA	2	DLLME	0.33	LC-UV	2-8/-	88–113	[28]
AM, MA, MDA, MDMA, MDEA	2	SPE	–	LC-MS/MS	1-5/1.3-20	81.2–/96.1	[29]

Table 3
Concentration ± SD (μg/mL) of ATS in blood (B) and urine (U) determined in 22 toxicological cases (n = 3). Only detected compounds were shown.

	AM		MA		MDA		MDMA	
Sample ID/Sex	B	U	B	U	B	U	B	U
				Non-fatal cases				
1/F	0.596 ± 0.039	–		–		–		–
2/F	0.473 ± 0.018	–		–		–		–
3/M	0.1951 ± 0.0070	–		–	34.1 ± 1.1	–	0.452 ± 0.012	–
4/M	0.302 ± 0.013	–		–		–		–
5/F	0.1956 ± 0.0039	–		–		–		–
6/M	0.1601 ± 0.0089	79.5 ± 2.8						
7/F	0.3912 ± 0.0066	–		–		–		–
8/F	0.2221 ± 0.0082	3.33 ± 0.14						
9/M	0.359 ± 0.021	–		–	0.00612 ± 0.00025		0.01925 ± 0,00075	–
10/M	0.381 ± 0.011	–		–		–		–
11/M	0.572 ± 0.020	89.7 ± 1.4						
12/F		–		–	0.00724 ± 0.00031	–	65.2 ± 1.2	–
13/M	0.0499 ± 0.0031	–		–		–		–
MEAN	**0.32**	**57.5**	–	–	**11.4**	–	**21.9**	–
				Fatal cases				
14/F	0.0125 ± 0.0011	0.895 ± 0.021					1.747 ± 0.061	37.7 ± 2.1
15/F	0.1982 ± 0.0049	1.102 ± 0.035						
16/M	0.01311 ± 0.00027	–		–		–		–
17/M	0.0361 ± 0.0023	1.119 ± 0.042						
18/F	1.767 ± 0.037	3.76 ± 0.11						
19/M	1.180 ± 0.022	3.77 ± 0.12						
20/M	1.243 ± 0.019	169.2 ± 4.9	<LOQ	0.933 ± 0.018			0.264 ± 0.011	0.1417 ± 0.0056
21/M	0.989 ± 0.035	6.81 ± 0.14						
22/M	58.5 ± 1.9	0.557 ± 0.029			27.9 ± 1.2	0.367 ± 0.011	2.310 ± 0.075	21.71 ± 0.65
MEAN	**7.1**	**23.4**	**<LOQ**	**0.93**	**27.9**	**0.37**	**1.4**	**19.9**

"–" – sample was not available for analysis, *F* – female, *M* – male.

the drugs may interact and exhibit increased toxicity in these cases (drug-drug interactions, DDIs) [15].

4. Conclusions

A simple and rapid LLE procedure with derivatization prior to GC–MS/MS analysis was developed for the quantification of six amphetamine-type stimulants using 200 μL of sample and a total of 2 mL of extraction solvent. The presented method was successfully validated according to internationally accepted guidelines and met all criteria for bioanalytical assays. The procedure allows the attainment of proper validation parameters, which makes it suitable for quantification even of trace amounts of ATS in the two biological matrices most commonly used for drug determination. The proposed protocol provided acceptable ranges of accuracy and repeatability, whereas the obtained low LODs and LOQs proved the sensitivity of the method. Moreover, the developed method requires less than 2 h for each sample analysis, which makes it suitable and useful for most toxicology laboratories. The proposed procedure was successfully applied for the determination of ATS concentrations in blood and urine in several fatal and non-fatal intoxication cases, which proved the utility of the method.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2017.09.020.

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