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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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ABSTRACT

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 spectrometry (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) transitions. 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 stimulants 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.

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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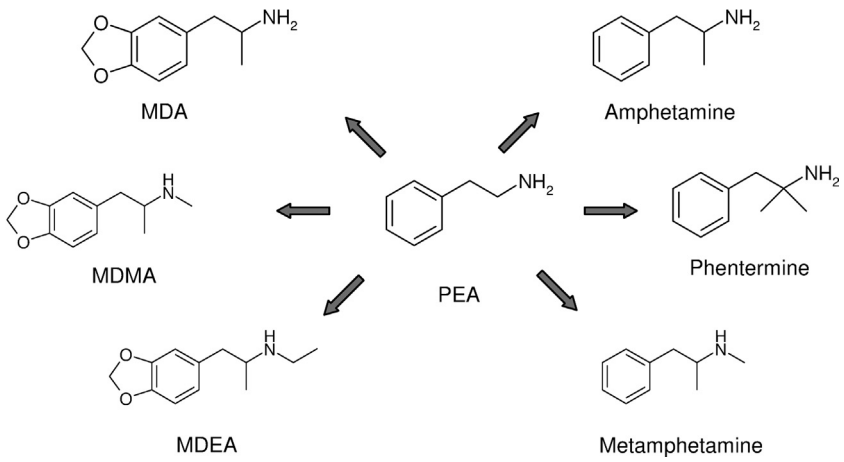

Fig. 1. Structures of six PEA derivatives included in the study.

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).