Novel procedures for determination of natural and synthetic drug amines

PhD thesis

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1. Introduction

According to the World Drug Report of United Nations Office on Drugs and Crime, 246 (± 83.5) million people (5.2 ± 1.8 %) between the ages of 15 and 64 used an illicit drug at least once in 2013. The new (semi-)synthetic designer drugs have slightly different in chemical structures compared to the banned ones, therefore they fall out of the low regulations, resulting in continuous challenge for researchers and law enforcement agencies. In Hungary, 108 designer drugs, especially synthetic AM-, CTN- and cannabinoid-type compounds have been identified since 2010.

The spread of drug abuse is associated with the multiplication of public health problems and social issues. One of the continuously more attention requiring consequences of drug use is that: the residues of illicit drugs are emerging in waters, similar to therapeutic medicines, especially in the densely populated areas. The researchers pay special attention to the determination of drugs in plants and other biological matrices as well as in environmental samples and seizures.

GC-MS is one of the most commonly applied analytical techniques for the qualitative and quantitative determination of organic compounds in complex matrices, therefore it has primary importance also in illicit drug analysis. The high volatility of the compounds is required for their gas chromatographic separation. Derivatization reduces the polarity of the components to acceptable level. In addition it results thermally stable products. Derivatization significantly improves the sensitivity and the selectivity of determination, as well as the efficiency of structure elucidation, consequently it is often used prior to GC-MS analysis, taking into account the increasing time and cost of sample preparation.

The aim of my work was to develop new sample preparation techniques for the quantitative determination of primary phenylalkylamines (PPAAs) – with special attention to illicit compounds (AM, MDA, MSC, CTN, CAT) – and CTN-type designer drugs (4-FMC, MCTN, PENT, 4-MEC, 3,4-DMMC, 4-EMC) in plants and other biological matrices.

2. Objectives

Based on the preliminary literature overview, the goals of my work were:

- **a.** extension of the multiresidue (> 100) analysis, previously developed by our research group, with PPAAs – with special attention to the illicit compounds (AM, MDA, MSC, CTN, CAT) – and the CTN-type designer drugs (4-FMC, MCTN, PENT, 4-MEC, 3,4-DMMC, 4-EMC); including:
- **b.** detailed derivatization and mass fragmentation studies: review the conditions of TMS-, 2TMS- or oxime-TMS-derivatization via the optimization of reagent (HMDS/MSTFA/BSTFA), catalyst (TFE/TMCS/TMIS), solvent (PYR/EtAc/ACN) as well as the reaction time and temperature applied;
- **c.** effective gas chromatographic separation of kathamines (CTN, CAT, NE), extending the GC methods previously described in the literature;
- **d.** recommendation of a novel sample preparation technique for the determination of phenylalkylamines in plants and other biological matrices in line with the principles of green chemistry, instead of the time consuming procedures published in the literature;
- **e.** the confirmation of the efficiency of the new methods through the calculation of the analytical performance characteristics and the comparison of the capabilities of the new methods with each other and with the recently applied techniques;
- **f.** the demonstration of the practical utility of new methods through the quantitative determination of drugs in plants and other biological matrices (*Lophophora williamsii, Catha edulis*, urine).

3. Experimental

3.1 Samples

The protein-free human urine samples were received from the Toxicology Laboratory of Department of Forensic and Insurance Medicine, Semmelweis University. The *Lophophora williamsii* cactus was given from the Department of Plant Anatomy, Eötvös Loránd University. *Catha edulis* leaves were received from Research Institute for Medicinal Plants Ltd. (Budakalász, Hungary).

3.2 Instrumentation

3.2.1 Materials

Freeze drying was performed on a Modulyo freeze dryer (Jencons, United Kingdom); Hamilton syringes with a precision of 1% (Bonaduz, Switzerland) were used for measuring the volume of the samples, model solutions, solvents, reagents and catalysts; the analytical balance with a readability of 0.01 mg was from Sartorius (Goettingen, Germany); the centrifuge machine (EBA 21) was the product of Hettich (Tuttlingen, Germany); ultrasonic extractions were performed on the Sonorex ultrasonic bath (Bandelin electronic, Berlin, Germany); the glass micro-fiber filters with a pore size of 1.6 µm were from Whatman (Maidstone, UK); solvent evaporation was performed on the Büchi Rotavapor R-200 (Flawil, Switzerland) rotary vacuum evaporator; the thermostable stove from Kutesz (Hungary) was used for heating during the derivatization.

3.2.2 Gas-chromatography

The apparatus consisted of a Varian 450 (Varian, Walnut Creek, CA, USA) GC system, equipped with a Varian 240 MS/MS iontrap detector, a Varian CP-8400 autosampler and a septum-equipped programmable injector (SPI). The column used was a product of SGE (Victoria, Australia); SGE forte capillary BPX5: 30m x 0.25mm; the film thickness was 0.25 µm. Helium of 6.0 (99.9999 %) purity was used as a carrier gas, with a flow rate of 1 mL/min.

3.2.3 Mass spectrometric conditions

The general parameters of the MS equipment were: mass range: 50-1000 amu; scan time: 5,000-10,000 amu/sec; filament current: 10-100 µA; maximum ionization time: 65.000 µs.

The detector was used in internal electron ionization mode. The temperature of the transfer line, ion trap and manifold were 300 °C, 210 °C and 80 °C, respectively. The electron energy was 70 eV, the Fil/Mul delay time was 3 minutes. Full scan was selected for acquisition method.

Varian MS Workstation 6.9. software was used for controlling the instruments.

3.3 Methods

3.3.1 Derivatization

Residues of standard solutions were perfluoroacylated, trimethylsilylated or oximated at 70-100 °C for 10-90 minutes.

After derivatization, the solutions were cooled down to room temperature, then 1-1 µL aliquots of the undiluted or five-tenfold diluted solutions were injected into the GC–MS system in three parallel measurements.

Three parallels and one blank sample were made in all derivatization methods.

3.3.2 Determination of drugs in urine without preliminary extraction

Ten µL of 10 % (w/w) HCl was added to 20-40 µL aliquots of centrifuged (1200 rpm, 5 minutes) urine samples, then the solutions were evaporated to dryness at 30 - 40 °C. After this step, the residues were derivatized directly.

For the determination of recovery, linearity and LOQ values, standard solutions of PPAAs and CTN-type designer drugs were added to 20 µL aliquots of blank urine samples. Further sample preparation steps were performed according to the details described in the previous paragraph.

3.3.3 Determination of drugs in plants

The *Catha edulis* (25.09 g) and *Lophophora williamsii* (2.95 g) samples were freeze dried. After lyophylisation, the dried mass of the *Catha edulis* leaves was 8.37 g, while 0.260 g for the *Lophophora williamsii* tissues.

3.3.3.1 Determination of drugs in Lophophora williamsii tissues

Two mL of 10 % (w/w) HCl containing MeOH was added to 2.00 mg of lyophilized sample, then it was sonicated at 60 °C for 30 minutes, applying a reflux condenser. After cooling and centrifugation (1200 rpm, 5 minutes), the solvent was quantitatively transferred to a 5 mL volumetric flask. The residue was extracted, centrifuged, then the solvent was transferred again. This procedure was repeated three times. The volume of this solution was made up to 5.0 mL. Aliquots of 5-50 µL were evaporated to dryness at 30 - 40 °C and derivatized.

3.3.3.2 Direct determination of drugs in Lophophora williamsii tissues and Catha edulis leaves

Low amounts (0.1-5 mg) of the lyophilized tissues were weighted into 2 mL reaction vials, then derivatized directly without preliminary extraction. After centrifugation (1200 rpm, 5 minutes), 1-1 µL aliquots of the undiluted or the five-tenfold diluted solutions were injected into the GC-MS system.

The analysis of *Catha edulis* leaves was extended by standard addition: the calculated aliquots of khatamin standard solutions were rotary evaporated to dryness at 30 - 40 °C, thereafter the dried residues were derivatized with the presence of the lyophilized leaves.

4. Results

I developed new sample preparation methods for the qualitative and quantitative determination of PPAAs – with special attention to illicit amines (AM, MDA, MSC, CTN, CAT) – and CTN-type designer drugs (4-FMC, MCTN, PENT, 4-MEC, 3,4-DMMC, 4-EMC).

I recognized the possibility of PPAAs' selective acylation by the HMDS+perfluorocarboxylic acid couples. The conditions and mechanism of the novel reaction pathway were described in details. I also confirmed that the response values obtained for the novel derivatization process (i) were independent of the perfluorocarboxylic acid applied; (ii) were uniformly high, due to the presence of the molecular ions ($[M]^{+\cdot}$) and/or the characteristic fragments ($[M+147]^{+}$) formed by self chemical ionization; (iii) were considerably larger compared to those obtained for TFAA. Latter observation is caused by the shortage of $[M+147]^{+}$ ions contribution as well as by the loss of derivatives occurs under reagent excess elimination, which was at least 46 % (2-PEA).

The second novel approach was applied for the selective and quantitative determination of PPAAs via their 2TMS-derivatives. The analytical advantages of the new process were proved by comparing the response values of the 2TMS-derivatives with the acylated products obtained by HMDS+perfluorocarboxylic acid reagent couples. On average, the ditrimethylsilylation resulted in about 1.7 times larger responses (AM-2TMS, MDA-2TMS and MSC-2TMS provided 1.9, 2.7 and 1.6 times larger responses, respectively) than the corresponding acylated species. The responses of AM-2TMS and MDA-2TMS were compared to the AM-TMS and MDA-TMS derivatives obtained by MSTFA in solvent free media: the AM-2TMS gave 2.5 times larger responses, while these values were 3.5 times higher for MDA-2TMS. The advantages of ditrimethylsilylation compared to monotrimethylsilylation were proved by the determination of AM in urine samples and MSC in *Lophophora williamsii* cactus tissues.

To fill gaps in the literature, I developed methods for the GC-MS determination of CTN, CAT and NE. As a novelty to the field, I suggested a two-steps derivatization procedure (1^{st} step oximation, 2^{nd} step trimethylsilylation) for determination of CTN.

The advantages of the new method were (i) the effective GC separation of khatamines and (ii) the better sensitivity of the GC-MS determination. Comparing the response values of CTN-TMS(TMS-oxime)$_{1,2}$ to those obtained for the CTN-TMS, it turned out that the two-steps derivatization is preferred. The fragment ions suitable for selective determination of kathamines were highlighted.

Similar to CTN, the CTN-type designer drugs were derivatized with the novel two-steps process. The advantage of the new procedure comparing to the trimethylsilylation is the stability of the TMS(TMS-oxime)$_{1,2}$ derivatives: the trimethylsilylation (without oximation) produced unstable species.

I recommended a novel sample preparation technique for the determination of drugs in plants and other biological matrices. The new procedure means the direct derivatization of the compounds of interest without any preliminary extraction in line with the principles of green chemistry, compared to the time consuming procedures published in the literature.

The analytical performance characteristics of the new principles were determined. The capabilities of the new methods were compared with each other and with the recently applied techniques

The practical utility of developed methods (extraction and derivatization) was demonstrated by the quantitative determination of drugs in plants and other biological matrices (*Lophophora williamsii* and *Catha edulis* tissues, urine samples).

5. Conclusions

New sample preparation techniques were developed for the quantitative determination of PPAAs – with special attention to illicit constituents (AM, MDA, MSC, CTN, CAT) – and CTN-type designer drugs (4-FMC, MCTN, PENT, 4-MEC, 3,4-DMMC, 4-EMC) in plants and other biological matrices. In the frame of it

1. new derivatization approaches were presented:
   (i) results suggest that the well-known silylating reagent HMDS+TFA is also an efficient acylating agent: the reaction can be applied for selective acylation of PPAAs' homologues series; this process provides significant higher response values than the counterpart products, derivatized with traditional acylating reagents;
   (ii) as novelty to the field, the selective quantitative determination of PPAAs via 2TMS-derivatives was also described; the method enables considerably better sensitivity than the monotrimethylsilylation or acylation;
   (iii) the oximation of the CTN and the CTN-type designer drugs followed by their trimethylsilylation was noted at the first time; the two-steps derivatization resulted in effective GC separation and stable derivatives;
2. the mass fragmentation patterns were described in detail for all derivatization principles;
3. a novel sample preparation technique was recommended for determination of drugs in plants and other biological matrices which means the direct derivatization of the compounds of interest without any preliminary extraction compared to the time consuming procedures published in the literature;
4. the practical utility of developed methods (extraction and derivatization) was demonstrated by the quantitative determination of drugs in plants and other biological matrices (*Lophophora williamsii* and *Catha edulis* tissues, urine samples).

6. Publications

Publications related to the PhD thesis

Molnár B, Fodor B, Boldizsár I, Molnár-Perl I. (2016) Trimethylsilyl speciations of cathine, cathinone and norephedrine followed by gas chromatography mass spectrometry: Direct sample preparation and analysis of khatamines. J Chromatogr A, 1440: 172-178.

Molnár B, Fodor B, Boldizsár I, Molnár-Perl I. (2015) Quantitative silylation speciations of primary phenylalkyl amines, including amphetamine and 3,4-methylenedioxyamphetamine prior to their analysis by GC/MS. Anal Chem, 87: 10188-10192.

Molnár B, Csámpai A, Molnár-Perl I. (2015) Hexamethyldisilazane as an acylation generator for perfluorocarboxylic acids in quantitative derivatization of primary phenylalkyl amines confirmed by GC/MS and computations. Anal Chem, 87: 848-852.

Molnár B, Molnár-Perl I. (2015) The role of alkylsilyl derivatization techniques in the analysis of illicit drugs by gas chromatography. Microchem J, 118: 101-109. *Review article*

Publications not related to the PhD thesis

Andrási N, **Molnár B**, Dobos B, Vasanits-Zsigrai A, Záray Gy, Molnár-Perl I. (2013) Determination of steroids in the dissolved and in the suspended phases of wastewater and Danube River samples by gas chromatography, tandem mass spectrometry. Talanta, 115: 367-373.

Perlné Molnár I, Zsigrainé Vasanits A, Sebők Á, Helenkár A, Andrási N, Faludi T, **Molnár B**, Záray Gy. (2012) Környezeti vizek szerves szennyezőinek azonosítása és meghatározása, trimetilszilil (oxim) éter/észter származékokként, a gázkromatográfia tömegsepktrometria felhasználásával. Magy Kém Foly, 118: 55-64. *Review article*