https://doi.org/10.5272/jimab.2017232.1603

Journal of IMAB - *Annual Proceeding (Scientific Papers).* 2017 Apr-Jun;23(2):

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Original article

AN OPTIMIZED GC-MS METHOD FOR AMPHETAMINES IDENTIFICATION

Georgi Bonchev[1], Snezha Zlateva[1,2], Petko Marinov[1,2], Ivaylo Vazharov[3]
1) Clinic for Intensive Treatment of Acute Intoxications and Toxicoallergies, Naval Hospital – Varna, Military Medical Academy, Bulgaria
2) Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University – Varna, Bulgaria
3) Clinic of Internal Diseases, Naval Hospital – Varna, Military Medical Academy, Bulgaria

ABSTRACT

Purpose: To develop a reliable method for qualitative determination of amphetamines in biological samples, that combines simplicity, sensitiveness and robustness. Optimization of the method should be carried out in order to meet the needs of forensic expertise as daily routine.

Material/Methods: Analytical identification was done by menas of gas chromatography – mass spectrometry. Validation was carried out using certified analytical standards.

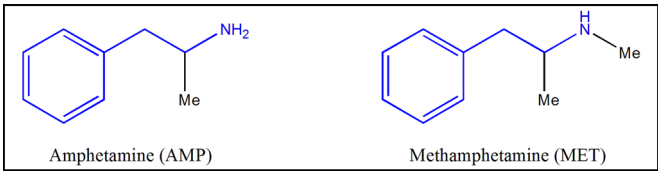
Results: Sample preparation procedure has been simplified. Only common reagents are needed. Solid phase extraction (SPE) has been used for initial purification of biological samples. Additional derivatization (PFPA) has been shown to be of major importance. Limit of detection (LOD) in blood samples has been determined to be as low as 25 ng/mL for amphetamine (AMP) and 10 ng/mL for methamphetamine (MET). Results are given within 100 minutes.

Keywords: amphetamine, methamphetamine, solid phase extraction, analytical toxicology, drugs of abuse, forensic expertise

INTRODUCTION

Amphetamine (AMP) and Methamphetamine (MET) are potent CNS stimulants which also produce anorectic and sympathomimetic actions [1]. Both substances belong to phenethylamine family (and more specifically, its phenylisopropylamine branch) of nitrogen-containing drugs (Fig. 1), chemically related to the naturally occurring trace amine neuromodulators, namely phenethylamine itself and N-methylphenethylamine. Although some of amphetamine's substituted derivatives occur in nature [2], usually amphetamines are synthetically produced.

Fig. 1. Chemical structures of AMP and MET (the common phenethylamine backbone is given in blue).



According to the official United Nations Office on Drugs and Crime (UNODC) statistic report [3], amphetamines are the second most popular drugs next to cannabis; there were more than 35 million people affected worldwide as of 2016 (ecstasy excluded). For the same period, amphetamines users in Europe were estimated to 2.4 million (0.4% of the population) [ibid.].

Amphetamines are illegal drugs in most countries and their detection is an often task in analytical toxicology. In Bulgaria amphetamine compounds fall under Group I of controlled substances – "Substances of high risk prohibited for medical use"). Pharmacokinetic properties include biological half-live of 6-15 h for MET and 4-8 h for AMP [4]. However, AMP is almost always present in cases of MET intoxication as it is one of its major metabolites. Low effective concentrations in body fluids and moderate biological half-life make detection of amphetamines in urine and blood samples usually possible within some 24 hours after intoxication, although a high dose may be detectable for up to several days [5].

Preliminary detection methods are mostly based on immunoassay; they give express results (usually in the order of minutes) and feature good cut-off concentration levels. For example, Dräger DrugCheck® 3000 routinely used by Bulgarian traffic police features a cut-off level of 50 ng/mL for amphetamines in oral fluid [6]. However, due to low analytical specificity and non-zero risk of false positive/false negative results, a confirmative method for analysis is mandatory in most cases. As a gold standard for this purpose, a gas chromatography mass-spectrometry technique is widely accepted and reviewed [7-11]. Some of the GS-MS methods achieve excellent results, although the rush for sensitivity complicates the procedure and increases the lab time. Indeed, there are simplified techniques, but purification of the sample is sacrificed along the way. Therefore, researching the most efficient approach by combining the advantages of already known studies and optimising the procedure is the main task of the study.

MATERIALS AND METHODS

Analytical grade or better chemical reagents were used. Preparation of the necessary solutions involves only

HPLC grade solvents and purified deionized water (0.067-0.100 ìS cm^{-1}, TKA™ Pacific water purification system). Certified reference material (Quick-Check™ Drug Solutions of 1 mg mL^{-1} AMP in MeOH and 1 mg mL^{-1} MET in MeOH) were purchased from Alltech Associates, Inc., USA. SPE Phenomenex® cartridges (Strata® Screen-C, 55ìm, 70Å, 150 mg/3mL tubes) were used. Pentafluoropropionic anhydride (for GC derivatization, 99%) was purchased from Sigma-Aldrich Co. LLC. Random samples of human blood and plasma from controlled stationary patients of Naval Hospital – Varna, available at Clinical Laboratory, were used for preparation of spiked samples.

GC-MS analysis was done on Agilent Technologies 7890B GC System & 5977A MSD module. Data acquisition and processing were controlled by Agilent MassHunter software package. Reference data from mass spectral library NIST version 2.0 g was used for comparison.

Statistical analysis was done using the OriginPro® software.

Tabl. 1. GC/MS Analysis conditions, SCAN mode.

Parameter	Value	Parameter	Value
Initial oven temp.	50°C	GC Column	HP-5ms
Initial time	0.5 min	Column dimensions	30 m × 0.25 mm
Oven ramp rate	3°C min^{-1}	Film thickness	0.25 ìm
Oven final first ramp	200°C	Inlet mode	splitless
Final time first ramp	0 min	Flow mode	constant flow
Oven ramp rate	4°C min^{-1}	Flow rate	1.5 mL min^{-1}
Oven final temp.	320°C	Carrier gas	He
Final time	10 min	Ion source temp.	230°C
Total run time	90.5 min	Inlet temp.	250°C

RESULTS AND DISCUSSION

Reference data

Before analysis of biological matrices, appropriately diluted standard AMP and MET solutions were used to record reference GC-MS data. Analysis conditions are listed in Table 1. As it has been proven inconvenient to switch software settings over and over again in state of emergency, all GC-MS parameters have been intentionally left unoptimized to match the screening procedure used on daily basis.

Amphetamines were analysed only after chemical derivatization by *N*-acylation. Commercially available agent (Pentafluoropropionic anhydride, PFPA) was used. Initial aliquot of standard amphetamines solutions (50 µL 20 µg mL^{-1} AMP/MET in MeOH) is evaporated under gentle stream of nitrogen (at < 60ºC) and reconstituted in 50 µL of toluene:acetonitrile (95:5). Then 50 µL PFPA were added and mixture was heated for 20 min at 70°C. After evaporation and reconstitution in 30 µL ethyl acetate, 1 µL of solution was injected into GC column. Pentafluoropropionyl derivatives were identified as follows: AMP × PFP at R_t = 14.24 min, mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): **190*** (100), **118** (99), **91** (75) and MET × PFP at R_t = 14.68 min, mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): **204*** (100), **160** (40), **118** (27). Qualifying ions are given in bold. Asterisk denotes the quantitation ions. The result is in excellent agreement with both software NIST library and reference MS spectrum data [12].

Experimental procedure

Purification of biological matrices before GC-MS analysis was done by mean of solid-phase extraction using silica-based strong type cation exchanger. The procedure is very close to already published technique for cocaine determination [13]:

(1) Condition the SPE column applying 2 mL MeOH followed by 2 mL water.

(2) Prepare the sample by diluting 1 mL blood serum/plasma with 2 mL of water, *or* by diluting 1 mL urine with 2 mL of 0.1 M phosphate buffer pH 6.0. Load the sample at rate as low as 1 drop per second.

(3) Wash the column consequently by 2 mL 0.1 M HCl and 2 mL MeOH.

(4) Dry at full vacuum for 2 minutes.

(5) Elute drop wise using 2 mL 5% ammonium hydroxide in MeOH.

(6) Thoroughly evaporate under nitrogen stream (at < 60ºC) to complete dryness.

(7) Reconstitute the residue in 50 µL of toluene: acetonitrile mixture (95:5) and add 50 µL acylation agent (PFPA).

(8) Heat for 20 min at 70°C.

(9) Evaporate resulting solution under nitrogen stream (at < 60ºC) again.

(10) Reconstitute in 30 µL ethyl acetate.

(11) Inject 1 µL of solution into GC column.

Method validation

There are increased demands on results' accuracy and precision because method is usually used as confirmative instrument in medico legal practice. We used fortified acceptance criteria before declaring any sample positive. Unusually severe set of requirements are *a priori* constructed and strictly followed during both validation and working

cycles. Simultaneous agreement is required on all next subjects:

(1) Sample signal R_t within 1% of calibration value *and* better than 20% qualifying ions ratios agreement;

(2) All qualifying ion maximum intensities obey three-sigma rule toward the background noise ($p < 0.01$);

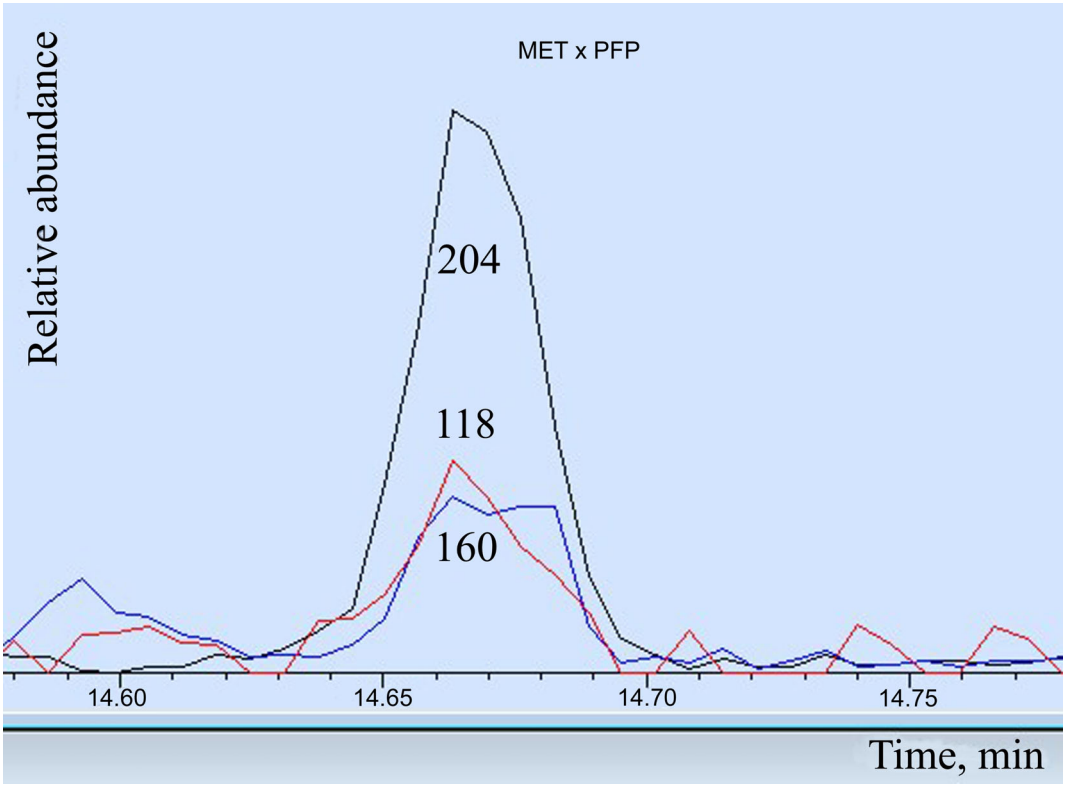
(3) Software identification agreement according to NIST database.

The strongly recommended empirical approach [14, 15] is represented by set (1). Statistical approach is represented by set (2). In attempt to reduce as much as possible type I (false positive) errors, we apply all sets cited.

LoD determination was done by menas of spiked blood samples (at levels 500, 400, 300, 200, 100, 50, 25, 10 ng mL^{-1}) for both substance of interest. LoD was associated with the lowest analyte concentration at which all of the acceptance criteria are still agreed. For AMP LoD was estimated to 25 ng mL^{-1}, and for MET – to 10 ng mL^{-1}.

Due to the severe requirements accepted, presence of amphetamines in test samples even at LoD levels was proven to produce clear visible evidence of well-defined peak area, as it is shown on Fig. 2 for example.

Fig. 2. Fragment of extracted ion chromatogram representing a clear evidence of MET presence at LoD level (10 ng mL^{-1} MET in blood, PFPA derivatization).



CONCLUSION

Available methods for amphetamines identification in biological matrices have been critically considered. An optimized approach, best fitted to given laboratory equipment has been proposed. Certified analytical standards have been used in method validation. It has been demonstrated that procedure gives accurate and precise results. Optimized method is robust, simple and relatively short (under 2 hours). Limits of detection are proven better than of immunoassay methods routinely used by traffic police, however running GC-MS in SIM mode could be a handy solution if yet better sensitivity is required. Starting the procedure with larger samples (e.g. 2 ml blood or more, if available) is another option of the same effect.

AKNOWLEDGEMENTS

The authors would like to thank the team of Analytical toxicology Lab, Military Medical Academy, Sofia, for valuable support and scientific guidance.

REFERENCES:

1. Glennon RA. Phenylisopropylamine stimulants: amphetamine-related agents. In: Lemke TL, Williams DA, Roche VF, Zito W, editors. Foye's principles of medicinal chemistry. 7th ed. Philadelphia, USA: Wolters Kluwer Health/Lippincott Williams & Wilkins. 2013. 646 p.

2.Hagel JM, Krizevski R, Marsolais F, Lewinsohn E, Facchini PJ. Biosynthesis of amphetamine analogs in plants. *Trends Plant Sci*. 2012 Jul; 17(7):404-12. [PubMed]

3. United Nations Office on Drugs and Crime. Statistics: Drug use and health consequences. Vienna: UNONDC; 2017. [cited 2017 Mar 31] [Internet]

4. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Crit Care*. 2012;16(4): R136. [PMC]

5.Clinical drug testing in primary care. Technical assistance publication series 32. United States Department of health and human services. Rockville: SAMHSA; 2012. [cited 2017 Apr 03] [Internet]

6. Dräger DrugCheck® 3000. Lubeck, Germany: Drägerwerk AG & Co. KGaA. 2017. [cited 2017 Apr 03] [Internet]

7. Cody JT, Foltz RL. GC/MS analysis of body fluids for drugs of abuse. In: Yinon J, editor. Forensic applications of mass spectrometry. Florida: CRC Press; 1995. p. 2–59.

8. Hsu MC, Chen D, Liu RH. Detection of abused drugs in urine by GC-MS. *J of Food and Drug Analysis*. 2009;17(4):233-45. [Internet]

9. Villamor JL, Bermejo AM, Fernández P, Tabernero MJ. GC MS determination of amphetamines in human urine. *Analytical Letters*. 2005; 38(5):781-90. [Internet]

10. Frison G, Tedeschi L, Favretto D, Reheman A, Ferrara SD. Gas chromatography/mass spectrometry determination of amphetamine-related drugs and ephedrines in plasma, urine and hair samples after derivatization with 2,2,2-trichloroethyl chloroformate. *Rapid Commun Mass Spectrom.* 2005; 19(7):919-27. [PubMed]

11. Peters FT, Samyn N, Wahl M, Kraemer T, De Boeck G, Maurer HH. Concentrations and ratios of amphetamine, methamphetamine, MDA, MDMA, and MDEA enantiomers determined in plasma samples from clinical toxicology and driving under the influence of drugs cases by GC-NICI-MS. *J Anal Toxicol*. 2003 Nov-Dec; 27(8):552-9. [PubMed]

12. NIST Mass Spectrometry Data Center. USA: U.S. Secretary of Commerce; 2016. [Internet]

13. Bonchev G. An optimized GC-MS method for identification of Cocaine and its major metabolites. *J of IMAB*. 2017 Apr-Jun;23(2):1523-6. [CrossRef]

14. Armbruster DA, Tillman MD, Hubbs LM. Limit of detection (LOD)/ limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clin Chem*. 40(7 Pt 1);1994:1233-8. [PubMed]

15. Lawson GM. Defining limit of detection and limit of quantitation as applied to drug of abuse testing: striving for a consensus. *Clin Chem*. 40(7 Pt 1);1994:1218-9. [PubMed]

Please cite this article as: Bonchev G, Zlateva S, Marinov P, Vazharov I. An optimized GC-MS method for amphetamines identification. *J of IMAB*. 2017 Apr-Jun;23(2):1603-1606. DOI: https://doi.org/10.5272/jimab.2017232.1603

Received: 03/04/2017; Published online: 26/06/2017

Address for correspondence:
Georgi Bonchev, PhD
Head, Laboratory of Analytical Toxicology, Military Medical Academy, Naval Hospital – Varna
3, Hristo Smirnenski Blvd., 9010 Varna, Bulgaria; Tel: + 359 (52) 386-204
E-mail: toxilab.varna@abv.bg