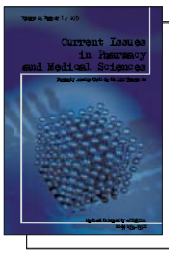

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Amphetamine in illegally produced phenylethylamine – intentional action or failed synthesis of a *designer drug*. Analysis of evidence material by LC/MS (APCI) and GC/MS (EI) methods

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ABSTRACT

On the Polish „drug scene", the products containing very low amounts of amphetamine offered as „pure" amphetamine are increasingly common. Occasionally, 1-phenylethylamine (1-PEA) is added to these products. The concentration of 1-PEA in such „street drugs" usually ranges from 2% to 90%. For the purposes of the ongoing prosecutor's proceedings, toxicological testing was performed in the Department of Forensic Medicine, Medical University of Lublin to detect narcotic agents and psychotropic substances in the white powder found in 23 plastic bags. LC/MS with atmospheric pressure chemical ionization method demonstrated only trace amounts of amphetamine and about 0.042–0.231% of 1-PEA in the samples analyzed. However, characteristic by-products of the synthesis of 1-phenylethylamine from acetophenone were indentified using GC/MS with electron ionization method. Results suggest that the analyzed „street drugs" were obtained by synthesis from acetophenone with slight amounts of benzyl methyl ketone (BMK) added intentionally, or using acetophenone contaminated with BMK

Keywords: amphetamine, 1-phenylethylamine, by-products of Leuckart reaction, LC/MS (APCI), GC/MS (EI)

INTRODUCTION

According to the report of the European Centre for Drugs and Drug Addiction (EMCDDA), Poland belongs to the leading producers of amphetamine in Europe [1]. Amphetamine is available on the „street drug market" in the form of diphosphate, hydrogen phosphate or sulphate salts, with some substances added to increase the volume of a „dust" [15]**.** The most common additives include glucose, saccharose and mannitol. In some cases, pharmacologically active substances are used, e.g. caffeine, paracetamol, acetylsalicylic acid, ethoxybenzamide or phenacetin [4,6,7]. In 2008, the average retail purity of amphetamine in Poland ranged from 2% to 98% [1]. According to some other data [14], the amphetamine salt content in the „street drugs" was 5-97%.

Moreover, some products contain the mixture of amphetamine sulphate, hydrochloride or phenylethylamine sulphate (1-PEA) the amounts of which range from 2% to 90% [4]. Illicit „pure" 1-PEA has also been offered [4,14]. Phenylethylamine occurs in the form of two isomers, one of which, i.e. 1-PEA, has stimulating effects similar to amphetamine although weaker and shorter. 1-PEA can be added to amphetamine to prolong its stimulating action as it is a monoamine oxidase B (MAO-B) inhibitor [4,6] and decreases amphetamine clearance. 2-PEA (*â*-PEA) is a biogenic amine – the neurotransmitter formed endogenously in the brain of humans and other mammals due to phenylalanine decarboxylation [5]. Moreover, 2-PEA belongs to putrefactive amines [9,10].

Amphetamine is frequently produced using the Leuckart reaction [13,16], which involves condensation of benzyl methyl ketone (phenyl-2-propane, BMK) with ammonium formate or formamide in the presence of formic acid. The resulting product, N-formylamphetamine, is hydrolyzed to amphetamine salt due to warming in the presence of hydrochloric or sulfuric acid (Fig. 1-A). During this synthesis, numerous contaminants are formed, namely residues of substrates and reagents (BMK, formamide, formates), half-products and by-products, i.e.

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4-methyl-5phenylpirymidine, 4-benzylpirimidine, 1-benzyl-phenylethylamine, N,N-di-(β-phenylisopropyl)amine, N,N-di-(β-phenylisopropyl) methylamine, N,N-di-(β-phenyl-isopropyl) formamide [2,6,8,14].

toring of trade in drug precursors between the Community and third countries[4].

In Poland, toxicological testing for narcotic drugs and psychotropic substances in evidence material secured by the police during investigations is conducted based on

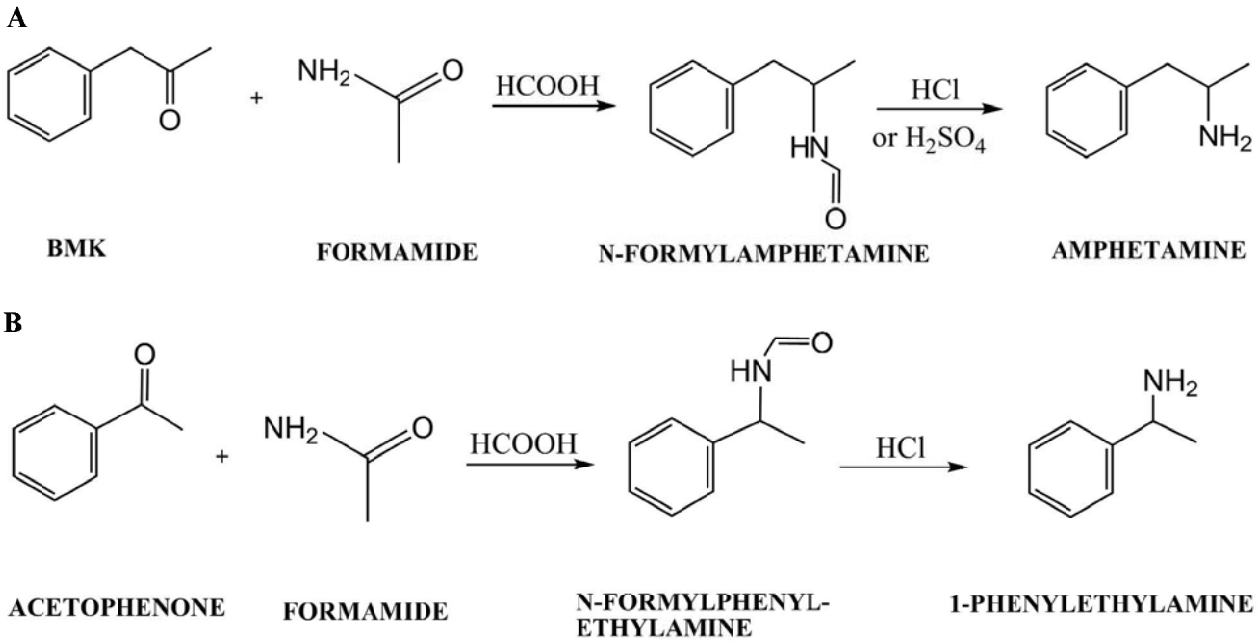

Fig. 1. Synthesis of amphetamine (A) and 1-phenylethyamine (B) using the Leuckart reaction

The Leuckart method can also be used to synthesize 1-PEA under the conditions similar to those for amphetamine (Fig. 1-B). The precursor of 1-PEA is acetophenone (phenyl methyl ketone) and the by-products are N,N-di-(1-phenylethyl)amine and N,N-di-(1-phenylethyl)formamide [4,6,10]. Thus, 1-PEA-containing amphetamine can be produced not only by mixing both substances but also by introducing the mixture of BMK and acetophenone (occasionally added to BMK to increase the final product volume) to the Leuckart reaction [4,7,14]. However, limited access to BMK may contribute to increased production of 1-PEA from acetophenone as it exhibits „psychoactive" effects similar to amphetamine. Importantly, acetophenone is not listed in the Art. 12 of the United Nations Convention (December 20, 1988) against the illicit traffic in narcotic drugs and psychotropic substances[1], the Commission Regulation (EC) No 1277/2005 (July 27, 2005)[2] laying down implementing rules for the Regulation (EC) No 273/2004 of the European Parliament and of the Council[3] on drug precursors and the Council Regulation (EC) No 111/2005 defining rules for the monitoring of trade in drug precursors between the Community and third countries[4].

In Poland, toxicological testing for narcotic drugs and psychotropic substances in evidence material secured by the police during investigations is conducted based on prosecutor's ruling. The tests should include all „controlled" substances mentioned in the Regulation on Counteracting Drug Addiction with later modifications[5] and narcotic precursors listed in the Regulation No 273/2004 of the European Parliament and of the Council.

The analysis of evidence material (white powder) carried out in 2010 at the Toxicology Division of the Department of Forensic Medicine, Medical University of Lublin demonstrated a low percentage of 1-PEA and trace amounts of amphetamine.

The aim of the study was to explain the chemical process that could lead to the formation of 1-PEA contained in the white powder offered as amphetamine. For this purpose, LC/MS with atmospheric-pressure chemical ionization (APCI) and GC/MS with electron ionization (EI) methods were used.

MATERIAL AND METHODS

Material: The white powder contained in 23 plastic bags, net weight 2148 - 4891 mg, was delivered to the Department of Forensic Medicine in Lublin for toxicological

[1] Coll. 95.15.69 Low of 20 Feb. 1995.
[2] Coll. OJ L 202 of 3 Aug. 2005.
[3] Coll. OJ L 47 of 18 Apr. 2004.
[4] Coll OJ L 22 of 26 Jan. 2005.
[5] Law gazette No 179 pos. 1485 of 29 Jul. 2005.

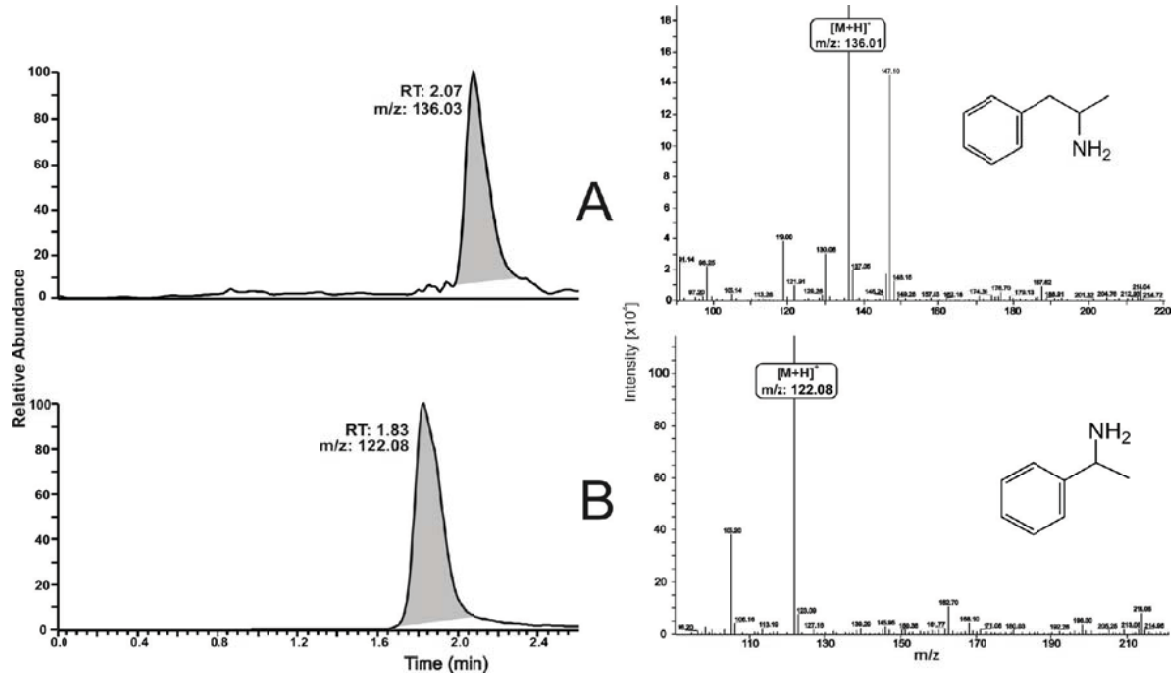

Fig. 2. LC/MS (APCI) chromatograms and mass spectra of amphetamine (A) and 1-phenylethyamine (B)

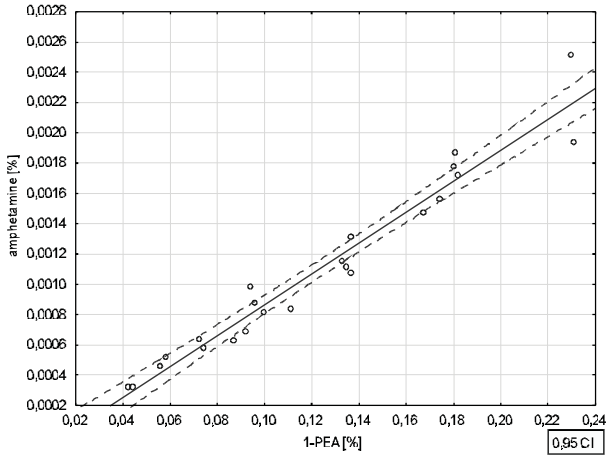

Fig. 3. Correlation of concentrations of amphetamine and 1-PEA in the test samples

analyses to detect narcotic drugs and psychotropic substances. From each sample, 300 mg of powder was collected, dissolved in 3 ml of methanol and centrifuged at 10°C for 10 minutes at 15000 rpm; supernatants were diluted 100- and 1000-fold with methanol and 25µl of internal standard solution, amphetamine-D11 was added (50 µg / 1 ml methanol).

***LC/MS analysis**:* Amphetamine and 1-PEA were determined quantitatively using the Accela ultra-pressure liquid chromatograph (UPLC) coupled with the LCQ Advantage Max ion trap mass spectrometer (Thermo Finningan, USA) controlled through the X'Calibur software. The conditions of chromatographic separation, spectrometer parameters and basic validation data of

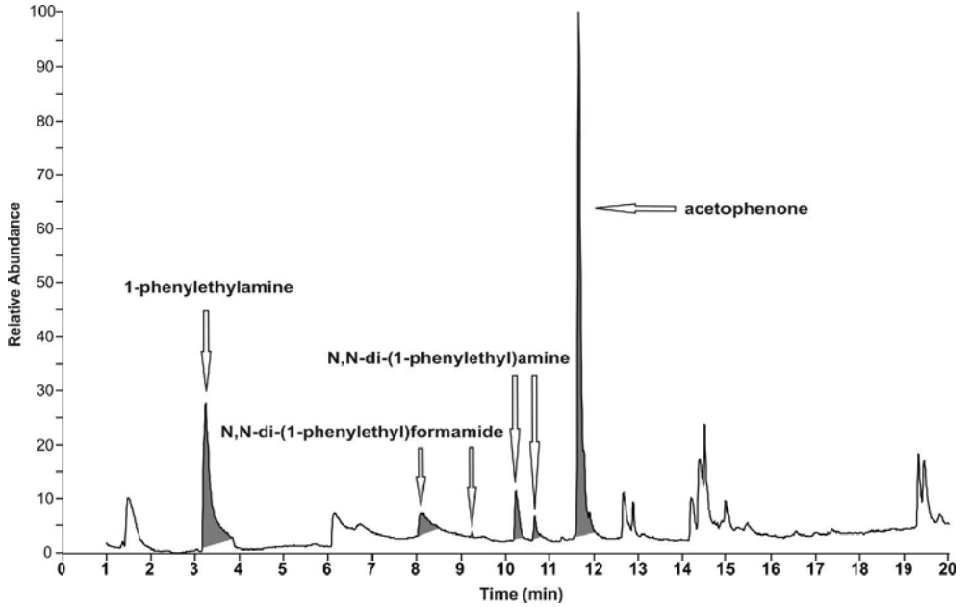

Fig. 4. GC/MS (EI) mass chromatogram of impurities characteristic of Leuckart synthesis of 1-phenylethylamine

LC/MS for determination of amphetamine and 1-PEA in the methanol solution of powders (compare 2.1) were presented in tables 1-3.

Table 1. LC/MS chromatographic conditions

Column	MERCK LiChroCART Purospher/RP-18e (5µm, 125 x 3 mm)		
Injection volume	5 µl		
Temperature	40°C		
Mobile phase composition	A – acetonitrile B – 25 mM ammonium formate, pH 4.5 in water		
Gradient profile	Time (min)	% A	Flow (ml/min)
	0.0	1	0.5
	2.1	100	0.5
	2.3	100	0.5

Table 2. LCQ Spectrometer operating parameters

Ionization	APCI (positive ions)
Scan mode	full scan
Mass range	50–650 *m/z*
Discharge current	5 µA
Capillary voltage	25 V
Temperatures	APCI probe – 450°C capillary – 180°C
Gases (N_2)	sheath – 65 ARB auxiliary – 10 ARB

Table 3. LC/MS validation parameters

	Amphetamine	1-PEA
Correlation coefficient	0.9957	0.9945
Linearity range	100–1000 ng/ml	100–1000 ng/ml
LOQ (in extracts)	100 ng/ml	100 ng/ml
LOD (in extracts)	50 ng/ml	50 ng/ml

***GC/MS analysis**:* The substances accompanying amphetamine were identified using the Trace GC Ultra gas chromatograph coupled with the Trace DSQ quadrupole mass spectrometer (Thermo Finningan, USA). The operation of the chromatograph and mass spectrometer was controlled by the X'Calibur software. N,N-di-(1-phenylethyl)amine and N,N-di-(1-phenylethyl) formamide were identified by comparing the spectra obtained with the NIST1'08 spectra. The conditions of chromatographic separation and operational parameters of the spectrometer were presented in table 4 and 5.

***Statistical analysis**:* Normality of distribution (Shapiro-Wilk test) and correlation coefficient (r- Pearson) were tested using the Statistica 10 Pl (StatSoft Inc., Poland).

Table 4. GC/MS chromatographic conditions

Column	J&W (USA) DB-5ms, 25m x 0.25mm x 0.13µm
Carrier gas	Helium, 1 ml/min
Injection (split/splitless)	1 µl; 1 min splitless
Injection temperature	280°C
Column temperature (gradient profile)	100°C (1 min) 290°C (10°C/min) 290°C (1 min)
Transfer line temperature	280°C

Table 5. DSQ spectrometer operating parameters

Ionization	EI (positive ions)
Electron energy	-70
Emission current	100
Detector Gain	3×10^5 V
Scan mode	full scan
Mass range	40–300 *m/z*
Ion Source temperature	250°C

RESULTS AND DISCUSSION

The studied powders contained amphetamine and 1-phenylethylamine. Using the Shapiro-Wilk test showed that the distribution of concentrations of amphetamine and 1-PEA in the test samples are consistent with a normal distribution ($p \leq 0.05$), and their concentrations were correlated (r-Pearson's, $p \leq 0.05$). The results were presented in table 6.

Table 6. Results of statistical analysis of the concentrations of amphetamine and 1-PEA in the test samples

N = 23	Amphetamine	1-PEA
C [%] min	0.00032	0.042
C [%] max	0.00251	0.231
Average	0.001094	0.1223
Standard deviation	0.000587	0.0562
Quartiles (25%–75%)	0.00063–0.00156	0.07400–0.17400
Correlation coefficient	0.9758 ($p \leq 0,05$)	
Shapiro-Wilk test	0.198	0.235

During qualitative analysis, full mass spectra of amphetamine and 1-PEA were recorded. For further quantitative examinations, pseudo-molecular ions of the highest signal intensity were selected at m/z 136 for amphetamine and 122 for 1-PEA [11] (Fig. 2). The samples contained only trace amounts of amphetamine – 0.0003–0.0025% and very low levels of 1-PEA – 0.042%–0.231%; moreover, their concentrations were correlated (Fig. 3), which suggests that it was obtained by synthesis from acetophenone with slight amounts of BMK added intentionally, or using acetophenone contaminated with BMK.

In the „products" synthesized in such a way, the amount of 1-PEA does not exceed 2% [4]. Moreover, two independent Leuckart reactions – one to obtain amphetamine and the other one to produce 1-phenylethylamine and mixing of their products, should result in endogenous impurities characteristic of both these substrates and intermediate products of both reactions [4,6]. However, GC/MS (EI) demonstrated only the presence of these substances that occur during the synthesis of 1-phenylethylamine in the Leuckart reaction (Fig. 4), i.e. acetophenone (the substrate of the reaction of 1-phenylethylamine synthesis) and its by- products, i.e. N,N-di-(1-phenylethyl)amine and N,N-di-(1-phenylethyl)formamide (Tab. 7). The substances formed as by-products during amphetamine synthesis (benzyl methyl ketone, 4-methyl -5-phenylpirymidine, N,N-di-(phenylisopropyl) amine) were not detected [3,12,13]. The by-products mentioned above were identified by comparing the spectra obtained with the NIST'08 spectra; their quantification was infeasible as certified quantitative analytical standards are not available.

Table 7. Chemical substances searched for in the test samples and basic parameters of analytical examinations by LC/MS (APCI) and GC/MS (EI) methods

Compounds	Retention time [min]		Characteristic ions* [*m/z*]
	LC/MS	GC/MS	
Amphetamine	2.11	—-	119; 130;136
Benzyl methyl ketone	n.f.	n.f.	43; 91; 134
4-methyl-5-phenyl-pyrimidine	n.f.	n.f.	102; 115; 170
N,N-di-(1-phenyliso-propyl)amine	n.f.	n.f.	91; 119; 162
Phenylethylamine	1.87	3.26	105; 122; 123
Acetophenone	n.f.	11.66	51; 77; 105
N,N-di-(1-phenylethyl) amine	n.f.	10.15; 10.57**	77; 105; 210
N,-N-di-(1-phenylethyl) formamide	n.f.	8.11; 9.25**	106; 134; 149

* – data obtained from the NIST'08 library (*National Institute of Standards and Technology)*; ** – retention time of diastereoisomeric pairs; n.f. – not found; --- – not determined

CONCLUSION

1. LC/MS (APCI) and GC/MS (EI) can be successfully applied for determination of amphetamine (even in trace amounts) and phenylethylamine as well as for qualitative identification of by-products formed during the Leuckart reaction.

2. The concentration of phenylethylamine in the samples was multifold lower than that in „street drugs" offered on the „drug market", which suggests that it might have been the product of the Leuckart reaction.

3. The hypothesis regarding the origin of amphetamine in the evidence material can be verified during quantitative analysis, which is currently limited due to the lack of certified analytical standards.

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