Original Article

Impurities profiling of seized crystals by GC/MS through 2016 in Iran

Hadis Musavi[1], Hajar Shokri-Afra[2], Hemen Moradi-Sardareh[2*]
[1]Department of Biochemistry, Faculty of Basic Sciences, Razi University, Kermanshah, Iran
[2]Department of biochemistry, faculty of medicine, Tehran university of medical sciences, Tehran, Iran
Received: 12 September , 2017; Accepted: 22 October, 2017

Abstract

Background: Methamphetamine is a highly addictive psychoactive substance which threatens the health of individuals and society. Crystal methamphetamine is an industrial synthetic illegal substance which is made from many chemical reagents. The aim of this study was to analyze methamphetamine samples qualitatively in order to determine the components of this substance. **Materials and Methods:** In this study 112 samples of crystalline methamphetamine were analyzed that obtained from Legal Medicine Organization of Iran during the year 2016. In order to determine the chemical characteristics of in samples, Gas Chromatography-mass Spectrometry (GC-MS) were carried out on the samples. **Results:** The results demonstrated that all samples contained methamphetamine. The most frequently occurring synthesis active ingredients, by-products and adulterants were acetic acid, amphetamine and dimethyl amphetamine. Other ingredients were N-formyl amphetamine, N-formyl methamphetamine, N-acetyl methamphetamine, N-acetyl amphetamine, benzyl amphetamine, 2-6-dimethyl-3,5-diphenyl pyridine, N-ethyl methamphetamine, ketamine, ephedrine and pseudoephedrine. About 70% of samples contained phenmetrazine. **Conclusion:** The chemical composition of crystal varies not only with methamphetamine content, but also in the adulterants. Illicit manufacture of methamphetamine results in the formation of some synthesis by-products. Identifying the composition of illicit amphetamines based on the presence or absence of other pharmaceuticals and by-products is presented in this study. All samples were methamphetamine. The fact that N-Benzyl 2-methylaziridine was the most frequently found impurity in the analyzed samples, indicates that most of the methamphetamine samples seized in Iran have been synthesized from ephedrine and pseudoephedrine as starting material.

Keywords: Methamphetamine, Gas chromatography-mass Spectrometry, Ephedrine, Pseudoephedrine, Nagai.

***Corresponding Author:** Hemen Moradi-Sardareh, biochemistry, faculty of medicine, Tehran university of medical sciences, Tehran, Iran, E.mail: hemen.moradi@yahoo.com ,Tel: (+98) 9399283093
Please cite this article as: Musavi H, Shokri-Afra H, Moradi-Sardareh H. Impurities profiling of seized crystals by GC/MS through 2016 in Iran. Arch Med Lab Sci. 2017;3(4):6-11.

Introduction

Methamphetamine (N-methyl-alpha-methylphenethylamine or "MA") as a stimulant drug is one of the most widely used addictive abused central nerve system (CNS) [1]. MA has different consequences on users; in short- term consumption it includes the following symptoms: tachycardia, hypertension, hyperthermia, hyperpnea, cardiovascular collapse, euphoria, insomnia and etc. and in long-term, it causes the narcolepsy, ketosis, formication, xerostomia, hallucination and etc. [2].

Profiling of MA impurities follows useful purposes as obtaining information about the trafficking networks, production methods, and precursors used for the synthesis [3]. For this reason, MA profiles have been obtained in several countries, including Iran [4], Japan [5], Philippines [3], Thailand [6], Australia [7, 8], China [9]. The abuse of MA has become a global dilemma, therefore international co-operation between

countries must be taken to prevent the spread and trafficking of psychoactive addictive drugs.

Because the different methods of MA synthesis lead to the production of impurities in it, which is important for diagnostic purposes, it is important to know the types of synthesis methods. Impurities give us valuable information about the conditions of synthesis, and the precursors materials used [5]. Common methods for the synthesis of MA are shown in Figure 1.

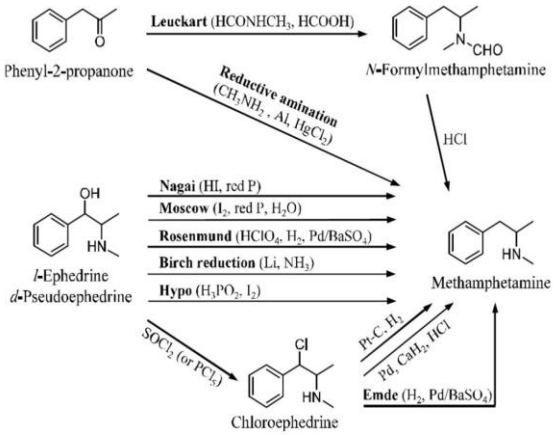

Figure 1. Methamphetamine synthesis pathways redrawn from Inoue et al. [10]

One of the best methods and strategies to prevent the producing of industrial drugs such as MA is to control their precursors [11]. Due to availability of materials and ease of making MA by these methods, the numbers of clandestine laboratories increased. Over the past few years, the production of amphetamine type stimulant (ATS) drugs such as MA is increasingly rising throughout the world. The amounts of seized ATS were 74 and 123 tons in 2010 and 2011 respectively (a 66 percent rise that the most proportion related to MA) [12]. Many researchers have recently investigated the impurities of MA seized in and out of Iran (1). This research is designed to prepare new statistics of psychoactive drugs impurities and discuss and compare its results to other studies in Iran and other countries.

Methods

112 crystal methamphetamine seized samples that referred to the forensic toxicology laboratories were selected through 2016 in Iran. Thereupon, physical properties of samples were examined. All samples with different colors (white, yellow, pink, brown and cream) divided into two classes, crystal and non-crystal.

Preparation and extraction samples. The liquid-liquid extraction method is used in present study with following sequences: 50 mg powder of each sample mixed with PH 10 phosphate buffer and vortex for 5 minutes, then 500 µl ethyl acetate (as organic phase) added to the mixture. The mixture, centrifuged at 300 rate per minute (RPM) for 5 minutes, next the samples were placed in a cold bath to freeze, after that the aqueous phase and the organic phase consisting the methamphetamine, were separated to GC-MS analysis [13, 14].

GC-MS analysis. The GC-MS apparatus was made by Agilent Company with following specifications: GC 7890A and MS 5975C, HP5-MS column (30 m × 0.25 mm internal diameter and 15 µm thickness). The helium as carrier gas was used at a rate 1.5 ml/min. The column temperature started and kept for one minute at 60 ˚C and later, it increased to 300 ˚C with 20˚C/min. The temperature of injection chamber, transfer line, ion source and quadrupole were adjusted at 250 ˚C, 300 ˚C, 230 ˚C and 150 ˚C respectively. The amount of 0.4 µl sample injected in the splitless mode.

Data analysis. Our GC-MS library is supported by Wiley, Pest and NIST libraries that can check and identify 50000 different components. The results analyzed with SPSS V16 software and the frequency of the impurities obtained from the samples, were determined.

Results

The psychoactive drugs that seized by the narcotics policemen were referred to the Forensics laboratories through 2015-2016 in Iran. Through these times, 112 samples were randomly selected and examined to find determination and identification of impurities and ingredients.

At first, the samples were analyzed based on color and shape. The results showed that the most frequency belongs to white and crystalline samples. The rest detailed results are shown in Table 1 and Table 2.

Table1. Frequency of colors in samples

color	Sample NO.	Frequency	Percent
Cream	**65,88,32,58,29,100,44**	7	6.25
Brown	**77,78,27**	3	2.67
Yellow	**66,74**	2	1.78
Pink	**91,89**	2	1.78
White	**Rest of sample**	98	87.5
Total	-	112	100

Table2. Division of samples based on physical shape

physical shape	Frequency	Percent
Crystalline	106	94.64
Non-crystalline	006	05.35
Total	112	100.0

The profiling of crystal impurities of all samples is summarized in Fig 2.

Each sample was examined for its impurities; thus 71 ingredients were identified. The following results obtained: methamphetamine (in 112 samples, 100%), Acetic acid (in 105 samples, 93.8%), amphetamine (in 106 samples, 94.6%), dimethyl amphetamine (in 107 samples, 95.5 %), N-Formyl amphetamine (in one sample, 0.9%), N-Formyl methamphetamine (in 102 samples, 91.1%), N-acetyl methamphetamine (in 108 samples, 96.4%), N-acetyl amphetamine (in 4 samples, 3.6%), benzyl amphetamine (in 3 samples, 2.7%), 2,4 dimethyl-6-phenylpyridine (in 4 samples, 3.6%), pyridine 4 (1,1-dimethyl) ethyl (in 2 samples, 1.8%), benzaldehyde (in 17 samples, 15.2%), 1,2-dimethyle 3-phenyleazidine (in 4 samples, 3.6%), N-acetylephedrine (in 4 samples, 3.6%), benzyl alcohol (in one sample, 0.9%), dibutyl phthalate (in 7 samples, 6.2%), phenmetrazine (in 76 samples, 67.9%), N-ethyl methamphetamine (in one sample, 0.9%), amphetamine (in 15 samples, 13.4%), chlorpheniramine (in 74 samples, 66.1%), N-benzyl-2-methylaziridine (in 95 samples, 84.8%), aziridine-1,2-dimethyl 3- phenyl (in 4 samples, 3.6%), Naphthalene 2,7-Dimethyl (in 88 samples, 79%), ketamine (in 4 samples, 3.6%), triprolidine (in 2 samples, 1.8%), N-methyl cyclohexylamine (in 3 samples, 2.7%), ephedrine and pseudoephedrine (in 9 samples, 8%), benzothiazole (in 6 samples, 5.4%), piperazine 1 (2,3 Dimethyl) phenyl (in 4 samples, 3.6%), 1- phenyl 2-propane oxime (in 2 samples, 1.8%), cyclohexanamine (in 5 samples, 4.5%), 3-methyl diphenyl amine (in one sample, 0.9%), 1,1-biphenyl 4,4-diol (in one sample, 0.9%), antracine (in 5 samples, 4.5%), naphthalene 1-

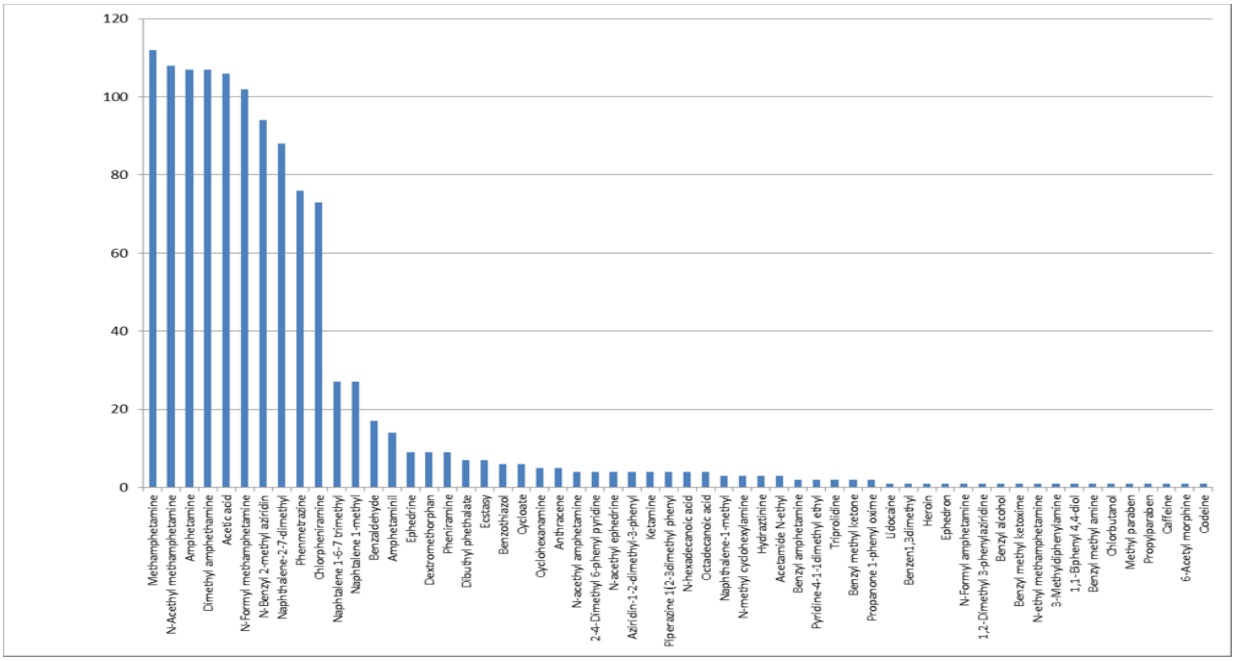

Figure 2. frequency Comparison of variety components of MA.

methyl (in one sample, 0.9%), naphthalene 1,6,7-trimethyl (in 27 samples, 24.3%), benzyl methylamine (in one sample, 0.9%), cycloat (in 6 samples, 5.4%), Chlorobutanol (in one sample, 0.9%), benzyl methyl ketone (in 2 samples, 1.8%), methylparaben (in one sample, 0.9%), propylparaben (in one sample, 0.9%), isobenzofuranone 6,7-dimethoxy (in one sample, 0.9%), hydrocotarnine (in one sample, 0.9%), pheniramine (in 6 samples, 5.4%), kaphein (in one sample, 0.9%), acridine 9-methyl (in one sample, 0.9%), Dextromethorphan (in 10 samples, 8.9%), codeine (in one sample, 0.9%), acetyl codeine (in one sample, 0.9%), papaverine (in one sample, 0.9%), acetyl morphine (in one sample, 0.9%), methylacetamide (in one sample, 0.9%), N-hexadecanoic acid (in 4 samples, 3.6%), octadecanoic acid (in 4samples, 3.6%), mephentermine (in one sample, 0.9%), ecstasy (in 6 samples, 5.4%), 3,4-methylene-dioxy-N-ethyl-amphetamine (in one sample, 0.9%), hydrastinine (in 3 samples, 2.7%), 3,4-methylene-dioxy-amphetamine (in one sample, 0.9%), methsuximide (in one sample, 0.9%), acetamide N-ethyl (in 3 samples, 2.7%), phentylamine (in one sample, 0.9%), acetamide N-phenyl ethyl (in one sample, 0.9%), lidocaine (in one sample, 0.9%), benzene 1,3-dimethyl (in one sample, 0.9%), heroine (in one sample, 0.9%), bisabolol (in one sample, 0.9%), canabidole (in one sample, 0.9%), dronabinol (in one sample, 0.9%) and ephedrone (in one sample, 0.9%).

Table3. The most commonly used drugs and narcotics in samples

Drugs	Frequency in 112 samples (%)
Methamphetamine	100
Amphetamine	94.6
Phenmetrazine	67.9
Chlorpheniramine	66.1
Dextromethorphan	8.9
Ephedrine and Psodueffedrin	8
Ecstasy	5.4
Pheniramine	5.4
Ketamine	3.6
Triprolidine	1.8
Acetyl morphine	0.9
Papaverine	0.9
Caffeine	0.9
Canabidole	0.9
Codeine	0.9
Lidocaine	0.9
Heroine	0.9
Acetyl codeine	0.9

The most commonly used drugs in Iranian crystals shown at table 3. The information of presence of drugs in crystal samples would be more useful and helpful for addiction stations to save addicted from addiction to crystals by doing more effective actions.

Discussion

The precursors used in the synthesis of crystal samples. There are many ways for crystal production and each of them needs specific precursors. The benzaldehyde and the benzyl chloride are used as precursors in Leukart and reductive amination routs for synthesis of methamphetamine. As well as, phenyl acetic acid converted to phenyl 2-propanone (P2P) when affected by acetic anhydride. P2P could be used as a precursor of methamphetamine in Leukart and reductive amination routs [11]. In this study benzaldehyde and phenyl 2-propanone were observed in 15.2% and 1.8% of crystal samples, respectively. The current results confirm that these two substances have been used as precursor for the synthesis of a number of crystals by the Leukart and reductive amination method.

In two Nagai and Emde routs, the ephedrine and pseudoephedrine utilized as methamphetamine precursors. In present study, about 85% of crystal samples were produced by Nagai method but ephedrine and pseudoephedrine recognized in 8% of samples; that it may be due to the result of incomplete purification process or failure to convert complete precursors into the final products.

Chlorpheniramine is used in antihistamine decongestant tablet as the antihistamine substance. On the other hand, pseudoephedrine is in antihistamine tablet and Dextromethorphan–p syrups, so it seems these two drugs have been used as precursors of crystal samples with Chlorpheniramine and Dextromethorphan impurities, respectively. Our results showed that Dextromethorphan (in 9% of

samples) and Chlorpheniramine (in 66% of samples) from antihistamine tablet and Dextromethorphan–p syrups used as great source of ephedrine and pseudoephedrine for crystal synthesis by Nagai rout.

The results of this study suggest that the ministry of health has to be more aware about abuse of these drugs and instruct pharmacies to refrain from the delivery of such drugs without a prescription and in high amounts; hereby, they will be able to reduce the abuse of antihistamine tablet and Dextromethorphan–p syrups as precursors of crystal.

Impurities in the crystal samples. Methamphetamine and its byproduct (amphetamine) were presented in 100% and 94% of samples, respectively. Acetic acid and N-Formyl methamphetamine may create as impurity during extraction of samples or may produce when the samples are affected by high temperature of infusion source of GC [4]. These two ingredients were observed in 93% and 91% of samples, respectively. Although one of the substrate of Leukart and amination reduction routs is 1-Phenyl-2-propanone oxime, as well as it can be produced as byproduct in methamphetamine synthesis from ephedrine and pseudoephedrine [11]. This substance was observed only in two samples of crystals.

Benzaldehyde is one of the precursors of methamphetamine that used in Leukart and amination reduction routs [11]. The presence of benzaldehyde in 15% of the samples can confirm its usage as a precursor or may produce during extraction of samples or when the samples are affected by high temperature of infusion source of GC-MS [4, 11].

N-benzyl amphetamine has presented in 3% of crystal samples which can produced when amphetamine react with benzaldehyde. Wille and Lambert reported that formaldehyde pollution in solvents such as methanol can convert the ephedrine to Phenmetrazine [15]. We observed Phenmetrazine in 68% of samples that supported the previous report of Wille and Lambert.

N-acetyl methamphetamine, which is present in 96% of crystal samples, may be formed as a byproduct during free base methamphetamine precipitation by adding hydrochloric acid in the presence of an acetate solvent such as acetic acid, ethyl acetate or ammonium acetate[4]. N-acetyl methamphetamine and N-formyl methamphetamine may be converted into methamphetamine during extraction or analysis with GC-MS, and may appear on chromatograms at small amounts [4].

Checking of availability of common drugs and narcotics in crystal samples. One of the hypothesis of current study was the presence of drugs and narcotics such as heroine, canabidole (type of marijuana), lidocaine, acetyl morphine, papaverine, acetyl codeine, codeine, ecstasy, Dextromethorphan, caffeine, triprolidine, ketamine, phenmetrazine and chlorpheniramine exist in crystal samples.

Investigation of the methods used to synthesis of methamphetamine. One of the objectives of this research is to determine the ways in which crystals can be synthesized by their raw materials, and also can be identified the countries producing crystals, the transit route and the smuggling, which depends on conducting simultaneous projects in cooperation with neighboring countries.

There are several methods for methamphetamine synthesis: Nagai, Edme, Leukart and Reductive amination (Fig 3). Each of these methods needs the specific precursors and each creates specific byproducts. The impurities Identification in crystal samples that are the tract of precursors and byproducts, led to realize which method is used to crystal synthesis. In present paper the routs that were

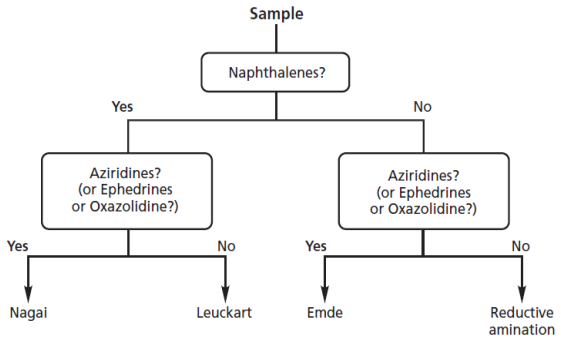

Figure 3. This chart explains that the Nagai rout is used to synthesis crystals with naphthalene's and aziridine precursors, but Leukart rout is used only to synthesis crystals with naphthalene without Aziridine. The Edme rout is used to synthesize crystals with Aziridine without naphthalene but for the reductive amination, it is crystals without both naphthalene and aziridine redrawn from Urano et al [11].In this paper, the majority of samples (85%) have Aziridine and naphthalene impurities which this means the Nagai rout is the predominant method for synthesis of crystal.

used to synthesis crystals, were determined based on presence of aziridine compositions (N-benzyl 2-methyl aziridine, 1,2-dimethyl 3-phenyl aziridine) and naphthalene compositions (naphthalene 2,7-dimethyl, naphthalene 1,6,7-trimethyl and naphthalene 1-methyl) in samples. The Figure 3 shows the simple chart to understand which rout is used to synthesis of crystals based on its impurities.

Conclusion

The main goal of the current study was to determine the impurities of Iranian illegal crystals. One of the more significant findings to emerge from this study is that methamphetamine was present in all experimental samples. The analysis of the data indicated that the most used method for crystal synthesis was Nagai rout.

Acknowledgment

Authors are thankful of all the forensic toxicology laboratories for preparing samples.

Conflict of Interest

The authors declare no conflict of interest.

References

1. National Police Agency of Japan, White Paper on Police, National Police Agency of Japan, Tokyo, 2005, pp. 191–192 (in Japanese).
2. Mehling R, Triggle D. Methamphetamine: Infobase Publishing; 2008.
3. Dayrit FM, Dumlao MC. Impurity profiling of methamphetamine hydrochloride drugs seized in the Philippines. Forensic science international. 2004;144(1):29-36.
4. Khajeamiri AR, Faizi M, Sohani F, Baheri T, Kobarfard F. Determination of impurities in illicit methamphetamine samples seized in Iran. Forensic science international. 2012;217(1):204-6.
5. Lee JS, Chung HS, Kuwayama K, Inoue H, Lee MY, Park JH. Determination of impurities in illicit methamphetamine seized in Korea and Japan. Analytica chimica acta. 2008;619(1):20-5.
6. Puthaviriyakorn V, Siriviriyasomboon N, Phorachata J, Pan-ox W, Sasaki T, Tanaka K. Identification of impurities and statistical classification of methamphetamine tablets (Ya-Ba) seized in Thailand. Forensic Science International. 2002;126(2):105-13.
7. Qi Y, Evans ID, McCluskey A. Australian Federal Police seizures of illicit crystalline methamphetamine ('ice') 1998–2002: impurity analysis. Forensic science international. 2006;164(2-3):201-10.
8. Qi Y, Evans I, McCluskey A. New impurity profiles of recent Australian imported 'ice': methamphetamine impurity profiling and the identification of (pseudo) ephedrine and Leuckart specific marker compounds. Forensic science international. 2007;169(2-3):173-80.
9. Zhang JX, Zhang DM, Han XG. Identification of impurities and statistical classification of methamphetamine hydrochloride drugs seized in China. Forensic science international. 2008;182(1-3):13-9.
10. Inoue H, Iwata YT, Kuwayama K. Characterization and profiling of methamphetamine seizures. Journal of health science. 2008;54(6):615-22.
11. Urano Y, Nagano T. Investigation of the origin of ephedrine and methamphetamine by stable isotope ratio mass spectrometry: a Japanese experience". Bulletin on Narcotics: Science in Drug Control-the Role of Laboratory and Scientific Expertise. 2007;57(1):63.
12. Burns L. World Drug Report 2013 By United Nations Office on Drugs and Crime New York: United Nations, 2013ISBN: 978-92-1-056168-6, 151 pp. Grey literature. Drug and Alcohol Review. 2014;33(2):216-.
13. Khajeamiri AR, Faizi M, Sohani F, Baheri T, Kobarfard F. Determination of impurities in illicit methamphetamine samples seized in Iran. Forensic science international. 2012;217(1-3):204-6.
14. Kuwayama K, Inoue H, Phorachata J, Kongpatnitiroj K, Puthaviriyakorn V, Tsujikawa K, et al. Comparison and classification of methamphetamine seized in Japan and Thailand using gas chromatography with liquid–liquid extraction and solid-phase microextraction. Forensic science international. 2008;175(2-3):85-92.
15. Zhang JX, Zhang DM, Han XG. Identification of impurities and statistical classification of methamphetamine hydrochloride drugs seized in China. Forensic science international. 2008;182(1):13-9.