ORIGINAL ARTICLE

HPLC Measurement of MDMA Content in Ecstasy Tablets Available in the Black Market of West Azerbaijan Province, Northwestern Iran

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Abstract

Background: Ecstasy, mainly composed of 3,4-methylenedioxymethamphetamine (MDMA), is one of the most popular addictive synthetic drugs. This study was aimed to investigate the amount of MDMA in the ecstasy tablets seized in West Azerbaijan province, northwest Iran and also to assess the relationship between the physicochemical and morphological characteristics of the tablets.
Methods: MDMA content of ecstasy tablets was analyzed using high-performance liquid chromatography method. Flow rate was 3 mL/min and mobile phase consisted of a mixture of acetonitrile and water containing triethylamine (pH = 3.2). The fluorescence spectrophotometer detector set at an excitation and emission wavelength of 220 and 306 nm, respectively. The retention time of ecstasy on this system was 2.2 minutes. The calibration curve was linear (R^2= 0.999) over the concentrations ranging from 0.2 to 2 µg/ml. The limit of detection and the limit of quantitation were found to be 0.06 µg/L and 0.19 µg/L, respectively with six times repetition.
Results: In this study, 85 ecstasy tablets were analyzed. Mean weight of the tablets was 275.6 ± 70.4 (range: 158.5-403.3) mg. Mean MDMA content of the tablets was 30.53 ± 23.23 (range: 0.05-70.7) mg. The tablets were classified into 8 groups based on their morphological features (color and logo). Considering the tablet groups, physicochemical features of the tablets (weight, MDMA content, and MDMA to weight ratio) were significantly different (P < 0.001). Correlation analysis showed that the MDMA content and weight of tablets were significantly correlated (P = 0.04).
Conclusion: There is variability in the physicochemical properties of ecstasy tablets available in the black market for illicit drugs in northwest Iran. This variability may potentially put abusers at increased risk of overdose due to inadvertent excess ingestion of the tablets to achieve desired effects and also experiencing more harm due to tablets adulterants.

Keywords: Amphetamines; High Pressure Liquid Chromatography; Iran; N-Methyl-3,4-methylenedioxyamphetamine; Street Drugs

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INTRODUCTION

Nowadays, drug abuse is considered as a social, health, economic and cultural problem and has caused a great deal of global concern as a destructive phenomenon (1). Addictive synthetic substances are of great importance because young population of the society is more inclined to abuse them. The prevalence of synthetic drugs abuse in Iran is lower compared with other countries; however, it is getting increasingly popular especially among youth (1,2).

3,4-methylenedioxymethamphetamine (MDMA), commonly known as ecstasy, is one of the most popular addictive synthetic drugs. It is an amphetamine-derived compound and due to its effects is categorized as a psychoactive drug by World Health Organization (3). American psychologists used this drug to facilitate psychotherapy in 1970. Since then and due the psychoactive properties of MDMA, many people have been encouraged using it for recreational purposes. Thus, the concern on the potential risks of non-medical use of these drugs urged the health authorities of the United States as well as other countries to restrict the use of MDMA and other amphetamine-derived medications (4,5). Nevertheless, these drugs are still illegally traded and abused across the world. In black market for illicit drugs, ecstasy is distributed in various forms, mostly capsules and oral tablets. Ecstasy tablets might be colorful and almost always some signs are imprinted on them (6).

Ecstasy is easily absorbed by the gastrointestinal system and its peak plasma concentration can be reached after 2 hours (7). This compound is mainly metabolized by CYP2D6 enzyme in the liver (7). MDMA can enhance release of monoamine and acetylcholine neurotransmitters from nerve endings (8). Therefore, it leads to a considerable increase in wakefulness, energy, sexual arousal and delay in

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fatigue (6,9-11). Moreover, increase in the muscles activity due to the direct effect of the drug on the thermoregulatory system can induce hyperthermia (9). In addition, headache, stiffness and pain at the lower-back and limb muscles, nausea, loss of appetite, blurred vision, mouth dryness, hypertension, tachycardia and insomnia has been reported during the first 2-3 days after consumption of ecstasy (9-12). In long term and in the most extreme cases, the use of these drugs can entail to hyperactivity, uncontrolled thoughts, illusions, loss of emotional intelligence, depersonalization and anxiety (6,9,10,12,13).

It has been found that no association may exist between the severity of ecstasy overdose and the number of tablets ingested, suggesting variation in the amount of MDMA in ecstasy tablets available in the black market (14-17). Although this issue has been investigated in other parts of the world (14-17), only limited number of studies worked out the variation of MDMA content in ecstasy tablets available in the black market of Iran (18,19). Measurement of MDMA in these compounds helps determining their toxicity risk. The present study was aimed to investigate the amount of MDMA in the ecstasy tablets seized in West Azerbaijan province, northwest of Iran, using high-performance liquid chromatography (HPLC) techniques, and also to assess the relationship between the physicochemical and morphological characteristics of the tablets.

METHODS

Materials and devices

The compounds utilized in this study had the appropriate purity for chromatography. All solvents were of HPLC grade and reagents were analytical grade. Acetonitrile, triethylamine and phosphoric acid were obtained from Merck® (Darmstadt, Germany). Deionized water was produced by using Elga deionized water system (Elga LLC., Illinois, USA). Chromatographic determinations were performed on a Cecil® high-performance liquid chromatograph (Cambridge, UK) equipped with a fluorescence spectrophotometer detector (E-Chrom Tech Co. Ltd., Taipei, Taiwan), a Biotech 2003 degasser (Onsala, Sweden) and CN columns (10cm, 0.8mm).

For weighing the tablets, digital analytical balance with 0.0001 g accuracy (ACCULAB, Bradford, MA, USA) was used. The pH of the solutions was measured by pH meter (827 pH lab, Metrohm AG Co., Herisau, Switzerland)

Process of measurement of standard solutions concentration

The samples concentrations were measured using HPLC technique with fluorescence spectrophotometer detector. The output data of HPLC were analyzed by power stream software (Cecil, Cambridge, UK). Firstly, 20 µL of the previously made standard solutions were injected into the chromatography column. Then, the mobile phase was assessed using a mixture of acetonitrile and deionized water (40:60-5:95, v/v) containing 0.01-0.05% triethylamine at the flow rate of 1-3 ml/min. Finally, a mixture of acetonitrile and water (40:60, v/v) containing 0.03% triethylamine adjusted at pH of 3.2 and flow rate of 3 ml/min was selected for the mobile phase. In addition, fluorescence spectrophotometer detector was set at an excitation and emission wavelength of 220 and 306 nm, respectively.

Investigation of the standard curve

In the optimal conditions, the calibration curve (H = 3.775 C + 3.566) was obtained which was linear within the range of 0.2-2 µg/L with R^2 = 0.999. In addition, the limit of detection of 0.06 µg/L and the limit of quantitation of 0.19 µg/L were achieved by 6 times repetition for each sample (Figure 1).

Investigation of accuracy and precision of the method

In order to determine the accuracy and precision of the analytical method, inter-day and intra-day changes were evaluated by calculation of standard deviation (SD) and coefficient of variation of the results obtained from investigation of the 3 selected concentrations. In order to monitor inter-day and intra-day changes, 3 different concentrations of MDMA standard solution were taken into account. The results of 5 replicates of each sample within one day (inter-day) are presented in table 1. The results of 5 replicates of each sample within 3 consecutive days (intra-day) are presented in table 2.

Collection of tablet samples and ethics

The ecstasy tablets analyzed in this study were collected by the anti-narcotics police in West Azerbaijan province, Iran, and were handed over to our research laboratory after obtaining license from the higher authorities. Approval of the local medical ethics committee was obtained for performing this study.

Stages of samples content analysis

Firstly, the ecstasy tablets were weighed using a digital scale. Then, the tablets were powdered using a porcelain mortar. Considering the difference in the tablets weights, 40 g of each tablet was put into appropriate falcon tubes. Subsequently, 50 mL monopotassium phosphate buffer solution containing 1% triethylamine with adjusted pH using

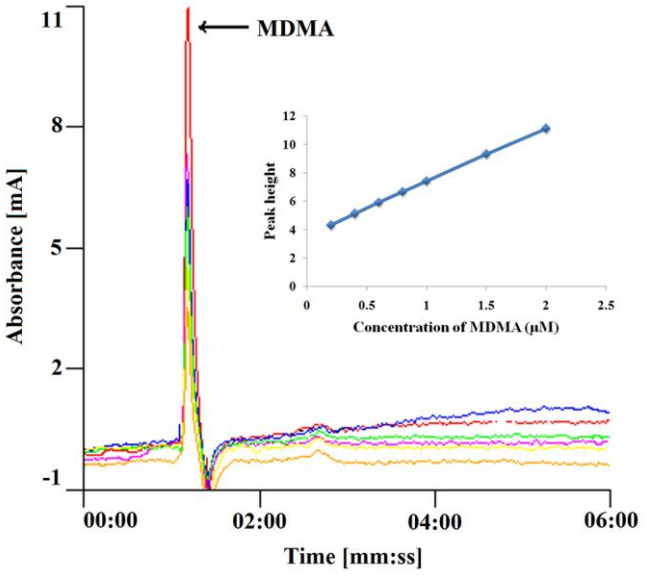

Figure 1. Calibration curve of the MDMA standard samples (concentration range: 0.2-2 µg/L)

Table 1. Inter-day changes of 3 concentrations of MDMA standard solution

	Standard concentration (µg/L)	Concentration (µg/L); mean ± SD	Precision (%)	Accuracy (%)
1	0.6	0.57 ± 0.06	10.2	95.0
2	1	1.08 ± 0.09	8.3	108.0
3	2	2.19 ± 0.05	2.3	109.5

Table 2. Intra-day changes of 3 concentrations of MDMA standard solution

	Standard concentration(µg/L)	Concentration (µg/L); mean ± SD	Precision (%)	Accuracy (%)
1	0.6	0.55 ± 0.02	3.5	91.0
2	1	1.04 ± 0.04	3.4	104.2
3	2	1.89 ± 0.05	2.9	94.5

phosphoric acid, 3/2 (1 M) was added. In order to effectively mix the materials and extract the MDMA, the mixture was put in ultrasonic device for 30 minutes. Then, the falcon tubes were centrifuged at 2000g for five minutes. Ultimately, the samples were filtered using syringe filters and were injected into the HPLC column. Each sample was analyzed in triplicate (Figure 2).

Statistical analysis

All the statistical analyses were performed using the SPSS statistical software (SPSS Inc., Chicago, USA). The tablets were classified into 8 groups based on their morphological features (color and logo). Kruskal-Wallis test was used to compare the physicochemical characteristics of tablets (weight, MDMA content, and MDMA content to weight ratio) among different groups. The correlation between two variables was analyzed by using Spearman's rank correlation coefficient. P values less than 0.05 were considered statistically significant

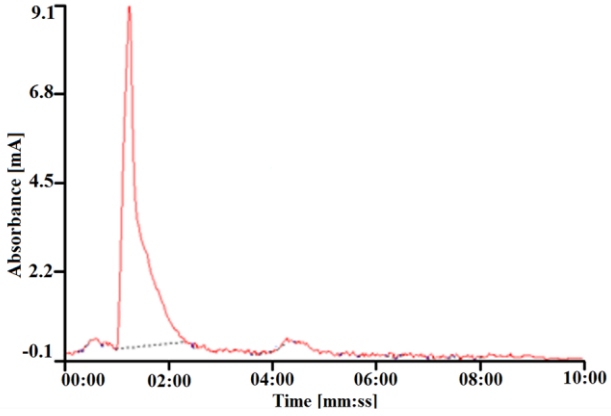

Figure 2. Chromatogram of brown captanor (retention time: 3 minutes, peak height: 9.0, area under the peak: 189.2)

RESULTS

In this study, 85 ecstasy tablets were analyzed. As mentioned previously, the tablets were classified into 8 groups based on their color and logo (Table 3). Mean weight of the tablets was 275.6 ± 70.4 mg ranging from 158.5 to 403.3 mg. The highest and lowest weights were related to the tablets with pink camel (366.30 ± 22.99 mg) and brown captanor (168.30 ± 6.49 mg) logos, respectively. Mean MDMA content of the tablets was 30.53 ± 23.23 ranging from 0.05 to 70.7 mg. The highest and lowest amounts of MDMA were related to the tablets with cross (59.60 ± 6.97 mg) and white captanor (0.06 ± 0.09 mg) logos, respectively.

Considering the tablet groups, weight, MDMA content, and MDMA to weight ratio of the tablets were significantly different (P < 0.001). Results showed that only 2 out of the 8 groups could be categorized into one group (dark blue camel and light blue camel) as they had similar weights and MDMA content. If the tablet logos were taken into account, the 5 resultant groups (camel, captanor, cross, Mitsubishi and crocodile) had significantly different weights (P < 0.001), which means different logos may represent different weights. Furthermore, in spite of the fact that tablets with one logo may have different colors, their MDMA contents may not be significantly different. In this respect, tablets with camel imprinting had almost similar amount of MDMA, while the tablets with captanor logo were considerably different. Given the MDMA to weight ratio, the tablets could be divided into 4 groups: (group 1: dark blue, light blue and pink camel; group 2: brown and white captonor and green Mitsubishi; group 3: yellow cross, group 4: blue crocodile). Correlation analysis showed that the MDMA content and weight of tablets were significantly correlated (P = 0.04).

DISCUSSION

The present study was aimed to find reasonable relationship between morphological (color and logo) and physicochemical (weight and MDMA content) features of ecstasy tablets seized in northwestern Iran. We found some links between the tablets morphology and MDMA content

Table 3. Comparison of the 8 groups of tablets according to weight and MDMA content

Color and logo of tablets	N. of tablets analyzed	Weight (mg); mean ± SD	MDMA content (mg); mean ± SD	Shape
Dark blue camel	10	342.50 ± 8.75	38.49 ± 4.68	
Light blue camel	10	340.50 ± 9.56	40.04 ± 4.85	
Pink camel	10	366.30 ± 22.99	40.18 ± 3.78	
Brown captanor	12	168.30 ± 6.49	2.62 ± 1.00	
White captanor	5	184.60 ± 3.51	0.06 ± 0.09	
Yellow cross	15	301.0 ± 11.28	59.60 ± 6.97	
Green Mitsubishi	13	259.50 ± 9.97	0.54 ± 0.24	
Blue crocodile	10	209.00 ± 6.50	47.58 ± 6.54	

as well as their weight. However, it should be noted that the weight and MDMA content of ecstasy tablets may not be precisely predicted if only their logo is taken into account because their weights and MDMA content even differs based on their colors despite having same logos. Nevertheless, as we found a significant correlation between MDMA content and tablet weight, it can be stated that the tablets weighing more contain greater amounts of MDMA. Khajeamiri et al similarly found partial relationship between MDMA content and weight of ecstasy tablets seized in different parts of Iran (18). Correspondingly, in a study by Cole et al on ecstasy tablets seized in the north-west of England during 1991 to 2001 significant positive association between the weight of the tablets and the MDMA content was established (17). However, in a study by Shetab Boushehri et al on the tablets captured in Tehran such correlation was not observed (19).

Ecstasy has been regarded as an illicit drug and its production and use has been prohibited in all countries. Hence, the production, distribution, and selling of this drug has been shifted to the black market for illicit drugs. Meanwhile, the production of ecstasy tablets has been expanded in illegal laboratories. Thus, these drugs are not controlled qualitatively and quantitatively, and so they are different regarding content, shape, color, and logo. Various studies have been performed on ecstasy tablets seized in different parts of the world and the findings of the most recent ones are summarized in table 4 (14,17-21). As can be seen, illegal ecstasy tablets have a wide range of MDMA content throughout the world and in each country. Moreover, it seems that despite having relatively similar weight of tablets, there has been a year-wise decline in the MDMA content of tablets in a single country. In this respect, the MDMA content of ecstasy tablets in Iran decreased from 60-180 mg in 2007-2008 as assessed by Khajeamiri et al (18) to 23.9-122.2 mg in 2009 as assessed by Shetab Boushehri et al (19) and ultimately to 0.05-70.7 mg in the present study. Likewise, in the United Kingdom, the mean MDMA content of ecstasy tablets decreased from 78.8 mg in 1991-2001 as shown by Cole et al (17) to 58.7 mg in 2006 as shown by Wood et al (14). This annual decrease in the MDMA purity of ecstasy tablets has also been shown independently in the study by Cole et al as they found mean MDMA content of ecstasy tablets seized in northwest

Table 4. Physicochemical features of ecstasy tablets seized across the world

Study, ref n.	Year	Country	N. of tablets analyzed	Analysis technique	Physicochemical features of tablets	
					Weight (mg)	MDMA content (mg)
Cole et al, 17	1991-2001	United Kingdom	80	HPLC-DAD[1]	Mean: 250 Range: 232-266	Mean: 78.8 Range: 73-89
Palhol et al, 20	1999-2002	France	106	GC-C-IRMS[2]	Mean: 270	Mean: 68
Teng et al, 21	2002-2005	Taiwan	181	GC-MS[3]	NS[*]	Range: 16-193
Wood et al, 14	2006	United Kingdom	101	HPLC	NS	Mean: 58.7 Range: 20-131
Khajeamiri et al, 18	2007-2008	Iran	50	LC-MS[4]	Range: 96-308	Range: 60-180
ShetabBoushehri et al, 19	2009	Iran	13	TLC[5]	Range: 220-380	Range: 23.9-122.2
Present study	2011-2013	Iran	85	HPLC	Mean: 275.6 Range: 158.5-403.3	Mean: 30.5 Range: 0.05-70.7

[1] High performance liquid chromatography with diode array detection
[2] Gas chromatography-combustion isotope ratio mass spectrometry
[3] Gas chromatography-mass spectrometry
[4] Liquid chromatography-mass spectrometry
[5] Thin-layer chromatography
[*] Not stated

England decreased from 102 mg in 1991 to 73 mg in 2001 (17). This can be due the fact that illegal producers in the recent years have adulterated the tablets with different impurities to gain more benefits (22-24). In the majority of cases, ecstasy tablets have been adulterated with caffeine, ephedrine and ketamine (23,24). Investigation of MDMA content in ecstasy tablets in Netherlands showed that reduction of MDMA purity led individuals to consume more tablets or high-dose preparations in order to achieve previous effects (25). Thus, having information about the amount of MDMA in the tablets distributed in the illegal markets can help health authorities assess the change in the pattern of consumption among abusers. On the other hand, identification of morphological and physicochemical features of ecstasy tablets can be beneficial in the emergency departments to assess the severity of poisoning especially when an unconscious patient is admitted to the hospital. Hence, preparing an information bank of the common ecstasy tablets seized in each geographical region including the tablets logo, shape, color, weight and MDMA content is recommended. Nevertheless, it should be noted that the morphological characteristics of tablets cannot definitely determine the MDMA content. Furthermore, given the fact that ecstasy tablets are produced illegally, information about their impurities and adulterants are necessary to be obtained to help clinicians predict the consequences of consumption. In fact, having knowledge about the ecstasy tablets adulterants can provide the physicians with valuable information about unwanted complications of poisoning with these drugs and facilitate decision making in the treatment process. This can be investigated in future studies.

LIMITATIONS

We could not evaluate and analyze impurities of the tablets in this study. The sample of ecstasy samples analyzed in this study was related to illicit drug market in northwest of Iran and thus is not nationally representative of the population of Iran ecstasy tablets. In addition, this sample is limited to Iran and therefore our findings may not be extrapolated to ecstasy tablets in other countries.

CONCLUSION

Long term effects of ecstasy tablets are indeed depended upon their MDMA content. There is variability in the physicochemical properties of ecstasy tablets available in the black market for illicit drugs in West Azerbaijan province, Iran. This variability may potentially put abusers at increased risk of overdose due to inadvertent excess ingestion of the tablets to achieve desired effects and also experiencing more harm due to tablets adulterants.

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