

RESEARCH ARTICLE

Quantitative determination of 3,4-methylenedioxymethamphetamine by thin-layer chromatography in ecstasy illicit pills in Tehran

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) is the major ingredient of ecstasy illicit pills. It is a hallucinogen, central nervous system stimulant, and serotonergic neurotoxin that strongly releases serotonin from serotonergic nerves terminals. Moreover, it releases norepinephrine and dopamine from nerves terminal, but to a lesser extent than serotonin. Poisoning and even death from abusing MDMA-containing ecstasy illicit pills among abusers is usual. Thus, quantitative determination of MDMA content of ecstasy illicit pills in illicit drug bazaar must be done regularly to find the most high dose ecstasy illicit pills and removing them from illicit drug bazaar. In the present study, MDMA contents of 13 most abundant ecstasy illicit pills were determined by quantitative thin-layer chromatography (TLC). Two procedures for quantitative determination of MDMA contents of ecstasy illicit pills by TLC were used: densitometric and so-called 'scraping off' methods. The former was done in a reflection mode at 285 nm and the latter was done by absorbance measurement of eluted scraped off spots. Limit of detection (LOD), considering signal-to-noise ratio (S/N) of 2, and limit of quantification (LOQ), regarding S/N of 10, of densitometric and scraping off methods were 0.40 µg, 1.20 µg, and 6.87 µg, 20.63 µg, respectively. Repeatabilities (within-laboratory error) of densitometric and scraping off methods were 0.5% and 3.6%, respectively. The results showed that the ecstasy illicit pills contained 24–124.5 mg and 23.9–122.2 mg MDMA by densitometric and scraping off methods, respectively.

Keywords: MDMA; ecstasy illicit pills; TLC; densitometry; scraping off

Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative which is classified as a hallucinogen, central nervous system (CNS) stimulant, and serotonergic neurotoxin (Ellenhorn 1997; Miller 2002; Flomenbaum et al. 2006). MDMA was discovered and patented in Europe as World War I began (1914), intended to make soldiers feel less hungry. In civilian usage the drug was supposed to help people lose weight, but other effects portended the product's commercial failure, and it never went on the market. Those other effects attracted attention in the 1960s and 1970s among therapists and recreational drug users similarly (Henry and Jeffreys 1992; Miller 2002).

Its clinical use was banned in the US in 1985 because of its neurotoxicity and its potential for misuse. In the

UK, MDMA has been listed since 1977 as a class A drug under the Misuse of Drugs Act, 1971 (Henry and Jeffreys 1992; Ellenhorn 1997). After recreational use became publicized, MDMA was made a Schedule I controlled substance in 1985 by the US Drug Enforcement Administration (Ellenhorn 1997; Miller 2002). At the end of the 1970s and early 1980s, it was introduced as an underground adjuvant drug for psychotherapy because the FDA did not approve it (Ellenhorn 1997).

Unlike amphetamines such as amphetamine and methamphetamine which release dopamine from nerves terminals, it is a powerful stimulant of serotonin release (Flomenbaum et al. 2006).

Severity of poisoning with MDMA obviously depends on the MDMA content of MDMA-containing preparations

such as pills and capsules. Thus, there is a possible risk for abusers of high MDMA-containing ecstasy illicit pills to be poisoned and even die (Aronson 2005; Bigelow 2006).

In low doses MDMA produces a pleasant altered state of mind, with enhanced emotional closeness, but it is also used in high doses and settings in which toxicity is often reported. Associated with increased physical activity and altered thermoregulation, ecstasy has been reported to cause unconsciousness, seizures, hyperthermia, tachycardia, hypotension, disseminated intravascular coagulation, and acute renal insufficiency, as well as death (Ling et al. 2001; Aronson 2005; Bigelow 2006). As recreational use of ecstasy has dramatically increased in recent years, deaths related to its use have been reported (Ling et al. 2001; Aronson 2005; Bigelow 2006). Thin-Layer Chromatography (TLC) is a simple, rapid, inexpensive, and relatively precise and sensitive method for analysis of drugs and poisons (Stahl 1969; Moffat 1986; Sherma 2003; Poole 2004; Wall 2005; Hahn-Deinstrop 2007). Simultaneous quantitative determination of drugs and poisons has made it a rapid method (Stahl 1969; Moffat 1986; Sherma 2003; Poole 2004; Wall 2005; Hahn-Deinstrop 2007).

Because one of the most important reasons of death from abusing ecstasy illicit pills is their MDMA content, continuous monitoring of MDMA contents of ecstasy illicit pills will help authorities to pay more attention to removing high-dose ecstasy illicit pills from the illicit drug bazaar.

In the present study, MDMA content of 13 most abundant ecstasy illicit pills in a Tehran illicit drug bazaar were determined by quantitative TLC. Two procedures for quantitative determination of MDMA contents of ecstasy illicit pills by TLC were used: densitometric and so-called 'scraping off' methods.

Methods

Chemicals

Methanol (Merck, Germany), concentrated ammonia solution (25%, Merck, Germany), hydrochloric acid (25%, Merck, Germany), 13 ecstasy illicit pills with different colors and symbols (from Drug police of Tehran, I.R. Iran), MDMA hydrochloride standard (Sigma, Dorset, UK), imipramine hydrochloride standard (Sigma), pre-coated TLC polyester plates (silica gel 60 UV₂₅₄, 20 × 20 cm, 0.200 mm layer thickness, MN), flat bottom TLC chamber for development of 20 × 20 cm TLC plates (CAMAG, Switzerland), a dual-wavelength (254/366 nm) UV cabinet (CAMAG, Switzerland), a TLC Scanning Densitometer (Shimadzu CS-9000, Japan), and a ultraviolet-visible spectrophotometer (Shimadzu, UV-160A, Japan) were used in this study.

Preparation of samples and standards solutions

Twenty-six ecstasy illicit pills, two-by-two were similar, were used (Figure 1). They were the most abundant ecstasy illicit pills in Tehran illicit drug bazaar.

Each pill was ground in a mortar followed by addition of 5 ml of methanol:ammonia (4:1) with mixing to convert the MDMA hydrochloride content of the pill to a respective base which is more soluble in organic solvent. The mixture was then centrifuged at 2000 rpm for 5 min and 1 ml of the supernatant was mixed with 1 ml imipramine hydrochloride (1 mg/ml, internal standard).

Thin-layer chromatography of prepared samples

Five microliters of resulted solutions were spotted on TLC plates by a micropipette. Moreover, 5 µl of solutions containing 2–6 mg/ml MDMA hydrochloride standards and 0.5 mg/ml imipramine hydrochloride (internal standard) were spotted on a separate TLC plate for use in the construction of calibration curves. Each experiment for each standard and sample concentration was in triplicate. The spotted plates were developed in a saturated TLC chamber containing methanol:ammonia (100:1.5) (Bussey and Backer 1974; Furnari et al. 1998; Moffat 2004).

Quantification of MDMA content of chromatographed samples

Developed plates were then dried and the MDMA values of spots and consequently ecstasy illicit pills were quantified by densitometric and a so-called 'scraping off' methods. The former was done by scanning of spots by a Shimadzu TLC Scanning Densitometer equipped with a deuterium lamp set at 285 nm in the reflection mode. The latter was done by finding locations of spots (samples and internal standard) under ultraviolet light at 254 nm in a UV cabinet. Spots-containing horizontal bands of TLC plates were divided into 2 × 2 cm squares so that each square completely comprised each spot. These divisions were performed by drawing horizontal and vertical lines using slight marking with a pencil, as shown in Figure 2.

A spot-free square was assigned to blank. Spots-containing squares were then scraped off from TLC plates with a razor blade followed by elution with 5 ml of distilled water:methanol:hydrochloric acid (55:40:5) to convert drugs to respected hydrochloride salts which are more soluble in aqueous solutions. This step is very influential for desorption of drugs (MDMA and imipramine) from stationary phase. The eluates were centrifuged at 2000 rpm for 5 min and absorbances of samples (MDMA-containing

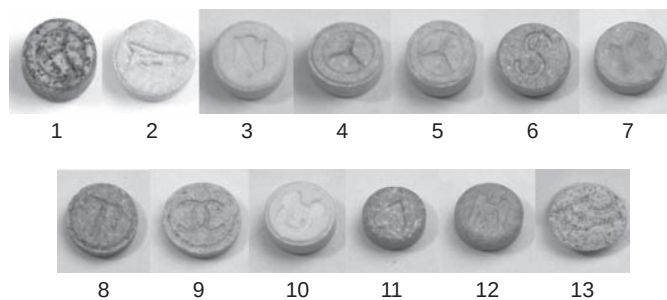


Figure 1. Ecstasy illicit pills with different colors and symbols whose MDMA values were measured in present study. Number under each pill indicates the number of pill.

squares) and internal standard (imipramine-containing squares) supernatants were measured vs blank at 285 and 251 nm, respectively. The spots of other probable constituents of ecstasy illicit pills did not interfere in densitometric or scraping off determination of MDMA values in this study. A calibration curve of the densitometric method was constructed by depicting ratios of peak areas of samples to internal standard vs concentration of respective MDMA standards, whereas a calibration curve of the so-called 'scraping off' method was constructed by depicting ratios of absorbances of samples (at 285 nm) to internal standard (at 251 nm) vs concentration of respective MDMA standards.

Statistical analysis

Statistical difference between MDMA values measured by densitometric and scraping off methods were determined by student *t*-test on the SPSS statistical package. Differences were regarded as significant at $p < 0.05$.

Results

R_f values for MDMA and imipramine were 0.33 and 0.48, respectively. The migration time was 90 min and migration distance was 17 cm.

Calibration curves of densitometric and scraping off methods are shown in Figures 3 and 4, respectively. The calibration curve of the densitometric method on the basis of peak area ratios ($r^2 = 0.99$, $p < 0.05$) was comparable to that of the scraping off method on the basis of absorbances ratios ($r^2 = 0.99$, $p < 0.05$), i.e. each standard curve showed good linearity over the range of MDMA concentrations examined. Furthermore, with a 95% confidence interval, a

significant correlation was observed for MDMA standard solutions between the two methods ($r^2 = 0.99$, $p < 0.05$) (Figure 5).

The MDMA values of ecstasy illicit pills using densitometric and scraping off methods are shown in Table 1. There is no significant difference between MDMA values measured by these two methods ($p < 0.05$). Furthermore, a significant correlation was observed for these values between two methods ($r^2 = 1$, $p < 0.05$) (Figure 6).

Limit of detection (LOD), considering signal-to-noise ratio (S/N) of 2, and limit of quantification (LOQ), regarding S/N of 10, of the densitometric and scraping off methods were 0.40 µg, 1.20 µg and 6.87 µg, 20.63 µg, respectively. Repeatabilities (within-laboratory error) of the densitometric and scraping off methods were 0.5% and 3.6%, respectively. Calibration sensitivities (slopes of calibration curves) of the densitometric and scraping off methods were 0.109 and 0.051, respectively.

Discussion

Procedures in which the substances are first separate with TLC (the so-called scraping off method), detected with a suitable method, eluted from the adsorbent and quantitatively determined, are methods for quantitative determination of drugs and poisons (Gänshirt 1969). Disadvantages in comparison with direct evaluation on the layer (such as densitometry) are the greater time necessary and a certain unrecoverable amount of substance. These disadvantages are however often accepted since the random errors are generally lower than in the direct methods. However previous studies have shown the efficiency of the 'scraping off' method in TLC (Gänshirt 1969;

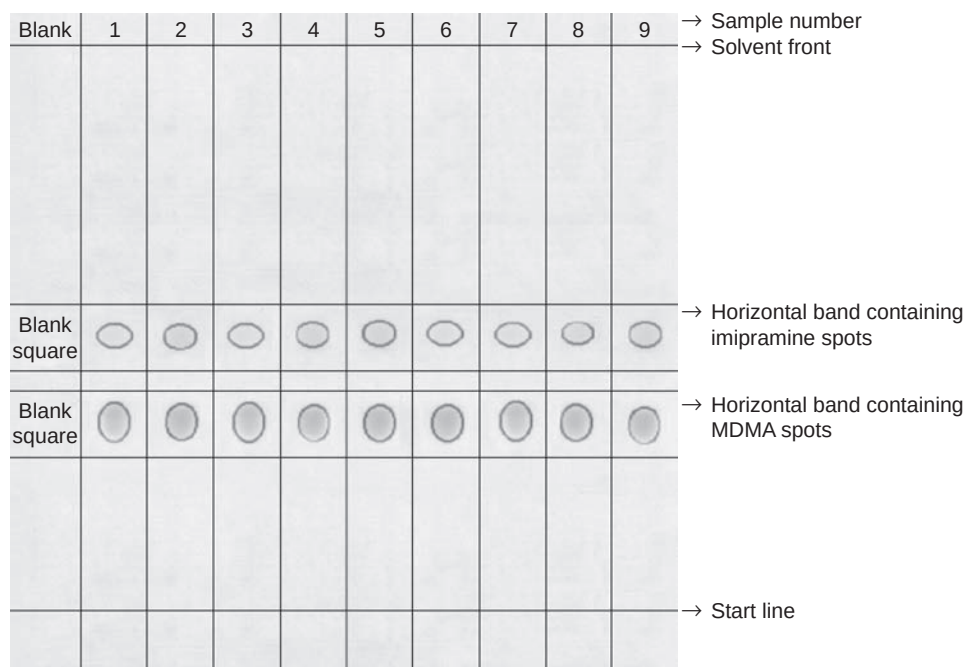


Figure 2. Schematic representation of preparation of TLC plates for scraping off method. Each horizontal band contains 2×2 cm spot-containing squares. One spot-free 2×2 cm square in each horizontal band was assigned to blank.

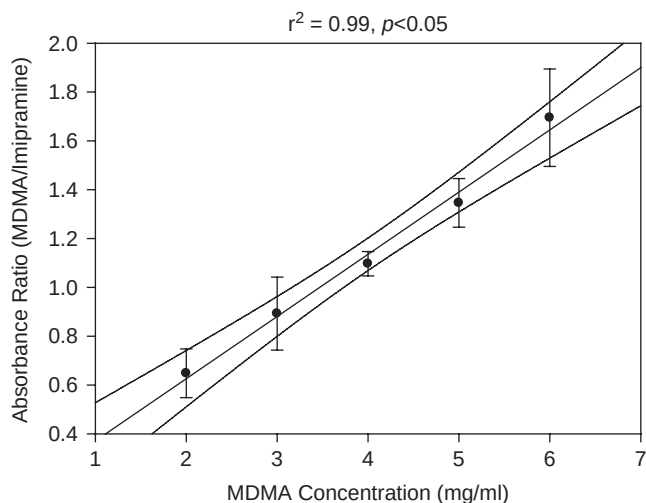


Figure 3. Calibration curve of scraping off method; 5 μ l of standard solutions containing 2–6 mg/ml MDMA hydrochloride standards and 0.5 mg/ml imipramine hydrochloride (internal standard) were spotted on TLC plate followed by elution, centrifugation, and absorbance measurement of each scraped off spot. Absorbances of MDMA and internal standard were measured at 285 nm and 251 nm, respectively.

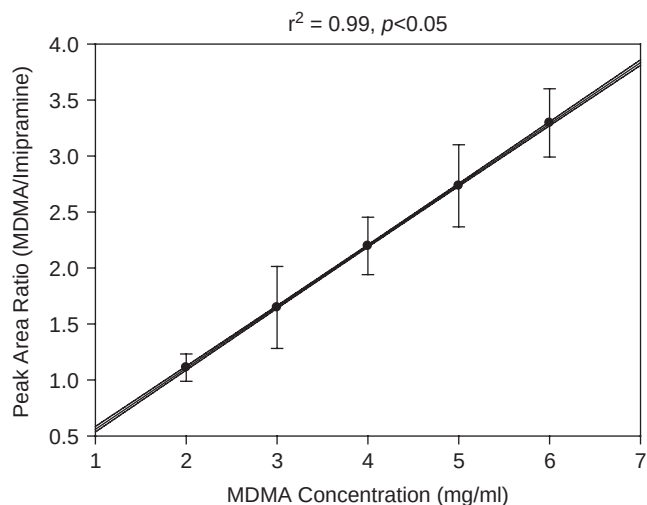


Figure 4. Calibration curve of densitometric method; 5 μ l of standard solutions containing 2–6 mg/ml MDMA hydrochloride standards and 0.5 mg/ml imipramine hydrochloride (internal standard) were spotted on TLC plate followed by densitometric quantification of MDMA content of spots in reflection mode at 285 nm.

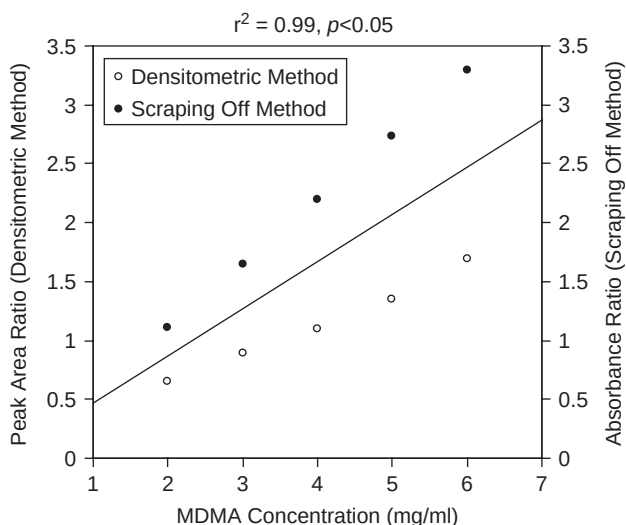


Figure 5. Linear relationship between densitometric and scraping off methods over the MDMA standard solutions examined.

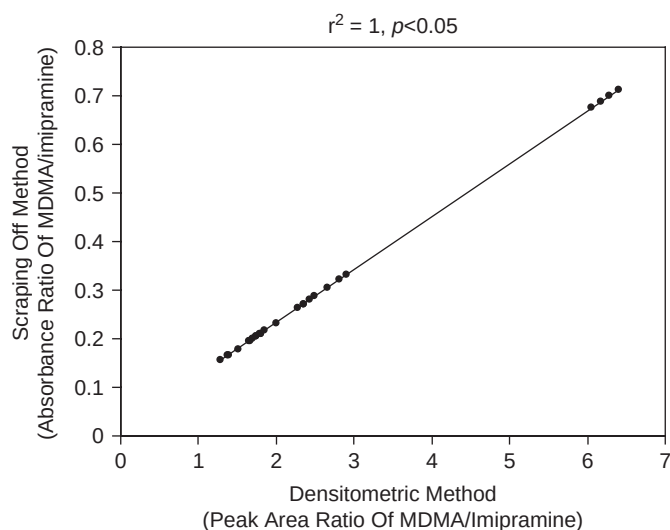


Figure 6. Correlation of MDMA contents measured by densitometric and scraping off methods.

Table 1. Characteristics and MDMA contents of examined ecstasy illicit pills.

Pill number	Weight of pill (gm)	Color of pill	MDMA value (mg) by densitometric method	MDMA value (mg) by 'scraping off' method
1	0.38	Cerulean with spotted black	43.5 $\pm$ 0.5	44.7 $\pm$ 0.7
2	0.30	Milky	24 $\pm$ 1.0	23.9 $\pm$ 0.9
3	0.22	Yellow	31.5 $\pm$ 1.5	31.9 $\pm$ 0.9
4	0.31	Green	31.5 $\pm$ 0.5	31.6 $\pm$ 1.3
5	0.29	Blue	31.5 $\pm$ 0.5	32.5 $\pm$ 0.5
6	0.24	Pink	51 $\pm$ 1.0	53.9 $\pm$ 0.9
7	0.31	Lotus	46 $\pm$ 1.0	48.2 $\pm$ 1.5
8	0.23	Jasper green	124.5 $\pm$ 2.5	122.2 $\pm$ 1.2
9	0.23	Blue	115.5 $\pm$ 1.5	117.8 $\pm$ 1.1
10	0.23	White	32.5 $\pm$ 0.5	30.9 $\pm$ 0.9
11	0.23	Red	32.5 $\pm$ 2.5	35.5 $\pm$ 1.5
12	0.24	Red	48.5 $\pm$ 1.5	43.1 $\pm$ 0.8
13	0.32	Red-white spotted	24 $\pm$ 2.0	26.1 $\pm$ 1.1

Shetab Boushehri et al. 2007). Comparative studies have shown that quantitative TLC procedures using elution of the separated substances have no greater range of error than other comparable analytical methods (Gänshirt 1969; Sherma 2003; Wall 2005).

Regular monitoring of MDMA contents of ecstasy illicit pills helps drug police and responsible authorities to find and pay more attention to high-dose ecstasy illicit pills and withdraw them from the illicit drug bazaar as soon as possible.

Table 1 shows that ecstasy illicit pill number 8 (green color and T symbol) had the highest value of MDMA (124.5 ± 2.5 mg), whereas pills number 2 (creamy color and shark symbol) and 13 (fish symbol and red-white spotted color) had the lowest values of MDMA (24 ± 1 mg and 24 ± 2 mg, respectively) within the studied ecstasy illicit pills. Furthermore, a significant correlation was observed for MDMA values between two methods ($r^2 = 1$, $p < 0.05$) (Figure 6).

Previous researches have shown that MDMA content of ecstasy illicit pills varies, but is generally in the range of 80–100 mg per tablet (King and McDermott 2004). The tablets normally carry a characteristic logo or imprint. These designs are not restricted to MDMA tablets, but may be found on amphetamine and other illicit products. In other words, the logo and other physical characteristics provide no reliable information on the drug content. Many hundreds of different impressions have been found (Miller 2002; King and McDermott 2004; Aronson 2005; Bigelow 2006).

The present study indicates that in spite of the aforementioned disadvantages, when a TLC Scanning Densitometer is not available the 'scraping off' method can be alternatively used and provides validated results.

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