Validation of a method for the quantitation of MDMA in seized materials by spectrophotometric method

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ABSTRACT

The identification and quantitation of MDMA in seized materials by police, gendarmerie, or customs is necessary for any legal case. Hence the results given must be rigorous, it is essential to work with validated procedures. Seeing that there is no reference method to validate the analysis of illicit substances in seized materials, a very rapid intern protocol was developed and validated according to the most recommended guidelines for analytical validation in Europe. This procedure helps verifying the linearity, limit of detection, limit of quantitationand precision of this method. Thus, twenty-sixtestedpills presentgood resultsforall of these parameters.

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KEYWORDS

MDMA;
Seized materials;
Analytical method;
UV-Vis spectrophotometry;
Validation.

INTRODUCTION

Although patented in 1914 as an appetite suppressant[1], 1-(3,4-methylenedioxyphenyl)-2-(N-methylamino)-propane (Figure 1), more commonly known as 3,4-methylenedioxymethylamphetamine (MDMA) or Ecstasy, is a commonly abused drug. In the 1980s, however, MDMA entered the lists of internationally controlled substances[2]. Actually, MDMA is predominantly a "club drug" and is commonly used at rave parties where it was referred as the "love pill"[3].

The first tablet with MDMA was found in 1975, the second in 1976, and the next several years saw a gradual increase in the number of MDMA tablets[4]. Shulgin and Nichols[5] were the first to describe the psychopharmacology of the MDMA, a compound with "occasionally incidence in the illicit street mar-

ket".

MDMA is a synthetic drug with both psychedelic and stimulant effects that may last between 4 and 6 h. MDMA is usually taken in oral tablet form. The psychological effects of MDMA include confusion, depression, anxiety, sleeplessness, drug craving, and paranoia. Adverse physical effects include muscle tension, involuntary teeth clenching, nausea, blurred vision, feeling faint, tremors, rapid eye movement, and sweating or chills. The risk of dehydration and hyperthermia increased by the excessive physical activity of dancing makes this combination a possible lethal cocktail[2].

Presently, ecstasy is illicitly sold in tablet form. This drug is typically looks very professional with a great variability of colors and symbols. Like all the abuse drugs illegally commercialized, the contents of this tablets are much diversified once they don't have quality con-

trol of tablets produced.

The illicit manufacturing of this drug results in MDMA doses that may vary from 0 to 200 mg per tablet[6].

Seeing that the consumption of one to five pills in a rave party, the total variation of MDMA dose could be sufficiently high to cause an overdose.

Also to MDMA, other psychoactive compounds may be present like amphetamine, heroin, cocaine, and others.

MDMA tablets analysis could be used to determine the presence or absence of MDMA in tablets called ecstasy, the variability of the contents and the dangerous chemical associations made on tablet's production by drug dealers on the same tablet. Certainly, tablets with similar physical appearances may have different chemical compositions.

Because of the growing abuse of this substance, a number of analytical methods such as immunoassays[7-11], gas chromatography (GC)[7-14], liquid chromatography (LC)[14-22], capillary electrophoresis (CE)[20,23,24] and more recently high-performance liquid chromatography (HPLC)[25,26] have been developed for its determination.

The purpose of this study was to develop and validate a very rapid, simple and sensitive method to characterize ecstasy tablets, seized in Morocco, especially with regard to MDMA contents variations. The results show that the method is applicable by the police.

METHODOLOGY

Reagents and samples

Analytical standard MDMA was obtained from LGC Standards France. Twenty-six samples of ecstasy tablets were provided by Moroccan Civil Police. Distilled water was used to prepare all solutions.

Physical analysis

Ecstasy tablets were separated by physical characteristics, color, logo, shape and mass prior to analysis.

Sample preparation

Ecstasy tablets were crushed to obtain a fine powder. 10 mg of each sample was dissolved in 10 mL of distilled water. The tubes containing the solutions were
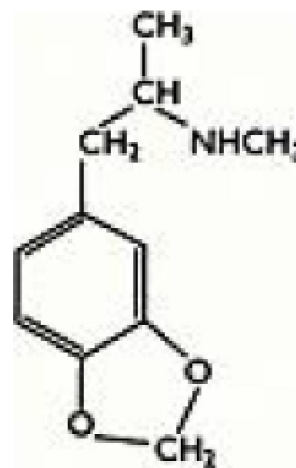

Figure 1 : Chemical structure of MDMA

stayed in ultrasound for 15 min at 40°C. After centrifugation, filtration and dilution a concentration of 100 μg/ml were prepared for each sample. The solutions were then read at selected wavelength.

Spectrophotometric analysis

Instrument used, for spectrum and absorbance measurements, was an UV-3100PC spectrophotometer with a pair of 1 cm matched quartz cells.

Validation of spectrophotometric method

The method was validated to comply with specified requirements using the most recommended guidelines for analytical validation in Europe[27,28], including the most widely applied analytical-performance characteristics such as linearity, limit of detection (LOD) and quantitation (LOQ) and precision.

Calibration curves

The calibration curve of MDMA (reference curve) was made using appropriate amounts of the MDMA standard diluted with distilled water to reach 10, 20, 50, 80 and 100 μg/mL MDMA concentrations.

Limit of detection and of quantification

The limit of detection (LOD) was set as the minimum compound concentration that could be detected with an acceptable level of precision. LOD samples were analyzed as unknown samples in ten replicates.

The limit of quantification (LOQ) was set as the last point of calibration curve (1.35 μg/mL) that presented an acceptable level of precision.

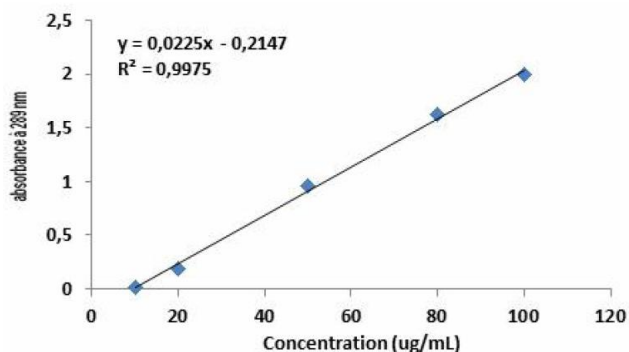**Figure 2 : Some ecstasy tablets seized and analyzed; Tablets show different colors and logos**

Figure 3 : Linear regression analysis of the calibration curve of MDMA in UV-Vis spectrophotometer. Each point is the mean ± SD of three experiments

TABLE 1 : ANOVA table

SS	df	Variance	F	$F_{critical}$	p-value	α
8.963818	1	8.963818	F=92410494.85	4.96	1.000	0.05
$4.03\ 10^{-6}$	3	$1.34333\ 10^{-6}$	$F_{nl} = 1.24633$	3.71	3.000	0.05
$9.7\ 10^{-7}$	10	$9.7\ 10^{-8}$				
8.963823	14					

TABLE 2 : Limit of detection and limit of quantification data

Number (n)	10
Mean (d)	2.54293
Standard Deviation (Sd)	0.1352983
CV %	5.32
Precision	82.02
LDM	0.406
LQM	1.35
R (Ratio of Conformity)	6.26

Precision

The precision of the method was tested in terms of replicability and repeatability.

Replicability result indicates the precision under the same operating conditions (same analyst, same apparatus and same day) over a short interval of time.

To evaluate replicability, concentrations used in the calibration curve were analyzed in ten repli-

TABLE 3 : Results of precision study

	Repeatability		Replicability
	1st day	2nd day	
Number (n)	10	10	10
Mean (d)	48.2531	49.05252	50
Standard Deviation (Sd)	0.0264673	0.056026	0.0168736
CV %	0.054851	0.11421617	0.03356
Precision	96.38%	98.068%	99.45%
t_{crit} (95;9)	2.262	2.262	
Error	3.62	1.931562	
Confidence Interval	48.253 ± 0.019	49.288 ± 0.196	
Mean of means	48.65281		
Mean of Standard deviation	0.04124665		
CV_{FI} %	$8.47775\ 10^{-4}$		

cates. Precision was determined as the %CV (coefficient of variation) of the MDMA concentration calculated for each of the ten replicates.

Repeatability result indicates the precision under the following operating conditions: same analyst, same apparatus and different days.

To evaluate repeatability, concentrations used in the calibration curve were analyzed in ten replicates on two different days. Precision was determined as the %CV of the MDMA concentration calculated for each of the ten replicates.

RESULTS

Identification of physical characteristics of illicit ecstasy tablets

Figure 2 shows some examples of tablets analyzed in this study. The ecstasy tablets had a large variety of physical characteristics. The tablets were embossed with logos (eg. Mitsubischi, Cherry, Star, Nike, Chanel, Crocodile, Champignon, Crushed, Heart) and dyed

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TABLE 4 : Characteristics and quantitative results of seized ecstasy tablets

N°	Logo	Color	Mass (mg)	MDMA content per tablet (mg)
1	-	yellow	206.1	40.29
2	Y	blue	253.8	101.14
3	Mitsubischi	yellow	298	161.34
4	-	blue	230	69.67
5	-	green	300	30.75
6	-	white	290	42.45
7	-	white	10	3.3
8	Cherry	white	220	82.21
9	Star	Orange	350	158.39
10	Y	blue	320	180.40
11	Nike	yellow	330	140.46
12	-	white	230	112.5
13	Chanel	pink	200	76.78
14	R	blue	240	106.86
15	Crocodile	blue	320	47.93
16	Crocodile	white	240	73.08
17	Nike	blue	180	76.32
18	R	blue	340	145.14
19	Champignon	yellow	288	116.84
20	B	yellow	210	96.49
21	Crushed	yellow	780	165.9
22	Champignon	yellow	288	128.25
23	Champignon	yellow	290	112.11
24	Champignon	yellow	290	133.31
25	Heart	blue	136	15.40
26	-	Orange	261	186.19

with various colors.

Consequently, general physical characteristics have shown no relationship with their inherent chemical contents. This is important information for police as it is not possible to associate a lot of tablets with a local drug dealer only by the physical appearance alone.

Validation of analytic method

The linearity of the calibration curve was confirmed over the MDMA concentration range (10-100 µgD mL) as shown in Figure 3. Typical regression equation for reference calibration curve was y = 0.022x - 0.214 (r^2=0.997).

To compare the results of three repetitions, Fischer test was used to validate the linearity range (TABLE 1). F_{nl} ratio is less than the critical value of F corre-

sponding to Fisher variable (0.05,3,10). Then, the results show that the linear range is validated. F_l ratio is greater than the critical value of F corresponding to Fisher variable (0.05,1,10). So, the regression model is acceptable.

The LOD (TABLE 2), defined as the lowest detectable concentration on the calibration curve where both accuracy and precision should be within the maximum tolerable CV of 5.32%, was deemed to be 0.406 ?gD mL. The LOD concentration was assayed in ten replicates. This LOD is adequate for the analysis of forensic samples, as this value falls within the concentration range of MDMA in many ecstasy tablets analyzed. Furthermore, Ratio of Conformity (6.26) is between 4 and 10[27], so LOD is validated.

The results of precision evaluation were found to be satisfactory and are reported in TABLE 3. The standard deviations and the percentage recoveries (not more than 2%) indicate good precision of the method.

Quantitative analysis of MDMA in ecstasy tablets

Twenty-six real forensic samples of ecstasy tablets from different batches containing MDMA were used in the quantification analysis.

The results had shown great variation in MDMA contents among the samples, from 3.3-186.19 mg/tablet. TABLE 4 shows the quantitative results of ecstasy tablets.

DISCUSSION

The proposed method for the quantitative analysis of MDMA in ecstasy tablets is sufficiently precise.

Chemical analyses of these tablets proved that some with similar physical appearance had different chemical compositions. They provide the variations in MDMA concentrations used in ecstasy tablets. But, that does not prevent different logos and colors may have the same composition.

In 1998, Handy declared: "It seems that the probability on an ecstasy tablet present only MDMA in its composition decreases as the popularity of the drug raises up"[29].

Although tablets with an association between MDMA and others psychoactive compounds may be

correspond to only a minority of the tablets, these associations could be dangerous.

An additional toxicological relevant feature of our study was the large variation of MDMA contents in different ecstasy tablets seized in Morocco. The tablets had MDMA concentrations varying from 3.3-186.19 mg/tablet.

Considering the great variation found in this study, we could suppose that the amount of tablets ingested during a rave party has also an enormous variation. This is most dangerous situation for the ecstasy abuser. The use of 10 tablets of sample 7, for example, during a long rave party corresponds only to 17.72% of MDMA dose presents in a single tablet of ecstasy sample 26.

This is particularly dangerous, because drug dealers often claim ecstasy tablets have the same composition. To the abuser, the control of ecstasy doses occurs in terms of number of tablets. In the previously example, the consumption of 10 tablets of ecstasy sample 7 of our study could give the false impression to the abuser of a secure dose with the ingestion of this number of ecstasy tablets. However, in other occasion, if the ecstasy supplier changes and sells a tablet with the chemical composition of ecstasy sample 26 in our study, the consumption of the same 10 tablets could give an MDMA blood level in the range of serious toxicity or fatality (from 0.5 mg/L to 10 mg/L).

CONCLUSION

A simple and rapid spectrophotometric method for determination of MDMA was developed and validated.

We have shown great variability in MDMA amounts in ecstasy tablets seized in Morocco, and the potential toxicity associated to the sum of this variation and the pattern of ecstasy abuse. Due to the usually unknown composition of the tablets, consumers are not aware of the quality and quantity of MDMA in ecstasy tablets, and due to their unpredictable effects, consumption of these drugs provides a considerable risk of severe intoxication.

Finally, the method is applicable by the police for quantitation of MDMA in tablets and powders.

REFERENCES

[1] E.Merck; VerfahrenzurDarstellung von Alkyloxyaryl, Dialyloxaryl- und Alkylenedioxy-arylaminopropanenbzw, derenAmstickstoffMonoalkyliertenDerivaten. German Patent, 274-350 **(1914)**.

[2] Office of National Drug Policy; MDMA (ecstasy), Microgram, **139(6)**, 149-56 **(2002)**.

[3] B.Jackson, A.Jr.Reed; JAMA, **211**, 830 **(1970)**.

[4] C.L.Renfroe; J.Psychoactive Drugs, **18**, 363 **(1986)**.

[5] A.T.Shulgin, D.E.Nichols; The pharmacology of hallucinogens. Characteristics of thress new psychotomimetics, (1stedn), Pergamon Press; New York, USA, **(1978)**.

[6] A.C.Parrott; Psychopharmacology (Berl), **173**, 234 **(2004)**.

[7] A.Dasgupta, S.Saldana, G.Kinnaman, M.Smith, K.Johansen; Clin. Chem., **39**, 104**(1993)**.

[8] A.Poklis, K.V.Hall, R.A.Eddleton, R.L.Fitzgerald, J.J.Saady, S.C.Bogema; ForensicSci.Int., **59**, 63 **(1993)**.

[9] P.Kintz, V.Cirimele, A.Tracqui, P.Mangin; J.Chromatogr.B, **670**, 162 **(1995)**.

[10] H.K.Ensslin, K.A.Kovar, H.H.Maurer; J.Chromatogr.B, **683**, 189 **(1996)**.

[11] G.W.Kunsman, B.Levine, J.J.Kulman, R.L.Jones, R.O.Hughes, C.I.Fujiyama, M.L.Smith; J.Anal.Toxicol., **20**, 517 **(1996)**.

[12] B.K.Gan, D.Baugh, R.H.Liu, A.S.Walia; J.ForensicSci., **36**, 1331 **(1991)**.

[13] P.Lillsunde, T.Korte; J.Anal.Toxicol., **15**, 71 **(1991)**.

[14] H.J.Helmin, K.Bracher, D.Bourquin, D.Voalanthen, R.Brenneisen, J.Styk; J.Anal.Toxicol., **20**, 432 **(1996)**.

[15] F.T.Jr.Noggle, C.R.Clark, A.K.Valaer, J.DeRuiter; J.Chromatogr.Sci., **26**, 410 **(1988)**.

[16] F.T.Jr.Noggle, C.R.Clark, J.DeRuiter; J.Chromatogr.Sci., **27**, 240 **(1989)**.

[17] E.R.Garett, K.Seyda, P.Marroum; Acta Pharm.Nord., **3**, 9 **(1991)**.

[18] F.T.Jr.Noggle, C.R.Clark, S.Andurkar, J.DeRuiter; J.Chromatogr.Sci., **29**, 103 **(1991)**.

[19] H.J.Helmin, R.Brenneisen; J.Chromatogr., **593**, 87 **(1992)**.

[20] R.E.Michel, A.B.Rege, W.J.George; J.Neurosci., **50**, 61**(1993)**.

[21] L.Tedeschi, G.Frison, R.Giorgetti, S.D.Ferrara; Int.J.Leg. Med., **105**, 265 **(1993)**.

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[22] M.Longo, C.Martines, L.Rolandi, A.Cavallaro; J.Liq.Chromatogr., **17**, 649 **(1994)**.

[23] M.Krogh, S.Brekke, F.Tonnesen, K.E. Rasmussen; J.Chromatogr. A, **674**, 235 **(1994)**.

[24] L.S.Lurie; in J.A. Adamovics (Editor), Analysis of Addictive and Misused Drugs, Marcel Dekker; New York, Basel, **(1995)**.

[25] F.Sadeghipour, J.L.Veuthey; J.Chromatogr.A, **787**, 137 **(1997)**.

[26] I.B.Müller, C.N.Windberg; J.Chromatogr. Sci., **43**, 434 **(2005)**.

[27] The Commission of the European communities. Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation,Off.J.Eur.Comm., **L221**, 8-36 **(2002)**.

[28] Eurachem; CITAC: Quantifying Uncertainty in Analytical Measurement, 2nd ed., http://www.eurachem.ul.pt/guides/ QUAM2000-1.pdf **(2000)**.

[29] C.Handy, R.Pates, A.Barroweliff; J.Subst Use, **3**, 82 **(1998)**.