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LC-MS/MS Analysis of MDMA in Ecstasy Tablets in Morocco

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Abstract

Ecstasy is a popular name designated for 3,4-methylenedioxymethamphetamine (MDMA). It displays effects related to amphetamine-type drugs and a set of distinctive effects like restlessness, well-being, and others effects. The aim of this work is to analyze ecstasy tablets seized in Morocco; studying their MDMA contents and their MDMA contents variations. We collected 12 batches of samples seized by Morocco Police that were analyzed by liquid chromatography coupled to tandem Mass Spectrometry to determine MDMA concentration presents in these tablets. The method was also validated in the following parameters: linearity, limits of detection and of quantification, precision and accuracy, to permit the MDMA quantification. A great variability among ecstasy tablets compositions was detected and the toxicological features were discussed.

Keywords: MDMA; Ecstasy; Quantification; LC-MS/MS; Validation

Introduction

Ecstasy is a street name originally designated for 3,4-methylenedioxymethylamphetamine (MDMA) (Figure 1) [1]. It is commonly used at rave parties for its psychedelic and stimulant effects that may last between 4 and 6 h [2]. This drug is generally sold in the form of tablets with good appearance, with great variety of colors, shapes, and sizes and printed with various types of pictures and logos [3-5].

Tablets sold as ecstasy primarily contain MDMA, although others amphetamine type stimulants (ATS) such as methylenedioxyamphetamine (MDA), methylenedioxyethamphetamine (MDEA) are present [6]. In addition, other psychoactive compounds may be present such as cocaine, heroin, caffeine, lidocaine and others [4]. Indeed, tablets with similar physical appearances may have different chemical compositions and the contents of MDMA in ecstasy tablets vary from 0 to 200.00 mg per tablet [7].

Due to the usually unknown composition of these tablets, consumers are not aware of the quality and quantity of MDMA in ecstasy tablets and consumption of these drugs provides a considerable risk of severe intoxication.

Then, ecstasy tablets analysis could be used to detect the presence of MDMA and dangerous chemical associations and to determine the variability of the contents. In fact, in the 1980s, ecstasy entered the lists of internationally controlled products [8].

Several analytical methods have been developed as ecstasy tablets analysis like immunoassays [9-13], gas chromatography (GC) [9-16], liquid chromatography (LC) [16-24], capillary electrophoresis (CE) [22,25,26], high-performance liquid chromatography (HPLC) [27,28], liquid chromatography coupled to mass spectrometer (LC-MS) [3] and gas chromatography coupled to mass spectrometer (GC-MS) [4].

Recently, we have characterized twenty six ecstasy tablets, seized in Morocco, with regard to MDMA contents variations using UV-Vis spectrophotometry [5]. We have shown great variability in MDMA amounts in ecstasy tablets seized in Morocco. Furthermore, our data also demonstrate that validated analytical procedure is applicable by the police for quantitation of MDMA in tablets and powders.

The present study develops a more precise, selective and sensitive method for the quantification of MDMA in ecstasy tablets. This method was validated using the most recommended guidelines for analytical validation in Europe [29,30]. The results show that the method is applicable by the scientific police.

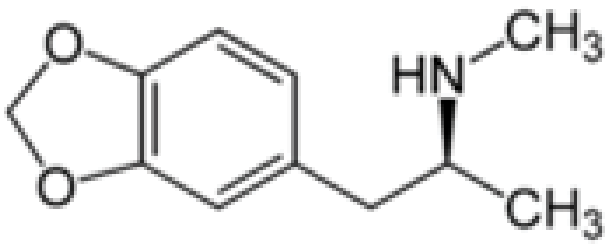

Figure 1: Chemical structure of 3,4-methylenedioxymethamphetamine.

Materials and Methods

Reagents and samples

Analytical standard MDMA was obtained from LGC Standards France. Twelve samples of ecstasy tablets were provided by Moroccan Civil Police. Ultrapure water of HPLC was prepared using dispositive labconco (Serial No: 130982243F).

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Standard solutions

The stock solution of MDMA (1.00 mg/mL) was prepared by dissolving the appropriate quantities in methanol. Two working solutions 10.00 µg/mL and 100.00 ng/mL of MDMA were prepared by dilutions in methanol. These solutions were used to prepare calibration standards with the concentrations (10, 20, 50, 80, and 100 ng/mL) in methanol.

As quality control (QC) sample three concentrations (15, 30, and 60 ng/mL) from calibration range was used. The resultant of calibration standards and quality control were stored at -20°C until analysis.

Sample preparation

Ecstasy tablets were crushed to obtain a fine powder. 10.00 mg of each sample was dissolved in 10.00 mL of distilled water. The tubes containing the solutions were stayed in ultrasound for 15 min at 40°C. After centrifugation, filtration and dilution a concentration of 100.00 µg/ml were prepared for each sample in methanol.

Apparatus

An HPLC MS/MS QTRAP 3200 system equipped with a Perkin Elmer Series 200 chromatographic pump and a Perkin Elmer Series 200 auto sampler (PE Sciex, Concord, Canada) was used for direct ESI- MS/MS determinations, operating in positive ion and multiple reactions monitoring (MRM) acquisition mode.

The chromatograms were acquired using the analyst software (version1.4.1).

Procedure

A liquid chromatographic system consisted of a solvent delivery system (pumps identified as A and B) and an autosampler was used. Chromatographic separation of the analytes was achieved on PHENOMENEX column (50 x 2, 2.00 mm; 4.00 µm) equipped with a pre-column filter. For preparation of mobile phase, 63 g of ammonium format was dissolved in 1 L water HPLC grade. Mobile phase A: 2.00 mL of 1.00 M ammonium format solution and 2.00 mL formic acid in 996.00 mL of water HPLC grade. Mobile phase B: 2.00 mL of 1.00 M ammonium format solution and 2.00 mL formic acid in 996.00 mL of acetonitrile HPLC grade. Eluents were sonicated before use. The flow rate was set at 0.50 mL/mn. The used gradient program time was presented in Table 1. The separation started with 10% B for 3 min, then B increased at 90% for 2 min, initial condition were achieved within 0.5min and maintained for 2.5 min before the next injection. The total run time was 8 mn and the column temperature was maintained at 40°C. The auto sampler injection needle was washed with methanol/water (1, v/v) after each injection. The mass spectrometer was run in positive ion ESI mode using multiple-reaction monitoring (MRM) to monitor the mass transitions. The ion spray voltage and the source temperature were set at 5500 V and 600°C, respectively. Nitrogen gas was used as the curtain gas and set at 10. The ion source gas 1 and 2 were set at 50. A summary of the ion transitions, declustering potentials, collision energies, and collision cell exit potentials for all range assays were presented in Table 2 [31]. The concentration of MDMA was determined automatically by the instrument data system using peak areas and external standard method.

Method validation

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), precision and accuracy.

Total time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0	0.50	80	20
3	0.50	10	90
5	0.50	10	90
5.5	0.50	80	20
8	0.50	80	20

Table 1: Program time.

Analyte	Q1mass (amu)	Q3mass (amu)	DP (V)	CE (V)	CXP (V)	EP (V)
MDMA	194.1	105.1	23	35	5	5

Table 2: Optimized mass spectrometric parameters [31]. Q1 mass, parent ion m/z; Q3 mass, daughter ion m/z ; DP, Declustering potentials; CE, Collision energies; CXP, cell exit potentials; EP, entrance potential(Q0 less).

Linearity: The calibration curve of MDMA was made using appropriate amounts of the MDMA standard diluted with methanol to reach 10, 20, 50, 80 and 100 ng/mL MDMA concentrations. The linearity of each calibration curves was determined by the peak area ratio (y) versus nominal concentration (x) of MDMA. The calibration curve was constructed by weighted (1/y) least squares linear regression.

Limit of detection and of quantification: LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It corresponds to 3 times the standard deviation of five replicates of samples.

LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy (less than 20% [23]). It corresponds to 10 times the standard deviation of five replicates of samples.

Precision and accuracy: The intra-day precision (expressed as coefficient of variation) and accuracy (expressed as relative difference between obtained and theoretical concentrations (%)) were determined by analyzing, on the same day, six replicates of three different samples from each standard (15, 30 and 60 ng/mL).

The inter-day precision was evaluated by repeating the intra-day precision study in 3 different days.

Results and Discussion

Validation of analytic method

The linearity of the calibration curve was confirmed over the MDMA concentration range. Coefficients of determination (r) are greater than 0.995 for the calibration curves of MDMA.

To verify the selectivity of the method are made with injections of low concentration standard solution until a well-resolved peak (Figure 2). No interfering peaks were observed at the retention times, confirming the good selectivity of the method.

The LOQ and LOD of MDMA were estimated following EMEA criteria [17]. LOD, defined as the signal-to-noise ratio ($\frac{S}{N}, 3$), is equal to 6.60 ng/ml and LOQ, defined as the signal-to-noise ratio ($\frac{S}{N}, 10$), is equal to 22.00 ng/ml.

The CV (%) values calculated for intra- and inter-day precision studies of MDMA did not exceed 15%. Thus, the developed method is considered precise for these analytes [25].

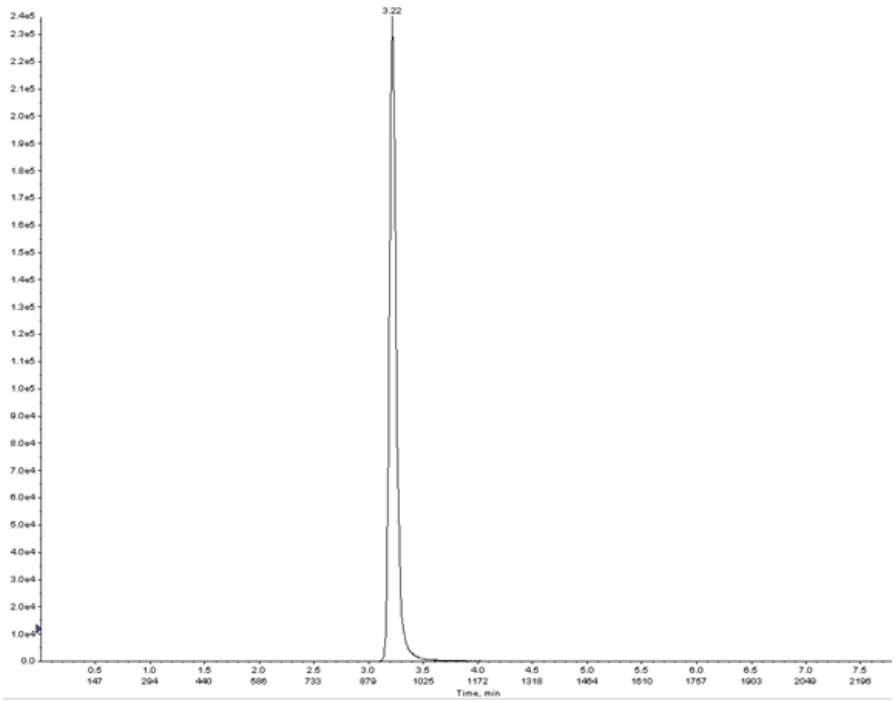

Figure 2: Chromatogram of a standard solution of MDMA with the concentration 50.00 ng/ml.

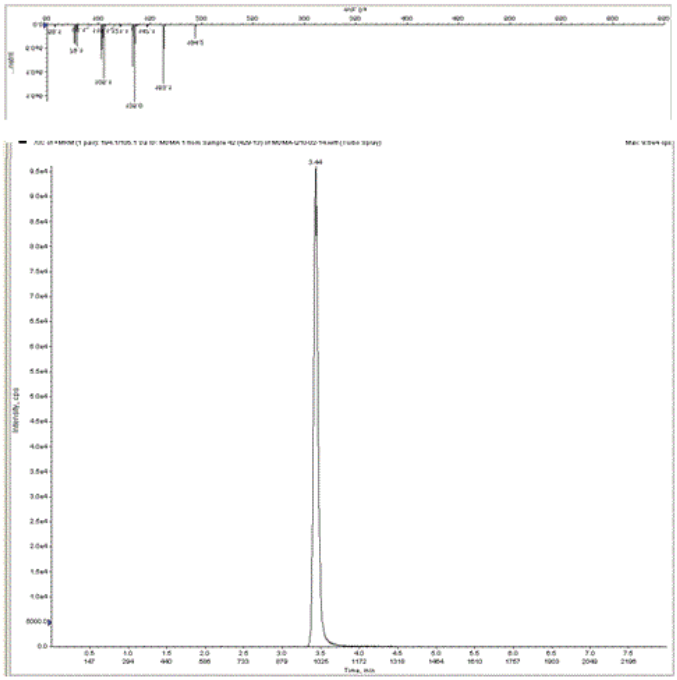

Figure 3: Representative chromatographic and mass spectra of MDMA, in tablet 2 of Table 3, analyzed using our quantitate method.

No	Picture	Logo	Color	Mass (mg)	MDMA content per tablet (mg)
1		Y	Bleu	253.8	161.3
2		Mitsubischi	Yellow	298.0	227.9
3		Heart	Blue	136.0	1.4
4		Nike	Blue	180.0	172.4
5		Crocodile	White	240.0	75.3
6		Cherry	White	220.0	182.3
7		Star	Orange	350.0	262.1
8		Y	Blue	320.0	251.6
9		Nike	Yellow	330.0	260.1
10		Chanel	Pink	200.0	80.1
11		R	Blue	240.0	120.8
12		Crocodile	Blue	320.0	161.1

Table 3: Characteristics and quantitative results of seized ecstasy tablets.

The % of accuracy varies from 98.7% to 109%, which is less than 120%. The calculated accuracy error is less than 5. So, it is considered insignificant [24]. These results show the high quality of quantitative results achievable with this method.

In order to reduce the total time of MDMA quantification in ecstasy tablets, the developed method excluded the initial basic extraction step generally described in the literature [32-37]. In comparison to other methods described in the literature [7,38,39], it is a low-cost preparative technique and a rapid technique, demanding only basic laboratory equipments and glassware. The method also proved to be effective for detecting the presence of contaminants (caffeine, lidocaine, and others) and other amphetamine derivatives in ecstasy street samples. At last, the proposed method for the quantitative analysis of MDMA in ecstasy tablets is sufficiently precise and accurate.

Quantitative analysis of MDMA in ecstasy tablets

Twelve forensic samples of ecstasy tablets were used in the quantification analysis. Figure 3 presents a chromatogram and mass spectra of MDMA in a tablet using our quantitate method. Table 3 shows the quantitative results of ecstasy tablets which show different colors, logos and sizes. These chemical analyses had shown great variation in MDMA contents among the samples, from 1.4-262.1 mg/tablet.

On the other hand, these results proved that some with similar physical appearance had different MDMA contents (samples 1 and 8 for example). Conversely, different logos and colors may have the same composition (samples 1 and 12 for example). This is particularly dangerous, because consumers often claim ecstasy tablets have the same composition. To them, the control of ecstasy doses occurs in terms of number of tablets.

However, the use of 10 tablets of sample 3, for example, during a rave party corresponds only to 5.57% of MDMA dose presents in a single tablet of ecstasy sample 7.

Therefore, the consumption of 10 tablets of ecstasy sample 7 could give an overdose of MDMA and others amphetamine type stimulants (ATS).

Conclusion

A rapid and low-cost method for quantitation of MDMA, in ecstasy pills, was developed and validated. The results showed that the method is selective. The linearity and accuracy were confirmed, and the precision was acceptable. The method may be used, by the scientific police, for quantitation of MDMA in pills and powders.

This study had provided MDMA contents in ecstasy tablets seized in Morocco. An enormous variability among ecstasy pills compositions was detected.

Acknowledgement

This work is dedicated to the memory of Professor M'Hamed CHARROUF, died on November 29th, 2014.

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