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 quantitation and precision of this method. Thus, twenty-six tested pills present good results for all 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 suppressant1, 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 substances2. 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 tablets4. Shulgin and Nichols5 were the first to describe the psychopharmacology of the MDMA, a compound with “occasionally incidence in the illicit street market”.

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 cocktail2.

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)\(^14-21\), 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 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.
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 replicates. 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. Mitsubishi, Cherry, Star, Nike, Chanel, Crocodile, Champignon, Crushed, Heart) and dyed...
Validation of a method for the quantitation of MDMA in seized materials

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TABLE 4: Characteristics and quantitative results of seized ecstasy tablets

<table>
<thead>
<tr>
<th>No.</th>
<th>Logo</th>
<th>Color</th>
<th>Mass (mg)</th>
<th>MDMA content per tablet (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>yellow</td>
<td>206.1</td>
<td>40.29</td>
</tr>
<tr>
<td>2</td>
<td>Y</td>
<td>blue</td>
<td>253.8</td>
<td>101.14</td>
</tr>
<tr>
<td>3</td>
<td>Mitsubishi</td>
<td>yellow</td>
<td>298</td>
<td>161.34</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>blue</td>
<td>230</td>
<td>69.67</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>green</td>
<td>300</td>
<td>30.75</td>
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<tr>
<td>6</td>
<td>-</td>
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<td>290</td>
<td>42.45</td>
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<tr>
<td>7</td>
<td>-</td>
<td>white</td>
<td>10</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>Cherry</td>
<td>white</td>
<td>220</td>
<td>82.21</td>
</tr>
<tr>
<td>9</td>
<td>Star</td>
<td>Orange</td>
<td>350</td>
<td>158.39</td>
</tr>
<tr>
<td>10</td>
<td>Y</td>
<td>blue</td>
<td>320</td>
<td>180.40</td>
</tr>
<tr>
<td>11</td>
<td>Nike</td>
<td>yellow</td>
<td>330</td>
<td>140.46</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>white</td>
<td>230</td>
<td>112.5</td>
</tr>
<tr>
<td>13</td>
<td>Chanel</td>
<td>pink</td>
<td>200</td>
<td>76.78</td>
</tr>
<tr>
<td>14</td>
<td>R</td>
<td>blue</td>
<td>240</td>
<td>106.86</td>
</tr>
<tr>
<td>15</td>
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</tr>
<tr>
<td>16</td>
<td>Crocodile</td>
<td>white</td>
<td>240</td>
<td>73.08</td>
</tr>
<tr>
<td>17</td>
<td>Nike</td>
<td>blue</td>
<td>180</td>
<td>76.32</td>
</tr>
<tr>
<td>18</td>
<td>R</td>
<td>blue</td>
<td>340</td>
<td>145.14</td>
</tr>
<tr>
<td>19</td>
<td>Champignon</td>
<td>yellow</td>
<td>288</td>
<td>116.84</td>
</tr>
<tr>
<td>20</td>
<td>B</td>
<td>yellow</td>
<td>210</td>
<td>96.49</td>
</tr>
<tr>
<td>21</td>
<td>Crushed</td>
<td>yellow</td>
<td>780</td>
<td>165.9</td>
</tr>
<tr>
<td>22</td>
<td>Champignon</td>
<td>yellow</td>
<td>288</td>
<td>128.25</td>
</tr>
<tr>
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<td>Champignon</td>
<td>yellow</td>
<td>290</td>
<td>112.11</td>
</tr>
<tr>
<td>24</td>
<td>Champignon</td>
<td>yellow</td>
<td>290</td>
<td>133.31</td>
</tr>
<tr>
<td>25</td>
<td>Heart</td>
<td>blue</td>
<td>136</td>
<td>15.40</td>
</tr>
<tr>
<td>26</td>
<td>-</td>
<td>Orange</td>
<td>261</td>
<td>186.19</td>
</tr>
</tbody>
</table>

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 \( F \) corresponding to Fisher variable \((0.05,3,10)\). Then, the results show that the linear range is validated. \( F_{nl} \) 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


