The Development of LC/MS Methods for Determination of MDMA (Ecstasy) and Metabolites in Biological Samples

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Introduction

The use of certain classes of drugs known collectively as "club drugs" has been increasing worldwide. This term refers to drugs being used by young adults at all-night dance parties such as "raves" or "trances," dance clubs, and bars. Among the more popular drugs used for this purpose is 3,4-methylenedioxy-methamphetamine (MDMA, Ecstasy). The most common methods of analysis for this compound and its metabolites utilize GC or GC/MS analysis after cumbersome derivatization steps. In this presentation, we will demonstrate LC-MS methods for a much more rapid and straightforward determination of GHB and MDMA and metabolites in biological fluids.
MDMA (Ecstasy) and Metabolites

An LC/MS method was developed for determination of MDMA (3,4-methylenedioxymethamphetamine) and its metabolites (MDA, 3,4, methylenedioxyamphetamine and HMMA, N,a-dimethyl-(3-methoxy-4-hydroxybenzene) ethanamine in urine. A mixed-mode cation-exchange SPE cartridge (Oasis® MCX) was utilized for extraction and cleanup prior to LC-MS analysis. The concentration range investigated spanned from 0.10 to 20 µg/mL with recoveries ranging from 88-108% for all analytes. Complete resolution of MDMA, MDA and HMMA as well as the internal standard was accomplished in less than 10 minutes. The quantitation limits (LOQ) were: MDMA = 0.06 µg/mL; MDA = 0.12 µg/mL; and HMMA 0.05 µg/mL.
Ecstasy (MDMA) is a synthetic, psychoactive drug with both stimulant (amphetamine-like) and hallucinogenic (mescaline-like) properties.

Its chemical structure (3-4 methylenedioxyamphetamine) is similar to methamphetamine, methylenedioxyamphetamine (MDA), and mescaline.

MDMA is neurotoxic. In high doses it can cause a sharp increase in body temperature (malignant hyperthermia) leading to muscle breakdown and kidney and cardiovascular system failure.
Prepare Sample glucuronidase, pH 5.2 8 hrs @ 37\degree

SPE Procedure for MDMA and Metabolites
Oasis® MCX (30 mg Cartridge)
Mixed-Mode Cation Exchange

1. **Condition**
   - 1mL methanol/ 1 mL water

2. **Load**
   - 0.5 mL sample

3. **Wash #1**
   - 1 mL 0.1 N HCl

4. **Wash #2**
   - 1 mL methanol

5. **Re-Equilibrate**
   - 1 mL water

6. **Wash #3**
   - 1 mL 50:50 5% NH₄OH/methanol

7. **Elute**
   - 1 mL methanol (10% NH₄OH)

Dilute the extract with 2 mL of 20 mM ammonium bicarbonate buffer (pH 9.0) and inject 15 µL
LC-MS for Ecstasy and Metabolites
HPLC Conditions

Analysis at pH 9 allows good peak shape and maximum retention for basic compounds with no modifiers that can interfere with LC-MS analysis.

Do not try this with traditional silica based columns!
## LC-MS for Ecstasy and Metabolites

### MS Conditions

#### LC/MS System

Waters 2690 Separations
Module interfaced to a Waters/Micromass ZQ™ mass spectrometer

#### Acquisition parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary (kV)</td>
<td>3.00</td>
</tr>
<tr>
<td>Extractor (V)</td>
<td>3.00</td>
</tr>
<tr>
<td>RF Lens (V)</td>
<td>0.1</td>
</tr>
<tr>
<td>Source Temp (°C)</td>
<td>150</td>
</tr>
<tr>
<td>Desolvation Temp (°C)</td>
<td>350</td>
</tr>
<tr>
<td>Cone gas Flow (L/hr)</td>
<td>50</td>
</tr>
<tr>
<td>Delsovation Gas Flow</td>
<td>500</td>
</tr>
<tr>
<td>High Mass resolution</td>
<td>15.0</td>
</tr>
<tr>
<td>Low Mass Resolution</td>
<td>15.0</td>
</tr>
<tr>
<td>Ion Energy</td>
<td>0.1</td>
</tr>
<tr>
<td>Multiplier (V)</td>
<td>650</td>
</tr>
<tr>
<td>Interchannel delay</td>
<td>0.1 s</td>
</tr>
<tr>
<td>Span</td>
<td>0.1 Da</td>
</tr>
<tr>
<td>Dwell</td>
<td>0.3 s</td>
</tr>
</tbody>
</table>

#### Ion (m/z) Cone (V) Delay (s)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion (m/z)</th>
<th>Cone (V)</th>
<th>Delay (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>194.11</td>
<td>25.0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>163.08</td>
<td>37.5</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>135.00</td>
<td>55.0</td>
<td>0.05</td>
</tr>
<tr>
<td>HMMA</td>
<td>196.16</td>
<td>20.0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>165.08</td>
<td>37.5</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>137.00</td>
<td>55.0</td>
<td>0.05</td>
</tr>
<tr>
<td>MDA</td>
<td>180.10</td>
<td>20.0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>163.08</td>
<td>37.5</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>135.00</td>
<td>55.0</td>
<td>0.05</td>
</tr>
<tr>
<td>MDMA-D₅</td>
<td>199.20</td>
<td>25.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>165.10</td>
<td>35.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>137.00</td>
<td>55.0</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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Mass Spectrometry
Fragmentation of MDMA (ESI+)

The following fragmentation was observed from in-source collision induced dissociation (CID) of MDMA. Similar fragmentation patterns were observed for the metabolites.

\[
\begin{align*}
\text{m/z} &= 194 \\
\text{m/z} &= 163 \\
\text{m/z} &= 135
\end{align*}
\]
Why is LC-MS attractive? Compare Methodologies

• **GC-MS Method** (recovery 50 – 75%, 2 hours)
  – perform SPE
  – evaporate to dryness
  – derivatize with heptafluorobutyric anhydride
  – evaporate again to remove excess derivatizing agent
  – take up residue with solvent
  – inject

• **LC-MS Method** (recovery 85 – 95%, 20 minutes)
  – perform SPE
  – dilute with mobile phase
  – inject

Enforcement methods require mass-spectral confirmation of identity (two fragment ions recommended)
## LC-MS for Ecstasy and Metabolites

### Results (n = 12 for each level)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery (%)</th>
<th>Concentration (µg/mL)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>108.0</td>
<td>0.10</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>89.3</td>
<td>0.50</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>88.1</td>
<td>1.25</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>98.8</td>
<td>2.50</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>99.9</td>
<td>5.00</td>
<td>5.7</td>
</tr>
<tr>
<td>MDA</td>
<td>103.0</td>
<td>0.10</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>84.2</td>
<td>0.50</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>83.8</td>
<td>1.25</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>95.4</td>
<td>2.50</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>104.5</td>
<td>5.00</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>93.7</td>
<td>20.00</td>
<td>13.1</td>
</tr>
<tr>
<td>HMMA</td>
<td>90.5</td>
<td>0.04</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>88.1</td>
<td>0.25</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>84.8</td>
<td>0.50</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>94.8</td>
<td>1.00</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>2.00</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>97.9</td>
<td>8.00</td>
<td>11.4</td>
</tr>
</tbody>
</table>
Real Forensic Sample

DUI Case:

A 21-year-old male was arrested for driving under the influence (DUI) of drugs after being involved in an accident.

A plastic bag containing approximately 5 g ecstasy was found under the driver’s seat.

The subject’s urine tested positive for benzoylecgonine (cocaine), 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (cannabis), and MDMA (ecstasy).

The Police Lab found 11.3 µg/mL of MDMA by GC-MS.

At Waters, we found 12.6 µg/mL of MDMA by LC-MS.
Real Forensic Sample
LC-MS Analysis

HMMA 6.1 µg/mL

MDMA 12.6 µg/mL

MDA 0.7 µg/mL

ISTD 10 µg/mL

TIC

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Can the SPE Method be Used for GC Analysis?
Yes, with minor modifications.

**SPE**

**Condition**
1 mL methanol/1 mL water

**Load**
0.5 mL sample

**Wash #1**
1 mL 0.1 N HCl

**Wash #2**
1 mL methanol

**Re-Equilibrate**
1 mL water

**Wash #3**
1 mL 70:30 5% NH₄OH/methanol

**Elute**
1 mL methanol (10% NH₄OH)

**Derivatization**

Slowly evaporate the methanolic ammonia extract at room temperature using a nitrogen evaporator to a volume of 200-300 µL

Add 100 µL of 1% HCl in methanol to each sample and completely remove the solvent using the nitrogen evaporator set at 50°C

Add 150 µL of MBTFA, cap the vial, and mix well (derivatize for 45 minutes at 70°C)

Inject 1 µL onto the GC/NPD or GC/MS
GC/NPD Analysis
5 µg/mL Spiked Urine

Instrument: HP5890 Series II with NPD

Column: Restek, RTX 30 m, 0.32 mm I.D., 0.25 µm film thickness

Carrier Gas: Helium @ 0.8 mL/min.

Temperature Program: 15°C/min from 100°C to 280°C

Injection: 1 µL using a split-splitless mode

1: MDA
2: HMMA
3: MDMA
4: MDPA (ISTD)
Conclusions

- LC/MS is an attractive alternative to GC based methods for ecstasy and metabolites
  - low quantitation limits
  - high sample throughput
  - fast sample preparation
    - no derivatization required

- An effective, reproducible SPE procedure has been developed that is suitable for LC or GC based analytical methods

- The LC/MS method has been demonstrated to give comparable results to the standard GC/MS method