Synthetic Cathinone Stability in Urine Using LC/Q-TOF-MS

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DISCLOSURE

The authors have no commercial disclosure

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SYNTHETIC CATHINONE
BACKGROUND

• Derived from cathinone, a naturally occurring compound in the leaves of the Catha edulis shrub

• Synthetic cathinones synthesized for the same effects
  • Effects similar to methamphetamine and MDMA

• Available on the internet and in head shops with labels such as:
  “not for human consumption”
  “bath salts”
  “Plant food”
Desired Effects:
- stimulant and euphoric symptoms
  - Increased energy, mood enhancement, empathy, sociability, concentration, euphoria

Adverse Effects:
- neurological, cardiovascular, and psychopathological symptoms
  - Hallucinations, delusions, confusion, violence, homicidal tendencies, death

Antemortem Cases: Motor Vehicle Accidents & Driving While Impaired
Postmortem Cases: Overdose, Suicide, Homicide
GENERAL STRUCTURE OF CATHINONE

Phenethylamines

R groups represent positions that can be substituted to create various synthetic cathinones
IMPORTANCE OF STABILITY

- Understanding the stability of a drug in biological matrices in essential

- Condition and length of storage can affect drug concentration

- Specimens stored for days, weeks, or months prior to analysis

- Subjected to various conditions during collection and shipping process
CATHINONE INSTABILITY

Plant Material

- Unstable in oxygen rich conditions (Szendrei, 1980)
- Unstable in alkaline conditions (Szendrei, 1980; Berrang, 1982)
- Dimer formation (Berrang, 1982; Chappell, 2010)
- Best to air dry and refrigerate

Thermal Degradation

- Thermal degradation in GC-MS
  - Methcathinone (DeRuiter, 1994)
  - α-PVP (Tsujikawa, 2013)
  - 19 synthetic cathanones (Kerrigan, 2015)
BIOLOGICAL MATERIAL

- **Paul and Cole (2001):**
  - Cathinone/Methcathinone
  - Urine
  - -18°C and 4°C
  - 3 months
  - -18°C: stable for 2 months
  - 4°C: stable for 3 days, unstable for 3 months

- **Sorensen (2011):**
  - Methcathinone, Ethcathinone, Mephedrone, Flephedrone, Methedrone, Methylone, Butylone
  - Blood (pH 7.4 and 5.9)
  - 5°C and 20°C
  - 7 days
  - More stable in pH 5.9 and 5°C over 7 day period

- **Tsujikawa (2012):**
  - Methcathinone, Mephedrone, 3-FMC, 4-FMC, Ethcathinone
  - Aqueous pH solutions
  - More stable at acidic pH, decomposition rate dependent upon chemical structure
BIOLOGICAL MATERIAL

- **Johnson and Botch-Jones (2013):**
  - MDPV/Mephedrone
  - Blood, Plasma, Urine
  - -20°C, 4°C, 22°C
  - 14 days
  - -20°C: stable in 3 matrices
  - Mephedrone unstable at 4°C and 22°C

- **Soh and Elliott (2014):**
  - 4-MEC
  - Blood and Plasma
  - 20°C and 5°C
  - 7 days
  - Unstable at both temperature

- **Busardo (2016):**
  - Mephedrone
  - Antemortem & Postmortem Blood
  - -20°C, 4°C, 20°C
  - 6 months
  - Unstable at 4°C and 20°C by 3 months
  - Stable at -20°C
Previous stability studies have been reported, however...

No systematic and fully comprehensive study addressing synthetic cathinone stability in biological evidence

- 22 synthetic cathinones
- 1 biological matrices
- 4 temperatures
- >6 months

Comprehensive study assessing stability as it relates

1. pH
2. Concentration
3. Temperature
4. Storage time
5. Chemical properties
RESEARCH DESIGN

- Urine pH 8
  - 100 ng/mL
  - 1000 ng/mL

- Urine pH 4
  - 100 ng/mL
  - 1000 ng/mL
**LC/Q-TOF-MS CONDITIONS**

**Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS**

**LC Separation**
- **Poroshell 120 EC-C18 Column** (2.1x100mm, 2.7 µm particle size)
- **Mobile Phase A**: 0.1% FA in diH$_2$O
- **Mobile Phase B**: 0.1% FA in ACN
- Flow Rate: 0.40 mL/min
- LC Gradient:
  - 96% A to 5 min, 90% A until 11 min, 60% A for 1 min, 0% A to equilibrate the column

**Mass Spectrometry**
- Capillary Voltage: 4000 V
- Fragmentor Voltage: 150 V
- Nozzle Voltage: 0 V
- **Collision Energy**: 30 eV, 20 eV
- MS Scan Rate: 8 spectra/sec
- MS/MS Scan Rate: 3 spectra/sec
- MS Scan Range: 40-1000 m/z
- **ESI Mode**: Positive

**Q/TOF Parameters**
- Gas Temperature: 200°C
- Gas Flow Rate: 13 L/min
- Sheath Gas Temperature: 250°C
- Sheath Gas Flow Rate: 12 L/min
- Nebulizer Pressure 20 psig

**Acquisition**
- Minimum of two ion transitions per drug
- Run Time: 13 minutes
LC/Q-TOF EIC
SWGTOX Standard Practices for Method Validation

**LOD**: 0.25 – 5 ng/mL

**LOQ**: 0.25 – 5 ng/mL

**Precision**: ±15%

**Bias**: ±15%

**Accuracy**: 84 – 104%

**Matrix Effects**: ±20%

**Dilution Integrity**: 2- and 4-fold

**Interferences**: No interferences (>50 interferents)
STABILITY STUDY ANALYSIS

Extraction
- Urine samples in duplicate (n=2)
  - 1000 ng/mL samples 1:4 dilution
- Calibrators extracted with every run
  - 10, 25, 100, 250, 350, and 500 ng/mL
- Negative and Positive (100 ng/mL) Controls

Analysis
- Concentration Mean (n=2)
- Error bars emitted for clarity
- Significant >20% loss

<table>
<thead>
<tr>
<th>Month</th>
<th>Samplings/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2-3</td>
<td>2-3*</td>
</tr>
<tr>
<td>4-6</td>
<td>1</td>
</tr>
<tr>
<td>6-12</td>
<td>1/month</td>
</tr>
</tbody>
</table>
SECONDARY AMINES, NO RING SUBSTITUENTS

<table>
<thead>
<tr>
<th>Methcathinone</th>
<th>Ethcathinone</th>
<th>Buphedrone</th>
<th>Pentedrone</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Methcathinone structure" /></td>
<td><img src="image2.png" alt="Ethcathinone structure" /></td>
<td><img src="image3.png" alt="Buphedrone structure" /></td>
<td><img src="image4.png" alt="Pentedrone structure" /></td>
</tr>
</tbody>
</table>
**SECONDARY AMINES, RING SUBSTITUTED**

<table>
<thead>
<tr>
<th>Mephedrone*</th>
<th>4-MEC</th>
<th>4-EMC</th>
<th>Methedrone</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Mephedrone chemical structure" /></td>
<td><img src="image" alt="4-MEC chemical structure" /></td>
<td><img src="image" alt="4-EMC chemical structure" /></td>
<td><img src="image" alt="Methedrone chemical structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flephedrone</th>
<th>3-FMC</th>
<th>3,4-DMMC</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Flephedrone chemical structure" /></td>
<td><img src="image" alt="3-FMC chemical structure" /></td>
<td><img src="image" alt="3,4-DMMC chemical structure" /></td>
</tr>
</tbody>
</table>
SECONDARY AMINES, METHYLENEDIOXY TYPE

<table>
<thead>
<tr>
<th>Methylone*</th>
<th>Ethylone*</th>
<th>Butylone*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Methylone" /></td>
<td><img src="image2" alt="Ethylone" /></td>
<td><img src="image3" alt="Butylone" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pentylone*</th>
<th>Eutylone*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4" alt="Pentylone" /></td>
<td><img src="image5" alt="Eutylone" /></td>
</tr>
</tbody>
</table>
# Tertiary Amines, Pyrrolidinidine Type

<table>
<thead>
<tr>
<th>Alpha-PVP*</th>
<th>MPBP</th>
<th>Pyrovalerone</th>
<th>Naphyrone*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Alpha-PVP*" /></td>
<td><img src="image2" alt="MPBP" /></td>
<td><img src="image3" alt="Pyrovalerone" /></td>
<td><img src="image4" alt="Naphyrone*" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDPV*</th>
<th>MDPBP</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5" alt="MDPV*" /></td>
<td><img src="image6" alt="MDPBP" /></td>
</tr>
</tbody>
</table>
CHEMICAL STRUCTURE DEPENDENCE:

pH 8 urine, 100 ng/mL, 4°C
TEMPERATURE DEPENDENCE
(PH 4, 100 NG/ML)

**32°C**

**4°C**

**20°C**

**-20°C**
TEMPERATURE DEPENDENCE
(PH 8, 100 NG/ML)

32°C

20°C

4°C

-20°C
PH DEPENDENCE
(100 NG/ML)

pH 4

pH 8

32°C
PH DEPENDENCE (100 NG/ML)

20°C

pH 4

pH 8
PH DEPENDENCE (100 NG/ML)

**pH 4**

**pH 8**

4°C
PH DEPENDENCE (100 NG/ML)

pH 4

pH 8

-20°C
CONCENTRATION DEPENDENCE

pH 4, 100 ng/mL

pH 4, 1000 ng/mL

pH 8, 100 ng/mL

pH 8, 1000 ng/mL

Target (%) vs Days for different pH and concentrations.
# DAYS TO >20% LOSS

<table>
<thead>
<tr>
<th>Cathinone Structural Group</th>
<th>32°C</th>
<th>20°C</th>
<th>4°C</th>
<th>-20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4</td>
<td>pH 8</td>
<td>pH 4</td>
<td>pH 8</td>
</tr>
<tr>
<td>Ring Substituted</td>
<td>7-78</td>
<td>&lt;1</td>
<td>24-172</td>
<td>≤1</td>
</tr>
<tr>
<td></td>
<td>&gt;172</td>
<td>1-5</td>
<td>&gt;172</td>
<td>3-12</td>
</tr>
<tr>
<td>Unsubstituted</td>
<td>21-68</td>
<td>&lt;1</td>
<td>42-115</td>
<td>≤1</td>
</tr>
<tr>
<td></td>
<td>&gt;172</td>
<td>1-5</td>
<td>&gt;172</td>
<td>7-19</td>
</tr>
<tr>
<td>Methyleneedioxy</td>
<td>68-143</td>
<td>≤1</td>
<td>&gt;172</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>&gt;172</td>
<td>5-21</td>
<td>&gt;172</td>
<td>16-172</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>&gt;143</td>
<td>1-14</td>
<td>&gt;172</td>
<td>3-42</td>
</tr>
<tr>
<td></td>
<td>&gt;172</td>
<td>19-172</td>
<td>&gt;172</td>
<td>16-172</td>
</tr>
</tbody>
</table>
### -20°C Instability

<table>
<thead>
<tr>
<th></th>
<th>Ring Substituted: &gt;6 months</th>
<th>Unsubstituted: &gt;6 months</th>
<th>Methylenedioxy: &gt;6 months</th>
<th>Pyrrolidine: &gt;6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring Substituted:</td>
<td>&lt;2 weeks</td>
<td></td>
<td>2 weeks - &gt;6 months</td>
<td></td>
</tr>
<tr>
<td>Unsubstituted:</td>
<td>1-3 weeks</td>
<td></td>
<td>2 weeks - &gt;6 months</td>
<td></td>
</tr>
<tr>
<td>Methylenedioxy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrrolidine:</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
<th>4 months</th>
<th>5 months</th>
<th>6 months</th>
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<tbody>
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<tr>
<td>Compound</td>
<td>Stability</td>
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<tr>
<td>Ring Substituted</td>
<td>&lt;1 week</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Unsubstituted</td>
<td>&lt;1 week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylenedioxy</td>
<td>&lt; 3 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylenedioxy</td>
<td>&gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>3 weeks - &gt;6 months</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Unsubstituted: &gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methylenedioxy: &gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyrrolidine: &gt;6 months</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
20°C INSTABILITY

Ring Substituted: ≤1 day

Ring Substituted: 3 weeks - > 6 months

Unsubstituted: ≤1 day

Unsubstituted: 1.5 – 4 months

Methylenedioxy: 1-3 days

Methylenedioxy: >6 months

Pyrrolidine: <1.5 mo

Pyrrolidine: >6 months

1 month 2 months 3 months 4 months 5 months 6 months
32°C INSTABILITY

- Ring Substituted: 1 wk. – 2.5 mo.
- Ring Substituted: <1 day
- Unsubstituted: <1 day
- Unsub: 3 wks – 2.5 mo.
- Methyleneedioxy: ≤1 day
- Methyleneedioxy: 2.5 – 5 mo.
- Pyrrolidine: < 2 weeks
- PYR: >5 mo.
CONCLUSIONS

- pH Dependence
  - Acidic > Alkaline
- Temperature Dependence
  - \(-20^\circ C > 4^\circ C > 20^\circ C > 32^\circ C\)
- No Concentration Dependence
- Significant Structural Dependence
  - MD/PYR > PYR > MD > Ring Substituted > Unsubstituted > 3-FMC
CONCLUSIONS

- Significant loss on the order of hours (alkaline urine, 32°C and 20°C)
  - 3-FMC: 32°C—undetectable after 6 hours
  - Substituted and Unsubstituted
    - 32°C: 20-88% loss after 6 hours
    - 20°C: 21-89% loss after 22 hours
- Instability at common storage conditions (4°C)

- Significant Structural Influence
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Questions

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