Comparison of UPLC-QTOF and GCMS for Detection of Designer Drugs in Urine Samples

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Disclaimer

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Project Background

- Attendees of electronic dance music festivals (EDM) demonstrate high rates of experimental drug use.
- Collection of biological specimens of EDM attendees increases treatment ability for those experiencing adverse reactions and increases ability of toxicology labs to detect compounds.
Project Objectives

• Analyze samples to obtain information regarding:
  – New drugs on the market
  – Prevalence of designer drugs
  – Identification of novel designer drugs and metabolites
  – Correlations and comparisons of designer drugs in blood, urine and oral fluid specimens
Sample Collection

• Approached participants on their way to EDM festival
• Location was ~100 yards from entrance gate
• Participants signed consent forms and were asked survey questions
• Samples collected included:
  – Oral Fluid Collection
    • Alere DDS2 Cartridge
    • Quantisal
  – Urine
  – Blood

Disclosure: Participants were not required to donate all 4 samples, and only donated samples based on their comfort level. The gift card incentive was only given if the participant donated a blood sample.
Urine Results

• Total number of urine samples collected: 104

• Samples underwent a battery of screen tests:
  – Immunoassay
  – Volatiles
  – RapidFire-MS/MS
  – GC/MS
  – LC-QTOF
  – LC-MS/MS
COMPARISON BETWEEN GC/MS AND LC-QTOF AS SCREENING TECHNIQUES
Sample Preparation (GC/MS)

• To 2 mL urine, add internal standard, 100 mM phosphate buffer (pH 6.0)

• To a copolymeric bonded phase extraction column:
  – **Condition**: Methanol, Water, 100 mM phosphate buffer
  – **Apply Sample**
  – **Wash**: Water, 20% Acetonitrile/Water, 100 mM Acetic Acid, then DRY
  – **Wash**: Hexane, Methanol, then DRY
  – **Elute**: Isopropanol, Ammonium Hydroxide, Methylene Chloride

• **Evaporate** (add 10% HCl) and **Reconstitute** with Acetonitrile
GC/MS Parameters

- Agilent GC (6890)/ MS (5975)
- Column: DB5MS 20m x 0.18mm x 0.18µm
- Split Ratio: 10:1
- Injection Temperature: 250°C
- Injection Volume: 2µL
- GC Oven Programming:
  - Initial 70°C (1 min)
  - Ramp 20°C/min
  - Final 300°C (5.5 min)
- Total Run Time: 17.5 min
- MS Acquisition: 42-550 m/z
Acceptability Criteria (GC/MS)

- Chromatographic peak must be clearly identifiable, as well as internal standard peak
- Chromatographic peak must be within ±2% of analyte in standard
  - If analyte is not present in a standard, standard is analyzed under same conditions to verify retention time
- Mass spectrum minimum confidence of 70% compared to reference library spectrum
Chromatogram of MS124 (GC/MS)

- Amphetamine
- 4-Fluoroamphetamine
- 5-APB
- MDA
- MDMA
- Methylone
Sample Preparation (LC-QTOF)

• To 0.5 mL urine, add internal standard, water, 100 mM phosphate buffer (pH 6.0)

• To a copolymeric bonded phase extraction column:
  – **Condition:** Methanol, Water, 100 mM phosphate buffer
  – **Apply Sample**
  – **Wash:** Water, 100 mM Acetic Acid, Methanol, then DRY
  – **Elute:** Isopropanol, Ammonium Hydroxide, Methylene Chloride

• **Evaporate** (add 10% HCl) and **Reconstitute** with Mobile Phase
LC-QTOF Parameters

- Waters Acquity I-Class UPLC Conditions:
  - Mobile phase A: 5mM ammonium formate (pH 3.0)
  - Mobile phase B: 0.1% formic acid in acetonitrile
  - Column: Waters Acquity HSS C18 150mm x 2.1mm x 1.8µm
  - Flow rate: 0.4 mL/min
  - Column Temperature: 50°C
  - Injection Volume: 2µL

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>0.5</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>10.0</td>
<td>50</td>
<td>50</td>
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<tr>
<td>10.75</td>
<td>5</td>
<td>95</td>
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<tr>
<td>12.25</td>
<td>5</td>
<td>95</td>
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<tr>
<td>12.5</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>15.0</td>
<td>87</td>
<td>13</td>
</tr>
</tbody>
</table>
LC-QTOF Parameters

• Xevo G2 QTOF Conditions:
  – Ionization: Positive electrospray
    • Capillary voltage: 0.8 kV
    • Sample Cone Voltage: 20 V
    • Extraction Cone Voltage: 4 V
    • Source Temperature: 140°C
    • Desolvation Temperature/Flow: 500°C/900 L/h
  – Resolution Mode: 50-1000 m/z
    • Collision Energy (Function 1) – 6eV
    • Collision Energy (Function 2) – 10-40eV
Acceptability Criteria (LC-QTOF)

- Chromatographic peak must be clearly identifiable, as well as internal standard peak
- Chromatographic peak must be within ±2% of analyte in standard or within ±0.3 min of analyte in database
  - If analyte is not present in a standard or database, standard is analyzed under same conditions to verify retention time
- Observed mass of molecular ion must be within ±5 ppm of mass in database
- Observed mass of fragment ion must be within ±5 ppm of mass in database
Chromatogram of MS124 (LC-QTOF)

- Amphetamine
- 4-Fluoroamphetamine
- 7-aminoclonazepam
- MDA
- MDMA
- Methylone
- α-PVP
- N-desmethyltramadol
GC/MS AND LC-QTOF RESULTS
GC/MS vs. LC-QTOF Positive Screens

- 5-FA
- MDMA
- Methamp/Amp
- Cocaine/Mets
- Methylone
- Dimethylone
- Ethylene/Butylene
- a-PVP
- 5-APB

Legend:
- GC
- LCQTOF
GC/MS vs. LC-QTOF Confirmation Rate

- 5-FA
- MDMA
- Methamp/Amp
- Cocaine/Mets
- Methylene
- Dimethylene
- Ethylene/Butylene
- a-PVP
- 5-APB

Legend:
- GC
- LCQTOF
<table>
<thead>
<tr>
<th>Analytes</th>
<th># Positives</th>
<th># Confirm Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methamp/Amp</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Cocaine/Mets</td>
<td>33</td>
<td>29</td>
</tr>
<tr>
<td>M ethylone</td>
<td>22</td>
<td>20</td>
</tr>
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</table>
## GC/MS vs. LC-QTOF

<table>
<thead>
<tr>
<th>Rate</th>
<th>GC/MS</th>
<th></th>
<th>LC-QTOF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Positivity Rate</td>
<td>49  80.3</td>
<td></td>
<td>63  103.3</td>
<td></td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>12  19.6</td>
<td></td>
<td>0   0.0</td>
<td></td>
</tr>
<tr>
<td>Total Positive Samples</td>
<td>61  --</td>
<td></td>
<td>61  --</td>
<td></td>
</tr>
</tbody>
</table>

Alcohol Only Positives:  8  
THC Only Positives:  16  

Total Number of Positive Sample:  $\frac{85}{104} = 82\%$
GC/MS Results

• Missed analytes:
  – Benzoylecgonine, THC, Cyclobenzaprine, DMAA, Alprazolam, Oxazepam, 7-aminoclonazepam, Psilocin, Buprenorphine, Azacyclonol, 3,4,5 Trimethoxy cocaine, PMMA, 2-CB

• Missed analytes due to sensitivity, no derivatization reagents used, poor chromatography on GC
LC-QTOF Results

- Missed or poor chromatography analytes:
  - Ecgonine Methyl Ester, THC, 5-APB, Nicotine, Cotinine

- Extra analytes detected due to: increased sensitivity of QTOF vs. confirmation technique, compounds not analyzed for in confirmation technique
Comparison Conclusion

- **GC/MS**
  - Decreased sensitivity
  - Library search capabilities
  - More false negatives
  - Identified less designer drugs
  - Data interpretation requires less training

- **LC-QTOF**
  - Increased sensitivity
  - Targeted screen
  - More unconfirmed positives
  - Identified more designer drugs
  - Data interpretation requires increased training
OVERALL RESULTS FOR ANALYTICAL TESTING
Combined % Confirmation Rate

THC
Benzodiazepines
Amines
Opiates
Cocaine
Basic Drugs
Designers
% Positive Rate in Sample Population

- THC: 50.0%
- Benzodiazepines: 20.0%
- Amines: 10.0%
- Opiates: 10.0%
- Cocaine: 20.0%
- Basic Drugs: 20.0%
- Designers: 30.0%
- Alcohol: 25.0%
Several participants indicated they had taken “Molly” in the last week.

Samples of subjects (9) who reported taking “Molly” contained:
- MDMA
- Methylone
- Alpha-PVP

Samples of subjects (15) who reported taking MDMA/Ecstasy contained:
- MDMA
- Methylone
- Dimethylone/Ethylone/Butylone
- Alpha-PVP
Thank You

- Thank you to everyone at AFMES for helping with all the aliquoting, extractions, data analysis, etc.

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**LC-QTOF Screen** – John Kristofic

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**Project Coordination** – CDR Bosy, Joseph Magluilo, Shawn Vorce, Justin Holler
Thank You

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Questions?

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