Optimization, validation, and application of LC-(QqQ)MS/MS for the determination and occurrence of new drugs of abuse in wastewater samples

Viola L. Borova
Constantinos Pistos and Nikolaos S. Thomaidis

What are “New Designer Drugs”

- **Designer Drugs:**
  - Synthetically changed natural substances
  - Completely designed molecular structures
  - Psychotropic effects

- **New Designer Psychotropic Drugs:**
  - Drug alternatives
  - Optimized effects of already existing drugs
    - Higher binding affinity with CB1, greater potency and adverse effects and longer duration of action
  - Not covered in most countries by controlled substance statutes
    - avoid detection and legal consequences
  - “Spice” drugs (smoking mixtures, herbal mixtures), bath salts on the recreational drug use market
Classes of New Designer Drugs

**Phenylalkylamines**
- Beta-keto (mephedrone, butylone (bk-MBDB), methylone (bk-MDMA))

**Piperazines**
- Phenylpiperazine (DCPP, mCPP, MeOPP, pCPP, TFMPP)
- Benzylpiprazine (BZP, MBZP, DBZP, MDBZP)

**Synthetic Cannabinoids**
- JWH cannabinoids (John W. Huffman),
- (JWH 018, JWH 073 aminoalkylindoles series and more than 400 cannabinoids)
- CP47, 497 (From Pfizer, cyclohexylphenol series) (analog CP47,497 and homologue C6,C7,C8,C9)

**Pyrrolidino phenones**
- Derivatives (PPP, MPPP, MPHP, MOPPP, MDPPP)

**INTRODUCTION**
**ANALYTICAL METHODOLOGY**
- OPTIMIZATION OF MS/MS
- OPTIMIZATION OF HPLC
- OPTIMIZATION OF SPE
  **METHOD VALIDATION**
  **APPLICATION IN REAL INFLENT WASTEWATER SAMPLES**
**CONCLUSIONS**
New Designer Drugs... a new class of emerging organic contaminants

- Not currently covered by existing water quality regulations
- Not investigated or little
- Newly identified or previously unrecognized
- Lack of environmental data
- Lack of analytical methods in wastewater
- Large volume of consumption and production
- Potential threats to ecosystems and human (limited information)

INTRODUCTION

ANALYTICAL METHODOLOGY

- Optimization of MS/MS
- Optimization of HPLC
- Optimization of SPE

METHOD VALIDATION

APPLICATION IN REAL INFLENT WASTEWATER SAMPLES

CONCLUSIONS

ANALYTICAL METHODOLOGY

Thermo Scientific Quantum Access

LC- (QqQ) MS/MS -ESI
INTRODUCTION

ANALYTICAL METHODOLOGY

Optimization of MS/MS
Optimization of HPLC
Optimization of SPE
Method Validation
Application in Real Influent Wastewater Samples

CONCLUSIONS

TARGET COMPOUNDS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M. Formula</th>
<th>MW</th>
<th>LogP</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>JWH-018</td>
<td>C24H23NO</td>
<td>341.5</td>
<td>6.51</td>
<td>-</td>
</tr>
<tr>
<td>JWH-018 D9</td>
<td>C23H21NO</td>
<td>327.4</td>
<td>6.07</td>
<td>-</td>
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<tr>
<td>JWH-073</td>
<td>C23H21NO</td>
<td>318.5</td>
<td>6.17</td>
<td>-</td>
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<tr>
<td>CP47,497</td>
<td>C11H16O2</td>
<td>177.2</td>
<td>2.12</td>
<td>8.69</td>
</tr>
<tr>
<td>Mephedrone / Mephedrone D3</td>
<td>C11H16N2</td>
<td>176.2</td>
<td>1.38</td>
<td>9.59</td>
</tr>
<tr>
<td>MPPP</td>
<td>C11H16NO</td>
<td>217.3</td>
<td>2.91</td>
<td>-</td>
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Method Development and Optimization of MS/MS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>[M+H]+</th>
<th>Product Ions (collision energy)</th>
<th>Tube Lens (V)</th>
<th>ESI</th>
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</thead>
<tbody>
<tr>
<td>JWH-018</td>
<td>342.1</td>
<td>155.0 (25V) 127.0 (44V)</td>
<td>81.8</td>
<td>+</td>
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<tr>
<td>JWH-018 D9</td>
<td>351.1</td>
<td>155.0 (26V) 127.0 (45V)</td>
<td>80.1</td>
<td>+</td>
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<tr>
<td>JWH-073</td>
<td>328.1</td>
<td>154.9 (25V) 127.0 (43V)</td>
<td>76.3</td>
<td>+</td>
</tr>
<tr>
<td>CP47,497</td>
<td>317.2</td>
<td>298.9 (24V) 244.7 (34V)</td>
<td>99.1</td>
<td>-</td>
</tr>
<tr>
<td>Mephedrone</td>
<td>178.1</td>
<td>160.0 (12V) 145.0 (19V)</td>
<td>48.1</td>
<td>+</td>
</tr>
<tr>
<td>Mephedrone D3</td>
<td>161.1</td>
<td>163.0 (12V) 148.0 (21V)</td>
<td>49.0</td>
<td>+</td>
</tr>
<tr>
<td>BZP</td>
<td>177.1</td>
<td>91.2 (28V) 65.3 (40V)</td>
<td>62.5</td>
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<tr>
<td>BZP D7</td>
<td>184.1</td>
<td>98.2 (27V) 70.3 (40V)</td>
<td>66.3</td>
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<tr>
<td>MPPP</td>
<td>218.1</td>
<td>119.1 (24V) 146.9 (18V)</td>
<td>61.1</td>
<td>+</td>
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</table>

Positive Ionization
Probe: C (0.5/1.5) Spray Voltage: 3500 V Sheath gas: 30 a.u. Aux gas: 10 a.u. Temp: 270°C

Negative Ionization
Probe: C (0.5/1.5) Spray Voltage: 2500 V Sheath gas: 20 a.u. Aux gas: 10 a.u. Temp: 300°C
Comparison of stationary phases

- **Stationary phases**: Atlantis T3 (C18) (Waters), XSelect (C18) (Waters) & PFP (Phenomenex)

- Compounds with –NH₂ or –NH- presented higher asymmetry (fronting) on C18 column.
- XSelect and Atlantis T3 suffer from peak shape issues and do not provide good separation of the compounds.
- **PFP**: excellent peak shape, good resolution and separation in short time with excellent MS sensitivity.

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Mobile Phase

- JWH 018, JWH 073, Mephedrone, Benzylpiperazine, MPPP

<table>
<thead>
<tr>
<th>Positive</th>
<th>H₂O (0.2% formic acid)</th>
<th>MeOH</th>
<th>pL/min</th>
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<tbody>
<tr>
<td>0.00</td>
<td>60</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>3.00</td>
<td>60</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>15.00</td>
<td>0.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25.00</td>
<td>0.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25.50</td>
<td>60</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>30.00</td>
<td>60</td>
<td>40</td>
<td>100</td>
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</table>

<table>
<thead>
<tr>
<th>Negative</th>
<th>MeOH</th>
<th>ACN</th>
<th>pL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>90</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>3.00</td>
<td>90</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td><strong>15.00</strong></td>
<td>90</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>
Effect of the organic modifier

Sample preparation - Comparison SPE

Recoveries with different sorbents (n=5)
Sample preparation

1. Wastewater samples were filtered on Glass fiber filters
2. 50 mL of filtrated sample were adjusted at a value of pH 2.5 with HCl (1 M)
3. Internal deuterated standards of the compounds were added to all samples
4. SPE (STRATA-X/ISOLUTE C18, STATA-XC)

Reconstitution: 500 μL of 40% MeOH and 60% ultra purified water with 0.05% v/v formic acid

Validation for wastewater samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear range pg/L</th>
<th>Corr. Coefficient R²</th>
<th>method LOD ng/L</th>
<th>Absolute Recovery %</th>
<th>RSD% (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZP</td>
<td>1 – 100</td>
<td>0.9993</td>
<td>4.8</td>
<td>29.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Mephedrone</td>
<td>1 – 100</td>
<td>0.9994</td>
<td>0.3</td>
<td>100</td>
<td>3.7</td>
</tr>
<tr>
<td>MPPP</td>
<td>1 – 100</td>
<td>0.9994</td>
<td>0.6</td>
<td>99.0</td>
<td>4.2</td>
</tr>
<tr>
<td>CP47.497</td>
<td>1 – 100</td>
<td>0.9991</td>
<td>37</td>
<td>97.0</td>
<td>15.2</td>
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<tr>
<td>JWH 018</td>
<td>1 – 100</td>
<td>0.9991</td>
<td>1.4</td>
<td>40.0</td>
<td>8.3</td>
</tr>
<tr>
<td>JWH 073</td>
<td>1 – 100</td>
<td>0.9992</td>
<td>0.8</td>
<td>42.0</td>
<td>15.3</td>
</tr>
</tbody>
</table>
Wastewater Samples from WWTP Santorini

Sampling
23/07/2013 - 29/07/2013

- Population: 10500 inhabitants
- Flow rate: 1500 m³/day

- Mediterranean climate
- High anthropogenic impact

RANGES:
- BZP → <LOD – 17.8 ng/L
- Mephedrone → 1.5 – 4.0 ng/L
- MPPP → <LOD ng/L
- CP47 → 179 – 1469 ng/L
- JWH 018 → <LOD – 2.2 ng/L
- JWH 073 → <LOD – 1.0 ng/L

Frequency of detection
Wastewater Samples from WWTP Santorini

Levels in influent wastewater of Fira

<table>
<thead>
<tr>
<th>Date</th>
<th>CP 47</th>
<th>MPPP</th>
<th>MEPHEDRONE</th>
<th>JWH 073</th>
<th>JWH 018</th>
<th>BZP</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/7/13</td>
<td>600.0</td>
<td>100.0</td>
<td>300.0</td>
<td>200.0</td>
<td>150.0</td>
<td>0.0</td>
</tr>
<tr>
<td>24/7/13</td>
<td>150.0</td>
<td>200.0</td>
<td>100.0</td>
<td>100.0</td>
<td>50.0</td>
<td>0.0</td>
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<tr>
<td>25/7/13</td>
<td>100.0</td>
<td>100.0</td>
<td>50.0</td>
<td>50.0</td>
<td>25.0</td>
<td>0.0</td>
</tr>
<tr>
<td>26/7/13</td>
<td>50.0</td>
<td>50.0</td>
<td>25.0</td>
<td>25.0</td>
<td>12.5</td>
<td>0.0</td>
</tr>
<tr>
<td>27/7/13</td>
<td>25.0</td>
<td>25.0</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
<td>0.0</td>
</tr>
<tr>
<td>28/7/13</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
<td>6.25</td>
<td>3.125</td>
<td>0.0</td>
</tr>
<tr>
<td>29/7/13</td>
<td>6.25</td>
<td>6.25</td>
<td>3.125</td>
<td>3.125</td>
<td>1.5625</td>
<td>0.0</td>
</tr>
</tbody>
</table>

CONCLUSIONS
CONCLUSIONS

- Development and validation of a novel method for new designer drugs in wastewater by LC-MS/MS
- Investigation of their occurrence in a WWTP in Santorini Island
- 5 out of the 6 compounds were detected at least in one day
- CP47 was detected for the first time in influent wastewater
- Not any special trend among the days was observed for mephedrone and CP47

Future Perspectives

- Integration into one multi-analyte method which provides the elution of all the compounds with one cartridge
- Identification of their transformation products
- Application of the method in the WWTP of Athens
THANK YOU VERY MUCH!!!

Questions?

Contact information: vborova@chem.uoa.gr
ntho@chem.uoa.gr

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