Overview

- Designer drugs are not detected by routine drug screens, and are more potent than traditional drugs.
- A disposable paper spray cartridge with SPE column can carry out analyte pre-concentration and ionization.
- Method optimized for detection of two synthetic cannabinoids JWH-200 and JWH-250.
- Most frequently abuse synthetic cannabinoids can be detected at sub-ng/mL levels.

Introduction

- Designer drugs mimic psychoactive effects of traditional drugs, however, they are typically more potent and can have unpredictable and severe health effects.
- They are cheap and marketed as 'legal highs', since they cannot be detected by routine drug screens.
- New (often more dangerous) drugs continue to emerge as known designer drugs become banned.
- There is a need for a rapid and sensitive analytical method to detect designer drugs.

- Paper spray mass spectrometry can directly analyze biological samples.
- Advantages: no sample preparation, small sample volume, small solvent volume, no solvent waste, no carry over, rapid analysis (1-2 minute run), automatable.
- Cartridge equipped with solid phase extraction (SPE) column can perform analyte pre-concentration and ionization.
- SPE helps improve detection limits by allowing larger sample volumes to be used, removing matrix interferences and pre-concentrating the analytes.

Methods

- Cartridges were made from Deltin® on a milling machine.
- Two parts of the cartridge join together via tongue and groove:
  - Bottom part dimensions: 40mm x 26mm x 6 mm (LWH)
  - Top part dimensions: 14mm x 22mm x 13mm (LWH)
- Bottom part has two separate regions to hold absorbent pad and paper spray substrate.
- Top part contains the SPE column (3.0 mm Whatman ET31 paper punch, SPE material, 3.0 mm nylon punch).
- Mass spectrometry analysis was performed using Thermo Scientific TSQ Vantage (TSQ) in the parallel reaction monitoring (PRM) mode.

Procedure

1. Sample is loaded at the top of the SPE column, and allowed to wick through.
2. Water is added to the top of the cartridge to help remove matrix components.
3. The cartridge is covered and allowed to dry.
4. Cartridge is positioned in front of the MS inlet and spray solvent is added to the top to extract the analytes.
5. Voltage is applied to the cartridge, and analyte signal is collected (2-5 minutes).

Figure 1: Structure of THC, and synthetic cannabinoids JWH-250 and SF-ADB

Figure 2: Cartridge positioned in front of the mass spectrometer inlet for analysis

Figure 3: SPE cartridge and column

Figure 4: Paper spray chronogram with MS/MS in MRM mode, Thermo Scientific TSQ Vantage

Figure 5: Paper spray chronogram with MS/MS in PRM mode, Thermo Scientific Q-Exactive Focus

Figure 6: Workflow for paper spray analysis with cartridge equipped with SPE

Table 1: TSQ MRM transitions, and the ISTD used for normalization

| Cannabinoid   | ISTD     | Transitions
|---------------|----------|-------------
| JWH-200      | AM-2201  | 385.3 → 114.1
|              | et d5   | 385.3 → 155.0
| JWH-250      | AM-2201  | 385.3 → 127.0
|              | et d5   | 385.3 → 155.0
| AM-2201      | AM-2201  | 385.3 → 127.0
|              | et d5   | 385.3 → 155.0
| AB-CHMINACA  | AB-CHMINACA | 385.3 → 145.0
| SF-FUBINACA  | SF-FUBINACA | 385.3 → 145.0
| SF-PB-22     | SF-PB-22 | 385.3 → 145.0
| XLR-11       | XLR-11   | 385.3 → 145.0
| THJ-2201     | THJ-2201 | 385.3 → 145.0

Table 2: QE PRM transitions, and the ISTD used for normalization

| Cannabinoid   | ISTD     | Transitions
|---------------|----------|-------------
| JWH-200      | AB-CHMINACA d4 | 385.3 → 155.0
| JWH-250      | AB-CHMINACA d4 | 385.3 → 155.0
| AM-2201      | AB-CHMINACA d4 | 385.3 → 155.0
| AB-CHMINACA  | AB-CHMINACA d4 | 385.3 → 155.0
| SF-FUBINACA  | SF-FUBINACA d4 | 385.3 → 155.0
| SF-PB-22     | SF-PB-22 | 385.3 → 155.0
| XLR-11       | XLR-11 | 385.3 → 155.0
| THJ-2201     | THJ-2201 | 385.3 → 155.0

- QE and TSQ produced different fragmentation and different MS/MS spectra.

- The fragments with the highest intensity were selected for quantitation.
**Results**

### Extraction Solvent

<table>
<thead>
<tr>
<th>SPE Material</th>
<th>JWH-200 (ng/mL)</th>
<th>JWH-250 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strata-X-RP</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>HybridSPE Phospholipid</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>HLB</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>SAX</td>
<td>0.1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1: Limits of detection using different SPE materials

- Acetonitrile with 0.1% formic acid improved analyte signal ~10x for both analytes
- The blank signal was not significantly affected
- Solid phase sorbent Strata-X-RP had the lowest detection limits

### Wash Step

- Rinsing SPE column with water after loading the sample helps remove matrix components
- Washing the paper substrate helps reduce the background signal
- For 100 µL of plasma, 10 mg of SPE material gave the best results
- Signal to Noise ratio (S/N) increases with larger sample volumes
- Optimized method was used to analyze samples on QE

### SPE Amount

- 5 mg
- 10 mg
- 20 mg

### Synthetic Cannabinoids Concentration in Plasma

<table>
<thead>
<tr>
<th>Synthetic Cannabinoids Concentration (ng/mL)</th>
</tr>
</thead>
</table>

Figure 7: Analyte signal obtained with SPE cartridges with various extraction solvents

Figure 8: Analyte signal improvement with the added wash step

Figure 10: Comparison between S/N and amount of SPE

Figure 11: Comparison between S/N and amount of sample loaded

### Synthesis of Cannabinoids

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>Direct Paper Spray TSQ (ng/mL)</th>
<th>SPE TSQ (ng/mL)</th>
<th>SPE QE (ng/mL)</th>
<th>SPE (TSQ)</th>
<th>SPE (QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JWH-200</td>
<td>3.0</td>
<td>0.03</td>
<td>0.22</td>
<td>0.938</td>
<td>0.9872</td>
</tr>
<tr>
<td>JWH-250</td>
<td>13.0</td>
<td>0.06</td>
<td>0.14</td>
<td>0.955</td>
<td>0.9975</td>
</tr>
<tr>
<td>AM-2201</td>
<td>5.0</td>
<td>0.014</td>
<td>0.2</td>
<td>0.9989</td>
<td>0.996</td>
</tr>
<tr>
<td>AB-CHMINACA</td>
<td>0.25</td>
<td>0.064</td>
<td>0.08</td>
<td>0.9991</td>
<td>0.9983</td>
</tr>
<tr>
<td>SF-ADB</td>
<td>0.3</td>
<td>0.033</td>
<td>0.27</td>
<td>0.9957</td>
<td>0.9904</td>
</tr>
<tr>
<td>SF-PB-22</td>
<td>8.5</td>
<td>0.016</td>
<td>0.25</td>
<td>0.9955</td>
<td>0.9840</td>
</tr>
<tr>
<td>XLR-11</td>
<td>7.3</td>
<td>0.02</td>
<td>0.15</td>
<td>0.9927</td>
<td>0.9940</td>
</tr>
<tr>
<td>THJ-2201</td>
<td>0.5</td>
<td>0.03</td>
<td>0.3</td>
<td>0.9939</td>
<td>0.9751</td>
</tr>
</tbody>
</table>

Table 4: Limits of detection and R² obtained from synthetic cannabinoid calibration curves

### Conclusions

- All synthetic cannabinoids could be detected sub-ng/mL levels
- Optimized SPE method decreased the detection limits ~100 times
- Good linearity from 0.1 – 10 ng/mL
- Some adjustments may be necessary to achieve the same LODs with the QE

### References


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