The Detection of Designer Drugs from Plasma

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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

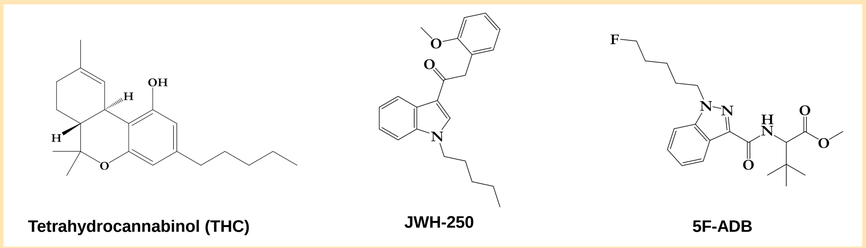

Figure 1: Structure of THC, and synthetic cannabinoids JWH-250 and 5F-ADB

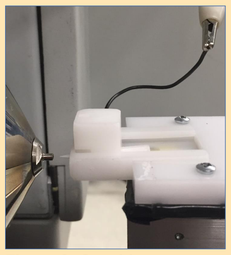

Figure 2: Cartridge positioned in front the of the mass spectrometer inlet for analysis

- 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 Delrin® 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)

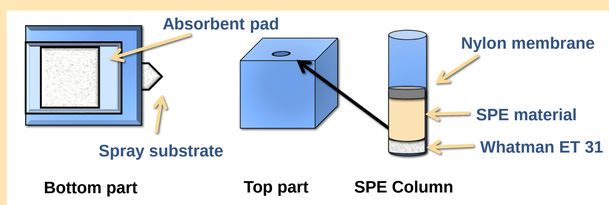

Figure 3: SPE cartridge and column

- 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 multiple reaction monitoring (MRM) mode and Thermo Scientific Q-Exactive Focus (QE) in the parallel reaction monitoring (PRM) mode

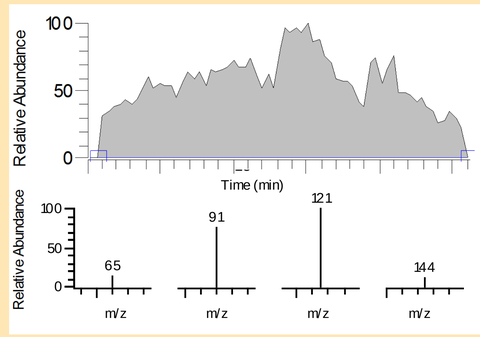

Figure 4: Paper spray chronogram with MS/MS in MRM mode, Thermo Scientific TSQ Vantage

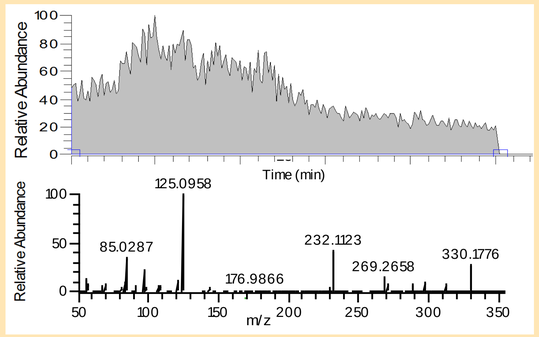

Figure 5: Paper spray chronogram with MS/MS in PRM mode, Thermo Scientific Q-Exactive Focus

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)

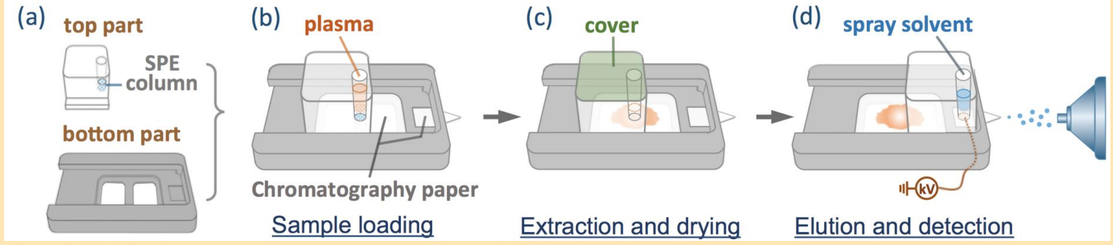

Figure 6: Workflow for paper spray analysis with cartridge equipped with SPE[1]

Cannabinoid	ISTD	Transitions*
JWH-200	AM-2201 d5 m/z 127	385.3 → 77.0 385.3 → 114.1 **385.3 → 127.0** 385.3 → 155.0
JWH-250	AM-2201 d5 m/z 127	336.281 → 65.1 336.281 → 91.1 **336.281 → 121.1** 336.281 → 144.1
AM-2201	AM-2201 d5	360.2 → 77.0 **360.2 → 127.0** 360.2 → 155.0 360.2 → 239.1
AB-CHMINACA	AB-CHMINACA d4	357.2→ 145.0 357.2→ 241.1 **357.2→ 312.2** 357.2→ 340.2
5F-ADB	AB-FUBINACA d4 m/z 257.1	378.2 → 145.0 **378.2 → 213.1** 378.2 → 233.1 378.2 → 318.2
5F-PB-22	AB-CHMINACA d4 m/z 149	385.3 → 89.0 385.3 → 116.0 385.3 → 144.0 **385.3 → 232.1**
XLR-11	AM-2201 d5 m/z 127	330.3 → 55.1 330.3 → 125.1 330.3 → 144.0 **330.3 → 232.1**
THJ-2201	AM-2201 d5 m/z 127	361.2 → 90.0 361.2 → 145.0 361.2 → 213.1 **361.2 → 233.1**

Table 1: TSQ MRM transitions, and the ISTD used for normalization
*Quantitation transition is bolded

Cannabinoid	ISTD	Transitions
JWH-200	AB-CHMINACA d4	385.3 → 155.0494
JWH-250	AB-CHMINACA d4	336.3→ 121.0652
AM-2201	AB-CHMINACA d4	360.2 → 155.0494
AB-CHMINACA	AB-CHMINACA d4	357.2→ 312.2076
5F-ADB	AB-FUBINACA d4	378.2 → 251.1193
5F-PB-22	AB-CHMINACA d4	385.3 → 232.1135
XLR-11	AB-CHMINACA d4	330.3 → 125.0966
THJ-2201	AB-FUBINACA d4	361.2 → 251.1193

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

- QE and TSQ produced different fragmentation and different MS/MS spectra
- The fragments with the highest intensity were selected for quantitation

a via Paper Spray Mass Spectrometry Cartridge

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Results

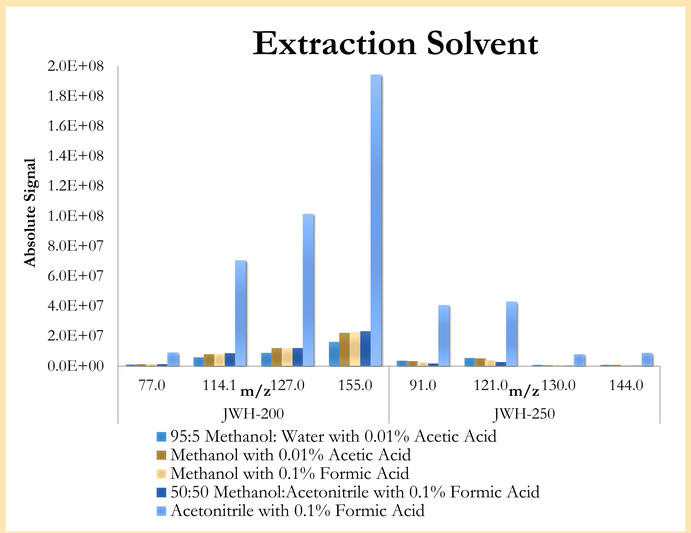

Figure 7: Analyte signal obtained with SPE cartridges with various extraction solvents

SPE Material	JWH-200 (ng/mL)	JWH-250 (ng/mL)
Strata-X-RP	0.03	0.1
HybridSPE Phospholipid	0.1	1
HLB	0.1	1
SAX	0.1	1

Table 3: 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

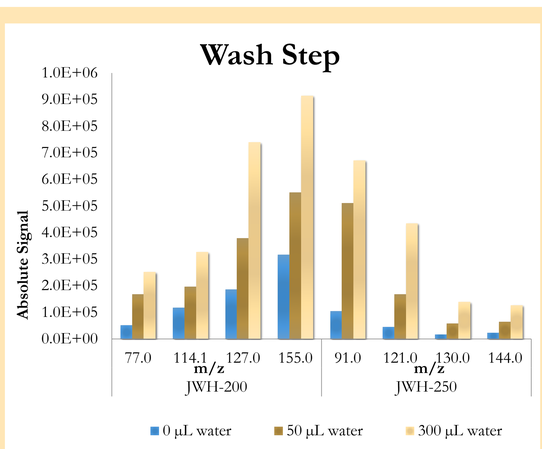

Figure 8: Analyte signal improvement with the added wash step

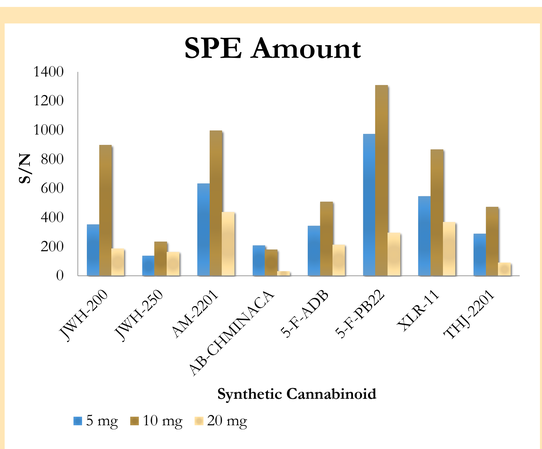

Figure 10: Comparison between S/N and amount of SPE

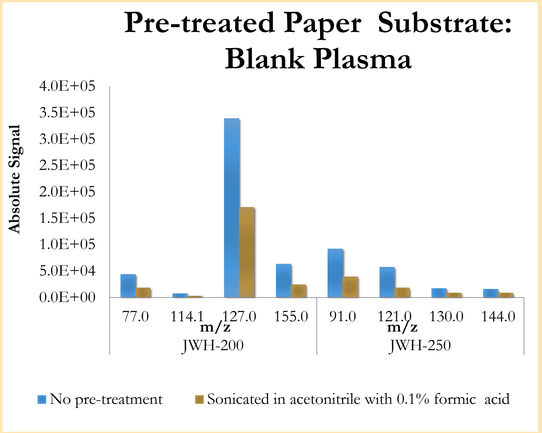

Figure 9: Blank signal for pre-treated and non-treated paper substrate

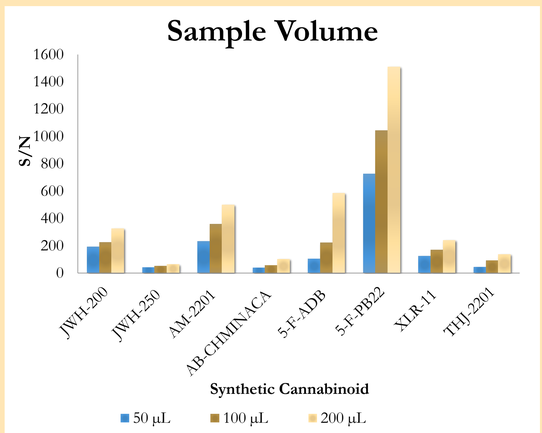

Figure 11: Comparison between S/N and amount of sample loaded

- 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

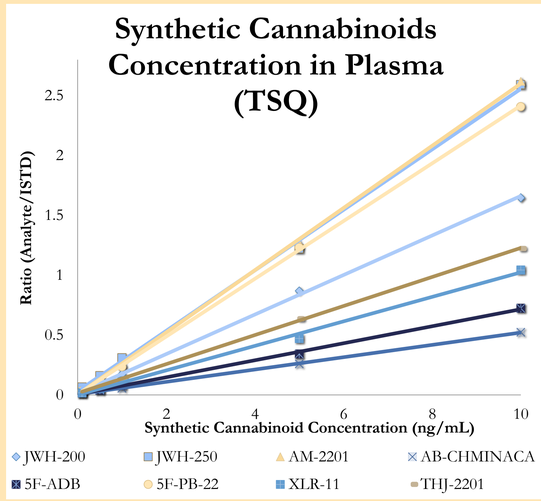

Figure 12: Calibration curve for synthetic cannabinoids, MS analysis performed using TSQ

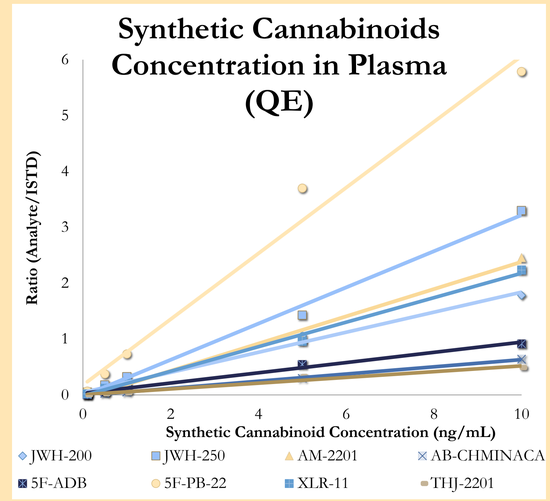

Figure 13: Calibration curve for synthetic cannabinoids, MS analysis performed using QE

Cannabinoid	Limit of Detection (LOD)			R^2	
	Direct Paper Spray TSQ (ng/mL)	SPE TSQ (ng/mL)	SPE QE (ng/mL)	SPE (TSQ)	SPE (QE)
JWH-200	3.0	0.03	0.22	0.9930	0.9872
JWH-250	13.0	0.06	0.14	0.9935	0.9975
AM-2201	5.0	0.014	0.2	0.9989	0.9906
AB-CHMINACA	0.25	0.064	0.08	0.9991	0.9983
5F-ADB	0.3	0.035	0.27	0.9957	0.9904
5F-PB-22	8.5	0.016	0.25	0.9955	0.9840
XLR-11	7.3	0.02	0.15	0.9927	0.9940
THJ-2201	0.5	0.03	0.3	0.9939	0.9751

Table 4: Limits of detection and R^2 obtained from synthetic cannabinoid calibration curves

- 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

Conclusions

- A method was developed and optimized for synthetic cannabinoids JWH-200 and JWH-250
  - Extraction solvent, SPE sorbent, sample volume, SPE amount and wash steps were investigated
- Method was able to detect several synthetic cannabinoids that were most commonly detected in US toxicology labs in the last two years at sub-ng/mL concentrations
- Synthetic cannabinoids can be quantified with the use of an ISTD
- The presented method allows for rapid, sensitive (sub ng/mL) detection of synthetic cannabinoids with minimal sample preparation and no chromatography.

References

1. Zhang, C. & Manicke, N. E. Development of a Paper Spray Mass Spectrometry Cartridge with Integrated Solid Phase Extraction for Bioanalysis. *Anal. Chem.* **87,** 6212–6219 (2015)

Acknowledgments

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