The Detection of Designer Drugs from Plasma via Paper Spray Mass Spectrometry Cartridge

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Overview

- Designer drugs are not detected by routine drug screens and are more potent than traditional drugs
- A method to detect designer drugs at sub-ng/mL levels was developed and validated using paper spray mass spectrometry with an integrated solid phase extraction cartridge
- 33 suspected overdose plasma samples were analyzed and several designer drugs were successfully detected. Results were compared to the screening results from a toxicology lab

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 (and often more dangerous) designer drugs emerge as others become banned
- There is a need for a rapid and sensitive analytical method to detect designer drugs

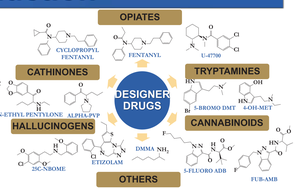

Figure 1: There are many different classes and structures of designer drugs

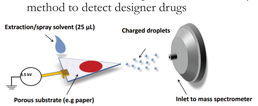

Figure 2: Paper spray analysis[1]

- 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 improves detection limits by allowing larger sample volumes to be used, removing matrix interferences and pre-concentrating the analyte

Methods

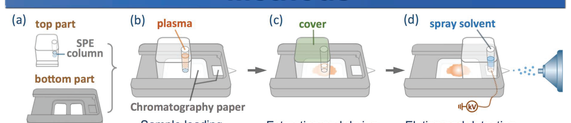

Figure 3: Workflow for paper spray analysis with cartridge equipped with SPE[2]

Procedure

1. Sample is loaded at the top of the solid phase extraction (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 signal is collected for 3 minutes

- Cartridges were 3D printed with Ultimaker 2 Extended+
- Mass spectrometry analysis was performed using Thermo Q-Exactive Focus
  - Non-targeted MS/MS mode using 7 m/z wide isolation window (34 windows 150-436 m/z) with stepped collision energy of 25, 35 and 65 eV

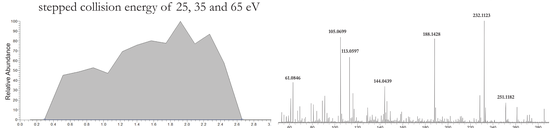

Figure 4: Paper spray chronogram and MS/MS spectra

Data Analysis:

- Data analysis was performed using TraceFinder 3.3
- In-house library was created with 300+ compounds, including new synthetic drugs and traditional drugs of abuse
- At least two fragment ions must be detected within 5 ppm m/z window

Results

Calibration Curves

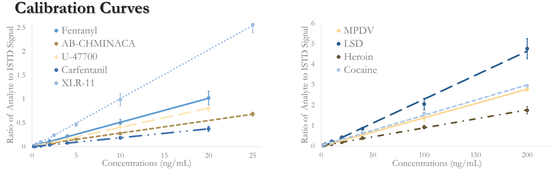

Figure 5: Calibration curves for selected designer and "classic" drugs

Limits of Detection

Analyte	LOD (ng/mL)	Rel. Error Slope	R^2	Analyte	LOD (ng/mL)	Rel. Error Slope	R^2	Analyte	LOD (ng/mL)	Rel. Error Slope	R^2
SYNTHETIC CANNABINOIDS				THJ-2201	0.50	3.3%	0.980	MDPV	0.5	1.8%	1.0
5F-ADB	0.10	1.9%	0.987	XLR-11	0.30	1.7%	0.989	Methylone	1	4.2%	0.9
5F-PB-22	0.10	4.0%	0.945	FENTANYL ANALOGUES				OTHER NPS			
AB-CHMINACA	0.30	1.1%	0.996	Acetyl Fentanyl	0.05	2.1%	1.0	25I-NBOMe	2	3.4%	1.0
AB-FUBINACA	0.10	3.2%	0.966	Carfentanil	0.05	2.5%	1.0	Benzylpiperazine	25	4.0%	0.9
AB-PINACA	0.30	1.9%	0.988	Cyclopropyl fentanyl	0.25	2.2%	1.0	Etizolam	1	2.6%	1.0
ADB-FUBINACA	0.1	3.9%	0.945	Fentanyl	0.05	2.3%	1.0	TRADITIONAL DRUGS			
AM-2201	0.10	2.7%	0.976	FIBF	0.3	1.8%	1.0	Alprazolam	0.5	2.2%	1.0
AMB-FUBINACA	0.1	2.3%	0.981	Furanyl Fentanyl	0.1	3.3%	1.0	Cocaine	2.25	0.8%	1.0
JWH-018	0.10	2.8%	0.974	Remifentanyl	0.05	2.6%	1.0	Heroin	1	1.8%	1.0
JWH-200	0.10	3.2%	0.967	U-47700	0.03	1.4%	1.0	Ketamine	1	2.3%	1.0
JWH-250	0.30	1.8%	0.989	CATHINONES				LSD	0.3	2.1%	1.0
MMB-CHMICA	0.50	4.1%	0.941	α- PVP	5	2.5%	1.0	Methamphetamine	20	4.3%	0.9

Table 1: Limits of detection for the calibrators

Bias and Precision

Analyte	Bias			Intraday Variation			Interday Variation		
	QC_{Low}	QC_{Medium}	QC_{High}	QC_{Low}	QC_{Medium}	QC_{High}	QC_{Low}	QC_{Medium}	QC_{High}
SYNTHETIC CANNABINOIDS									
5F-ADB	-4.5%	4.0%	14.3%	29.6%	14.2%	22.0%	8.9%	12.9%	5.5%
5F-PB-22	6.2%	-17.3%	4.2%	29.2%	17.7%	41.7%	7.9%	2.4%	26.5%
AB-CHMINACA	-0.7%	2.6%	2.9%	39.0%	4.5%	8.7%	4.4%	4.6%	4.4%
AB-FUBINACA	1.3%	-14.5%	-4.7%	25.4%	17.6%	25.9%	21.2%	19.9%	6.6%
AB-PINACA	5.0%	-3.0%	9.9%	27.2%	15.8%	15.8%	8.8%	25.2%	15.8%
ADB-FUBINACA	13.0%	-5.9%	7.6%	29.0%	25.7%	22.9%	5.5%	15.1%	12.1%
AM-2201	8.9%	9.1%	12.8%	25.9%	15.9%	11.8%	11.9%	14.8%	7.8%
AMB-FUBINACA	24.0%	0.6%	1.3%	32.9%	21.8%	20.6%	4.9%	11.4%	14.0%
JWH-018	-0.1%	7.6%	4.3%	18.4%	14.2%	17.1%	12.4%	17.4%	8.0%
JWH-200	21.2%	-12.2%	6.6%	35.5%	22.4%	31.7%	21.1%	32.5%	28.4%
JWH-250	-3.2%	5.6%	4.9%	21.4%	11.8%	17.7%	14.9%	8.4%	7.2%
MMB-CHMICA	6.3%	-16.1%	0.1%	13.7%	18.9%	21.0%	5.8%	28.4%	19.3%
THJ-2201	-0.6%	12.5%	0.7%	37.7%	21.8%	33.6%	16.3%	14.6%	18.6%
XLR-11	10.7%	-12.4%	4.3%	21.2%	13.7%	11.5%	15.8%	14.2%	15.7%
FENTANYL ANALOGUES									
Acetyl Fentanyl	11.1%	1.5%	3.6%	26.1%	10.1%	16.0%	14.6%	13.6%	6.3%
Carfentanil	5.7%	-9.7%	1.9%	32.2%	26.8%	16.9%	11.5%	17.4%	12.8%
Cyclopropyl fentanyl	24.2%	11.3%	16.9%	27.6%	7.9%	14.3%	4.2%	5.8%	3.4%
Fentanyl	6.7%	10.7%	8.2%	29.0%	9.8%	14.4%	16.9%	13.3%	9.8%
FIBF	-6.3%	-1.4%	6.6%	35.8%	14.2%	13.4%	51.7%	12.3%	8.4%
Furanyl Fentanyl	10.7%	3.0%	1.5%	30.0%	23.9%	21.4%	12.9%	44.8%	22.0%
Remifentanyl	-4.2%	4.1%	-5.2%	26.6%	13.2%	23.6%	9.8%	13.4%	14.0%
U-47700	0.0%	-3.8%	6.4%	12.0%	11.4%	8.2%	8.0%	15.2%	9.7%
CATHINONES									
α- PVP	7.7%	2.4%	6.0%	16.8%	13.8%	22.3%	17.6%	7.4%	8.6%
MDPV	9.4%	6.1%	1.5%	20.9%	12.1%	22.0%	18.6%	5.2%	4.2%
Methylone	20.9%	9.7%	1.9%	23.1%	26.8%	33.1%	9.2%	13.6%	10.8%
OTHER NEW PSYCHOACTIVE SUBSTANCES									
25I-NBOMe	9.7%	-16.7%	6.6%	14.7%	17.7%	23.8%	11.5%	21.6%	10.4%
Benzylpiperazine	19.1%	-10.6%	6.7%	30.1%	46.8%	49.2%	7.1%	33.4%	58.3%
Etizolam	4.2%	3.5%	-1.7%	16.0%	20.4%	16.9%	4.0%	8.6%	4.9%
TRADITIONAL DRUGS									
Alprazolam	3.8%	2.2%	2.6%	18.4%	9.5%	8.5%	23.2%	4.7%	5.8%
Cocaine	8.5%	7.1%	5.5%	14.6%	2.2%	7.6%	2.3%	0.6%	2.1%
Ketamine	6.7%	9.6%	-8.9%	26.1%	16.7%	23.5%	6.6%	27.2%	10.3%
LSD	14.9%	-0.7%	13.2%	19.7%	15.5%	18.6%	10.3%	30.1%	3.3%
Methamphetamine	7.1%	-7.2%	-0.5%	14.8%	15.8%	29.6%	10.1%	13.3%	14.5%

Table 2: Bias and Precision were evaluated over a five day period, and at least three replicates were run for each QC

Relative Matrix Effects

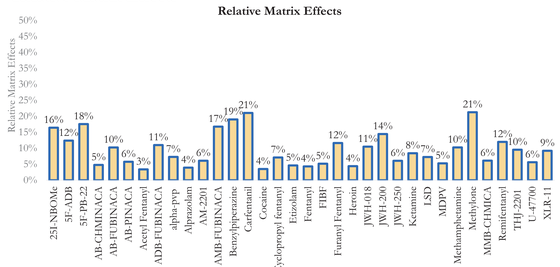

Figure 6: Relative Matrix Effects for calibrators. Calibration curves were prepared in ten individual donor plasma samples. Variation of the calibration curve slopes were used to evaluate relative matrix effects.

Ion Suppression

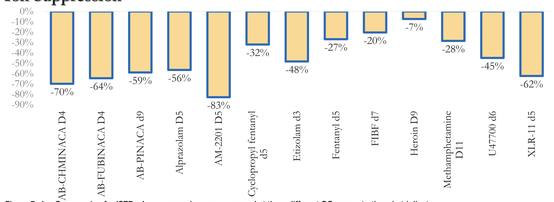

Figure 7: Ion Suppression for ISTDs. Ion suppression was measured at three different QC concentrations in triplicate.

Suspected Overdose Samples

- Suspected overdose samples were collected from two local emergency departments
- To date, 32 suspected overdose samples were analyzed, and more will be analyzed in the near future

Paper Spray Results

- **31/32** samples tested positive for a least one drug
- **30/32** samples tested positive for more than one drug
- **84** unique drugs were detected
- Methamphetamine (12), Fentanyl (10), Lorazepam (8) were most commonly detected drugs

Toxicology Lab Results

- Only one class of compounds could be tested due to small sample volume
- Several synthetic cannabinoids, cathinones, tryptamines and fentanyl analogues were not screened for
- No false negatives
- 19 False positives were detected
  - 10 drugs were detected were below the Toxicology lab's cut off levels
  - 9 drugs were detected due to interference

Drug Class	Detected in # Samples
Synthetic Cannabinoids	15
Benzodiazapines	12
Methamphetamine	12
Opiates (non-fentanyl)	12
Fentanyl	11
Fentanyl analogues	8
Cathinones	8
Cocaine	5
Tryptamines	3
NBOMe	1

Table 3: Number of times a drug class was found in a unique sample

Conclusions

- A method was developed, optimized and validated for detection of designer drugs in plasma with paper spray mass spectrometry
- The presented method allows for rapid, sensitive (sub ng/mL) detection of designer and "classical" drugs with minimal sample preparation and no chromatography
- Method successfully identified designer and "classic" drugs in multiple clinical samples, and the results were confirmed by an independent forensic toxicology lab

References

1. Shi, R. Z., El Gierari, E. T. M., Manicke, N. E. & Faix, J. D. Rapid measurement of tacrolimus in whole blood by paper spray-tandem mass spectrometry (PS-MS/MS). *Clin. Chim. Acta* **441,** 99–104 (2015).

2. 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).

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