Comparison of SWATH, DDA, and PRM Methods for Screening Novel Psychoactive Substances in Plasma by Paper Spray Mass Spectrometry

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Introduction and Background

- **Manicke Group Mission:** Use mass spectrometry to find solutions to difficult real-world problems.
- Mass spectrometry is an analytical tool that is versatile, sensitive (ppb range), able to be coupled to other instruments, have high accuracy, and be coupled to other instruments.
- Synthetic drug use is at an all time in the United States.
- We compare the sensitivity and specificity of three commonly used mass spectrometry acquisition techniques: **Sequential Windowed Acquisition of All Theoretical Mass (SWATH), Parallel Reaction Monitoring (PRM), and Data Dependent Acquisition (DDA).**
- Typical clinical workflow for biofluid includes plasma storage, sample cleanup/pretreatment, before chromatography and MS analysis.
- Paper spray allows for analysis of crude biofluid.

Paper Spray Mass Spectrometry

- Analytical technique first developed by the Cooks and Ouyang Groups at Purdue.
- Mentioned in over 1800 papers since 2010.

Advantages
Small sample volume
No solvent waste
No carryover
Rapid analysis (1-2 minutes)
No sample preparation
Analyze both small and large molecules
Ambient ionization
Automatable

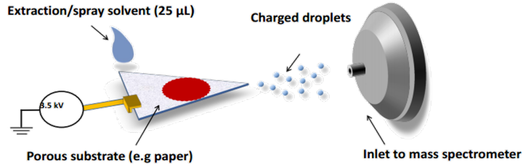

Figure 1. Schematic of paper-spray mass spectrometry[2]

Project Goals

Most work comparing these methods is done with proteins utilizing LC-MS. This project focuses on comparing the sensitivity and specificity of these methods utilizing paper spray ionization mass spectrometry and small molecule drugs.

Hypothesis:

PRM will be most sensitive with lowest incident of false positives.

SWATH will be more sensitive than DDA but have some false positives.

DDA will have comparable sensitivity to PRM on the peaks it performs MS/MS on, if it misses the target peak, specificity is worse than PRM/SWATH.

Method Development and Analyte Selection

MS Methods

Mass Spectrometer: Thermo Q-Exactive Focus
Sample Volume Spot: 5 µL
Spray Solvent: Acetonitrile with 0.1% Formic Acid
Instrument Resolution: 35,000
Stepped Collision Energy: 20,35,55
Polarity: Positive
Spray Voltage: 4.5 kV
Data Collection Time: ~2 minutes per sample

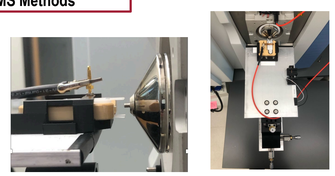

Figure 2. PS-MS Apparatus

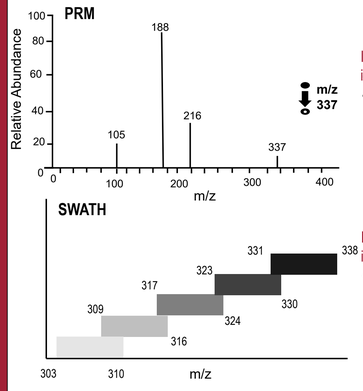

Figure 4: PRM, SWATH, and DDA Mass Spectrometry Methods

PRM MS Method:

Instrument Mode: **Targeted** MS/MS with 1.5 m/z isolation windows with 12 windows (50-400 m/z)

- Performs full scan of each transition from exact mass inclusion list, then fragments each precursor ion resulting in an MS^2 spectrum.

SWATH MS Method:

Instrument Mode: **Untargeted** MS/MS with 7 m/z isolation windows with twenty windows

- Twenty isolation windows with 7 m/z isolation with all molecular ions captured in windows are fragmented and produce MS^2 spectrum.

DDA MS Method:

Instrument Mode: Data Dependent analysis, exclusion list with known background compounds from solvent and plasma

- The most abundant (starred) peaks are selected by the mass spectrometer for MS^2 analysis.

DDA MS^2 spectrum is completely dependent on the data. Performs full-scan first then MS^2 on most abundant ions from full scan spectrum. Most intense ions (15-20 peaks) in MS^1 are fragmented one by one. This cycle is repeated 3 times.
Potential Limitation: Compounds of interest may never be selected for MS^2 analysis.

m/z	Source
239.1274	Solvent Cluster
284.3306	Matrix
391.2823	Paper

Table 1: Example of Compounds on DDA Exclusion List

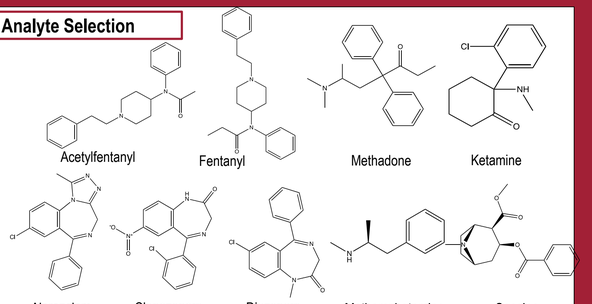

Figure 3. Stimulant Drugs of Interest

Compound	Calibration Range (ng/mL)	Internal Standard
Acetylfentanyl	4.0-200.0	Acetylfentanyl $^{13}C_6$
Alprazolam	4.0-200.0	Alprazolam D_5
Clonazepam	4.0-200.0	Clonazepam D_4
Cocaine	4.0-200.0	Cocaine D_3
Diazepam	4.0-200.0	Diazepam D_5
Fentanyl	0.4-20.0	Fentanyl D_5
Ketamine	4.0-200.0	Ketamine D_4
Methadone	4.0-200.0	Methadone D_3
Methamphetamine	4.0-200.0	Methamphetamine D_{11}

Table 2: Calibrator Concentration Range and Internal Standards

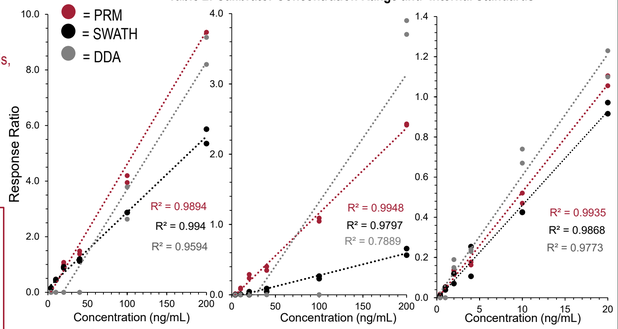

Figure 5: Representative Calibration Curves for Acetylfentanyl, Alprazolam, and Fentanyl. Where the red line represents the PRM calibration curve, the black line represents the SWATH calibration curve, and the grey line represents the DDA calibration Curve.

Sensitivity: LOD

Compound	PRM (ng/mL)	SWATH (ng/mL)	DDA (ng/mL)
Acetylfentanyl	4.43	6.86	100
Alprazolam	3.20	12.72	200
Clonazepam	4.90	6.31	0
Cocaine	6.63	6.10	20
Diazepam	6.85	6.11	100
Fentanyl	0.35	1.02	2
Ketamine	3.42	16.92	40
Methadone	6.29	6.17	40
Methamphetamine	7.07	12.71	100

Table 3: Limit of Detection (LOD) for all analytes for each method. Tracefinder 3 was used for data analysis and provided numbers for PRM and SWATH methods. DDA samples were analyzed manually

Specificity: Patient Samples

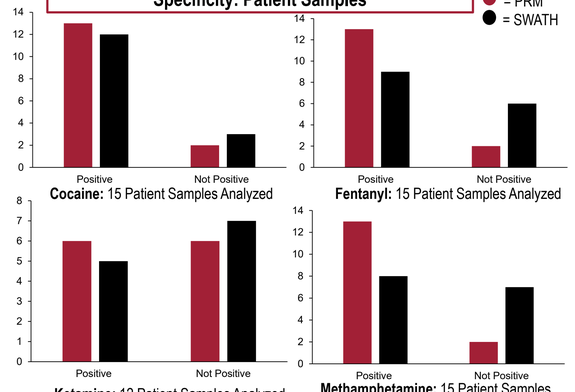

Figure 6. Patient Sample Results for PRM and SWATH Methods

Acknowledgements

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Manicke Research Group

References

1. He, W. et al., Angewandte Chemie International Edition 2010, 49 (5), 877-880.
2. Shi, R. Z. et al. Clinica Chimica Acta 2015, 441, 99–104.
3. Zhang, C. et al., Analytical Chemistry 2015, 87(12), 6212-6219.