Studying Paper Properties to Improve Detection Limits of Synthetic Cannabinoids and Fentanyl Using Paper Spray Mass Spectrometry

Brandon J. Bills, Jeffrey Kinkade, Greta Ren and Nicholas E. Manicke*
Department of Chemistry and Chemical Biology. Indiana University-Purdue University Indianapolis. Indianapolis, IN.
*nmanicke@iupui.edu

Overview

- The surge of synthetic cannabinoids and fentanyl poses an analytical problem
- Analytes can be at low concentration with the possibility of degrading further
- Paper spray is rapid, but, detection limits are hindered by matrix effects
- The properties of the substrate impact recovery and ionization efficiency
- A systematic approach measures the impact of individual matrix effects
- Detection limits can be improved by exploiting spray substrate properties
- Adding sesame oil to the paper can reduce degradation of THC in dried urine

Introduction

- Paper spray involves flowing solvent through a sample on paper and applying a voltage to generate a spray similar to ESI
- The spray substrate (paper) has an impact on the matrix effects
- Relative ionization efficiency can be measured by the change in signal of the internal standard in the spray solvent when comparing a biofluid to neat
- Relative recovery can be measured by comparing the ratios betweenthe analyte extracted from the sample and the internal standard in the solvent for biofluid and neat

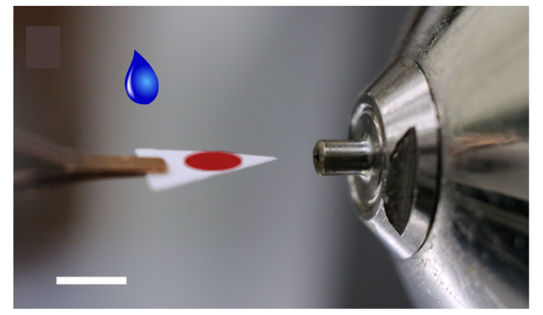

Figure 1: Paper Spray Set up[1]

$$\text{Relative Ionization Efficiency} = \frac{(AUC_{\text{SIL in solvent}})_{\text{biofluid}}}{(AUC_{\text{SIL in solvent}})_{\text{neat}}}$$

$$\text{Relative Recovery} = \frac{\left(AUC_{\text{Analyte from sample}} / AUC_{\text{SIL in solvent}}\right)_{\text{biofluid}}}{\left(AUC_{\text{Analyte from sample}} / AUC_{\text{SIL in solvent}}\right)_{\text{neat}}}$$

- Goals:
  - Determine relationship between paper properties and matrix effects
  - Utilize paper properties to lower detection limits with paper spray
  - Modify paper to improve detection of difficult analytes (like THC)
  - Modify paper to concentrate analytes from urine

Table 1: Paper Properties
Properties from the manufacturer website. (*values that were determined experimentally)

Paper	Pore Size (µm)	Thickness (µm)	Weight (g/m²)	Flow Rate (mm/30 min.)	Flow During PS-MS (µL/minute)*
Whatman Grade 4 Filter Paper	25	210	92	-	3.1
Whatman Grade 5 Filter Paper	2.5	200	100	-	1.1
Grade 3MM CHR Paper	-	340	186*	130	2.4
Grade 31 ET CHR Paper	-	500	183*	225	5.8

Methods

- Spray substrates were selected in pairs based on either pore size (filter paper), flow rate (chromatography paper)
- To determine the impact of thickness Cellulose powder TLC plates were made
- A universal cartridge was designed (figure 2) to accomodate different types of paper and TLC plates
- Paper and solvent with better ionization efficiency were compared to paper/solvent with better recovery for detection limits in urine (which has worse ionization efficiency)

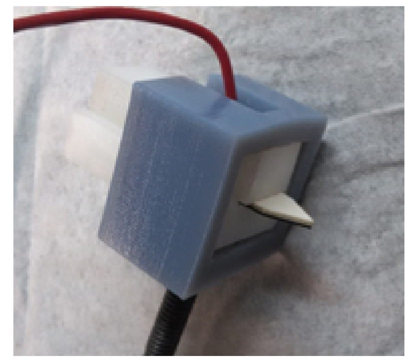

Figure 2: Universal spray cartridge

Figure 3: Sesame Oil

- 3MM chromatography paper was modified by adding sesame oil as a preservative[3] for tetrahydrocannabinol (THC) and its metabolites 11-nor-carboxy-THC (COOH-THC) and the glucuronide
- The ratio between analyte in the urine and internal standard spotted on immediately after drying and after 24 hours was used to determine if the analytes were preserved
- Sesame oil was compared to mineral oil and oleic acid for preserving ability
- Urine was flowed through paper with and without sesame oil to determine if hydrophobic analytes would concentrate on the paper or in the oil
- Urine was spotted at one end of a strip of paper containing sesame oil and allowed to wick through
- After drying, the strip was cut into 5 mm increments (figure 4)
- Each segment was spiked with internal standard and analyzed
- A set of cal curves prepared with and without oil (the first 5 mm segment) and were analyzed over the span of a month

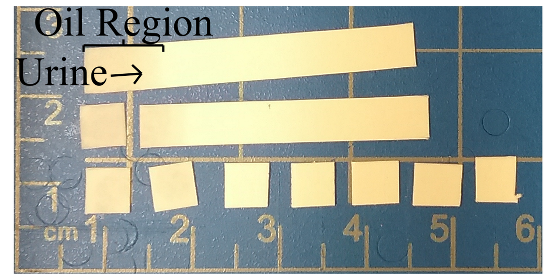

Figure 4: Cut up paper with oil

Results and Conclusions

- Figure 5 shows the change in relative recovery and ionization efficiency with slower flow rates (comparing CHR paper), smaller pore size (comparing filter paper), and thicker substrates (TLC plates)
- Properties that make paper a better filter lower recovery but improve ionization efficiency
- Differences in detection limits when comparing paper/solvent combinations for better recovery (filter 4/methanol) and ionization (3MM/acetonitrile) are shown in table 2
- Charged analytes showed less improvement

Table 2: Comparing detection limits LoD values using 2 different solvents and 2 different papers.

	F4 Methanol	3MM Acetonitrile
AB-CHMINACA	37	0.94
AM-2201	1.9	1.4
Fentanyl	1.0	0.86
Hydrocodone	2.5	2.6

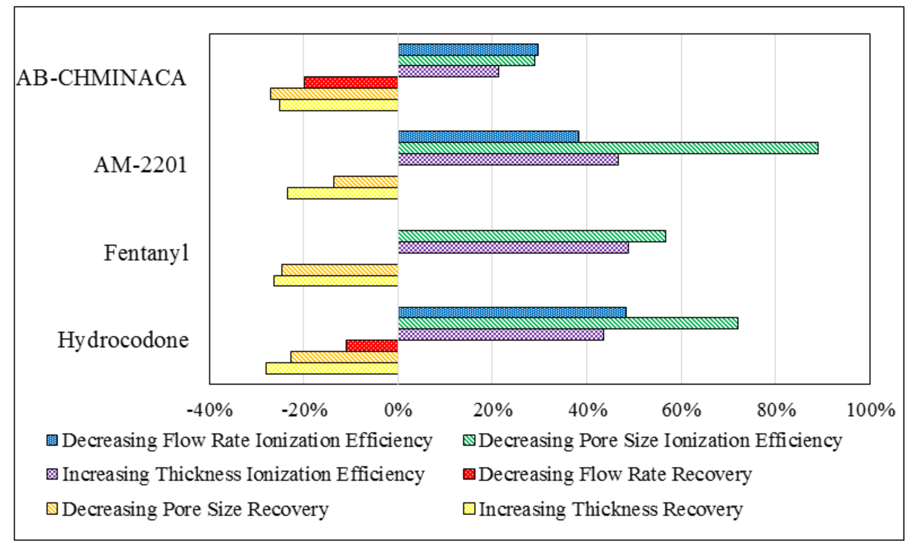

Figure 5: Difference in relative recovery and ionization efficiency.

Table 3: Decrease in analyte/internal standard ratio after 24 hours of drying on the counter with oil

	Decrease from day 1 to day 2		
	THC	COOH THC	THC glucuronide
Mineral Oil	-95%	-53%	-20%
Oleic Acid	-100%	-96%	-68%
Sesame Oil	-7%	-6%	-29%
No Oil	-92%	-88%	-95%

- Table 3 shows the change in analyte/post-spiked internal standard ratio after 24 hours
- Of the oils tested, sesame oil made the biggest impact on preserving THC and carboxy THC at room temperature on the benchtop
- Figure 6 shows the normalized (to the first 5 mm increment) change in analyte/internal standard ratio from the starting edge to the end with and without sesame oil

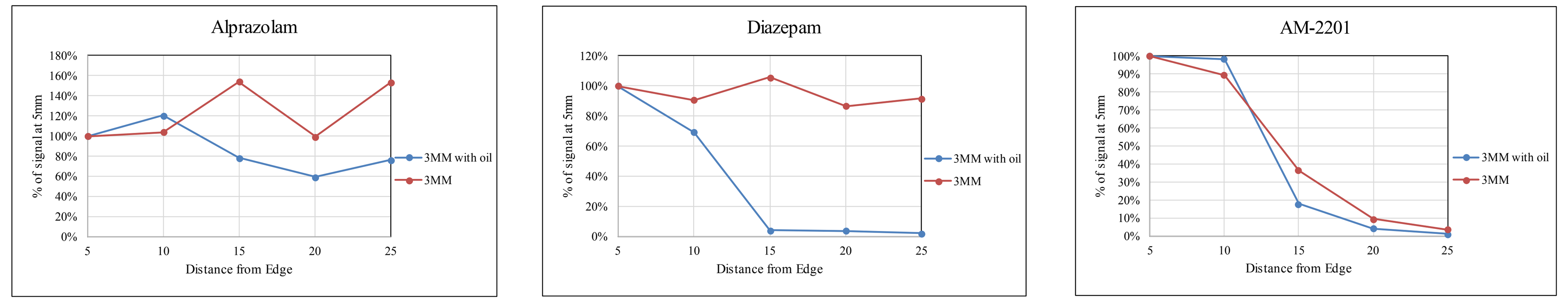

Figure 6: Decreases in analyte/internal standard ratio for 3 drugs as a function of distance traveled through a strip of paper with or without sesame oil at leading edge. Results normalized to ratio of first 5 mm segment

Table 4: Decrease in analyte/internal standard ratio after 24 hours of drying on the counter with oil

	Day 1 LoD		Day 27 LoD	
	Urine	Oil	Urine	Oil
THC	3.3	0.15	110	2.2
OH THC	5.7	0.68	54	7.7
COOH THC	3.7	8.2	-	12
AB-CHMINACA	1.1	6.8	2.5	3.5
AM-2201	0.20	0.20	0.44	0.18
Fentanyl	1.1	3.3	0.61	6.9

- Calibration curves using sesame oil to concentrate/preserve the samples had noticeably lower detection limits for THC and its metabolites, but, not for synthetic cannabinoids or fentanyl (table 4)
- AM-2201 had lower detection limits in urine using unmodified 3MM paper in table 4 than table 2 due to the fact that it concentrates as it passes through a strip of paper (as shown in figure 6)
- It was shown that by using paper properties and sesame oil lower detection limits could be achieved for certain analytes

Acknowledgments and Citations

Funding for this research was provided by a grant from the National Institute of Justice, Office of Justice Programs under award number 2016-DN-BX-007

1. Wang, H., et al., Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. Angew. Chem., Int. Ed. 2010, 49 (5), 877-880, S877/1-S877/7.
2. Vega, C., et al., Ionization Suppression and Recovery in Direct Biofluid Analysis Using Paper Spray Mass Spectrometry. J. Am. Soc. Mass Spectrom., 2016. 27(4): p. 726-734.
3. Wempe, M. F., et al., Stability of dronabinol capsules when stored frozen, refrigerated, or at room temperature. Am. J. Health-Syst. Pharm. 2016, 73 (14), 1088-1092