Investigating paper properties for ion suppression and recovery in paper spray mass spectrometry

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Overview

- Paper Spray Mass Spectrometry utilizes a porous spray substrate when generating ions
- The properties of the porous substrate impact analyte recovery and ion suppression
- A systematic approach allows for the study of the impact of individual matrix effects
- Detection limits can be improved by careful selection of spray substrate

Introduction

- The spray substrate is a wedge of paper or similar porous substrate with a macroscopic point
- Solvent is applied to the paper and a dried sample and an applied voltage produces a cone of charged solvent droplets similar to ESI (shown on the right)

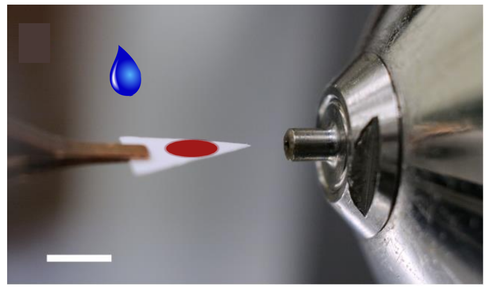

Figure 1: Paper Spray Set up[1]

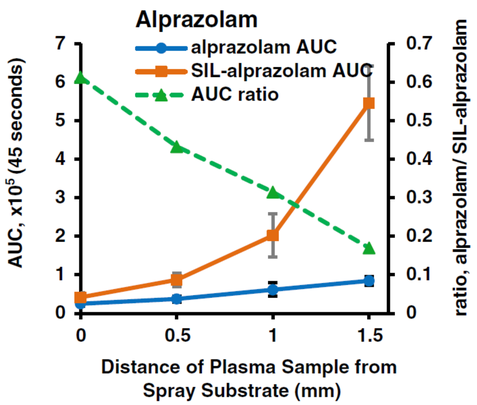

Figure 2: Change in recovery, ion suppression and analyte signal in regards to distance of paper passed through[2]

- The porous spray substrate has an impact on the matrix effects
- Measuring the signal of a stable isotopic label (SIL) in the solvent measures ion suppression (orange line in figure 2)
- Comparing the ratio between the signal from an eluted analyte and the SIL measures recovery (green line in figure 2)
- Increased travel distance through paper improves ion suppression and reduces recovery (shown on left)

- Past studies of the type of spray substrate often compare papers with multiple different properties
- To understand how different properties impact recovery and ion suppression papers must be selected that are as similar as possible with only one property drastically different
- Manufacturing cellulose TLC plates allows for more control of the properties of the spray substrate
- Spray substrates can be made hydrophobic to understand the impact of surface properties

Methods

- Pharmaceuticals with variable properties were selected to better understand trends

	MW	logP	pKa (acid)	pKa (base)	Physiological Charge
Alprazolam	308.77	2.23	18.3	5.08	0
Atenolol	266.336	0.57	14.08	9.67	1
Carbamazepine	236.269	2.1	15.96	-3.8	0
Diazepam	284.7	2.63	NA	2.92	0
Fentanyl*	336.471	4.12	NA	8.77	1
Flunitrazepam*	313.3	2.2	NA	1.7	0
Gabapentin	171.237	-1.9	4.63	9.1	0
Hydrocodone	299.368	2.13	18	8.61	1
Phenylephrine	167.205	-0.69	9.07	9.69	1

Table 2: Properties of analytes used in study
*Fentanyl was substituted for flunitrazepam for later trials due to poor signal

- Paper was selected in pairs with as many properties similar as possible except one
- Filter papers were selected with different pore size
- Chromatography papers were selected with different flow rates
- Different spray substrates were given a hydrophobic treatment[3]

Paper	Pore Size (µm)	Thickness (µm)	Weight (g/m²)	Flow Rate (mm/30 min.)
Whatman Grade 4 Filter Paper	25	210	92	-
Whatman Grade 5 Filter Paper	2.5	200	100	-
Grade 3MM Chromatography Paper	-	340	186*	130
Grade 31 ET Chromatography Paper	-	500	183*	225

Table 1: Paper properties. *Weights not given by manufacturer were measured using a scale

- TLC plates were manufactured using cellulose and cut using a laser engraver
- A spray cartridge was designed that could be used on spray substrates of different thickness
- Cartridge consists of a top and bottom part milled from plastic and 3D printed clamp

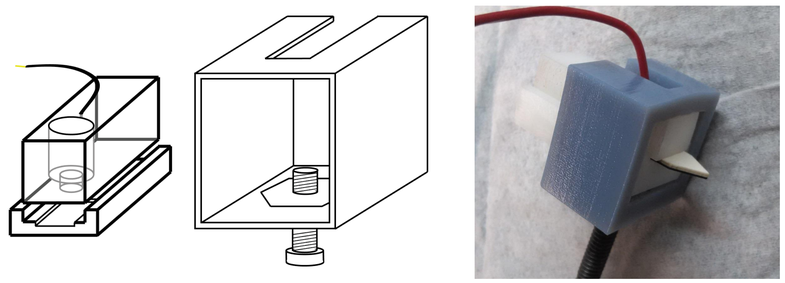

Figure 3: Universal Spray Cartridge

$$Relative\ Recovery = \frac{AUC_{\text{Analyte } biofluid} \Big/ AUC_{SIL\ biofluid}}{AUC_{\text{Analyte } matrix\ free} \Big/ AUC_{SIL\ matrix\ free}} \quad (1)$$

$$Relative\ Ion\ Suppression = \frac{AUC_{SIL\ biofluid}}{AUC_{SIL\ matrix\ free}} \quad (2)$$

$$\%Change = \frac{(A - B)}{A} * \%100 \quad (3)$$

- Spray substrates with similar properties were analyzed in pairs
- Relative recovery and ion suppression was calculated from the area under the curve (AUC) using equations 1 and 2
- Changes in relative results were calculated using equation 3

Results

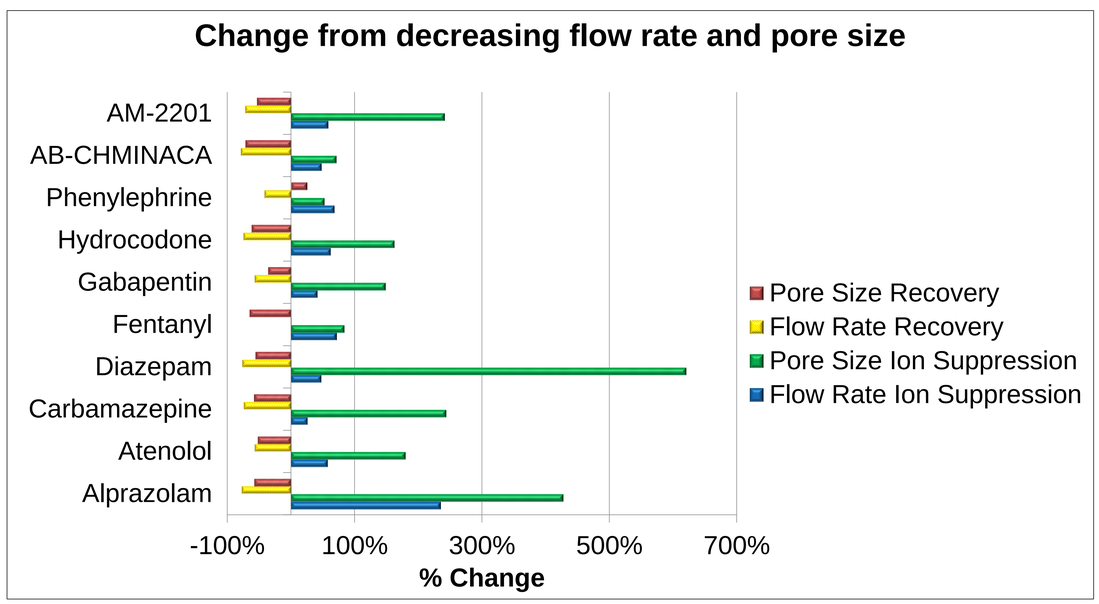

Figure 4: Change in relative recovery and ion suppression when comparing grade 4 to grade 5 filter paper (large to small pores) and 31ET to 3MM chromatography paper (fast to slow flow rate)

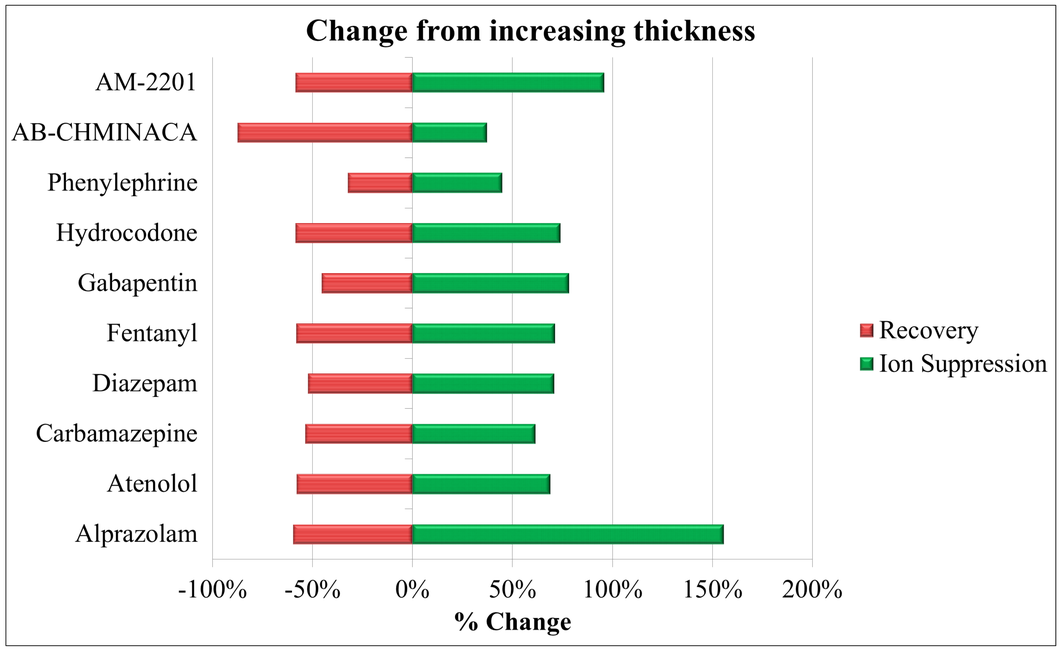

Figure 5: Change in relative recovery and ion suppression when comparing thin to thick TLC stationary phase

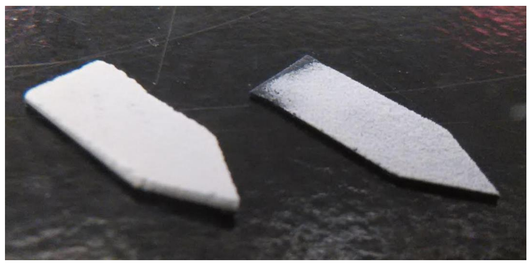

Figure 6: Thick and thin TLC spray substrates

- Using a thicker TLC stationary phase had a similar effect as using a smaller pore size
- There is a trend of a trade off between ion suppression and recovery
- The general tendency appears to be that a higher resistance to flow decreases recovery and improves ion suppression

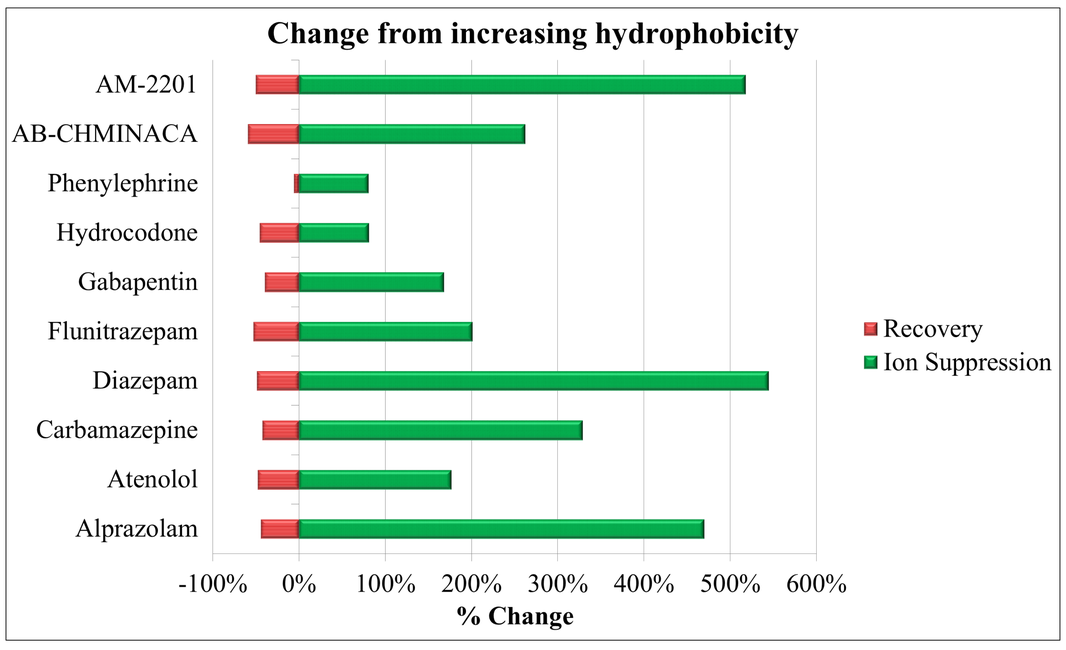

Figure 7: Change in relative recovery and ion suppression when comparing hydrophilic to hydrophobic grade 4 filter paper

- A more hydrophobic spray substrate appears to hurt recovery while improving ion suppression
- In theory a spray substrate could be optimized for the biological matrix being used
- Urine was selected as a matrix that has problems with ion suppression, but, minimal problems with recovery

Optimized conditions for urine

- Whatman grade 4 filter paper (a thin substrate with large pores) was paired with methanol for optimal recovery, but, poor ion suppression
- 3MM chromatography (a thicker substrate with a slow flow rate) was paired with acetonitrile for optimal ion suppression
- Detection limits were determined using the standard error of the y-intercept of a calibration curve

Solvent	methanol	acetonitrile
Material	Filter 4	3MM
Alprazolam	0.39	0.78
Atenolol	53	26
Carbamazepine	11	1.4
Diazepam	2.0	1.2
Fentanyl	5.8	3.8
Gabapentin	-	3.2
Hydrocodone	190	95
AB-CHIMINACA	65	4.4
AM-2201	0.84	0.61

Table 3: Change in detection limits when comparing optimal ion suppression with optimal recovery conditions and a urine matrix. Gabapentin showed no signal for filter 4 at low concentration

Conclusions

- A universal spray cartridge was manufactured to test a variety of porous spray substrates of variable thickness and composition
- Small pore size, slow flow rate, thick spray substrate, and hydrophobic cellulose were all found to improve ion suppression while hurting recovery
- Optimal conditions for ion suppression showed an improvement in the limits of detection for a urine matrix
- Future work entails studying the components of urine to determine if the spray substrate can be modified to further enhance ion suppression
- Improved methods will be applied to a method for the detection of synthetic cannabinoids

Acknowledgments

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Works Cited

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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. Damon, D.E., et al., *Direct Biofluid Analysis Using Hydrophobic Paper Spray Mass Spectrometry.* Anal. Chem. (Washington, DC, U. S.), 2016. **88**(3): p. 1878-1884.