Optimization and Quantitation of Antibiotics in Dried

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OVERVIEW

PURPOSE: The goal is to develop the first rapid, user-friendly paper spray mass spectrometry (PS-MS) assay for the quantitation of anti-bacterial agents

METHODS: Serum samples spotted on chromatography paper and analyzed via paper spray

RESULTS: Solvent-substrate conditions must be optimized in order to achieve appropriate detection limits

INTRODUCTION

- Suboptimal dosing of beta-lactam anti-microbial agents increases the likelihood of therapeutic failure and resistance
  - Dosing optimization is an attractive approach to combat these issues; however, they are difficult to implement
  - Different medical conditions can significantly alter the pharmacokinetics making a "one size fits all" dosing strategy inadequate
- Therapeutic drug monitoring (TDM) can be used to overcome these issues if rapid and robust methods can be developed
- Paper spray mass spectrometry (PS-MS) was used to detect and quantify four anti-microbial agents (linezolid, meropenem, ampicillin, and piperacillin) in plasma

METHODS

- Monitored in plasma
- Spiked with an internal standard containing stable isotope labeled analogs of the drugs
- Spotted onto Verispray cartridges containing various substrates and allowed to dry
- Dried biofluid spots were analyzed via paper spray using 90-10-0.1 organic-water-formic acid spray solvent
- Experiments were performed on a Thermo TSQ Altis

Figure 1: TSQ Altis™ Mass Spectrometer with Verispray™ Autosampler

RES

Antibiotic Structures

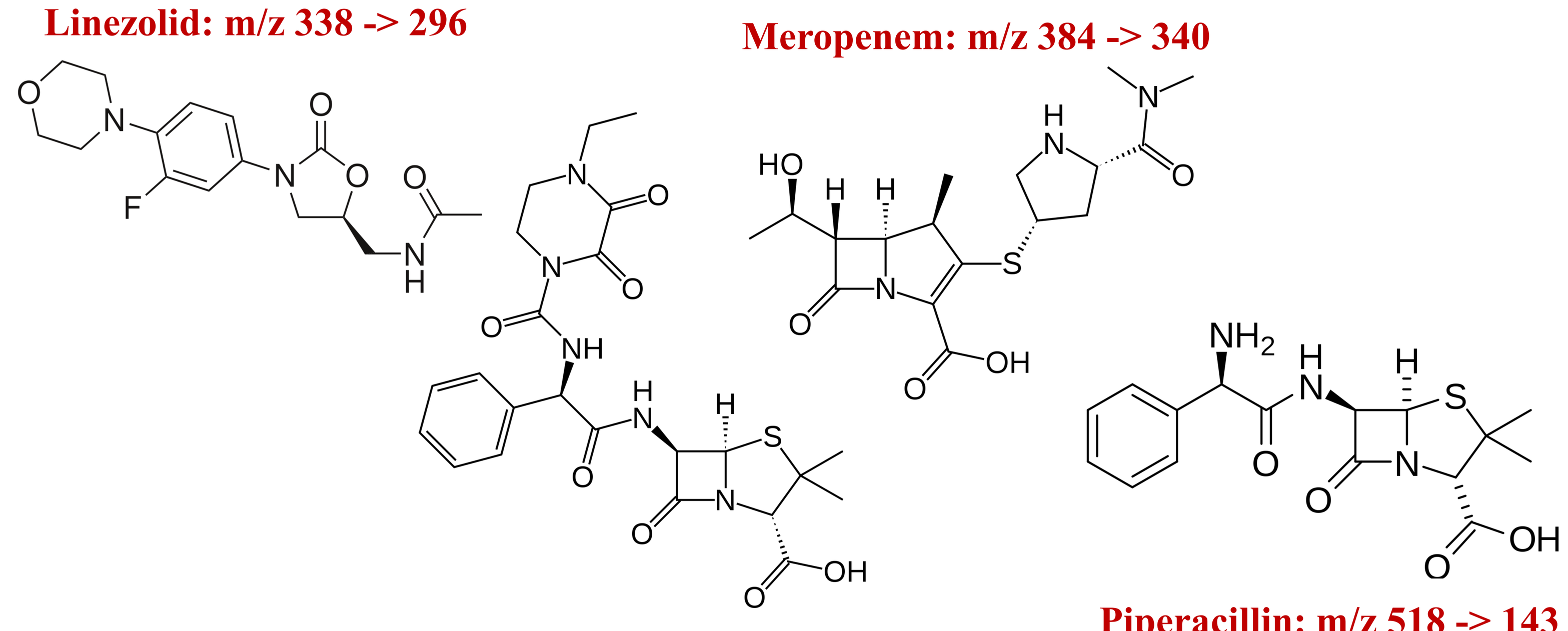


Figure 2: Structures of antibiotics with precursor and transition m/z

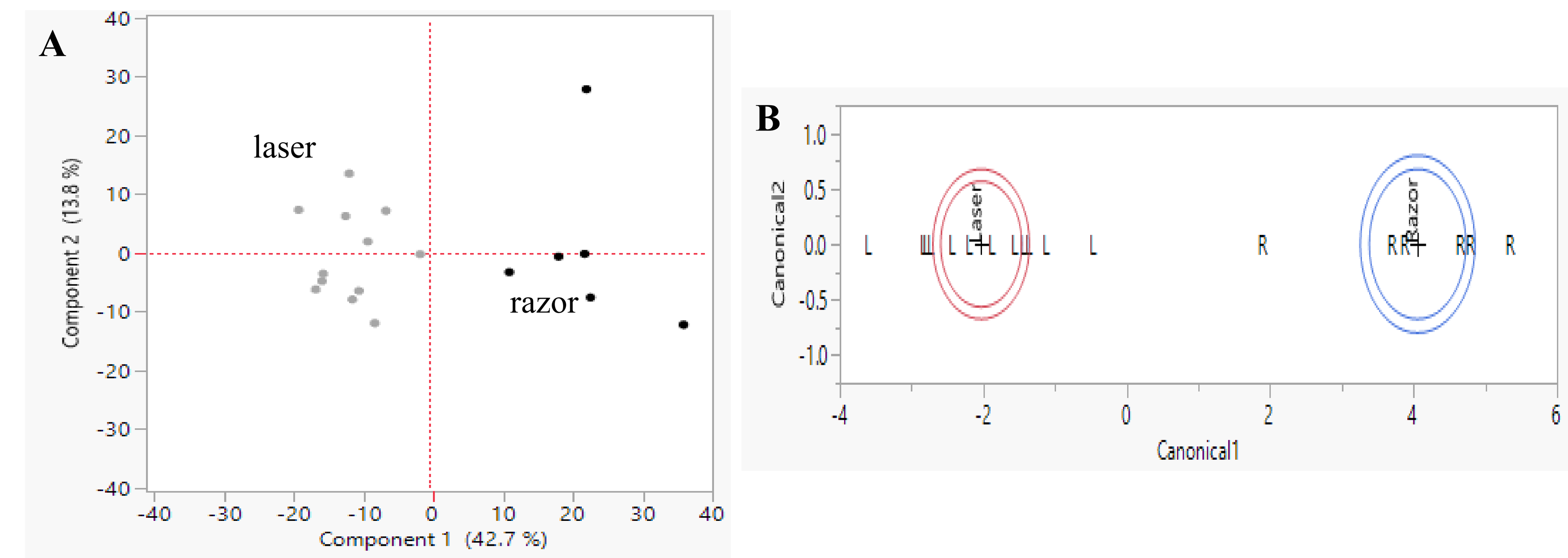


Figure 4: Principal Component Analysis (A) and Discriminant Analysis (B) depicting differences in blank signal for 55% power laser cut versus razor cut paper tips. Razor cut paper tips showed overall lower blank signal than razor cut paper tips.

Optimization of Paper Spray Conditions

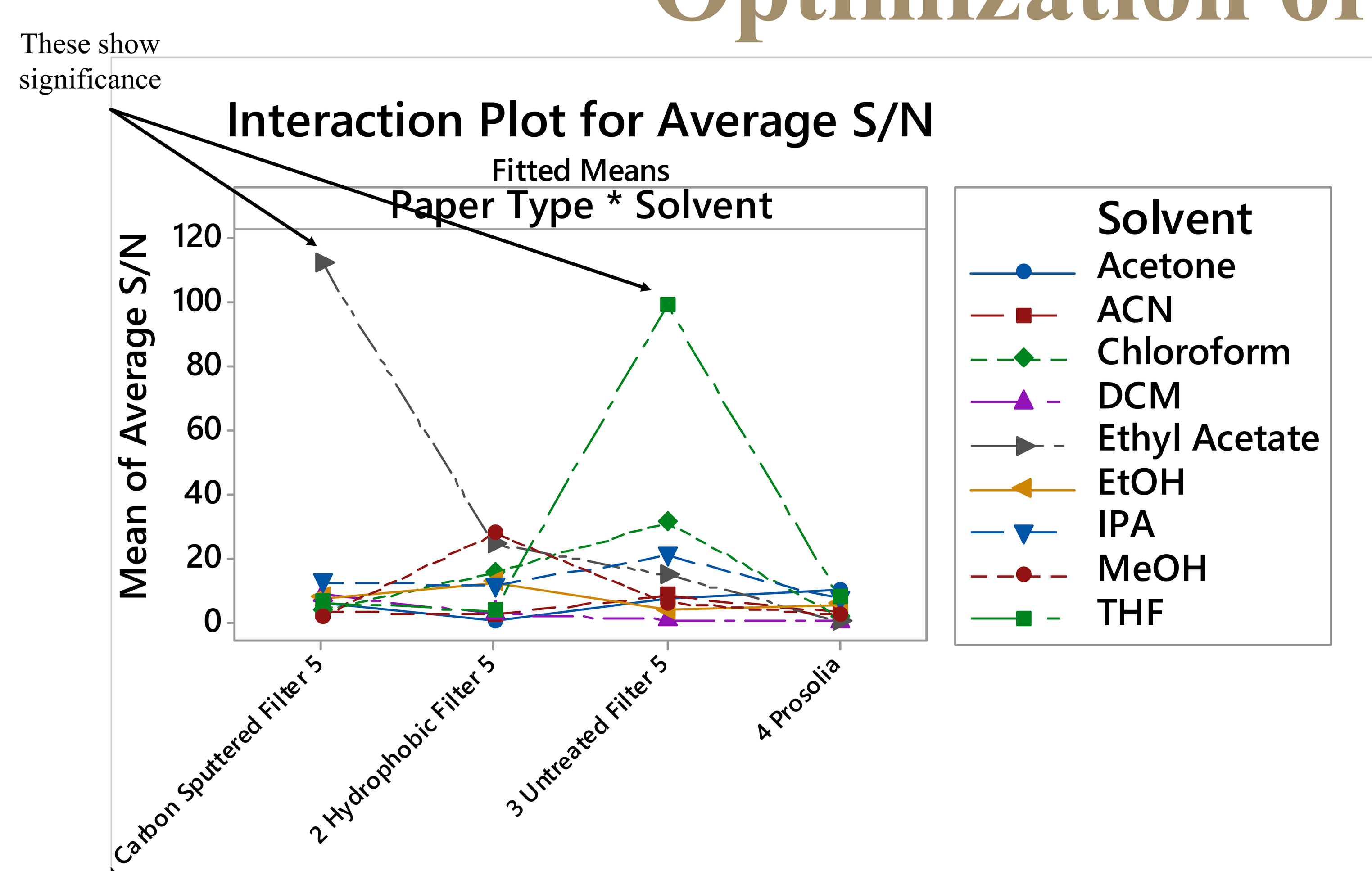
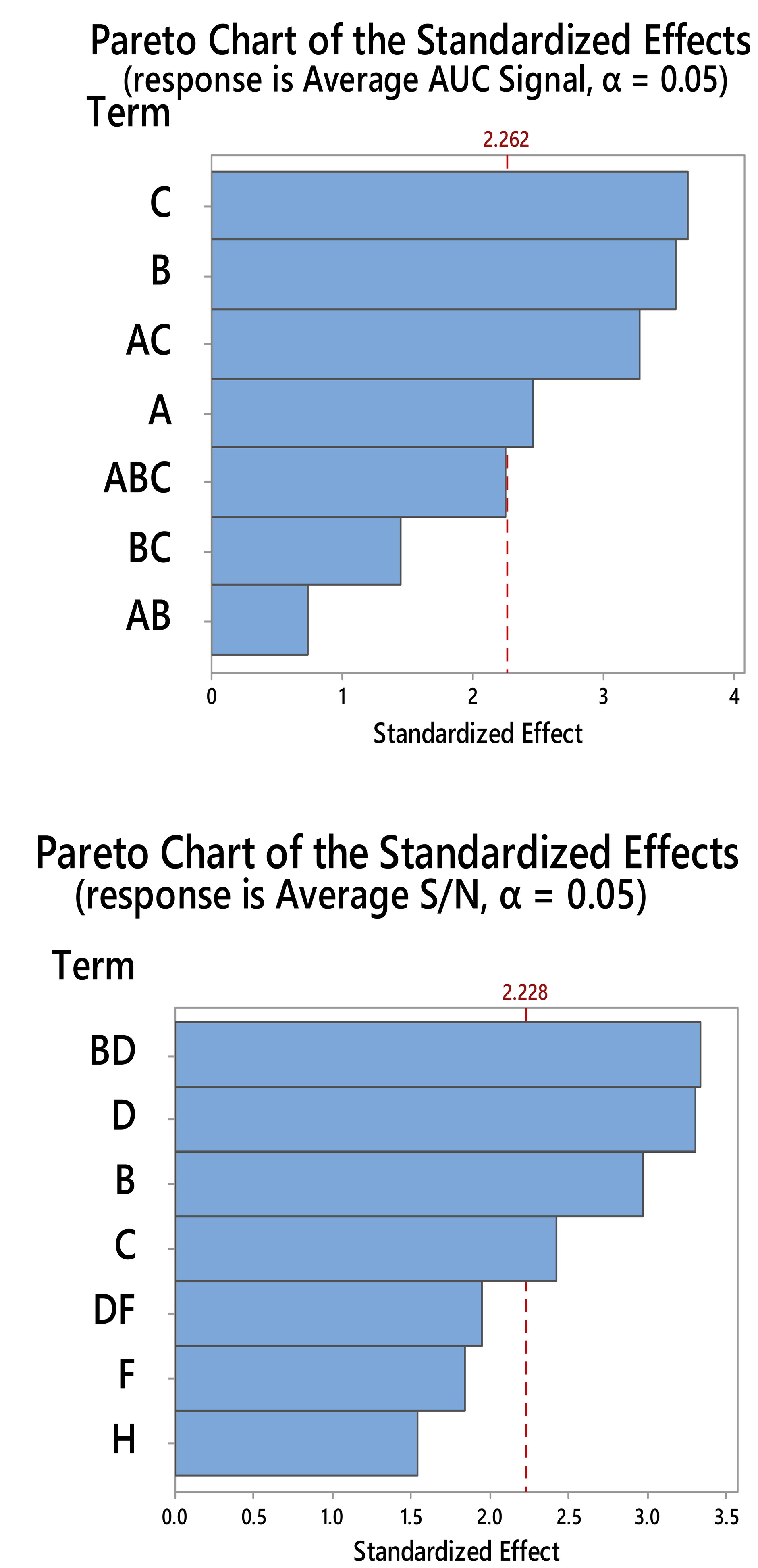


Figure 3: Interaction plot depicting optimal S/B for ampicillin based on various solvent-substrate combinations. THF-untx and CS-EA showed optimal S/N



Factor	Name
A	Pore Size
B	Sample Volume
C	Solvent Volume
D	Paper Spray Mount
E	Solvent Mixture
F	Washing Paper
G	Cut Paper
H	Solvent Location

Figure 5: Pareto of standardized effects depicting statistically significant factors affecting AUC and S/N for ampicillin. Pore size, sample volume, solvent volume, and PS mount all showed significance.

Factors

Pore Size	filter 1575	31ET
Sample Volume	1	3
Solvent Volume	40	100
Paper Spray Mount	Alligator clip	Prosolia Cartridge
Solvent	60:30:10 ACN:THF:H2O 0.1% FA	90:10 THF:H2O 0.1% FA
Washing Paper	no wash	wash
Cut Paper	bad	perfect
Solvent Location	front	back

Table 1: Eight unique paper spray factors varied to find optimal conditions for maximum AUC and S/B

Plasma Spots Utilizing Paper Spray Mass Spectrometry

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ULTS

Calibration Curves

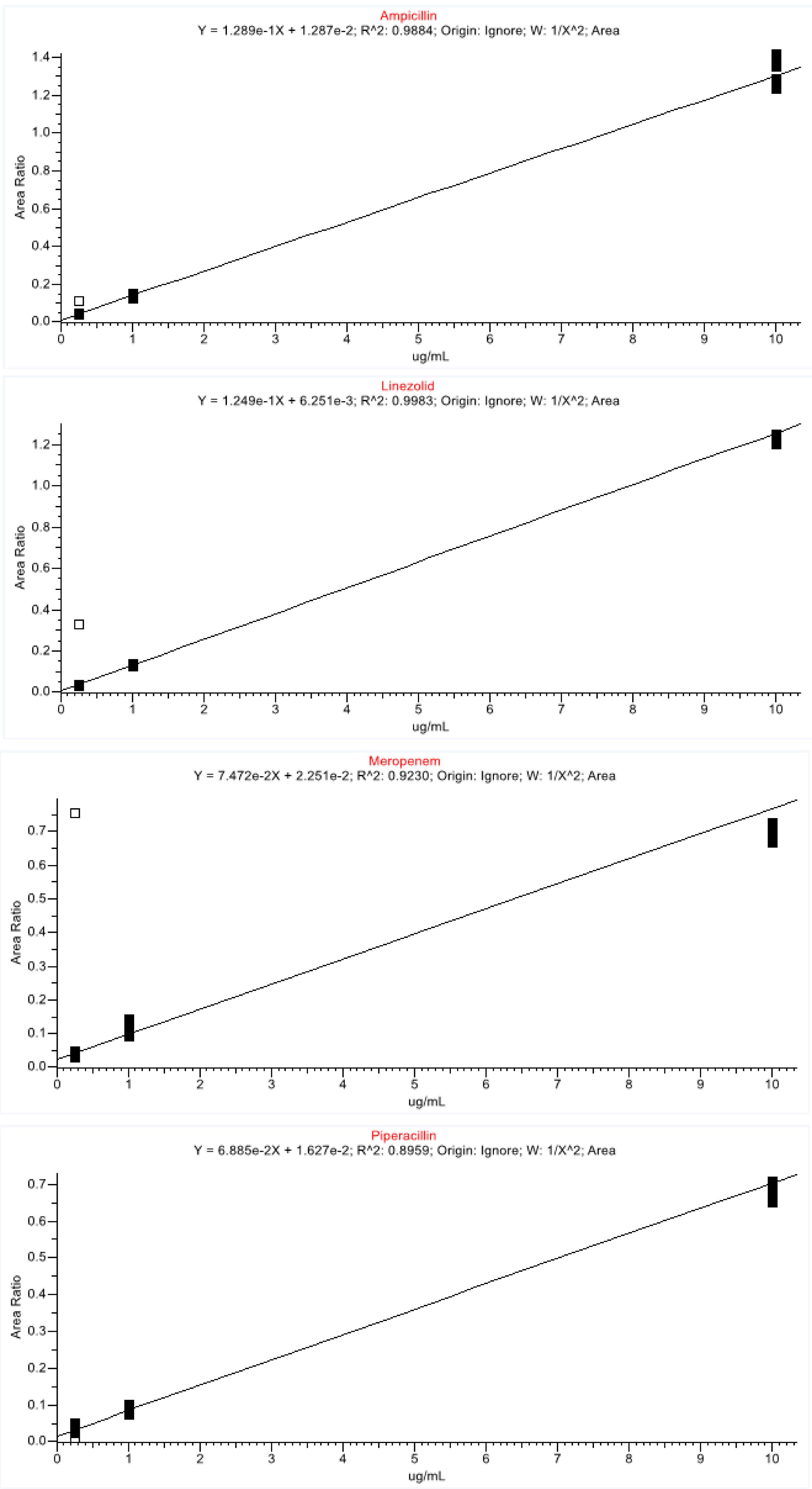

Figure 6: Calibration curves from 0.25 ug/mL – 10ug/mL utilizing razor cut paper in Verispray[TM] cassettes

Estimated LOD/LOQ

Compound	Ampicillin	Linezolid	Meropenem	Piperacillin
LOD (k=3)	0.03	0.01	0.07	0.09
LOQ (k=10)	0.09	0.03	0.24	0.28
Rel. Error of Slope (%)	2.08%	0.78%	5.56%	6.56%

Table 2: Estimated LOD and LOQ values in ug/mL for each analyte

Evaluation of Blank Signal

1	Laser_55%Power_1%acetic_1.raw
4	Laser_55%Power_1%formic_2.raw
7	Laser_55%Power_1%nitric_1.raw
10	Laser_55%Power_10%acetic_1.raw
13	Laser_55%Power_10%Formic_1.r...
15	Laser_55%Power_10%nitric_1.raw
18	Laser_55%Power_100Cbake_1.raw
21	Laser_55%Power_125Cbake_1.raw
26	Laser_55%power_acetone_3.raw
28	Laser_55%Power_Control_2.raw
32	Laser_55%Power_Hexane_1.raw
36	Laser_55%Power_IPA_1.raw

Figure 7: Treatment groups tested to reduce blank signal

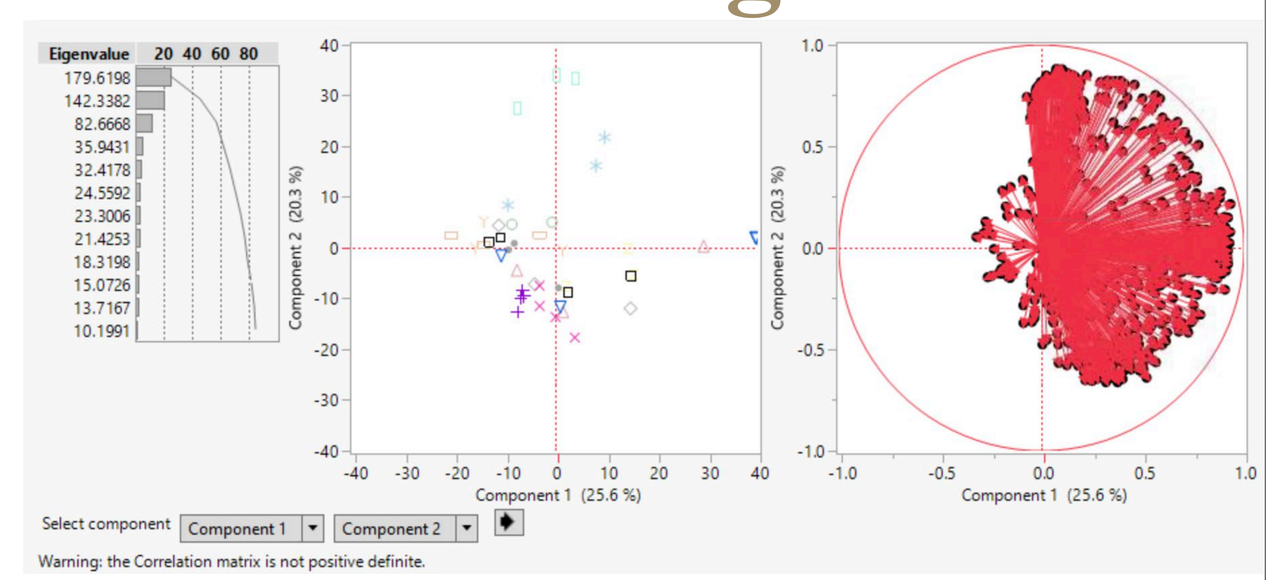

Figure 8: Scree Plot, score plot, and loading plot for all laser cut treatment groups for PC1 and PC2

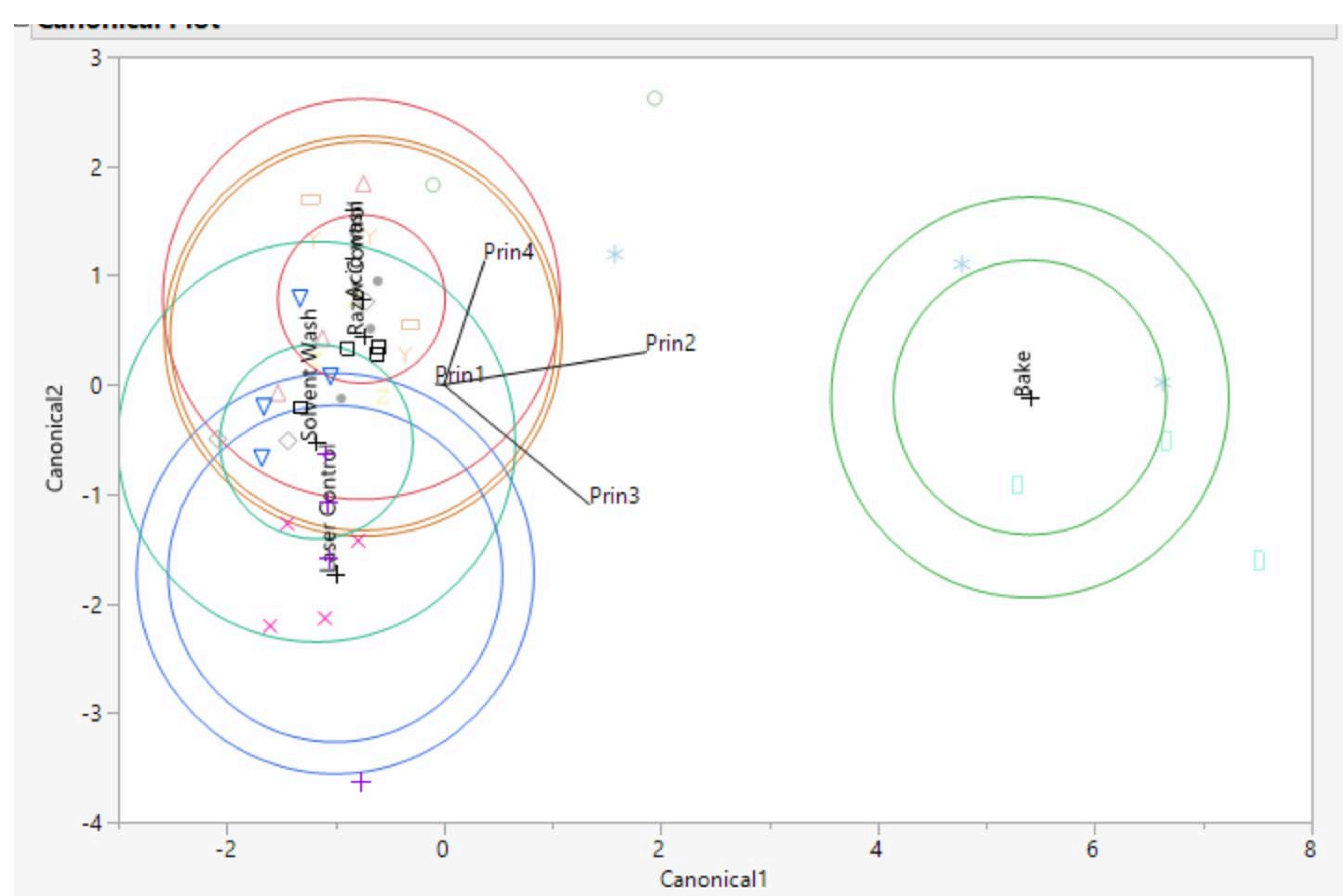

Figure 9: Canonical plot for all laser cut treatment groups showing differences in acid wash, solvent wash, and bake treatment groups. Baking the paper resulted in increased blank signal.

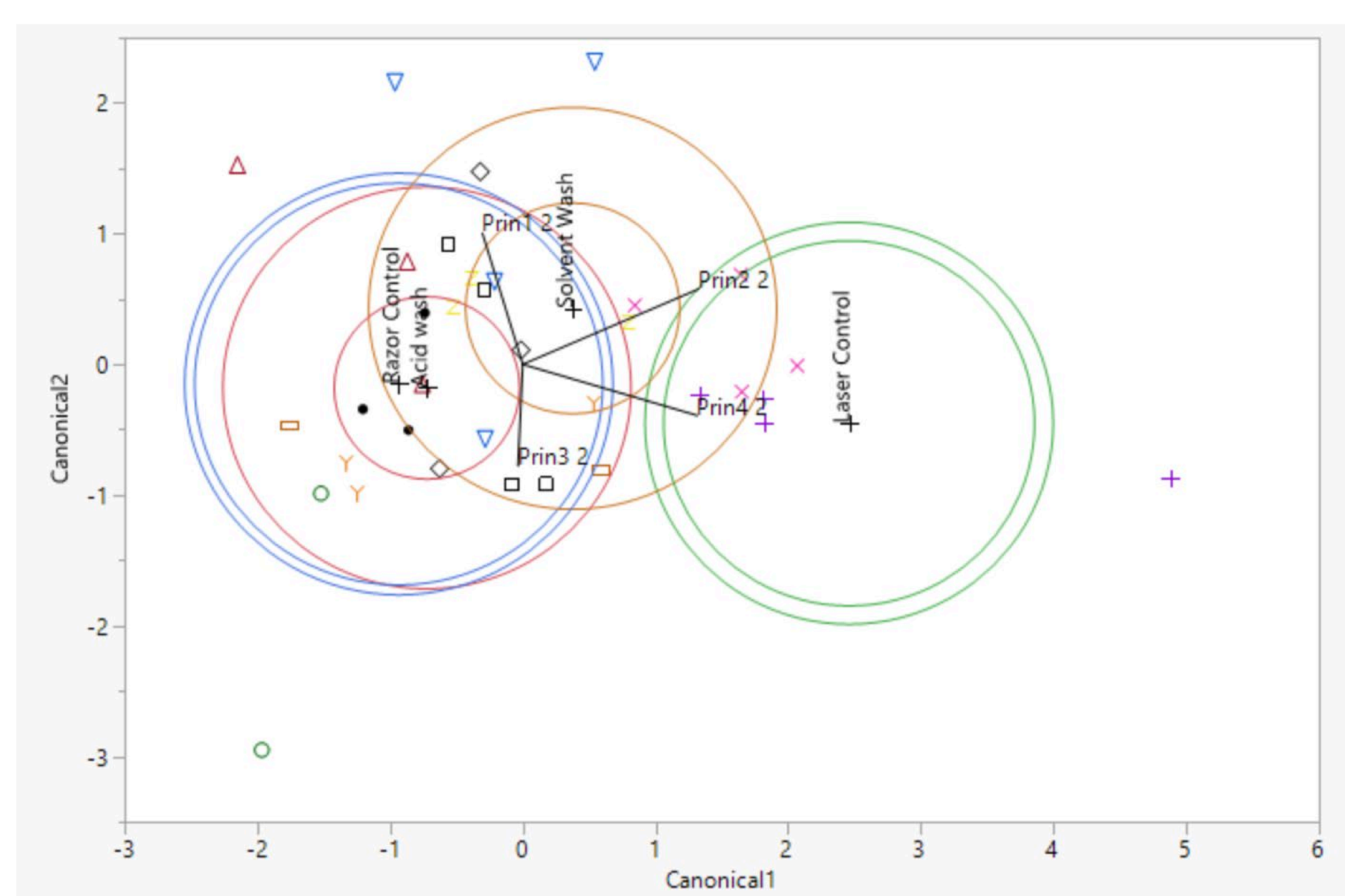

Figure 10: Canonical plot for acid wash and solvent wash treatments verus the laser and razor cut controls. Both resulted in decreased blank signal with the acid wash being close in blank signal to the razor control.

CONCLUSIONS

- Antibiotics are hydrophilic molecules and have a strong binding affinity to cellulose paper
- Carbon sputtered – ethyl acetate and untreated - THF showed highest S/N
  - Possible silanyzed paper could have better outcomes if the procedure was altered
- Pore size, sample volume, and solvent volume must be set at the highest practical limits to produce optimal signal
  - A 60:30:10 ACN:THF:H2O 0.1% FA solvent produced the most stable spray
  - Laser cut paper shows elevated blank signal most likely due to pyrolysis products and can also result in ion suppression preventing appropriate detection limits from being achieved
    - Razor cutting paper indicates that therapeutically relevant LODs will be attainable
  - Washing the laser cut paper shows promising results for decreased effects from the laser cutting process
    - A 1% acid wash in addition to a polar protic solvent wash could eliminate problems from blank signal altogether
  - Follow up studies should be performed to confirm findings from the blank study and results should be compared to the razor cut control and both optimized substrate-solvent combinations

REFERENCES

[1] Espy RD, Manicke NE, Ouyang Z, Cooks RG. Rapid analysis of whole blood by paper spray mass spectrometry for point-of-care therapeutic drug monitoring. *Analyst.* 2012;137:2344-9.

[2] Manicke NE, Abu-Rabie P, Spooner N, Ouyang Z, Cooks RG. Quantitative Analysis of Therapeutic Drugs in Dried Blood Spot Samples by Paper Spray Mass Spectrometry: An Avenue to Therapeutic Drug Monitoring. *Journal of the American Society for Mass Spectrometry.* 2011;22:1501-7.

[3] Manicke NE, Yang Q, Wang H, Oradu S, Ouyang Z, Cooks RG. Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. *International Journal of Mass Spectrometry.* 2011;300:123-9.

[4] Wang, H., Liu, J., Cooks, R., & Ouyang, Z. (2010). Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angewandte Chemie, 122*(5), 889-892. doi:10.1002/ange.200906314

[5] Wang H, Ren Y, McLuckey MN, Manicke NE, Park J, Zheng LX, Shi RY, Cooks RG, Ouyang Z. Direct Quantitative Analysis of Nicotine Alkaloids from Biofluid Samples using Paper Spray Mass Spectrometry. *Analytical Chemistry.* 2013;85:11540-4.

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