RESEARCH ARTICLE

A statistical approach to optimizing paper spray mass spectrometry parameters

Christine Skaggs[1] | Lindsey Kirkpatrick[3] | William R.A. Wichert[1] | Nicole Skaggs[4] | Nicholas E. Manicke[1,2]

[1] Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

[2] Forensics and Investigative Sciences, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

[3] Department of Pediatrics, Division of Pediatric Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN 46202, USA

[4] Technical Problem Solver, General Motors Proving Grounds, Milford, MI 48380, USA

Correspondence
Nicholas E. Manicke, Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis. Indianapolis, IN 46202, USA .
Email: nmanicke@iupui.edu

Rationale.

Paper spray mass spectrometry (PS-MS) was used to analyze and quantify ampicillin, a hydrophilic compound and frequently utilized antibiotic. Hydrophilic molecules are difficult to analyze via PS-MS due to their strong binding affinity to paper substrates and low ionization efficiency among other reasons.

Methods: Solvent and paper parameters were optimized to increase the extraction of ampicillin from the paper substrate. After optimizing these key parameters, a Resolution IV 1/16 fractional factorial design with two center points was performed to screen eight different design parameters simultaneously.

Results: Pore size, sample volume, and solvent volume were the most significant factors affecting average peak area under the curve (AUC) and the signal-to-blank (S/B) ratio for the 1 µg/mL ampicillin calibrant. After optimizing the key parameters, a linear calibration curve with a range of 0.2 µg/mL to 100 µg/mL was generated ($R^2 = 0.98$) and the limit of detection (LOD) and lower limit of quantification (LLOQ) were calculated to be 0.07 µg/mL and 0.25 µg/mL, respectively.

Conclusions: The statistical optimization procedure undertaken here increased the mass spectral signal intensity by more than a factor of 40. This statistical method of screening followed by optimization experiments proved faster and more efficient, and produced more drastic improvements than typical one-factor-at-a-time experiments.

1 | INTRODUCTION

Desorption electrospray ionization (DESI)[1] and direct analysis in real time (DART)[2] paved the way for a variety of ambient ionization techniques for mass spectrometry, which are utilized for the direct analysis of samples without extensive sample preparation or separations.[2-4] Paper spray mass spectrometry (PS-MS) is one such example as it provides a rapid and cost-effective method for the analysis of small organic molecules, complex mixtures, peptides, and intact proteins in a variety of environmental and biological matrices with little to no sample preparation.[5-8] Samples can be preloaded onto triangular-shaped paper spray substrates or the substrates themselves can be used to wipe the sample off of various

surfaces.[5,9] Pre-loading analyte onto the paper substrates allows for potential long-term storage of the sample prior to analysis, which is beneficial when sample collection occurs in the field.[10] Dried sample can be directly analyzed from the paper substrates by simply wetting the paper substrate with an organic spray solvent and applying a high voltage while in close proximity to the mass spectrometer inlet (1–4 mm). Analysis times typically range from 30 seconds to one minute.[8,11] This methodology is particularly beneficial in clinical and forensic applications for the analysis of crude bio-fluids (i.e. whole blood, plasma, and urine) or when access to miniature mass spectrometers is practical.[6,12-16] When analyzing biofluids, the applied spray solvent allows the analyte of interest to be quickly extracted while leaving the bulk of the bio-fluid behind due to the

affinity of the analyte and spray solvent, as well as mobility of the molecules in the sample.[8,10,11] Other benefits of PS-MS include low sample and solvent consumption, improved detection limits, and inexpensive substrates.[5,8]

There is a need to improve analytical approaches for therapeutic drug monitoring (TDM). Mass spectrometry-based methods for TDM tend to be and technically complex. Patient care can be further delayed when samples must be sent to an outside reference laboratory for analysis. Paper spray MS assays have been reported for a number of different therapeutic drugs.[17-19] Nearly all the paper spray MS literature on therapeutic or abused drugs concerns the analysis of hydrophobic molecules.[11] While most drugs are hydrophobic molecules, there are still a significant number of hydrophilic drugs, including beta-lactam antibiotics such as ampicillin. Detection and quantification limits for hydrophilic compounds are generally significantly higher than for hydrophobic molecules, however, making water-soluble drugs a challenging drug class for paper spray mass spectrometry. The relatively poor detection limits are caused by a combination of the strong binding affinity of these molecules to the paper substrate, poor recovery in organic solvents, co-extraction of matrix components in polar spray solvents leading to greater ion suppression, and lower intrinsic electrospray ionization efficiency of hydrophilic molecules. To overcome this, optimizing solvent and substrate properties is imperative to obtain adequate sensitivity, accuracy, and reproducibility.

This work presents a fractional factorial statistical design approach where eight different paper spray experimental factors were screened simultaneously to improve quantitation of ampicillin. This optimization procedure significantly improved detection limits and reproducibility, which enabled quantitative analysis of ampicillin in dried plasma with a detection limit of 0.07 µg/mL. This paper represents the first report of a beta-lactam antibiotic by paper spray MS, and offers a comprehensive, statistical, and systematic approach to method optimization.

2 | METHODS

2.1 | Safety

Flammable solvents are used throughout the method. Special care, including the use of personal protective equipment, should be taken when analyzing bio-fluids.

2.2 | Chemicals and reagents

Whatman paper (31ET, Filter 5, Filter 1575 and Filter 4) (Sanford, ME), polyethylene paper (Fisher Scientific, Pittsburg, PA, USA), graphene paper (Sigma Aldrich, St. Louis, MO, USA), 3 M Durapore paper (Fisher Scientific), Whatman silica-coated paper (Sanford, ME, USA), and pre-made laser cut cartridges (Prosolia, Inc, Indianapolis, IN, USA) were all tested as paper substrates in the paper screening study. Analytical grade acetonitrile, N,N-dimethylformamide, ethyl

acetate, chloroform, dichloromethane, methanol, ethanol, isopropanol, acetone, and water were purchased from Fisher Scientific. A 90:10 acetonitrile:water solution was used for the spray solvent in all paper substrate screening experiments. Formic acid at a concentration of 0.1% was added to all spray solvents to aid in ionization. Ampicillin and Ampicillin-D5 were purchased from Sigma Aldrich and Toronto Research Chemicals, Inc., (North York, ON, Canada) respectively.

2.3 | Modifying of paper substrates

The Whatman chromatography paper (31ET, Filter 5, Filter 1575, and Filter 4) was coated using a silanization reagent reported by Damon et al. [20] Carbon-nanotube coatings were prepared as described.[21] Polyethylene, 31ET, Filter 5 and Filter 1575 were coated in a layer of carbon exceeding 100 nm in thickness using a Denton Vacuum Desk V sputtering system (Moorestown, NJ, USA) as previously described.[22] Briefly, paper substrates were cut to fit within the sputter chamber, approximately 20 cm². Carbon rods were sharpened, held in contact via a spring-loaded mount and a current (~15 amps) was applied to slowly deposit the carbon, which yielded a uniform layer on the paper substrates.

2.4 | Sample preparation

Because ampicillin can be methanolized or hydrolyzed in methanol or water, respectively, it was dissolved in N,N-dimethylformamide (1 mg/mL). The 1 mg/mL ampicillin solution was further diluted to 10 µg/mL and 1 µg/mL solutions in acetonitrile. Paper tips for the thirteen substrate types were cut for manual paper spray. A volume of 8 µL of neat solution was spotted onto each of the paper substrates at three concentrations: 10 µg/mL, 1 µg/mL, and a blank. All experiments were run in triplicate, and samples were allowed to dry for one hour after spotting. For studies that took place in crude bio-fluid media, this same procedure was followed; however, only 3 µL of each concentration was spotted to avoid overloading the paper substrate with bio-fluid.

2.5 | Paper spray setup

The samples were analyzed on a Thermo Fisher Scientific LTQ XL linear ion trap mass spectrometer (San Jose, CA, USA). The mass spectrometry parameters were optimized during tuning and utilized as follows: 250°C capillary temperature, 43 V capillary voltage, 90 V tube lens voltage, 4000 V spray voltage, 1 microscan, and 15 eV collision energy. All spectra were acquired in positive ion mode and MS/MS with collision-induced dissociation was utilized for analyte identification. Xcalibur software (Xcalibur Software, Inc, Arlington, VA, USA) was used for collecting and processing. The transitions used were the $[M + H]^+$ m/z 350 ➔ 160 for ampicillin and the $[M + H]^+$ m/z 355 ➔ 160 for ampicillin-D5.

2.6 | Statistical analysis

The two concentrations (10 μg/mL and 1 μg/mL) and blanks for the thirteen different paper substrate and coating combinations were compared statistically at each level. A one factor analysis of variance (ANOVA) test compared the peak AUCs for the fragmented m/z 350 data followed by a post-hoc Fisher Least Significant Difference (LSD) test. Statistical analyses were performed using SPSS (IBM, Armonk, NY, USA) and Minitab (Minitab, Inc, State College, PA, USA). After optimizing the paper substrate, spray solvent solutions containing THF, ethyl acetate, dichloromethane, chloroform, acetonitrile, acetone, methanol, isopropyl alcohol, or ethanol were mixed in a 90:10 ratio with water and were analyzed for optimal peak AUC, S/B ratio, blank signal, and blank standard deviation. In this study, signal-to-blank is defined as the AUC of the m/z 160 in the extracted ion chromatogram for the 1 μg/mL ampicillin calibrant versus the AUC of the m/z 160 in the extracted ion chromatogram for the blank.

2.7 | Optimization of ampicillin

Optimization of experimental conditions was carried out to maximize the S/B ratio and average AUC for the 1 μg/mL ampicillin calibrant after selecting a paper substrate and solvent composition. A screening Design of Experiment (DOE) tool was used after the factors were properly identified. This experiment is classified as a screening DOE due to the highly fractionated design used. From this screening, solvent volume (40 μL and 100 μL), sample volume (1 μL and 3 μL), paper pore size (2 μm and 16 μm), PS mounting type (alligator clip and pre-made plastic paper spray cartridges from

Prosolia), paper that was washed (sonicated in THF for 10 minutes and allowed to air dry) or unwashed paper, the cut quality of the paper (bad with frayed or dulled edges and good cut quality with no fraying and sharp tips), and the location of the solvent when applied to the dried bio-fluid spot (back half of the dried biofluid spot and front half of the dried biofluid spot) were identified as the most likely causes of changes seen in intensity, S/B, blank signal, and variation. A randomized Resolution IV 1/16 fractional factorial design with two center points was performed to identify main effects with low order interactions between factors more efficiently (Table 1). A factorial design uses a statistical technique to analyze which variable (s) affect the specified response. This design is ideal when interactions between factors can occur as the conditions of one factor will require a specific condition of another factor to have the appropriate response. Factorial designs fit a regression model:

$$y = \beta 0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{j=1}^{k} \beta_j x_j + \sum \sum_{i<j} \beta_{ij} x_i x_j + \ldots + \varepsilon \quad (1)$$

where y is the response, β_0 is constant, β_i is the coefficient for factor A, x_i represents the level of factor A effects, β_j is the coefficient for factor B, x_j represents the level of factor B effects, β_{ij} is the coefficient of the interaction between factors A and B, and ε indicates the experimental errors.[23] More terms can be added with an increasing amount of factors.[23]

These variable terms are coded −1, 0, and 1 to represent low, center, and high points, respectively. The physical values for the factors are specified above. It is important to note that due to the high fractionality of the design, some factors and interactions will be indistinguishable. A Resolution IV design was used instead of a full factorial to minimize the amount of experimental trials without losing

TABLE 1 Eight factor DOE on untreated 31ET paper using THF in dried plasma spots. The units −1, 0, and 1 represent low, medium, and high values respectively

Run order	Pore size	Sample volume	Solvent volume	Paper spray mount	Solvent mixture	Washing paper	Cut paper	Solvent location
1	0	0	0	0	0	0	0	0
2	1	1	1	−1	−1	−1	1	−1
3	−1	−1	−1	−1	−1	−1	−1	−1
4	−1	1	1	−1	−1	1	−1	1
5	1	−1	1	1	−1	1	−1	−1
6	−1	1	1	1	1	−1	−1	−1
7	−1	1	−1	1	−1	1	1	−1
8	0	0	0	0	0	0	0	0
9	1	1	−1	−1	1	1	−1	−1
10	1	1	1	1	1	1	1	1
11	−1	−1	1	1	−1	−1	1	1
12	1	−1	1	−1	1	−1	−1	1
13	1	−1	−1	−1	−1	1	1	1
14	−1	−1	1	−1	1	1	1	−1
15	−1	−1	−1	1	1	1	−1	1
16	1	1	−1	1	−1	−1	−1	1
17	−1	1	−1	−1	1	−1	1	1
18	1	−1	−1	1	1	−1	1	−1

the lower order interactions, which better classifies this design as a screening experiment.

2.8 | Calibration curve preparation

A calibration curve ranging from 0.20 μg/mL to 100 μg/mL was generated from ampicillin spiked into pooled human plasma. To reduce carryover and blank signal, the plastic pieces from the paper spray cartridges were pre-rinsed by sonicating the cartridges for 60 min in water twice while rinsing with methanol between steps and allowing them to air dry prior to reassembly. Whatman 31ET paper was razor cut to fit in pre-made plastic paper spray cartridges. Ampicillin was spiked into plasma, and 8 μL of the ampicillin or blank plasma was spotted onto the respective cartridge. The sample was allowed to dry for 1 hour. The cartridge was secured in close proximity to the mass spectrometer inlet using a manual paper spray set-up, and 60 μL of the spray solvent containing 60:30:10 ACN: THF:H2O with 0.1% FA and high voltage (4 kV) were applied. The data was fitted using a weighted least square regression analysis with a weight of $1/x^2$. The limit of detection (LOD) was defined as three times the standard deviation of the AUC in drug-free plasma divided by the slope of the calibration curve ($3*s_b/m$).[24] The lower limit of quantitation (LLOQ) was calculated as $10*s_b/m$.

2.9 | Results and discussion

### 2.9.1 | Selection of paper substrates and spray solvent

An initial screening on neat ampicillin standards was performed using Whatman 31-ET laser cut paper, carbon-sputtered polyethylene paper, hydrophobic filter 5 paper, hydrophobic filter 4 paper, porous polyethylene paper, carbon-nanotubes coated paper, carbon-sputtered 31ET paper, 3 M Durapore paper, silica-coated paper, and graphene paper. Upon initial analysis, the laser cut 31ET paper had the highest AUC for a neat sample. However, the carbon-sputtered polyethylene had the highest S/B (data not shown). Neither the silica-coated nor graphene paper substrates could be analyzed due to difficulties with spray stabilization and corona discharge. The substrates with the highest AUC and S/B ratio were used in further studies containing dried biofluids. This eliminated the various polyethylene papers, 3 M Durapore paper, carbon-nanotube coated paper. When comparing the peak AUC and S/B ratio of the 1 μg/mL ampicillin calibrant in dried plasma spots among various coatings and paper types, the untreated Filter 1575 paper had the highest peak AUC (Figure 1A) while the carbon-sputtered 31ET paper had the highest S/B ratio (Figure 1B). Both carbon-sputtered Filter 1575 paper and untreated Filter 1575 paper were also quantifiable meaning the S/B was calculated to be 10 or greater. No clear
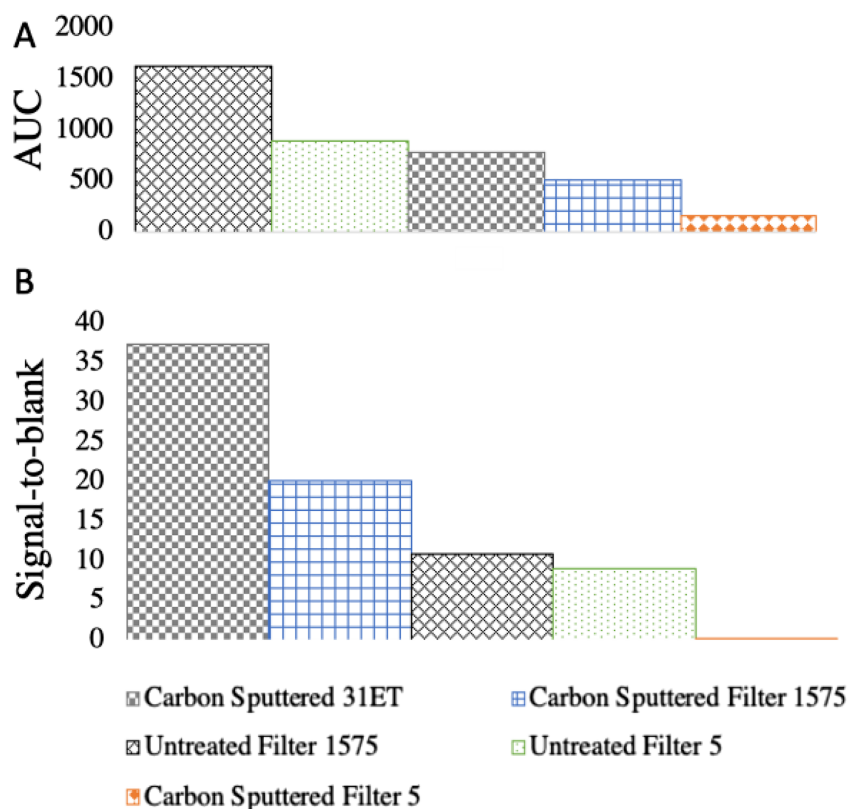
FIGURE 1 Area under the curve (a) and S/B (B) for ampicillin (1 μg/mL) in dry plasma spot on various paper types [Color figure can be viewed at wileyonlinelibrary.com]

determination could be made as to whether it was the paper type or coating type that improved the AUC and S/B ratio.

As previously reported, the spray solvent composition affects both the recovery of the analyte and ion suppression.[25-27] To evaluate the spray solvent, a screening experiment using the middle pore size paper (Filter 5; 2.5 μm) and a medium solvent composition, 90:10 organic solvent:water with 0.1% formic acid, was performed for nine different solvents on four different paper coating types (hydrophobic, carbon-sputtered, untreated razor cut Filter 5 paper, and laser cut Whatman 31ET). The results showed an interaction between paper type and solvent type; the same solvent behaved differently depending on the paper coating type (Figure 2). The highest AUC for the 1 μg/mL ampicillin calibrant in dried plasma was obtained with the carbon-sputtered Filter 5 paper using 90:10 ethyl acetate:water with 0.1% formic acid ($P = 0.002$) and untreated razor
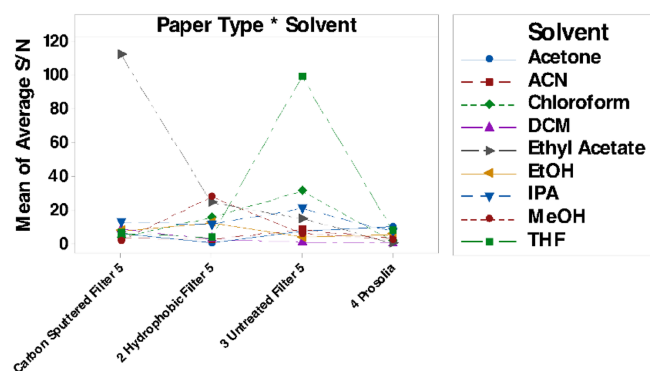
cut Filter 5 with 90:10 THF:water with 0.1% formic acid ($P = 0.005$). The THF solvent showed poor signal stability despite having the highest average AUC. Methanol and acetonitrile were added to this solvent to attempt to maintain the favorable extraction properties of THF while stabilizing the spray. Both acetonitrile and methanol stabilized the signal, but the S/B ratio was close to 1 when methanol was used. Addition of acetonitrile, on the other hand, did not have a deleterious effect on the ampicillin signal.

2.9.2 | Optimization of experimental conditions

A screening DOE was conducted to test the effects of paper pore size, sample volume, solvent volume, the paper spray mount, paper wash, quality of tip cut, solvent mixture and solvent location on ampicillin detection from dried plasma. Solvent volume, sample volume, and pore size significantly affected the peak AUC of the 1 μg/mL ampicillin calibrant (Figure 3A). There was an observable two-way interaction between pore size and solvent volume, meaning that one factor was directly affected by the other factor. There was also one three-way interaction between pore size, sample volume, and solvent volume. Similar results were observed for the S/B ratio (Figure 3B). A cube plot was used to show interactions between the three factors and the predicted response of each of the factor combinations. The predicted value was at its highest point when the sample volume (3 μL), solvent volume (100 μL), and pore size (31 ET paper) were at their high levels (Figure 4), meaning that these factors should be set at their high values to obtain the highest signal. This finding implied that these factors could be further optimized to increase the signal of the 1 μg/mL ampicillin calibrant. The pre-made plastic cartridge holder with razor cut 31 ET paper was chosen for ease of use and the

FIGURE 2 Interaction plot of coating and solvent type for the average S/B of ampicillin (1 μg/mL) in dried plasma spot [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Pareto chart of the standardized effects for the a) average AUC and B) the average S/B of the 1 μg/mL ampicillin calibrant in dried plasma. Standardized effect refers to the t-statistics gathered for each factor when testing if the factor will have no effect (effect = 0) on the average AUC or S/B. effects below the red dashed line indicate this null hypothesis being rejected with an alpha ≥0.5 (i.e. no significant effect). The standardized effect is an absolute value and therefore only the magnitude of the response can be determined, not the direction (see cube plot for direction) [Color figure can be viewed at wileyonlinelibrary.com]
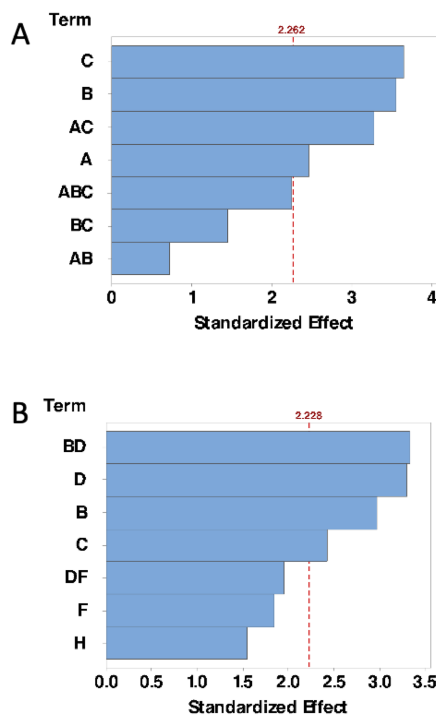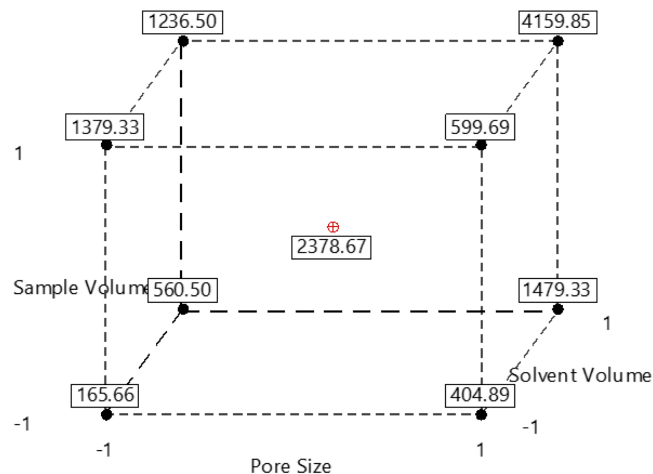
FIGURE 4 Cube plot of three way interaction between pore size (x), solvent volume (z), and sample volume (y) for average AUC of 1 µg/mL ampicillin. The boxes on each vector depict the AUC of the 1 µg/mL ampicillin for each factor combination run at the high[1] and low (−1) factor parameter [Color figure can be viewed at wileyonlinelibrary.com]

60:30:10 ACN:THF:water with 0.1% FA solvent mixture was chosen due to the improved reproducibility as discussed previously. The remaining three factors (paper washing, quality of tip cut, and solvent location) were confirmed as not statistically significant. The high and low values of the three-way interaction were run to confirm these results. Pore size of the paper was not investigated further as the Whatman 31 ET paper is a widely used paper in dried blood cards and for paper spray MS. Sample volume and solvent volume were investigated further to see if increasing the volumes of these would increase the AUC and S/B. It was found that sample volume was the only factor that was statistically significant, indicating that higher sample volume produces higher analyte signal (data not shown). Our model indicated that more sample volume could be used but, due to the physical limitations of the paper substrate, the sample volume was not increased past 8 µL. Too much solvent can "overload' the paper and cause leaking which will cause high variability in the AUC of the analyte. To prevent solvent leakage from the cartridge, 10 µL of solvent was added onto the dry plasma spot followed by adding 60 µL to the back of the cartridge rather than utilizing the maximized solvent volume from the model. This "prewetting" step of adding solvent in the front of the cartridge was to reconstitute the analyte in solvent to allow capillary action to still be effective even with the reduced solvent volume. This decrease in signal did not affect the ability to detect the 1 µg/mL ampicillin calibrant. None of the eight factors reduced the signal for the blank or reduced the standard deviation of the blank.

2.9.3 | Analysis of dried plasma samples

To confirm the parameter optimization model, a calibration curve was generated (Figure 5). The data was linear for the range of 0.2–100 µg/mL (R^2 = 0.98) with a LOD of 0.07 µg/mL and a LLOQ of 0.25 µg/mL.
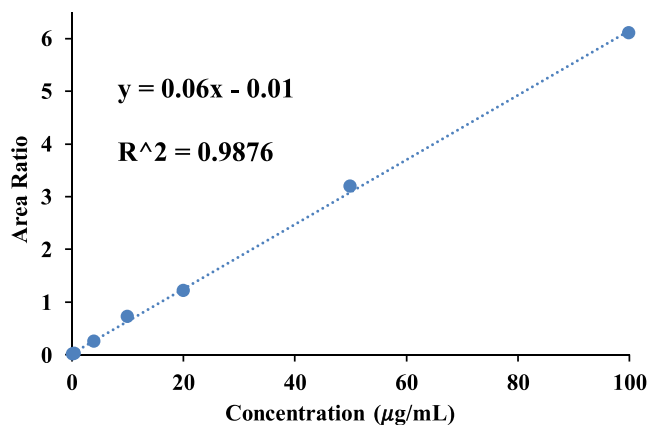
FIGURE 5 Calibration curve of ampicillin in a dry plasma spot using PSI-MS. each data point is the average of two analytical replicates [Color figure can be viewed at wileyonlinelibrary.com]

The range of this calibration curve is consistent with other methods in the literature for monitoring ampicillin plasma concentrations for TDM.[28] The average AUC for the 1 µg/mL ampicillin plasma calibrant increased from 500 to 21,000 with the optimized conditions compared with the original method. Likewise, the S/B ratio at the 1 µg/mL level increased from 2 to 58.

3 | CONCLUSION

A fractional factorial design was employed for the analysis of the hydrophilic compound, ampicillin, in a dried plasma spot using PS-MS. Pore size, sample volume, solvent volume, paper spray mount, and solvent mixture were all optimized to improve the average peak AUC of the 1 µg/mL ampicillin calibrant and the S/B ratio compared swith a blank signal. The cut of the paper, paper wash, and the solvent location were not statistically significant factors, indicating that they would not contribute greatly to changes in signal. This finding highlights the robustness of the process. A seven-point calibration curve was performed and showed linear values (R^2 = 0.98). An LOD of 0.07 µg/mL and a LLOQ of 0.25 µg/mL was calculated, which is well below the minimum inhibitory concentration (MIC) reported for all pathogens sensitive to this antibiotic. The area under the curve of ampicillin increased by a factor of 42, and the S/B ratio increased by a factor of 29 using our optimized conditions. This statistical method of screening followed by optimization experiments is faster, and more efficient, and produces more drastic improvements than typical one-factor-at-a-time (OFAT) experiments.

ORCID

Nicholas E. Manicke https://orcid.org/0000-0002-2296-0497

REFERENCES

1. Takats Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science*. 2004;306:471-473.

2. Cody RB, Laramee JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. *AnalChem*. 2005;77:2297-2302.

3. Cooks RG, Ouyang Z, Takats Z, Wiseman JM. Ambient mass spectrometry. *Science*. 2006;311:1566-1570.

4. Huang MZ, Yuan CH, Cheng SC, Cho YT, Shiea J. Ambient ionization mass spectrometry. *In Annual Review of Analytical Chemistry* 3, Yeung ES, Zare, RN. Eds. Palo Alto, 2010. 3:43-65.

5. Liu JJ, Wang H, Manicke NE, Lin JM, Cooks RG, Ouyang Z. Development, characterization, and application of paper spray ionization. *Anal Chem*. 2010;82:2463-2471.

6. McKenna J, Jett R, Shanks K, Manicke NE. Toxicological drug screening using paper spray high-resolution tandem mass spectrometry (Hr-Ms/Ms). *J Anal. Toxicology*. 2018;42:300-310.

7. McKenna J, Dhummakupt ES, Connell T, et al. Detection of chemical warfare agent simulants and hydrolysis products in biological samples by paper spray mass spectrometry. *Analyst*. 2017;142:1442-1451.

8. Wang H, Liu JJ, Cooks RG, Ouyang Z. Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angew Chem Int*. 2010;49:77-880.

9. Evard H, Kruve A, Lohmus R, Leito I. Paper spray ionization mass spectrometry: Study of a method for fast-screening analysis of pesticides in fruits and vegetables. *J Food Compos Anal*. 2015;41:221-225.

10. 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;37:2344-2349.

11. Manicke NE, Bills BJ, Zhang CS. Analysis of biofluids by paper spray MS: Advances and challenges. *Bioanalysis*. 2016;8:589-606.

12. Wang H, Manicke NE, Yang QA. Zet al. Direct analysis of biological tissue by paper spray mass spectrometry. *Anal Chem*. 2011;83:1197-1201.

13. Kissinger PT. Thinking about dried blood spots for pharmacokinetic assays and therapeutic drug monitoring. *Bioanalysis*. 2011;3:263-2266.

14. Maurer HH. Mass spectrometry for research and application in therapeutic drug monitoring or clinical and forensic toxicology. *Ther Drug Monit*. 2018;40:89-393.

15. Spooner N, Lad R, Barfield M. Dried blood spots as a sample collection technique for the determination of pharmacokinetics in clinical studies: Considerations for the validation of a quantitative bioanalytical method. *Anal Chem*. 2009;81:1557-1563.

16. Michely JA, Meyer MR, Maurer HH. Paper spray ionization coupled to high resolution tandem mass spectrometry for comprehensive urine drug testing in comparison to liquid chromatography-coupled techniques after urine precipitation or dried urine spot workup. *Anal Chem*. 2017;89:11779-11786.

17. Shi R-Z, El Gierari ETM, Faix JD, Manicke NE. Rapid measurement of cyclosporine and Sirolimus in whole blood by paper spray–tandem mass spectrometry. *Clin Chem*. 2016;62:95-297.

18. Shi R-Z, El Gierari ETM, Manicke NE, Faix JD. Rapid measurement of tacrolimus in whole blood by paper spray-tandem mass spectrometry (Ps-Ms/Ms). *Clin Chim Acta*. 2015;441:99-104.

19. Yannell KE, Kesely KR, Chien HD, Kissinger CB, Cooks RG. Comparison of paper spray mass spectrometry analysis of dried blood spots from devices used for in-field collection of clinical samples. *Anal Bioanal Chem*. 2017;409:121-131.

20. Damon DE, Davis KM, Moreira CR, Capone P, Cruttenden R, Badu-Tawiah AK. Direct biofluid analysis using hydrophobic paper spray mass spectrometry. *Anal Chem*. 2016;88:878-1884.

21. Zhang CS, Glaros T, Manicke NE. Targeted protein detection using an all-in-one mass spectrometry cartridge. *J am Chem Soc*. 2017;139:10996-10999.

22. Wichert WRA, Dhummakupt ES, Zhang C, et al. Detection of protein toxin simulants from contaminated surfaces by paper spray mass spectrometry. *J am Soc Mass Spectrom*. 2019;30:1406-1415.

23. Design MDC. *Analysis of Experiments*. NY: Wiley; 2001.

24. Long GL, Winefordner JD. Limit of detection: A closer look at the Iupac definition. *Anal Chem*. 1983;55:712A-724A.

25. Bills BJ, Kinkade J, Ren G, Manicke NE. The impacts of paper properties on matrix effects during paper spray mass spectrometry analysis of prescription drugs, fentanyl and synthetic cannabinoids. *Forensic Chemistry*. 2018;11:15-22.

26. Vega C, Spence C, Zhang C, Bills B, Manicke NE. Ionization suppression and recovery in direct biofluid analysis using paper spray mass spectrometry. *J am Soc Mass Spectrom*. 2016;27:726-734.

27. Wang T, Zheng Y, Wang X, Austin DE, Zhang Z. Sub-Ppt mass spectrometric detection of therapeutic drugs in complex biological matrixes using polystyrene-microsphere-coated paper spray. *Anal Chem*. 2017;89:7988-7995.

28. El-Najjar N, Hösl J, Holzmann T, Jantsch J, Gessner A. Uplc–MS/MS method for therapeutic drug monitoring of 10 antibiotics used in intensive care units. *Drug Test Anal* 2018;10:584–591.