



Breaking Down the Barrier to Rapid Anti-bacterial Drug Levels with Paper Spray-Mass Spectrometry: Simultaneous Quantitation of Five β -lactams from Plasma

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SCHOOL OF MEDICINE

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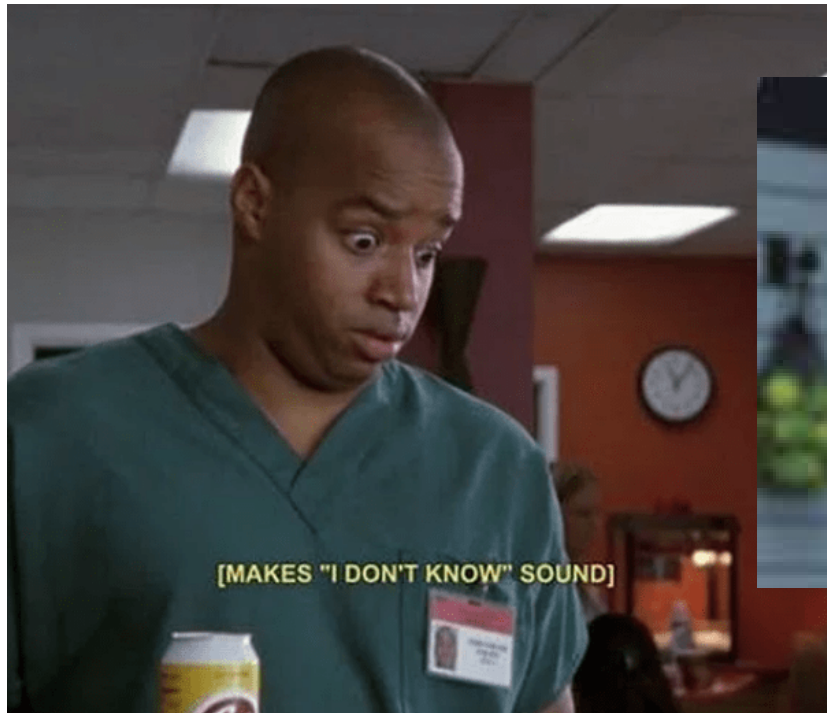


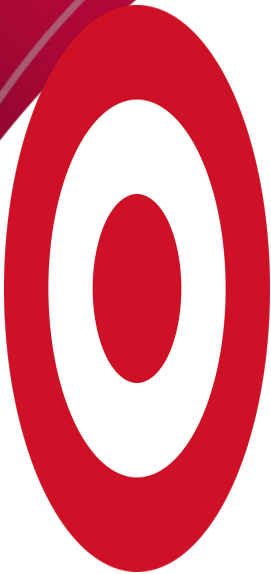
Learning Objectives

- Describe the problems facing clinicians in terms of ensuring appropriate drug levels in special populations, such as children
- Learn the most common methods for drug quantitation currently utilized by laboratories, as well as their strengths and limitations
- Name some ambient ionization methods that allow for the detection of analytes directly from crude samples
- Understand how paper spray mass spectrometry works and some of its potential applications for drug quantitation in the clinical laboratory



How Much Drug Is The Patient Really Seeing?

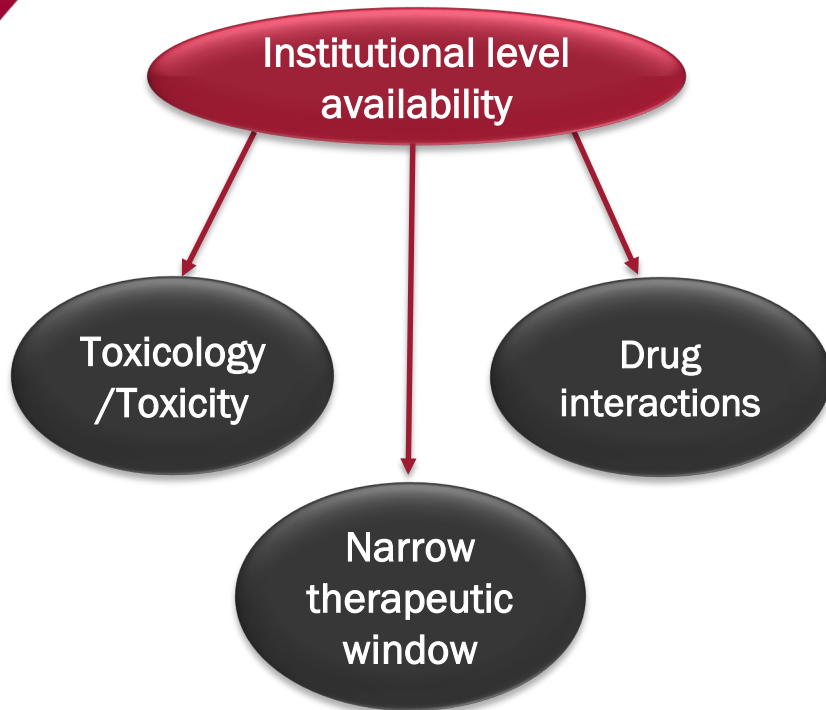




Goal: *Develop better predictive models that take into account variable physiology, ontogeny, and disease state to inform more precise dosing in pediatric patients.*



Drug Quantification Challenges in Medicine:



Acetaminophen
Aspirin
Ibuprofen
Alcohols
Caffeine
Amikacin
Gentamicin
Tobramycin
Vancomycin
Aminophylline
Carbamazepine
Valproic acid

Phenytoin
Lamictal
Phenobarbital
Topamax
Lidocaine
Digoxin
Procainamide
Cyclosporine
Methotrexate
Sirolimus
Tacrolimus
Theophylline

Most Common Methods for Drug Quantification:



<https://www.thermofisher.com/order/catalog/product/0373910>

Immunoassays

High Performance-Liquid Chromatography (HPLC)



<https://www.thermofisher.com/order/catalog/product/98630000>



<https://www.agilent.com/en/products/liquid-chromatography/infinitylab-analytical-lc-solutions/>



The Ideal On-site MS Analyzer of Biological Samples:

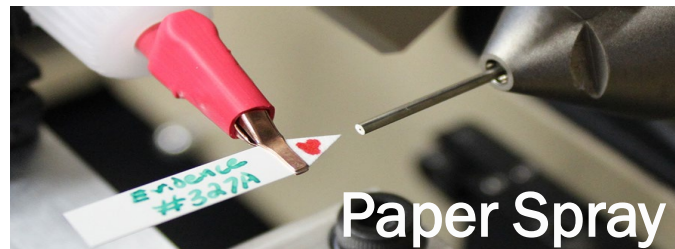
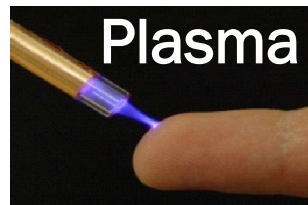


- Desktop size
- Fast turnaround time: <5 minutes
- Disposable cartridge
- Minimal sample volume

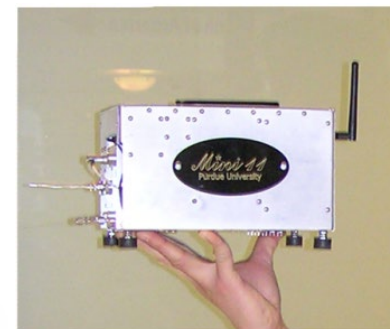


Widening the Scope of Chemical Analysis:

Extraction and Ionization



Mass Spectrometry



Paper Spray – Mass Spectrometry:

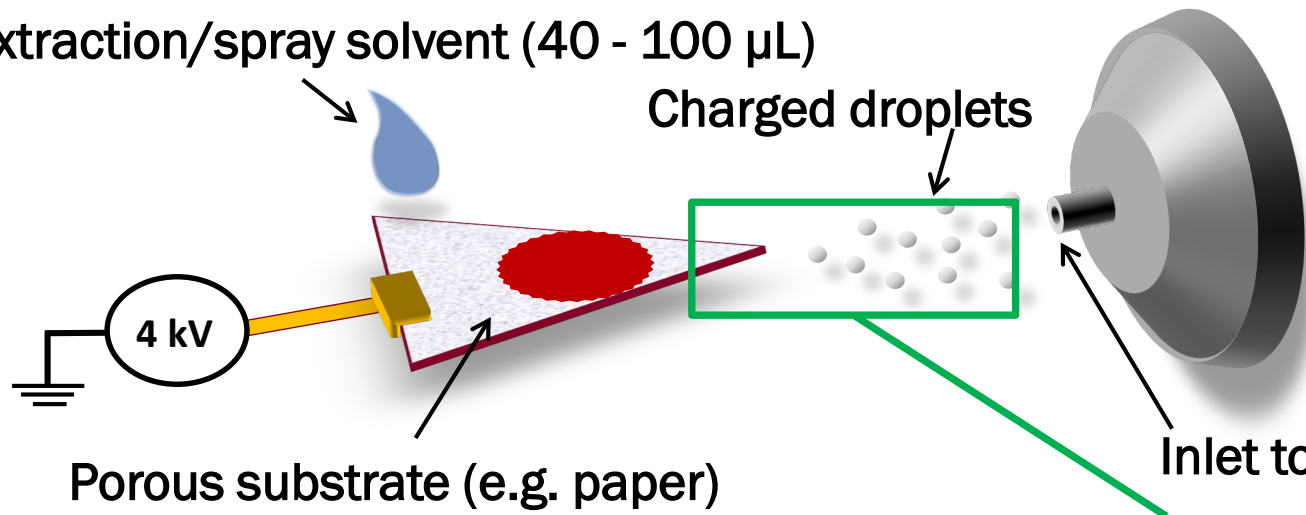
Extraction/spray solvent (40 - 100 μL)

Charged droplets

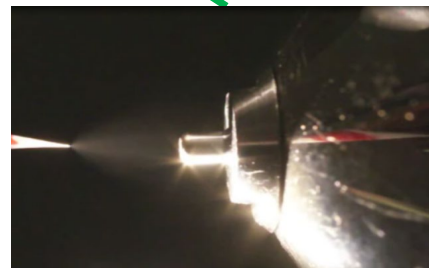
Inlet to mass spectrometer

Porous substrate (e.g. paper)

4 kV

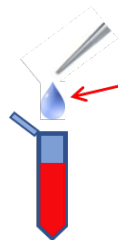
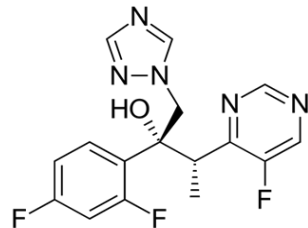


He Wang, Jiangjiang Liu, R. Graham Cooks, and Zheng Ouyang. *Angewandte Chemie* 2010. vol 49, pg 877.



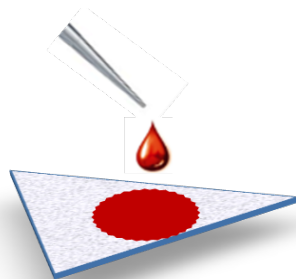
Quantitative Analysis by Paper Spray Mass Spectrometry:

Voriconazole: m/z 350



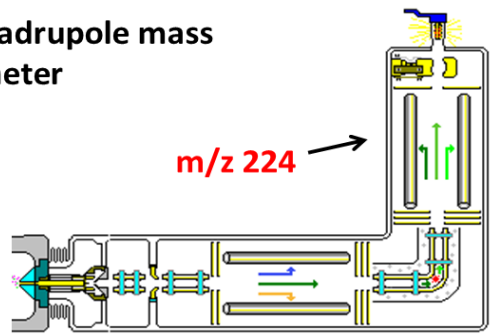
Internal standard solution

Spike internal standard into sample



Triple Quadrupole mass spectrometer

PS-MS



m/z 350

Data Acquisition and Quantitation

Paper Spray Advantages and Disadvantages:



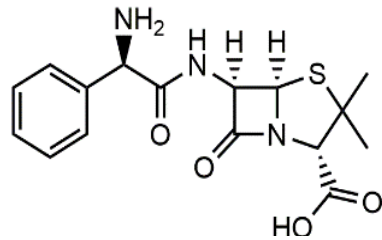
- No carryover
- Small sample volume
- Small solvent volume
- No solvent waste
- No sample prep
- Quick analysis
- No LC or GC system to maintain or troubleshoot



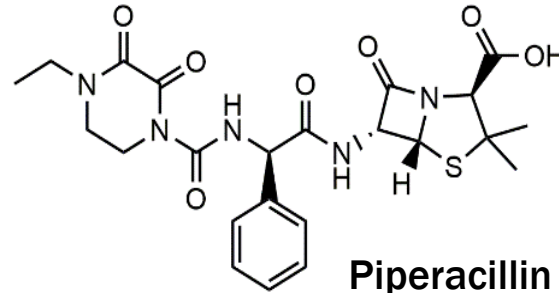
- No analyte separation
- Detection limits are not as good as some methods
- Fewer described methods than LC- or GC-MS/MS

Goal LOD = 0.25 $\mu\text{g/mL}$
Goal LLOQ = 1 $\mu\text{g/mL}$
Goal ULOQ = 50 $\mu\text{g/mL}$

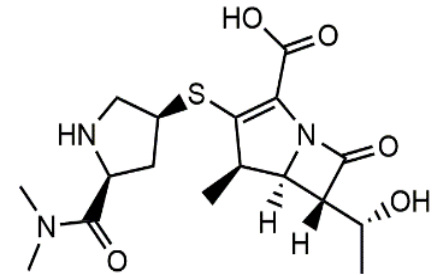
Target Analytes:



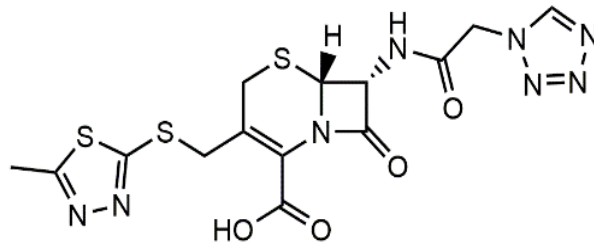
Ampicillin



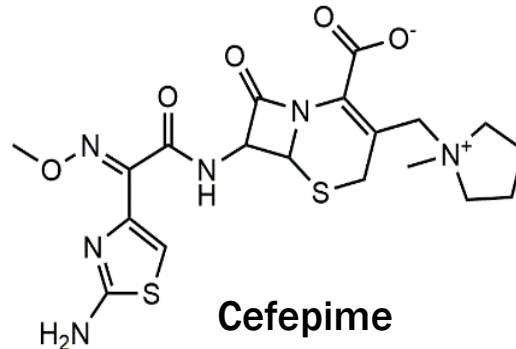
Piperacillin



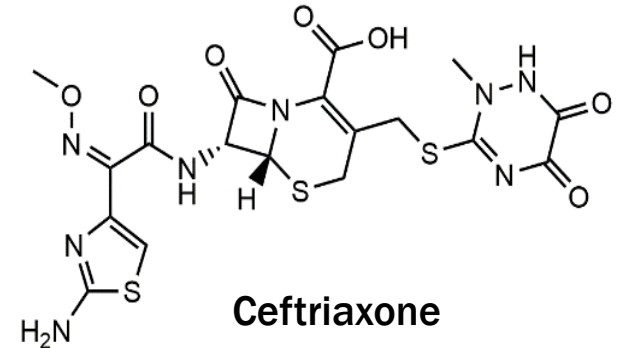
Meropenem



Cefazolin

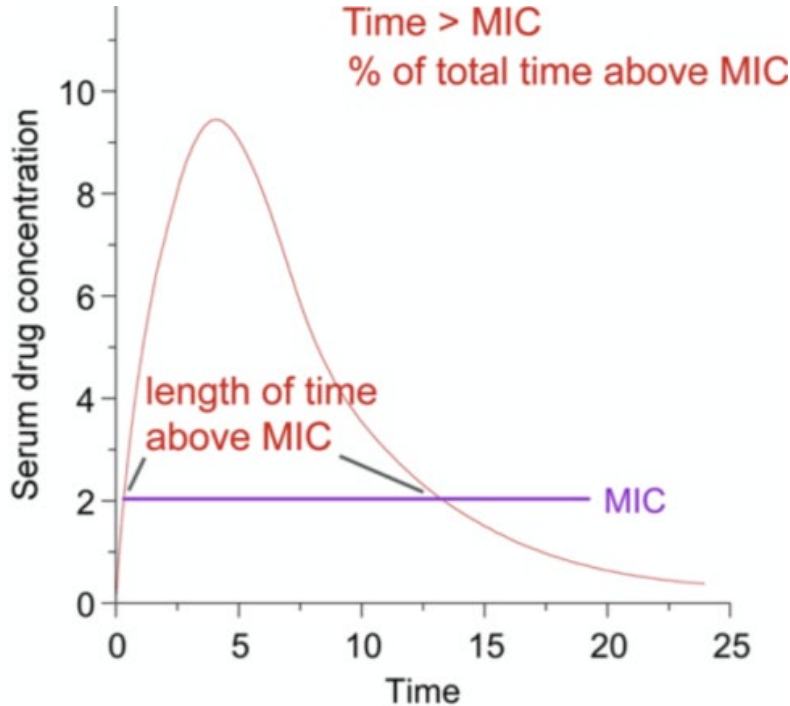


Cefepime



Ceftriaxone

Time-Dependent Killing: β -lactams



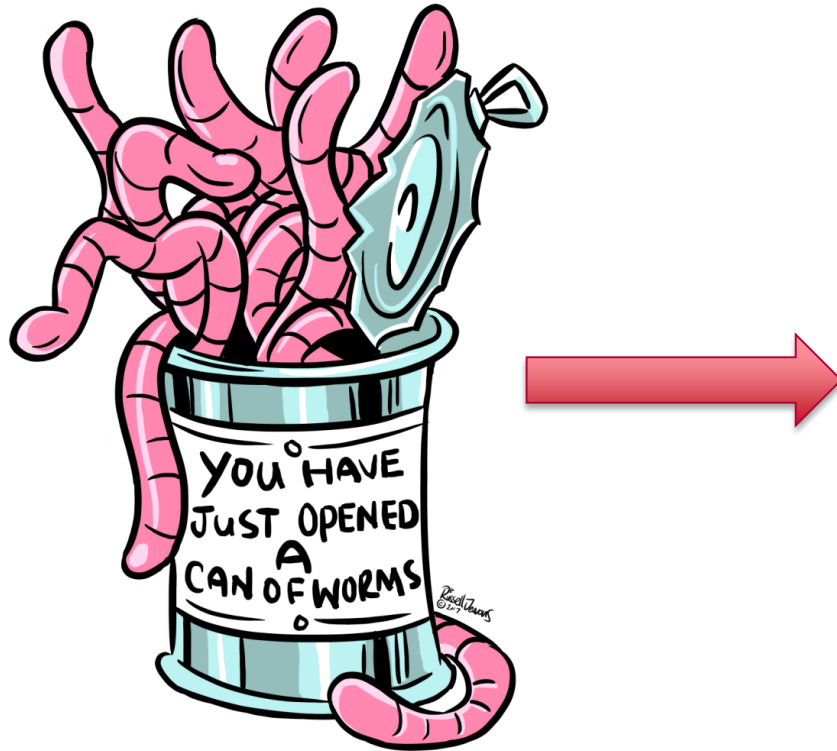
Goals for Maximum Bactericidal Activity

40 - 70% T>MIC depending on the drug

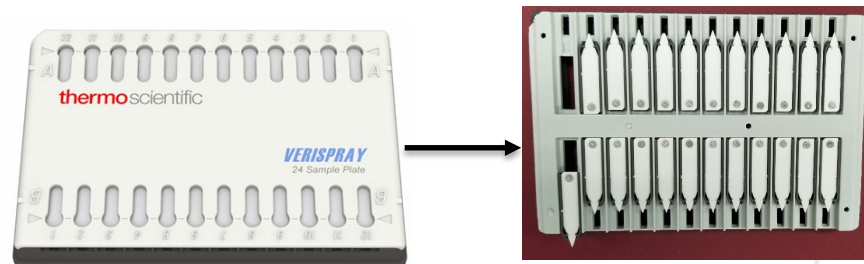
2 - 4x above the MIC



What About Anti-bacterial Agents?



Thermo Fisher TSQ Altis with Verispray source



Work the Problems: Six Sigma Lean



Challenges with Optimization: Fractional Factorial Design

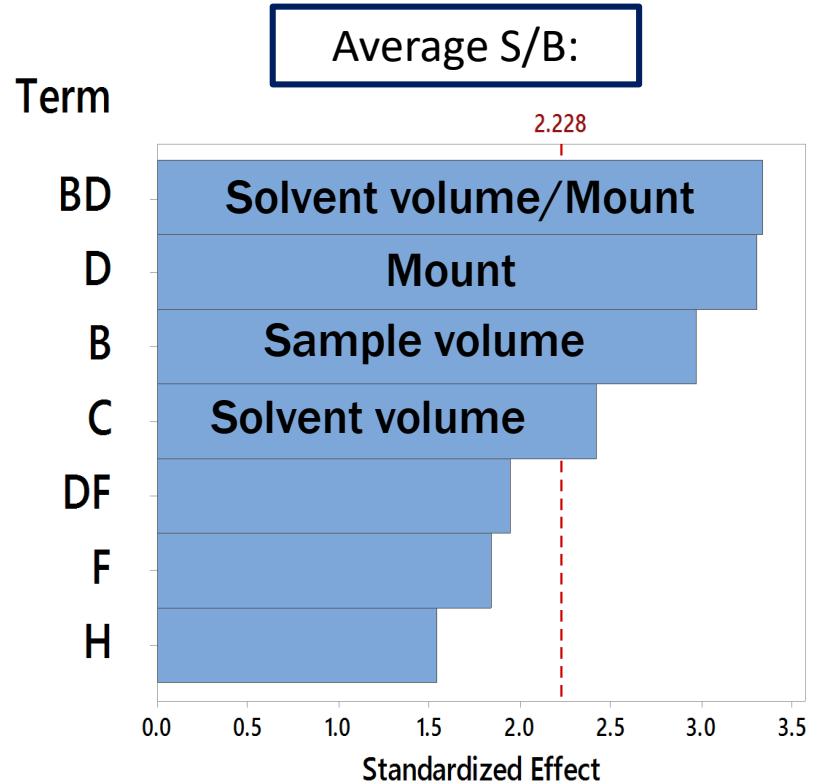
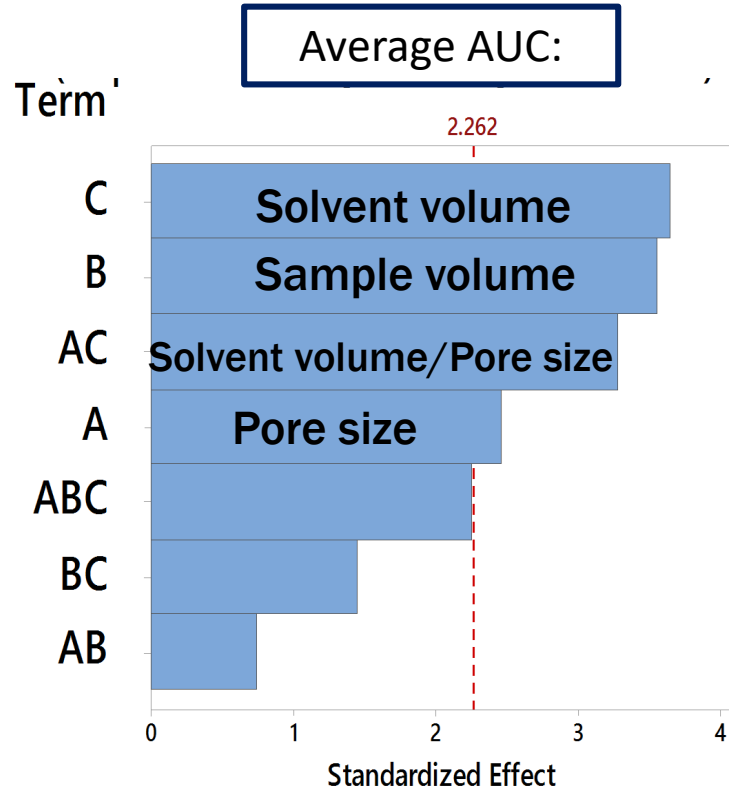
Goal: Maximize analyte signal (AUC) and signal/blank ratio

Ampicillin
= 1 $\mu\text{g}/\text{mL}$
in plasma

Reduced
number of
experimental
runs from
thousands to
<200

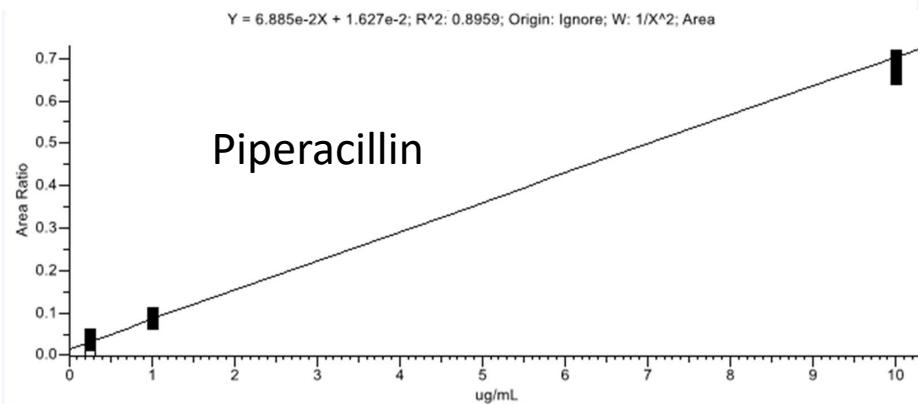
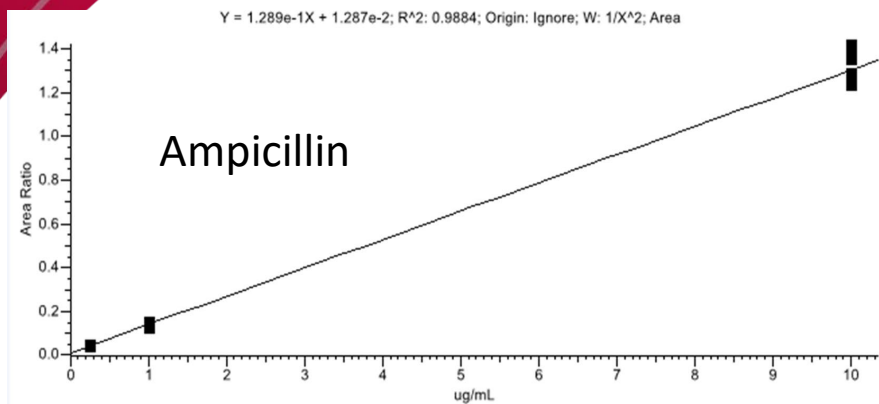
Factors	Factor 1	Factor 2
Pore size	<2 μm (Filter 1575)	16 μm (31-ET)
Sample volume	1 μL	3 μL
Solvent volume	40 μL	100 μL
Solvent Type	60/30/10 ACN/THF/H ₂ O w/ 0.1% FA	90/10 THF/H ₂ O w/ 0.1% FA
Paper wash	Wash (THF)	No wash
Cut of paper	Bad	Good
Solvent location	Front of paper	Back of paper

Challenges with Optimization: Fractional Factorial Design

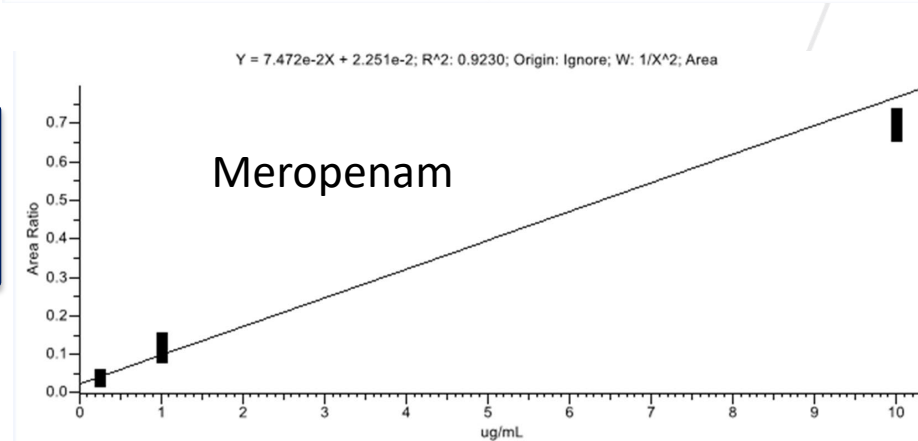


Did It Work?

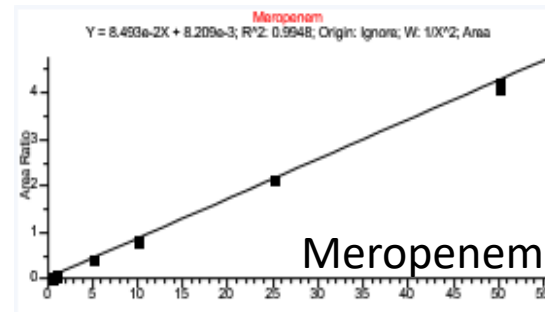
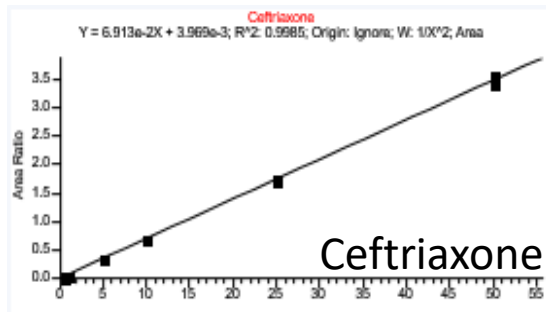
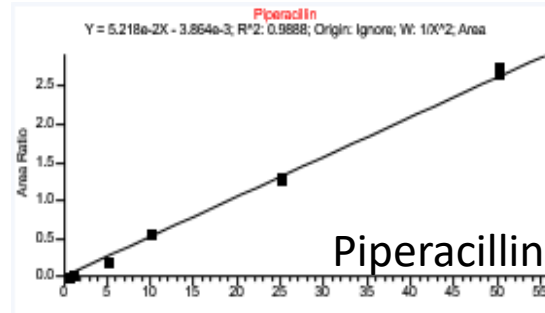
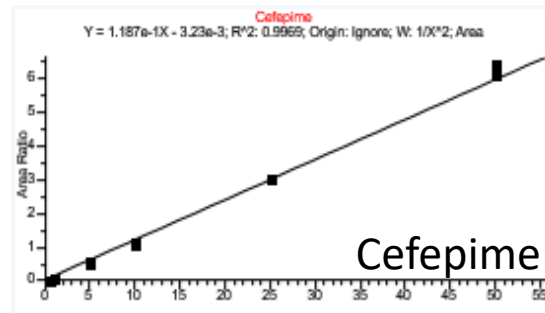
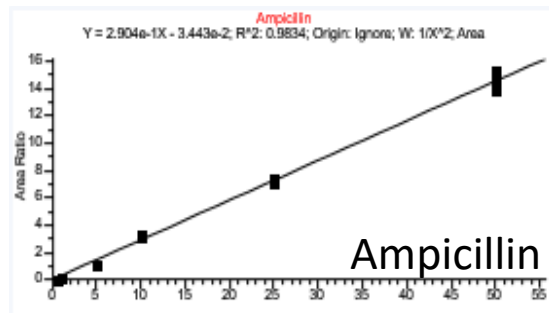
Goal LOD = 0.25 mcg/mL
Goal LLOQ = 1 mcg/mL



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



Challenges: Feasibility of Using Razor Cut Paper



LOD: 0.03 – 0.1 ug/mL
LLOQ: 0.1 – 0.35 ug/mL





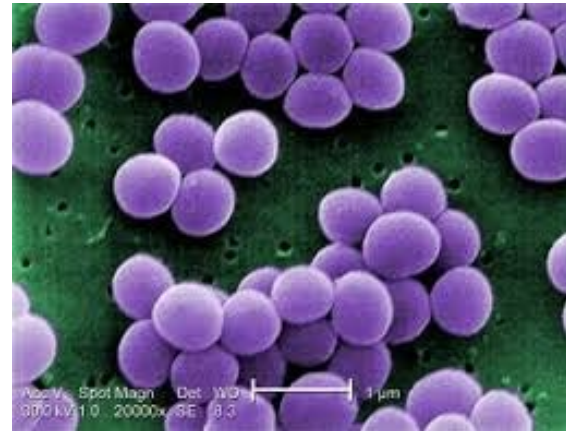
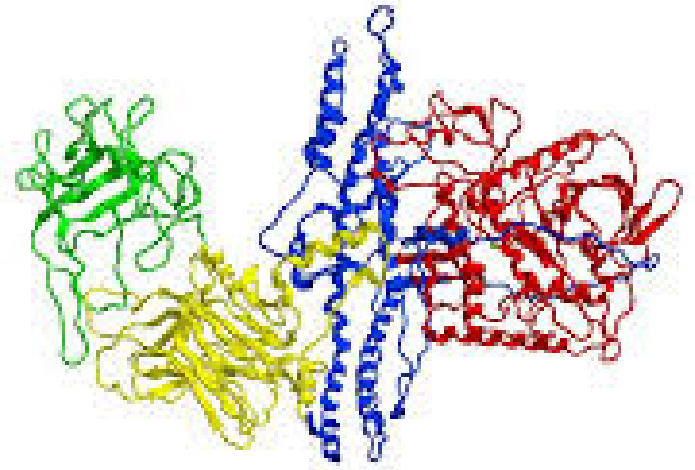
Quick Recap:

- Paper spray MS: a method for rapid drug quantitation by mass spectrometry with feasibility for near point-of-care implementation at the institutional level
 - Legal and illicit drugs
 - Immunosuppressants
 - Tri-azole anti-fungal agents
 - Many more applications...

- Several limitations with hydrophilic drugs still need to be overcome

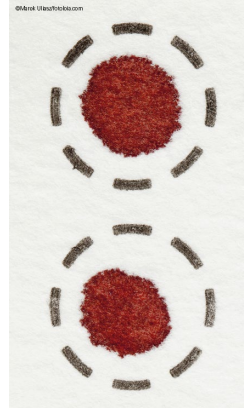
Other Applications:

- Other drug classes, including other anti-infectives
- Proteomics
- Biomarkers
- Metabolics/Lipidomics
- Bacterial and fungal differentiation from culture (phospholipids)
- And many more...



Future Directions:

- Clinical application of the tri-azole anti-fungal and beta-lactam methods, as well as developing methods for other anti-microbials, to further develop physiology-based PK/PD and precision dosing models in pediatric populations
- Advancements in automation: Integrated paper spray sources, plug and play technology
- Advancements in micro-sampling: VP shunt sampling, heel sticks, VAMs



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SRM Transition Selection for Quantitation: **Selectivity** is **KEY!**

- Isobaric analytes, isomeric analytes, and drug metabolites may be difficult to distinguish by MS/MS
- Fragment ions that will give good selectivity rather than most intense fragment ions should be chosen
- Small fragment ions tend to be less selective and should be avoided
- Fragments with common neutral losses, such as loss of water or ammonia, should be avoided due to poor selectivity and tendency to be higher in the blank signal

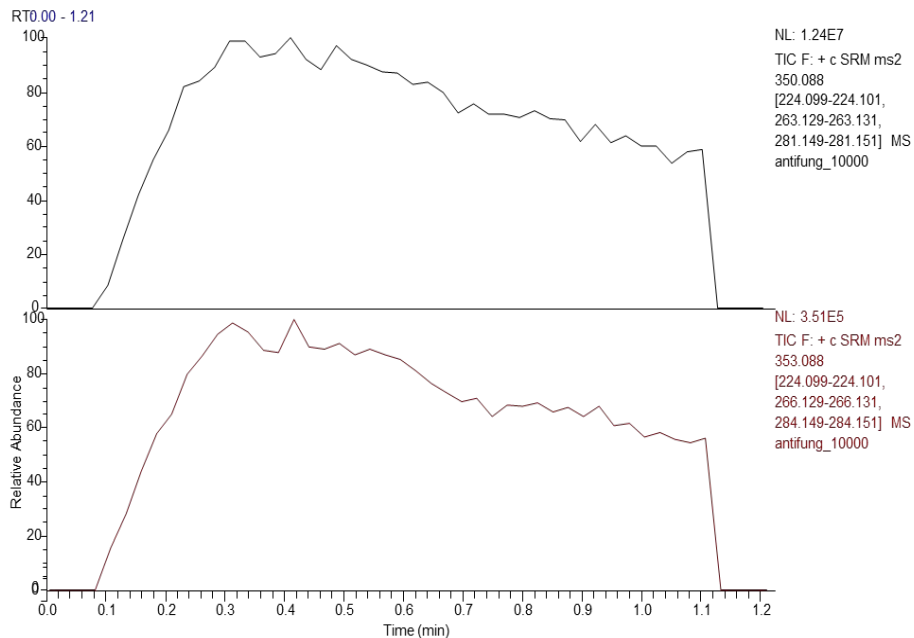


How Many Analytes?

- Each SRM channel must have an adequate number of scans
 - 15 is typically sufficient for quantitation
- Number of scans depends on the analysis time, the dwell time for each SRM, and the number of SRM channels
- Longer ion dwell times improve sensitivity at the expense of the number of scans collected
- Analyst can vary analysis time
 - Longer times (2 minutes) for larger panels
 - Shorter times (~ 30 seconds) for small panels
- More Information: *Jett, et al., Analytical Methods 2017*



Chronograms:



Integrate to
obtain peak
AUC of chosen
analyte fragment
ions and IS
fragment ions



Obtain peak area
ratio of analyte:IS
to normalize and
quantify against
concentration