Paper Spray Mass Spectrometry for Screening

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OVERVIEW

PURPOSE: To develop a proper methodology to allow health care providers to properly determine drug dosage for individual patients.

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

RESULTS: There is no difference in samples between different patients for this method.

INTRODUCTION

- Difficult to determine the correct drug dosage for individual patients
  - Environmental and external factors cause variation
- Paper spray ionization is fast, accurate, and reproducible.
  - Uses low sample and solvent volume
- Paper spray MS/MS method for analysis of four anti-fungal drugs
  - Treat fungal infections in immunocompromised patients

METHODS

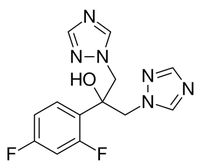
- Monitored in serum
- Spiked with an internal standard containing stable isotope labeled analogs of the drugs.
- Spotted onto Whatman 31ET chromatography paper and allowed to dry.
- Dried serum spots were analyzed via paper spray using 90-10-0.01 acetonitrile-water-acetic acid.
- Extract the drug of interest.
- Experiments were performed on a Thermo TSQ Vantage.

Figure 1: Autosampler for TSQ

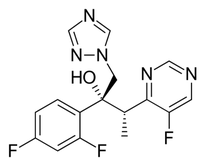
Figure 2: Paper Spray Cartridge

RES

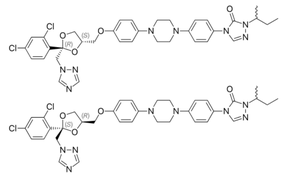
Fluconazole: 307 m/z



Voriconazole: 350 m/z



Itraconazole: 705 m/z



Posaconazole: 701 m/z

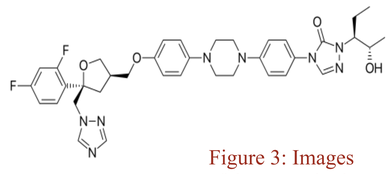
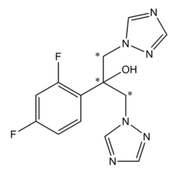
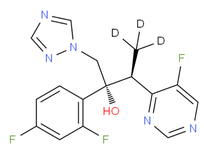


Figure 3: Images of drug standards

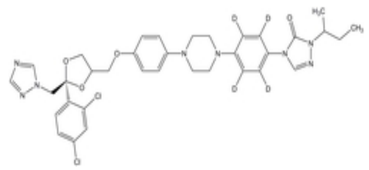
Fluconazole-13C3: 310 m/z



Voriconazole-D3: 353 m/z



Itraconazole-D4: 709 m/z



Name	Formula	Parent m/z	Fragment m/z
Posaconazole	$C_{37}H_{42}F_2N_8O_4$	701	614, 546, 370, 344
Itraconazole	$C_{35}H_{38}Cl_2N_8O_4$	705	392, 371, 348, 335
Voriconazole	$C_{16}H_{14}F_3N_5O$	350	281, 263, 224
Fluconazole	$C_{13}H_{12}F_2N_6O$	307	238, 220, 169
Itraconazole-D4	$C_{35}H_{34}D_4Cl_2N_8O_4$	709	396, 380, 339, 352
Voriconazole-D3	$C_{16}H_{11}D_3F_3N_5O$	353	284, 266, 224
Fluconazole-13C3	$C_{10}[13]C_3\ H_{12}F_2N_6O$	310	241, 223, 172

Table 1: m/z for drug standards

MRM DATA FOR CAL-3 LOT 7

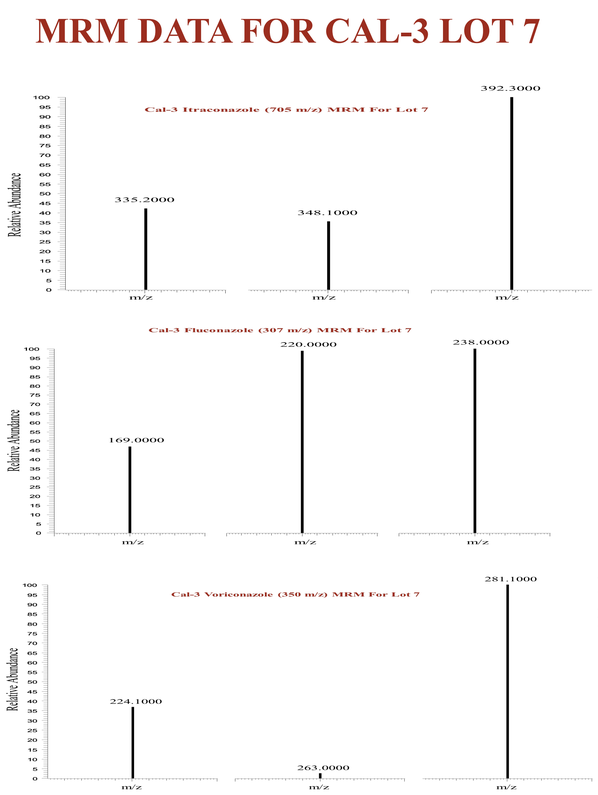


Figure 4: Multiple Reaction Monitoring (MRM) for Lot 7 for each drug.

XCALIBUR WINDOW FOR PROCESSING DATA

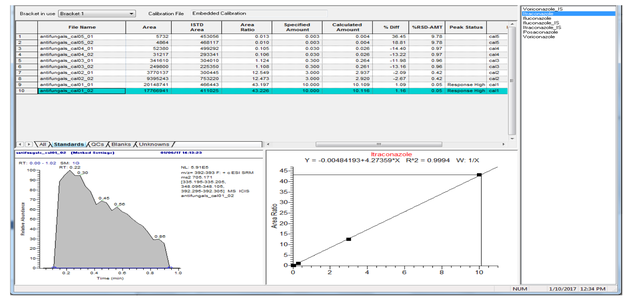


Figure 5: Processing window in Xcalibur depicting area integration for calibration curve.

CALIBRATION CURVES FOR LOT 7

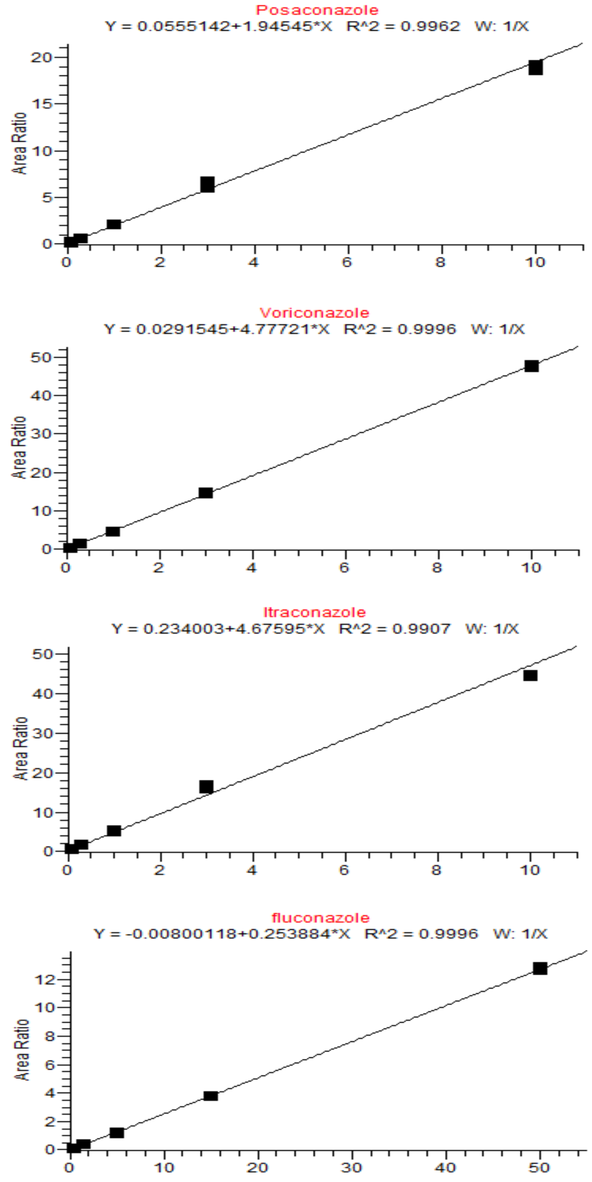


Figure 6: Calibration Curves for Lot 7 for all drugs including slope, and R square. The calibration curve shows good, linear values.

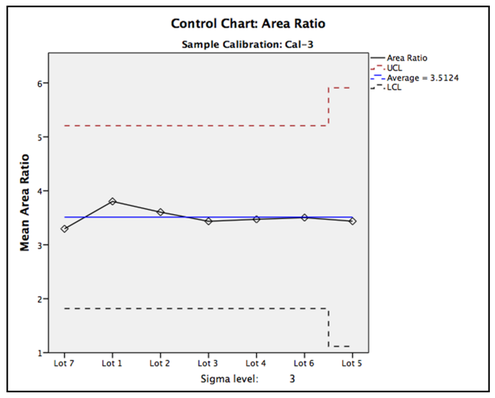
# of Antifungal Drugs from Plasma Samples

and Nick Manicke

Indiana University-Purdue University Indianapolis

ULTS

X BAR/R CONTROL CHARTS FOR CAL-3



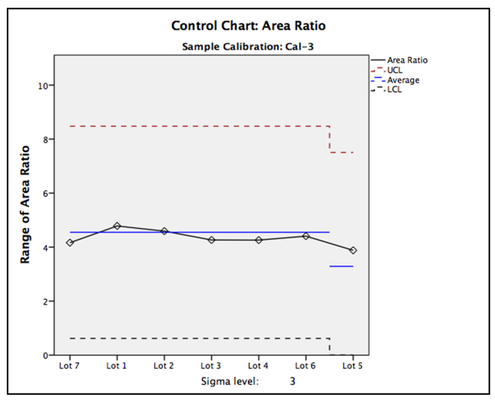


Figure 7: Control charts depicting the repeatability and reproducibility of paper spray for the individual human samples from the calibration curve.

CHROMATOGRAM OVERLAY OF FLUCONAZOLE FOR EACH LOT

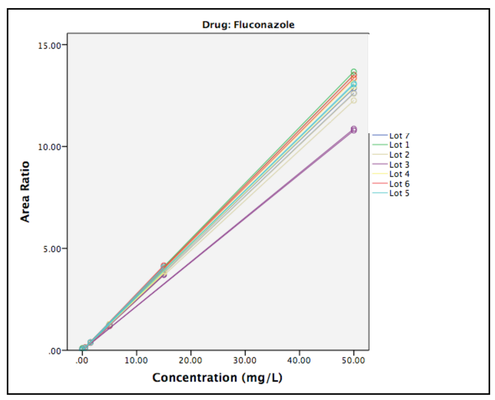


Figure 8: Overlay of calibration curve chromatograms for each human sample for the drug Fluconazole depicting the consistent linearity of the curves for each lot.

Sample Number	Drug	R^2	Slope	LOD (ng/mL)
Lot 1	Fluconazole	1.00	0.32	0.05
Lot 2	Fluconazole	1.00	0.31	0.06
Lot 3	Fluconazole	1.00	0.29	0.12
Lot 4	Fluconazole	1.00	0.31	0.03
Lot 5	Fluconazole	1.00	0.32	0.06
Lot 6	Fluconazole	1.00	0.32	0.06
Lot 7	Fluconazole	1.00	0.32	0.10
Lot 1	Itraconazole	0.99	5.45	0.03
Lot 2	Itraconazole	0.98	5.27	0.05
Lot 3	Itraconazole	0.98	4.83	0.05
Lot 4	Itraconazole	0.99	5.15	0.03
Lot 5	Itraconazole	0.99	5.18	0.03
Lot 6	Itraconazole	0.99	5.18	0.03
Lot 7	Itraconazole	0.99	5.37	0.03
Lot 1	Posaconazole	0.99	2.71	0.03
Lot 2	Posaconazole	0.99	2.30	0.04
Lot 3	Posaconazole	0.98	2.14	0.04
Lot 4	Posaconazole	0.99	2.50	0.03
Lot 5	Posaconazole	0.99	2.47	0.04
Lot 6	Posaconazole	0.99	2.47	0.04
Lot 7	Posaconazole	1.00	2.49	0.02
Lot 1	Voriconazole	1.00	5.07	0.00
Lot 2	Voriconazole	1.00	4.97	0.01
Lot 3	Voriconazole	1.00	4.51	0.02
Lot 4	Voriconazole	1.00	4.83	0.01
Lot 5	Voriconazole	1.00	5.05	0.01
Lot 6	Voriconazole	1.00	5.05	0.01
Lot 7	Voriconazole	1.00	5.07	0.02

Table 2: Summarized calibration curve information for each human sample.

LOD Across Seven Donors

Target	%CV of Slope	Average LOD Across 7 donors (ng/mL)	Therapeutic range
Fluconazole	3%	69	4 – 20 μg/mL
Itraconazole	4%	37	>500 ng/mL (local infection) >1000 ng/mL (systemic infection)
Posaconazole	10%	31	~ 1 μg/mL (trough)
Voriconazole	4%	13	~ 1 μg/mL (steady-state)

Table 3: LOD showing that each drug is below the therapeutic range (1 µg/mL).

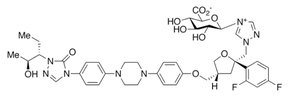
Variation of Slope and Detection Limits

Target	LOD (ng/mL)	Min LOD (ng/mL)	Max LOD (ng/mL)	LOD RSD
Fluconazole	69	32	117	43%
Itraconazole	37	27	50	21%
Posaconazole	31	18	43	27%
Voriconazole	13	3	21	44%

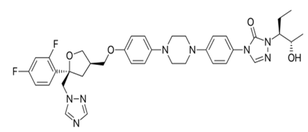
Table 4: Variation of detection limits for each drug across 7 lots of plasma.

METABOLITE INTEREFERENCES

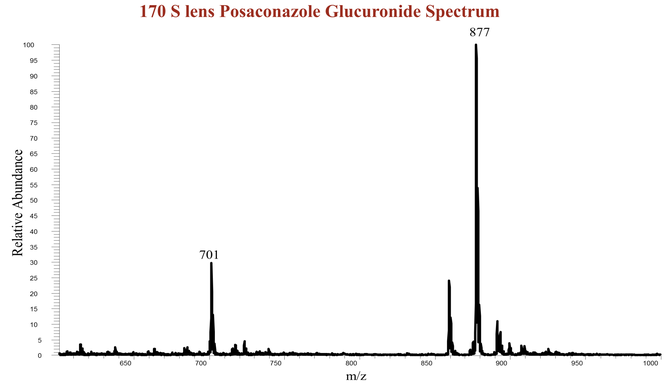
Posaconazole Glucuronide: 877 m/z



Posaconazole 701 m/z



170 S lens Posaconazole Glucuronide Spectrum



HPLC Separation of Posaconazole Glucuronide Standard

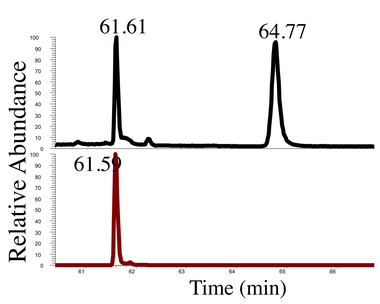


Figure 10a: Extracted ion chromatograms of m/z 701 (top) and m/z 877 (bottom)

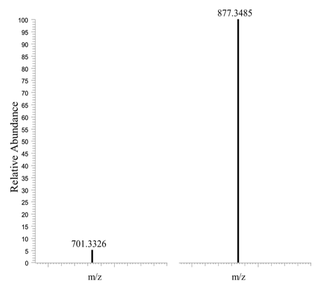


Figure 10b: Ratio between intact glucuronide metabolite and parent drug at 61 minutes. Interference is < 3%.

Voriconazole-n-oxide: 366 m/z

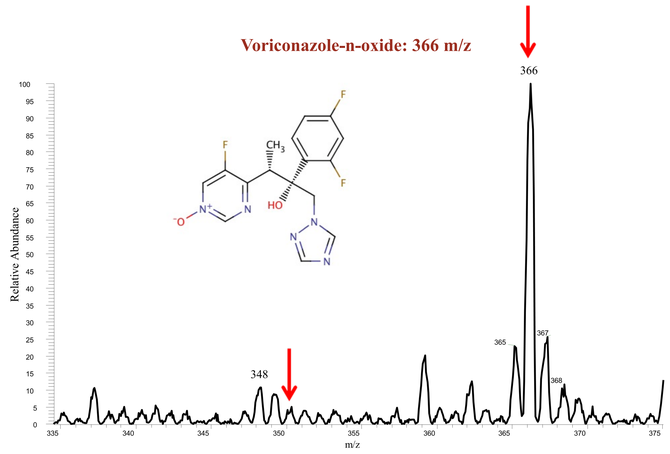


Figure 11: Full MS of voriconazole-n-oxide (m/z 366). Contribution to intact drug (m/z 350) appears negligible.

CONCLUSIONS

There is no significant difference between different human samples making paper spray a good, reproducible method.

Future studies:
Further work can be done to explore metabolites of the antifungal drugs in order to determine the limitations of this method due to interference from these metabolites.

We can also evaluate the performance of the paper spray MS method by comparing the results to a clinical laboratory reference method.

Matrix Effects can also be investigated to determine their impact on the paper spray methodology as a whole.

REFERENCES

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ACKNOWLEDGEMENTS

We would like to thank the Indiana University- Purdue University Indianapolis Department of Chemistry for allowing us to perform these experiments.