

Optimizing Extraction Recovery for Volumetric Adsorptive Microsampling (VAMS)

Paige A Malec, Aaron Ledvina

March 9, 2021

Copyright © 2021 Covance Inc. All Rights Reserved.



COVANCE
by labcorp

Outline

- 1 Patient-centric sampling
- 2 VAMS
- 3 Experimental design
- 4 Extraction optimization
- 5 Predicting extraction performance
- 6 Conclusions and future directions

Why “Patient-Centric” Sampling?

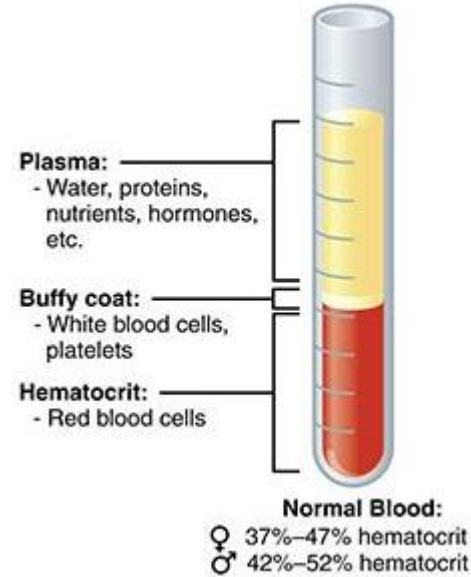
- ▶ At-home testing reduces time commitment
- ▶ Less invasive sampling creates less discomfort for patients
- ▶ Reduced exposure to sick individuals
- ▶ Increased access for patients in remote areas
- ▶ Increased retention in clinical trials



Images provided by Neoteryx, LLC

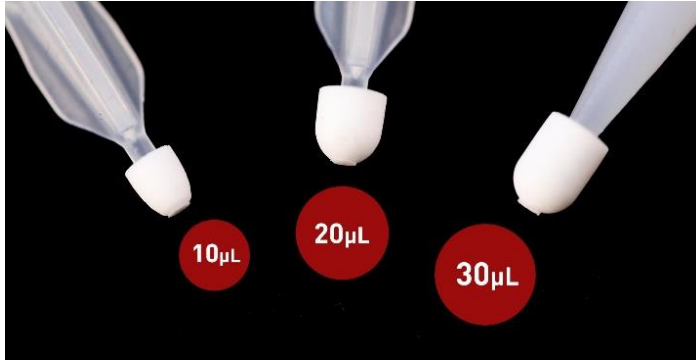
Dried Blood Spot (DBS) Analysis

- ▶ Typically good stability relative to biofluids
- ▶ Easy to store and transport
- ▶ Difficult to relate back to absolute concentration
 - Volume?
 - Hematocrit?



Volumetric Adsorptive Microsampling (VAMS)

- ▶ Mitra[®] devices (Neoteryx) are an alternative to DBS
- ▶ Hydrophilic polymer tips wick up specific volume of blood
- ▶ Samples are dried and shipped to lab
- ▶ Volume is independent of hematocrit



Images provided by Neoteryx, LLC

Challenges with Clinical Analysis of Mitra[®] Samples

- ▶ Recovery over time
 - Analytes often become harder to extract
 - Extraction must be optimized
 - Time consuming method development
- ▶ Hematocrit affects recovery
 - High hematocrit samples tend to have lower recovery
 - More optimization needed
- ▶ Impact of anticoagulant
 - Incurred samples have no anticoagulant
 - How to account for anticoagulant in lab generated samples?

Experimental Design

- ▶ Does anticoagulant matter?
 - K₂EDTA and Sodium Heparin
- ▶ Does hematocrit matter?
 - ~30% and ~60%
- ▶ Extraction optimization
 - Methanol
 - Acetonitrile
 - Heated incubation
 - Longer incubation
 - + Water
 - + Base
- ▶ Recovery over time
 - Three time points

Analyte	pKa	logP	MW
Iohexol	11.73	-2	821.1
Tolbutamide	4.33	2.3	270.3
Rifampin	-1.6	3.22	822.9
Paclitaxel	10.36	3.54	853.9
Itraconazole	3.92	7.31	705.6

Extraction Procedure

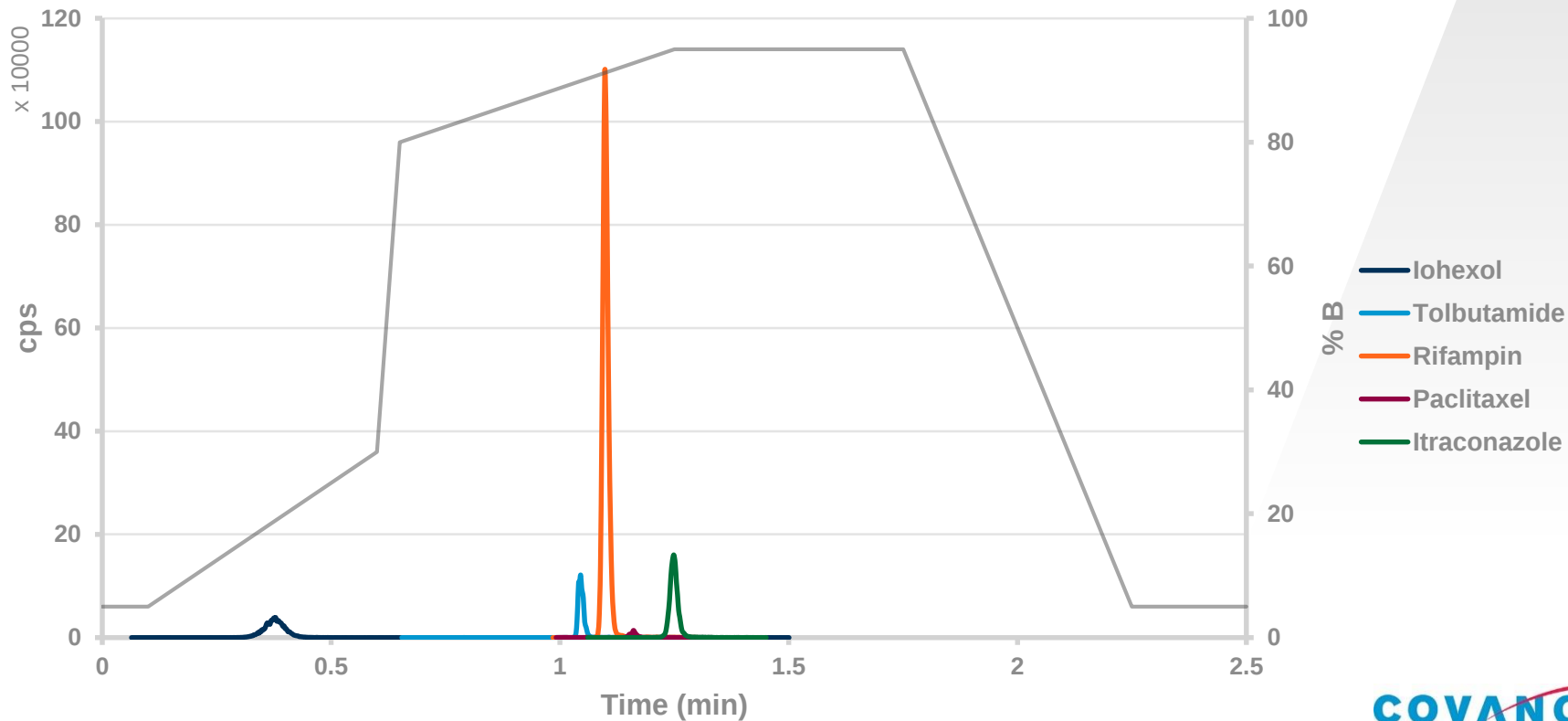
- ▶ Remove head of Mitra tip and place into 96-well plate
- ▶ 10 μ L ISTD
- ▶ 400 μ L extraction solvent
- ▶ Sonicate 15 min
- ▶ Vortex-mix at 1200 RPM for 1-2 hours
- ▶ Centrifuge 5 min
- ▶ Transfer 200 μ L supernatant; dry down
- ▶ 150 μ L recon solution

Instrument Conditions

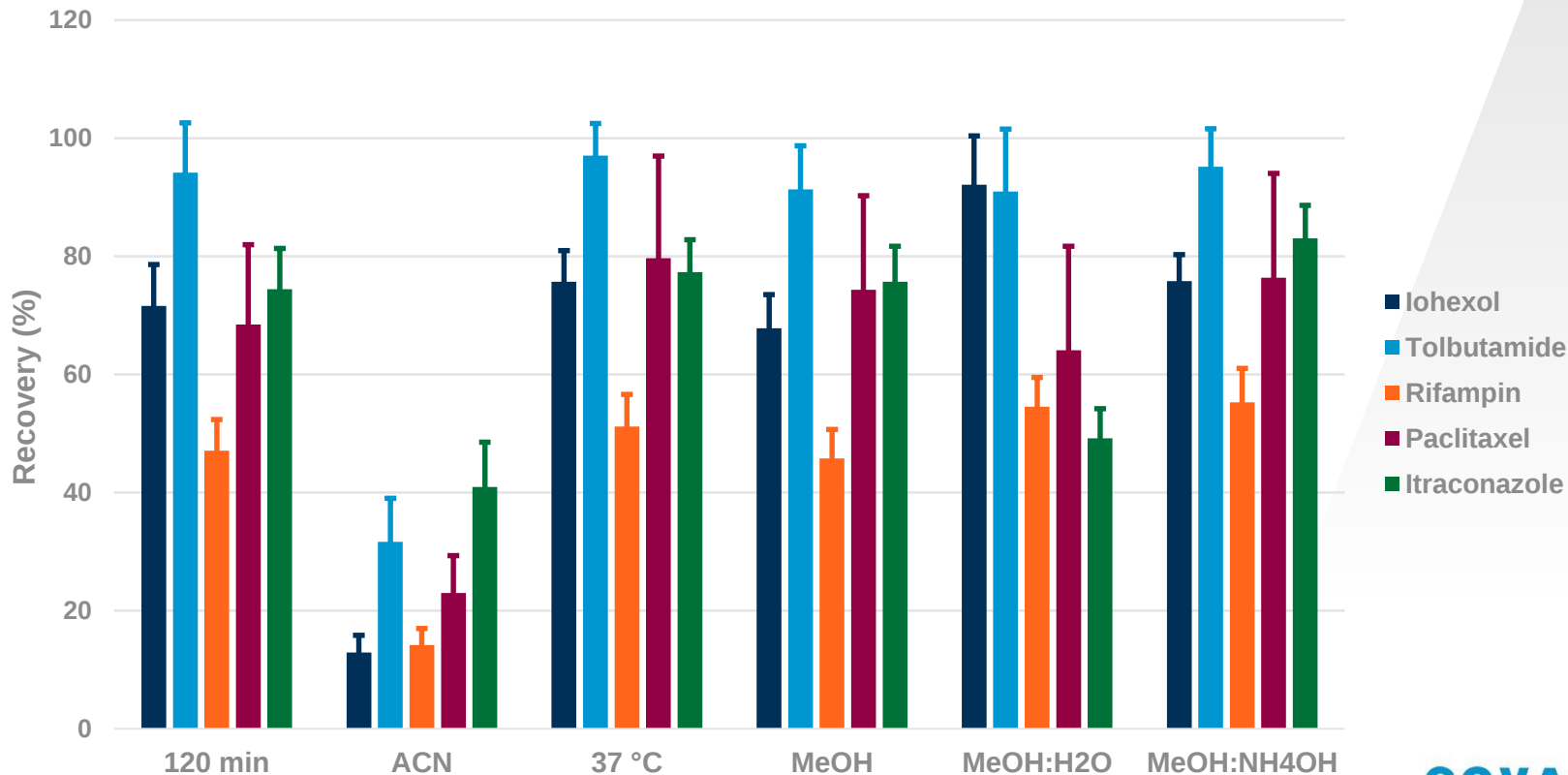
LC	Shimadzu Prominence, 20 Series
Column	Phenomenex Gemini NX-C18, 50 x 2 mm, 5 µm
Column temperature	40 °C
Autosampler temperature	5 °C
Mobile phase A	Ammonium formate 10 mM (aq): Ammonium hydroxide (100:0.1)
Mobile phase B	Methanol
Injection volume	5 µL
Flow rate	1.00 mL/min

Mass spectrometer	Sciex API 5000
Ionization	ESI +
Mode	sMRM
IonSpray voltage	2500 V
Temperature	500 °C
MRM detection window	18 sec
Target cycle time	0.200 sec

Example Chromatogram



Initial Recovery



Does Anticoagulant Matter?

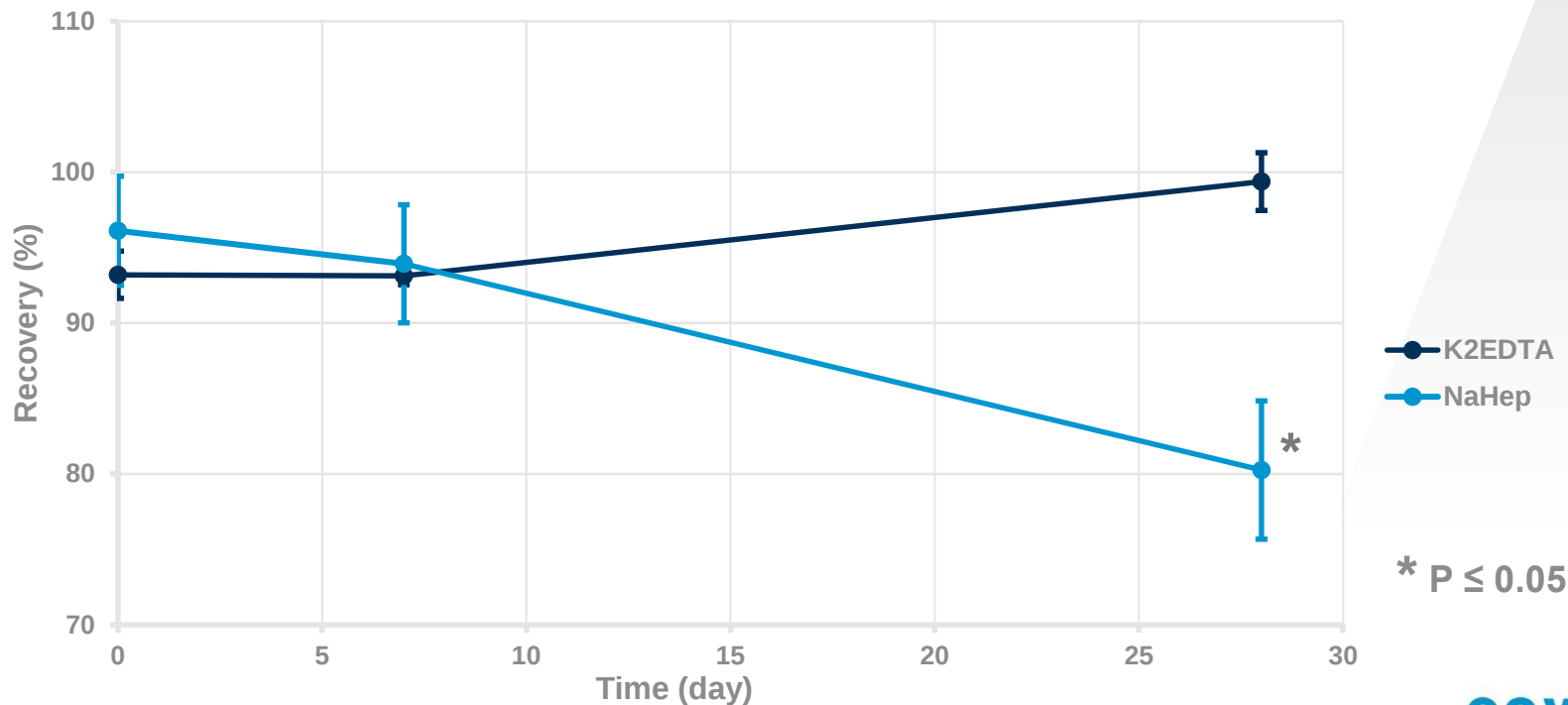
- ▶ No significant differences observed

	Iohexol	Tolbutamide	Rifampin	Paclitaxel	Itraconazole
120 min	✓	✓	✓	✓	✓
37°C	✓	✗	✓	✓	✓
ACN	✓	✓	✓	✓	✓
MeOH	✓	✓	✓	✓	✓
MeOH:H ₂ O	✓	✗*	✓	✗*	✓
MeOH:NH ₄ OH	✓	✓	✓	✗*	✓

* “Failure” caused by outlier

Differences with Anticoagulant

- Tolbutamide, high hematocrit, 37°C



* $P \leq 0.05$

Impact of Hematocrit

- At least one extraction performs well for each analyte

	Iohexol	Tolbutamide	Rifampin	Paclitaxel	Itraconazole
120 min	✓	✓	✓	✓	✓
37°C	✓	✓	✓	✗	✓
ACN	✓	✓	✓	✓	✓
MeOH	✓	✗	✓	✓	✓
MeOH:H ₂ O	✓	✗	✓	✓	✓
MeOH:NH ₄ OH	✓	✓	✓	✓	✓

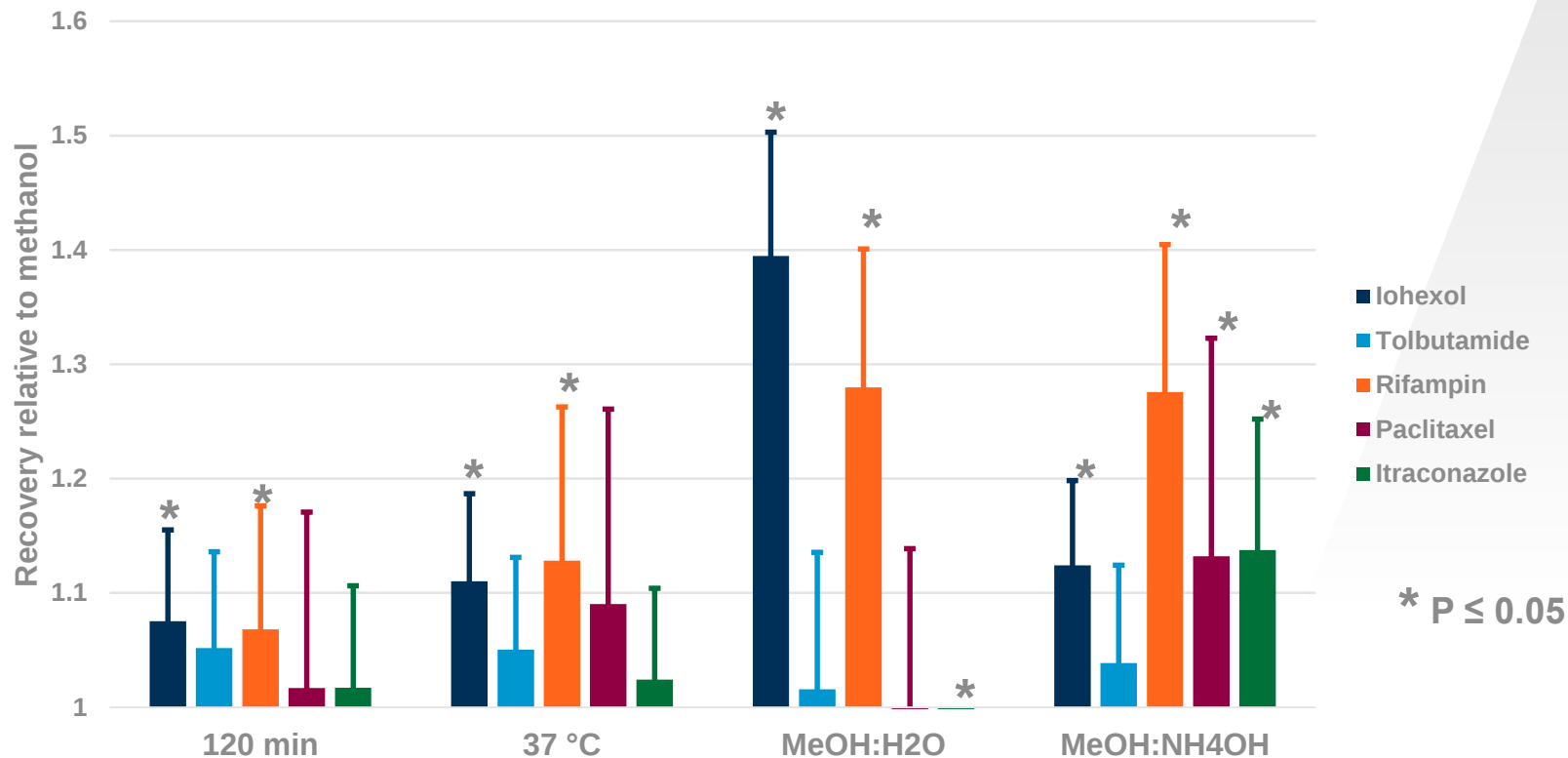
Recovery Over Time

- Further optimization is likely needed for itraconazole

	Iohexol	Tolbutamide	Rifampin	Paclitaxel	Itraconazole
120 min	✓	✓	✓	✓	✓
37°C	✓	✓	✓	✓	✗
ACN	✓	✗	✓	✗	✗
MeOH	✓	✓	✓	✓	✗
MeOH:H ₂ O	✓	✓	✓	✓	✓
MeOH:NH ₄ OH	✓	✓	✓	✓	✗

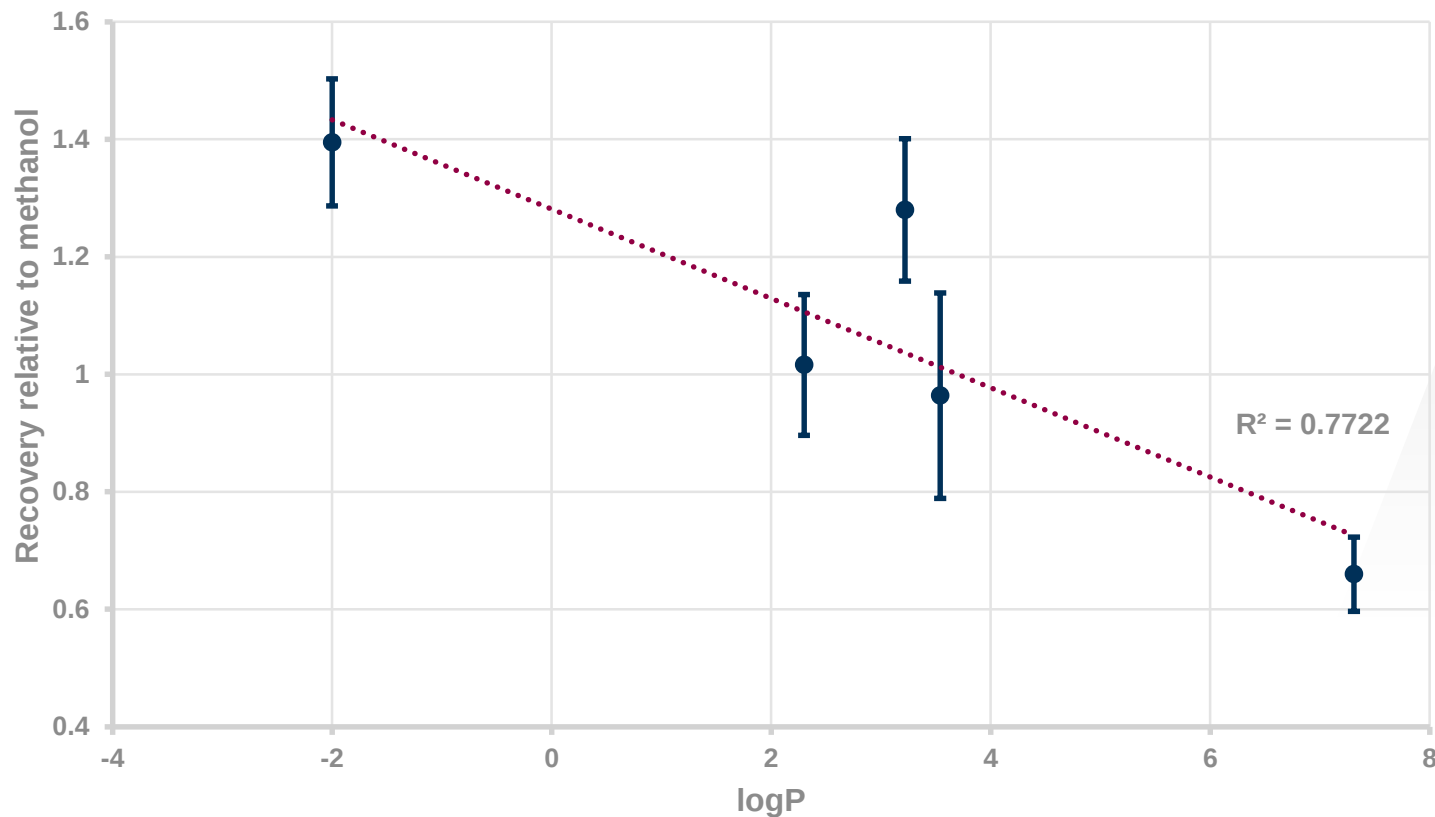
Can We Predict Optimal Extraction Conditions?

Recovery Relative to Methanol

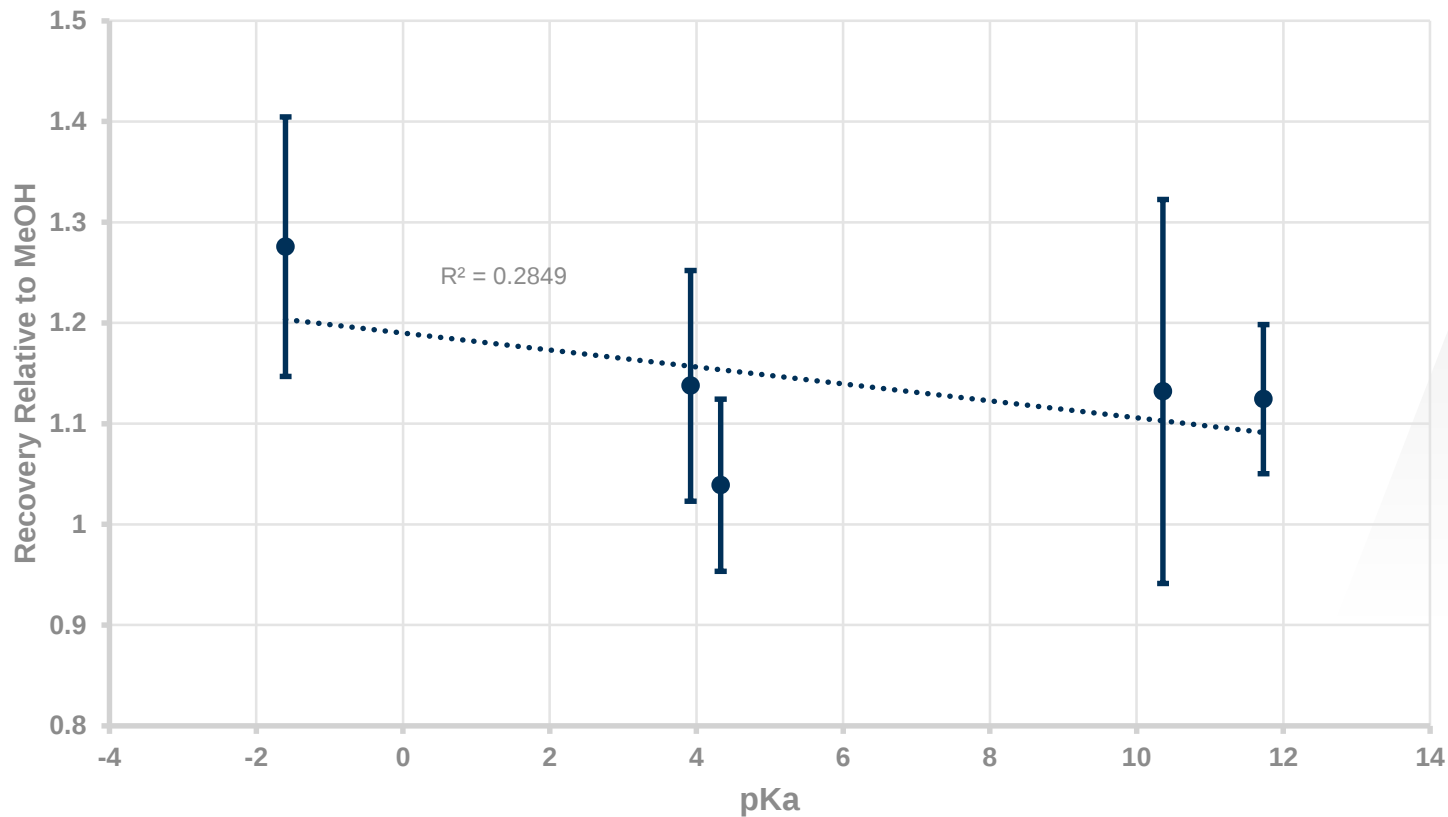


* $P \leq 0.05$

Methanol: Water Recovery vs Polarity



Methanol: NH_4OH Recovery vs pKa



Conclusions

- ▶ In general, anticoagulant does not make a difference
- ▶ Extraction can be optimized to account for hematocrit differences
 - Analyte dependent
- ▶ Consistent long term recovery was not achieved for itraconazole
- ▶ Further optimization needed to increase recovery for rifampin
- ▶ Physical properties may provide some guidance towards extraction strategies
 - Add water for polar analytes
 - Add base based on pKa

Future Directions

- ▶ Impact-assisted recovery
- ▶ Expand upon extractions and analytes
- ▶ Compare to blood with no anticoagulant
- ▶ Create reference for extraction optimization

Acknowledgements

- ▶ Covance
 - Aaron Ledvina
 - Zach Ebben
- ▶ Neoteryx
 - Traci Mizuno

Please direct additional questions to:
paige.malec@covance.com



Covance by Labcorp is a leading global life sciences company, which provides contract research services to the drug, medical device and diagnostics, crop protection and chemical industries. COVANCE is a registered trademark and the marketing name for Covance Inc. and its subsidiaries around the world.

www.covance.com