•Dried blood Spot ● LC-MS ● Untargeted metabolomics

Democratizing Metabolomic Studies: Remote Blood Sampling for Compound Kinetics with Pre-analytical Normalization Strategies and Sampling Site Insights

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Background

Remote sampling technologies, allowing for blood collection outside of a clinical environment, provide a powerful means to "digitize biology" – capturing compound exposure and differential kinetics and tracking metabolic changes over an individual's lifetime. With technologies offering diverse sampling locations, sample volumes, and matrix materials, variations in compound distribution and concomitant concentration estimates due to sampling site differences must be accounted for. Pre-analytical normalization strategies are essential to these effects. This project demonstrates individual-level compound distribution and metabolism kinetics using Neoteryx Mitra, Whatman 903 ProteinSaver cards, and the OneDraw blood collection device contrasting collection from the upper arm and fingertip as sampling sites at collection frequencies beyond what is easily capable with venous collection.

LC-MS Methods

An Agilent Infinity II UPLC system connected to a Bruker TIMSTOF Pro2 mass spectrometer was employed as the LC-MS platform. For reverse phase liquid chromatography (RPLC) runs, separation was carried out on a ZORBAX Eclipse Plus C18 column (50 * 2.1 mm, 1.8 µm, Part # 00D-4475-AN, Torrance, CA) at a flow rate of 300 µL/min with the column compartment temperature set to 40 °C. For Hydrophilic Interaction Liquid Chromatography (HILIC) runs, an Agilent InfinityLab Poroshell 120 HILIC-Z column (100 * 2.1 mm, 2.7 µm, Part # 00D-4475-AN, Torrance, CA) was employed at a flow rate of 400 µL/min with the column compartment temperature set to 10 °C. To make mobile phase A, 100 mL of freshly prepared 200 mM ammonium acetate (Sigmaaldrich, CAS-No: 631-61-8) solution at PH 9.3 is diluted in H2O to 1L with the addition of 1 mL of the Agilent InfinitiyLab deactivator solution (Part No: 5191-3940), while mobile phase B is pure Acetonitrile (UPLC grade, Honeywell, 1L).

For MS acquisition, autoMS/MS @12 Hz with m/z range of 50 - 1300 m/z was employed with the following VIP-HESI Source parameters and Tune parameters: Capillary Voltage: 4500 V, Nebulizer: 2.0 Bar, Sheath Gas: 275 °C at 4.0 L/min, dry gas: 230 °C at 8.0 L/min Transfer Time: 54.0/65.0 µs for NEG/POS mode.

Compound identification was performed at multiple levels. For all exogenous compounds and their metabolites, exact m/z at MS1 level and MS/MS library match along with the consistency in the measured compound kinetics profile with the compound reported half-life were used. Additionally, for phase II metabolites, the presence of either a fragment ion matching the structural moiety of the compound or a fragment ion with m/z of 175.0237 matching the glucuronide ion moiety was used when MS/MS spectra is available.

Highlights

- Consistent compound kinetics profile captured using three remote sampling devices targeting two different sampling sites.
- Drug dose and formulation effect on metabolism
- Sampling site differential kinetics demonstrated through energy drink exogenous compounds

Results

Remote sampling technologies are demonstrated as robust and efficacious methods to capture exogenous compound kinetics profile without clinical infrastructure. While most compounds surveyed indeed displayed kinetics comparable to the reported half-lifes, 4-pyridoxate, which is the Vitamin B main shunt metabolite that has a half-life of days, showed a rise-and-fall kinetic profile within 9 hours.

Future Work

Future work could involve enrolling volunteers to study questions of "real-world" metabolism.

Overview of drug metabolism pathway involving the formation of phase I and phase II metabolites.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8657965/

Compound	Tests	half life	Note
caffeine	cyp1a2	4-5h[c]	
dextromethorphan	cyp2d6	2-4h[c]	
Omeprazole (70% normal)	cyp2c19	0.5-1h[a]	5-Hydroxyomeprazole -- must do ratio of 5-hydroxyomeprazole to omeprazole
Omeprazole (30% normal)	cyp3a4	0.5-1h[a]	omeprazole sulfone
ibuprofen	cyp2c9	1-3h[b]	
Midazolam	cyp3a4	2-4h[b]	*gold standard for 3a4 -- but also Rx*
Acetaminophen	cyp2e1 (minor)	1.5-3h[c]	APAP-GSH
Acetaminophen	phase II	1.5-3h[c]	APAP-S, APAP-Glc

Compounds of interest to interrogate CYP450 enzymes

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[c]Agrawal S, Murray BP, Khazaeni B. Acetaminophen Toxicity. [Updated 2025 Apr 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441917/

Acknowledgements

Funding in part for these efforts was provided from Uniformed Services University of the Health Sciences (USUHS) award from the Defense Health Program to the Murtha Cancer Center Research Program (HU00011820032, JE Katz/JSH Lee) administered by the Henry M. Jackson Foundation for the Advancement of Military Medicine. Disclaimer: The contents of this publication are the sole responsibility of the authors and do not necessarily reflect the views, opinions, or policies of the USUHS, the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., the Department of Defense (DoD), the Departments of the Army, Navy, or Air Force. Mention of trade names, commercial products, or organization does not imply endorsement by the U.S. Government. Further, we would like to extend our gratitude to Dr. Andrew Morris and Dr. Terra Vincent-Hall. Their valuable time and insightful discussions have significantly contributed to these efforts.

Conflicts of Interest

There are no conflicts of interest to report.

Remote Sampling Strategy for Compound Kinetics Evaluation

Remote Sampling Technologies to Target Different Sampling Sites

(a)

Upper Arm Sampling

Volumetric Sampling
Whatman 903 comparable matrix
Self-contained sampling/storage unit

Drawbridge Onedraw device

Fingertip Sampling

Non-Volumetric sampling
Well studied paper matrix
Hard to collect reproducible blood spots

Whatman 903 Protein Saver Card

Volumetric sampling
Less studied sorbent matrix
Easy to collect samples reproducibly

Neoteryx Mitra Collection Kit

Exogenous Compound Reported Half-life

(b)

Compound	Half-life	Source/Note
Taurine	1~1.5 hours[1]	Rip It and Monster Energy drink ingredient
Caffeine	4~5 hours[2]	Rip It and Monster Energy drink ingredient
4-pyridoxate	days[3]	Main shunt metabolite of vitamin B6(energy drink)
Guaifenesin	~1 hour[4]	Cough suppressant liquid/pil expectorant
Dextromethorphan	2 ~ 4 hours[5]	Cough suppressant liquid/pill
Ibuprofen	1.8 ~ 2 hours[6]	Advil pills (200 mg) NSAIDs
Naproxen	12 ~ 17 hours[7]	L368 pill 1* 220 mg NSAIDs

(c)

Intervention Events: Rip It Energy Drink (16 oz) CVS Adult DM Cough Suppressant Liquid (Dextromethorphan HBr 20 mg, Guaifenesin 400 mg) Ibuprofen (Advil, 2 * 200 mg pill)

1st Kinetics Experiment (Fasting Administration)

Sampling Events	0h	0.5h	1h	3h	5h	7h	9h
	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)	OD (1) MT (2) WM(2)

(d)

Intervention Events: Monster Energy Drink (16 oz) CVS Mucus Relief DM(44 533 pill, dextromethorphan hydrobromide 20 mg/ guaifenesin 400 mg) Ibuprofen (Advil, 1 * 200 mg pills) Naproxen Sodium(L368, 1* 220 mg) Lemon (1) Broccoli (steamed, ~100g) Apple Cider Vinegar (15ml) 4h Tofu, Corn Tortilla, Cheese, Oil (~50g each)

2nd Kinetics Experiment (Fasting Administration + Food Intervention)

Sampling Events	0h	0.5h	1h	3h	5h	7h	9h
	OD(1) MT(2) WM(2)	OD(1) MT(2) WM(3)	OD(1) MT(4) WM(5)	OD(1) MT(2) WM(3)	OD(1) MT(2) WM(2)	OD(1) MT(2) WM(2)	OD(1) MT(4) WM(3)

Figure 1: Overview of the selected remote sampling technology/devices and kinetics experiment. (a) microsampling collection devices targeting fingertip and upper arm for skin puncture blood draws. **(b) The half-lifes of the OTC drug and energy drink marker compounds involved in the kinetics experiments. (c) and (d) are the experimental design for two kinetics sampling experiments.** The same individual was involved for sampling with 0h representing sampling after fasting overnight.

OneDraw Strip Weight Measured for Blood Volume Estimation, Extraction Volume Normalization and Data Normalization

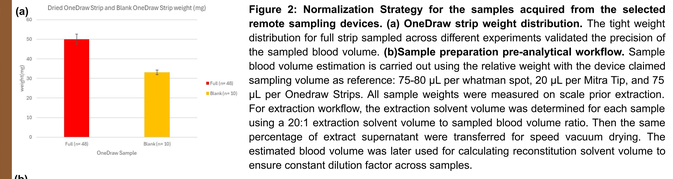


Figure 2: Normalization Strategy for the samples acquired from the selected remote sampling devices. (a) OneDraw strip weight distribution. The tight weight distribution for full strip sampled across different experiments validated the precision of the sampled blood volume. **(b)Sample preparation pre-analytical workflow.** Sample blood volume estimation is carried out using the relative weight with the device claimed sampling volume as reference: 75-80 µL per whatman spot, 20 µL per Mitra Tip, and 75 µL per Onedraw Strips. All sample weights were measured on scale prior extraction. For extraction workflow, the extraction solvent volume was determined for each sample using a 20:1 extraction solvent volume to sampled blood volume ratio. Then the same percentage of extract supernatant were transferred for speed vacuum drying. The estimated blood volume was later used for calculating reconstitution solvent volume to ensure constant dilution factor across samples.

Naproxen Metabolism

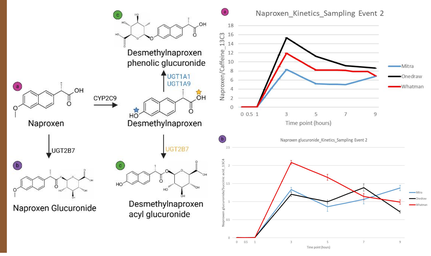


Figure 4: Naproxen metabolite kinetics profile from the 2nd kinetics measurement. The naproxen reported half-life (12~17h) is beyond the kinetics experiment sampling time span (9 hours), which is consistent with the observed kinetics profile.

Frequent Sampling via Remote Sampling Approach enables Drug Metabolism Insights

Ibuprofen Metabolism: Dose Dependence on Kinetics

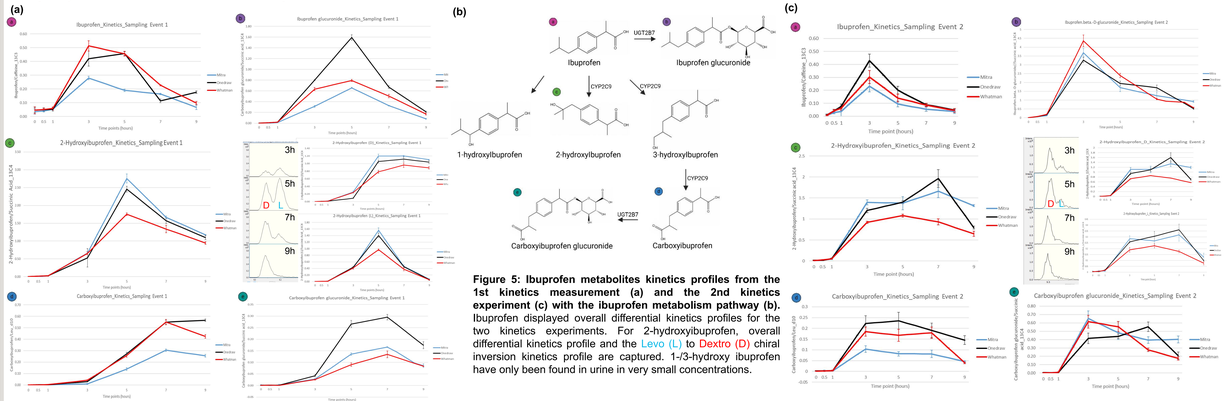


Figure 5: Ibuprofen metabolites kinetics profiles from the 1st kinetics measurement (a) and the 2nd kinetics experiment (c) with the ibuprofen metabolism pathway (b). Ibuprofen displayed overall differential kinetics profiles for the two kinetics experiments. For 2-hydroxyibuprofen, overall differential kinetics profile and the Levo (L) to Dextro (D) chiral inversion kinetics profile are captured. 1-/3-hydroxy ibuprofen have only been found in urine in very small concentrations.

Dextromethorphan Metabolism: Drug Formulation Dependence on Kinetics

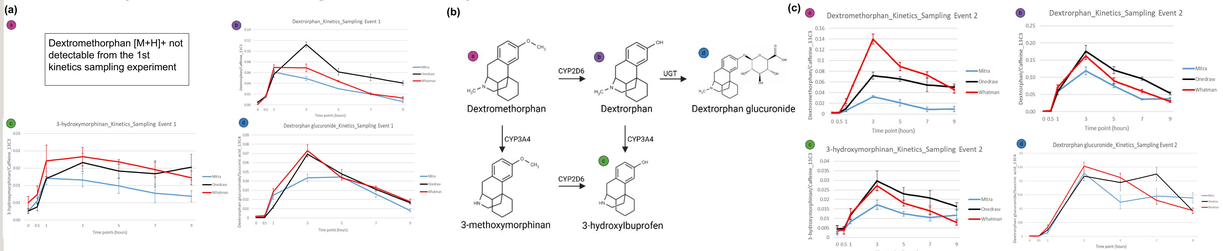
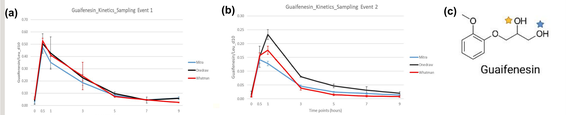


Figure 6: Dextromethorphan metabolite kinetics profiles from the 1st kinetics measurement (al) and the 2nd kinetics experiment (c) with the ibuprofen metabolism pathway (b). Dextromethorphan is not detected in the first kinetics event, which could be due to faster absorption and metabolism to dextrorphan as evidenced by the rise in dextrorphan intensity at 0.5h in the 1st kinetics experiment, whereas the rise in dextrorphan intensity is observed at 1h in the 2nd kinetics experiment..

Guaifenesin Metabolism

Formulation (Liquid vs pill) Impact on Release/Absorption



Glucuronidation on Primary and Secondary Aliphatic OH

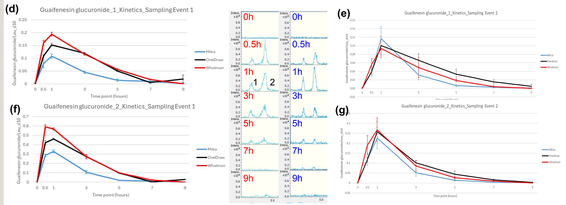


Figure 7:Formulation impact on guaifenesin absorption/ release(a,b) and guaifenesin glucuronidation kinetics. Guaifenesin showed peak intensity at 0.5 h in the 1st kinetics experiment whereas it showed peak intensity at 1h in the second kinetics experiment, suggesting slower release/absorption of guaifenesin from the pill as compared to liquid form. Guaifenesin has two aliphatic OHs possible for glucuronidation (c). EIC of the [M-H]- ion of guaifenesin glucuronide HILIC run showed two peaks (d, 1st/2nd kinetics experiment), suggesting the formation of glucuronide at both -OH with comparable kinetics profiles (d,e for peak 1; f,g for peak 2).

Energy Drinks Compounds Reveal Sampling Site Differential Kinetics

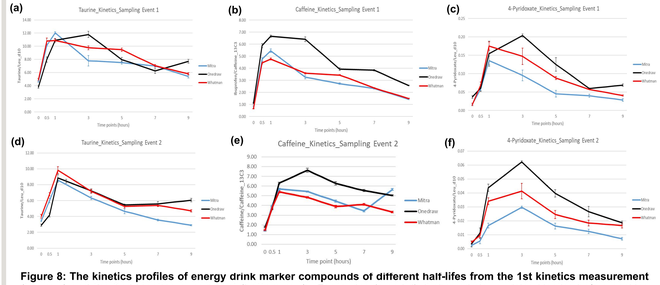


Figure 8: The kinetics profiles of energy drink marker compounds of different half-lifes from the 1st kinetics measurement (top row) and the 2nd kinetics experiment (bottom row). For taurine (a and d), it showed slower rising kinetics before peaking from upper arm sampling (OneDraw) as compared to fingertip sampling(Mitra & Whatman). For Caffeine (b and e), its peak concentration lasts longer at the upper arm capillary as compared to at the fingertip capillary.

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