Blood-Plasma Partitioning Effects During Dried Blood Spotting-Plasma Cross-Validation

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Overview

PURPOSE

- Cross-validate dried blood spotting (DBS) against traditional plasma sample collection for determination of pharmacokinetics of an orally-administered carboxylic acid-containing compound and its acyl glucuronide metabolite in female Sprague-Dawley rats (n=4).
- Determine 16-day stability of the carboxylic acid and its acyl glucuronide metabolite on the DBS card when stored at ambient conditions in an envelope in a lab drawer.

METHODS

- Venous tail blood was collected at 0.5, 1, 2, 4, 8, or 12 hours. For DBS, 40 µL of blood was directly spotted onto an ID-Biologicals Bioanalysis Card and allowed to dry under ambient conditions. For plasma, 200 µL of blood was placed into a K_2-EDTA-treated microcentrifuge tube, spun to plasma, acidified, and stored at – 20 deg C.
- A punch of the DBS card or an aliquot of plasma was added to a 96-MTP containing solvent with deuterated internal standards to extract analytes. DBS extracts were diluted. Plasma extracts were spun in a centrifuge to yield particulate-free supernatant prior to dilution.
- The diluted supernatant was analyzed via positive Turbospray reversed-phase LC-MS/MS.

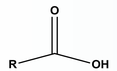
RESULTS

- DBS was successfully cross-validated with rat plasma for determination of PK parameters for the carboxylic acid-containing compound; however, the cross-validation was not successful for the acyl glucuronide metabolite.
- Both the carboxylic acid-containing compound and the acyl glucuronide metabolite were shown to be stable on the DBS card for 16 days when stored under ambient conditions in an envelope in a lab drawer.
- Blood-plasma partitioning effects were demonstrated with in vitro incubations of the acyl glucuronide metabolite and appear to contribute to the negative bias observed with DBS sample collection.

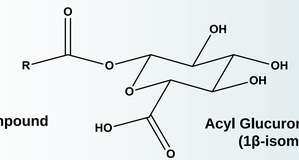
Introduction

A major in vitro and in vivo metabolite of a small molecule carboxylic acid-containing compound was its corresponding acyl glucuronide. Acyl glucuronides (AGs) are potentially labile species capable of undergoing hydrolysis, acyl migration, transacylation with nucleophiles, and/or interaction with biomolecules[1,2]. To prevent AG rearrangement and reversion to parent compound, EDTA-treated plasma samples were acidified prior to frozen storage[3]. To circumvent these hydrolysis and preparation burdens, dried blood spotting (DBS) was investigated as an alternative sample collection technique for the determination of preclinical pharmacokinetics[4,5,6]. Prior work validated the 1-week room-temperature stability of three O-glucuronide androgen metabolites on DBS cards and the subsequent use of the testosterone glucuronide / testosterone ratio obtained from these DBS cards as a suitable alternative to plasma[7]. Another lab reported on the utility of DBS for acylcarnitine and free carnitine determinations during neonatal screening for carnitine transporter deficiency and concluded that acylcarnitines were stable for at least 330 days at – 18 ºC and that room temperature storage beyond 2 weeks liberated free carnitine and the corresponding fatty acids; thus, retrospective analyses would need to correct for the sample decay during storage to be valid[8]. It was hoped that immobilizing blood on the cellulose support of DBS at room temperature would immediately stop AG degradation without the need for blood centrifugation, acidification of plasma, and frozen storage yielding a simplified sample collection and storage procedure for routine preclinical pharmacokinetics within a drug discovery environment operating on a 2-week bioanalytical turnaround time.

Structures



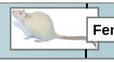
Carboxylic Acid-Containing Compound



Acyl Glucuronide Metabolite (1β-isomer shown)

Methods – Pharmacology, Sample Collection & Storage

Single Oral Dose of Carboxylic Acid-Containing Compound at 15 or 50 mg/kg
Vehicle: 0.1% Pluronic F68, 0.5% CMC in 10 mM Na_2HPO_4, pH 8, aq
White Uniform Suspension with Dose Volume of 5 mL/kg

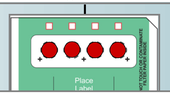


Female Sprague-Dawley Rat (n=4)

Whole Blood Withdrawn from Tail Vein via Syringe (25 gauge needle)

DBS

Direct Spotting (~ 4 drops / spot)



ID-Biologicals Bioanalysis Card

Air Dry > 3 hrs

Place in Envelope at Ambient

PLASMA

K_2-EDTA Collection Tube (< 30 min)

Centrifuge: 13 kG x 4 ºC x 10 min

Aliquot Plasma into Polypropylene Tubes
Acidify (plasma : 5% formic acid (aq), 4:1)

Cap & Mix Tube

- 20 ºC Storage

Methods – Calibrator and QC Creation

Create Dosing Stocks for Calibrators and QCs (PBS:DMSO, 9:1)

10% Dosing Spike

Naïve EDTA-Treated Whole Blood

Gentle Rock x RT x 5 min

Spot Card with 40 µL Aliquot from Pipette

Air Dry > 3 hrs & Place in Envelope at Ambient

Naïve EDTA-Treated Plasma (plasma:5% formic acid, aq; 4:1)

Moderate Vortex x RT x 5 min

- 20 ºC Storage

Methods - Bioanalytical Prep

DBS

Add 3.0-mm DBS Punch to 96-MTP

Add 60 µL of MeOH + 80 ng/mL D7-ISTD

Seal x Shake x RT x 20 min

Transfer 30 µL of Extract to 96-MTP Containing 30 µL of 0.1% Formic Acid + 1% IPA (aq)

THAWED PLASMA

Add 20 µL to 96-MTP

Add 60 µL of ACN + 0.1% FA + 80 ng/mL D7-ISTD

Seal x Shake x RT x 5 min

Centrifuge: 3 kG x 4 ºC x 10 min

Transfer 20 µL of Supernatant to 96-MTP Containing 60 µL of 0.1% Formic Acid + 1% IPA (aq)

LC-MS/MS

Methods – LC-MS/MS Bioanalysis

LC-MS/MS Hardware

- ABI Sciex 4000 Qtrap Triple Quadropole Mass Spectrometer
- Agilent 1200 HPLC
- CTC Technologies HTC-PAL Autosampler with 100 µL Syringe
- Valco Divert Valve

HPLC Conditions

- Column: Waters Xterra MS C18, 3.0 x 30 mm, 3.5 µm.
- Column Temperature: Ambient
- Mobile Phase A: 0.1% Formic Acid + 1% IPA (aq)
- Mobile Phase B: 0.1% Formic Acid in acetonitrile
- Flow Rate: 1 mL/min
- Injection volume: 10 µL (plasma) or 20 µL (DBS)

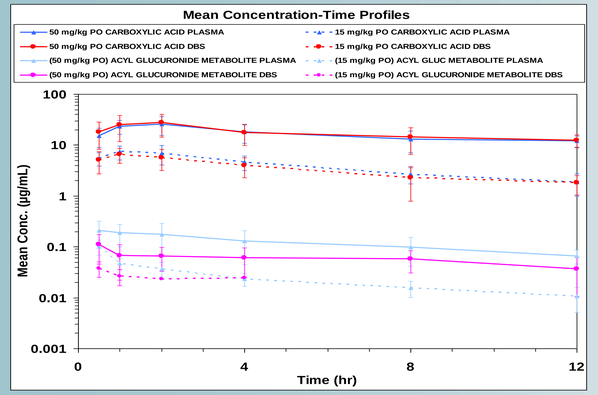
Time (min)	% B	Valve to MS (min)
0.0 – 0.4	5	1.0 – 3.0
0.4 – 1.2	5 → 95	
1.2 – 2.2	95	
2.2 – 2.3	95 → 5	
2.3 – 3.3	5	

Mass Spectrometry Parameters

Polarity/Mode	Ion Spray Voltage	Temp (ºC)	GS1/GS2	Curtain Gas	CAD Gas
Positive Turbo Spray	5500	550	40 / 40	30	Medium

Analyte	DP	EP	Q1 (m/z)	Q3 (m/z)	CE	CXP
Carboxylic Acid	90	10	516	331	30	10
Carboxylic Acid D7-ISTD	90	10	523	335	30	10
Acyl Glucuronide	90	10	692	331	53	11
Acyl Glucuronide D7-ISTD	90	10	699	335	53	11

Results – Main Pharmacokinetic Parameter Comparison



CARBOXYLIC ACID-CONTAINING COMPOUND, 15mg/kg PO (n=4)				
Analyte	Parameter	AVG DBS	AVG PLASMA	DBS % BIAS
Carboxylic Acid	C_{max} (µg/mL)	6.65 ± 2.25	7.65 ± 2.56	-15.0%
	AUC_t (µg-hr/mL)	40.7 ± 17.2	44.2 ± 7.9	-8.6%
Acyl Glucuronide	C_{max} (µg/mL)	0.040 ± 0.011	0.102 ± 0.033	-155%
	AUC_t (µg-hr/mL)	0.052 ± 0.044	0.280 ± 0.039	-438%

CARBOXYLIC ACID-CONTAINING COMPOUND, 50mg/kg PO (n=4)				
Analyte	Parameter	AVG DBS	AVG PLASMA	DBS % BIAS
Carboxylic Acid	C_{max} (µg/mL)	27.6 ± 13.0	28.0 ± 7.3	-1.4%
	AUC_t (µg-hr/mL)	204 ± 77	182 ± 76	10.0%
Acyl Glucuronide	C_{max} (µg/mL)	0.117 ± 0.058	0.227 ± 0.091	-94.0%
	AUC_t (µg-hr/mL)	0.690 ± 0.276	1.35 ± 0.82	-95.7%

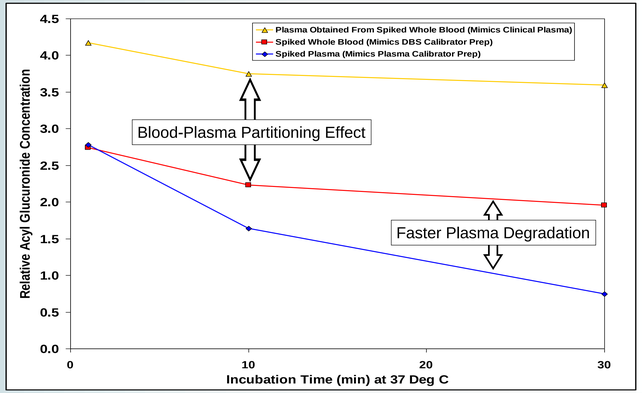
Results – Long-Term DBS Stability & Blood-Partitioning

Mean Percent Accuracy & Precision Values (%) for CARBOXYLIC ACID-CONTAINING COMPOUND at 1µg/mL in Dried Rat Blood on ID-Biologicals Bioanalysis Cards Following 16-Day Storage in a Lab Drawer at Room Temperature in an Envelope (n=3)

Whole Blood Incubation Time (min)	Prep 1	Prep 2	Prep 3	Mean	Std. Dev.	RSD (%)
5	98.1%	92.4%	116%	102%	12%	12%
15	110%	111%	104%	108%	4%	3%
30	102%	108%	111%	107%	5%	4%

Mean Percent Accuracy & Precision Values (%) for ACYL GLUCURONIDE METABOLITE at 1µg/mL in Dried Rat Blood on ID-Biologicals Bioanalysis Cards Following 16-Day Storage in a Lab Drawer at Room Temperature in an Envelope (n=3)

Whole Blood Incubation Time (min)	Prep 1	Prep 2	Prep 3	Mean	Std. Dev.	RSD (%)
5	94.7%	94.3%	84.3%	91.1%	5.9%	6.5%
15	91.4%	84.9%	80.8%	85.7%	5.3%	6.2%
30	78.6%	85.2%	75.9%	79.9%	4.8%	6.0%



Summary

- DBS was cross-validated against plasma for PK determination of an orally-administered carboxylic acid-containing compound in Sprague-Dawley rat. However, DBS was not successfully cross-validated with plasma for the acyl glucuronide metabolite due to a negative bias in PK exposure parameters.
- In vitro blood-plasma partitioning demonstrated that the acyl glucuronide partitioned into the plasma sub-compartment of whole blood more favorably than the erythrocyte. This may partially explain the negative bias observed with DBS and suggest the need for a partitioning correction factor[5]. Literature has shown that a multispecific organic-anion transporter for glucuronides (MOAT4) exists in human erythrocytes[9]. Other literature reported that parent drug to metabolite ratios for morphine, morphine-3-glucuronide, and morphine-6-glucuronide were strongly influenced by hematocrit and water ratios[10].
- 16-day room-temperature stability on DBS cards was demonstrated for both the carboxylic acid-containing compound and its acyl glucuronide metabolite eliminating degradation on DBS paper as the culprit.

References

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