Poster
ThP 339

A 10-Point Time Course Pharmacokinetic Study Collected from a Single Mouse by use of the Mitra Microsampling Devices

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Introduction

Conducting PK studies using dried blood spot (DBS) sampling provides some advantages over conventional plasma sampling, most notably minimal sample volume requirements, prolonged stability at ambient temperature and testing for RBC partitioning becomes unnecessary. The novel patent pending Mitra® microsampling device offers additional advantages over DBS since there is no concern of introducing variability from different sample sizes due to hematocrit (Hct) or manually punching out the filter paper sample. This poster presents quantitative data from a full 10-point time course obtained from a single mouse, followed by use of the Mitra tip extraction prototype and LC-MS/MS analysis. The ability to conduct an entire PK study using a single mouse reduces variability and provides a cost savings with reduced labor, animal use and test article requirements.

Methods

Compound IWP-051, which was evaluated for both RBC partitioning and whole blood and plasma stability, was dosed PO at 0.1 mg/kg to twenty-one CD-1 mice. The Mitra whole blood samples were collected at 10 time points over 8 hours by tail nick under a heat lamp. Mitra sampler tips were removed by means of the Mitra tip extraction prototype and then quenched in 200 uL ethyl acetate:methanol (50:50, v/v), dried down and then reconstituted in 20% acetonitrile containing 0.1% formic acid. Plasma samples were collected from retro orbital bleeds from the same mice and time points, extracted with 200 uL acetonitrile, dried down and reconstituted in the previously mentioned solution. Both plasma and Mitra whole blood samples were analyzed on a Sciex QTrap 5500™.

Figure 1: Picture displaying the use of the Mitra tip extraction prototype to remove the whole blood sample tips from the Mitra and drop into a 96-well plate containing the 200 uL ethyl acetate:methanol (50:50, v/v) for extraction

- Species: male CD-1 mice (fed)
- Compound: IWP-051
- Dose: 0.1 mg/kg PO, single dose
- Vehicle: PEG400

Methods (Cont.)

- Time points: 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 hours
- Group 1: (2 time points/mouse) collect both the whole blood from a tail nick, under a heat lamp, with the Mitra and plasma from eye bleed (3 mice/time point)
- Group 2: (1 time course/mouse) collect whole blood from a tail nick, under a heat lamp, with the Mitra (6 mice/time point)
- Standard curves were linear from 1 to 1000 ng/mL with all concentrations within 15% of the theoretical concentration

Whole blood and plasma stability results

Compound ID	Assay Concentration (µM)	Matrix	Anticoagulant	Species	$T_{1/2}$ (min)	% Remaining at 360 min
IWP-051	1	Plasma	K2EDTA	CD-1 Mouse	>360	99.5
Propantheline	1	Plasma	K2EDTA	CD-1 Mouse	72.2	2.7
Enalapril	1	Plasma	K2EDTA	CD-1 Mouse	95.1	7.4
IWP-051	1	Whole Blood	K2EDTA	CD-1 Mouse	>360	110.4
Propantheline	1	Whole Blood	K2EDTA	CD-1 Mouse	127.6	13.2
Enalapril	1	Whole Blood	K2EDTA	CD-1 Mouse	111.6	10.1

IWP-051 was found to be stable in both mouse whole blood and plasma at room temperature for 6 hours; hence, the possibility that different sample handling or matrices could contribute to differences in results was ruled out

RBC partitioning results

Compound ID	Assay Concentration (µM)	Species	(Conc. in Spiked Plasma) / (Conc. in Plasma from Spiked Blood)	Ratio Adjusted for Hematocrit	$K_{p(RBC/PL)}$	% Bound to RBCs	% Plasma Stability
IWP-051	1	CD-1 Mouse	0.973	1.80	0.940	44.5	105.7
Chloroquine	1	CD-1 Mouse	1.66	3.08	2.44	67.5	84.9
Chlorthalidone	1	CD-1 Mouse	33.1	61.3	70.8	98.4	105.5

Notes: Adjusted Ratio takes hematocrit into account = (Conc. Spiked Plasma / Conc. in Plasma from Spiked Blood) x 1/(1-hematocrit)
RBC binding/partitioning is indicated if the Adjusted Ratio is > 1.0, and if $K_{RBC/PL} > 0$.
$K_{RBC/PL}$ = [(Conc in spiked plasma/Conc in plasma from spiked WB) - 1] x (1/Hematocrit) + 1

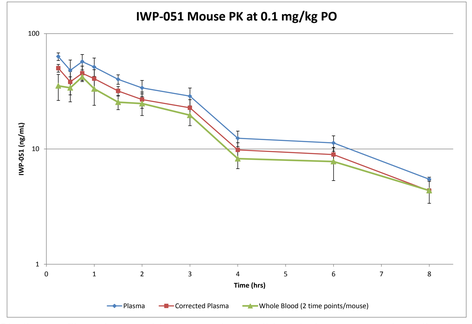
Assay Conditions:	1 hour incubation; 37 °C
Hematocrit:	46.0%

IWP-051 showed some measurable partitioning to mouse red blood cells. Therefore, in order to compare plasma concentrations to whole blood concentrations, the following correction using % bound to RBCs and Hct was applied to the plasma results to convert to whole blood concentrations

$$[\text{whole blood}] = ([\text{plasma}]*\text{Hct}) / (1 - \%\ \text{bound to RBCs})$$

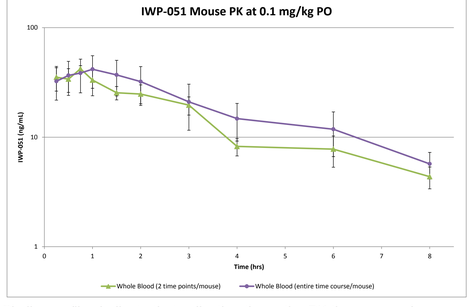
PK results

Figure 2: Traditional plasma collection vs. Mitra whole blood microsampling



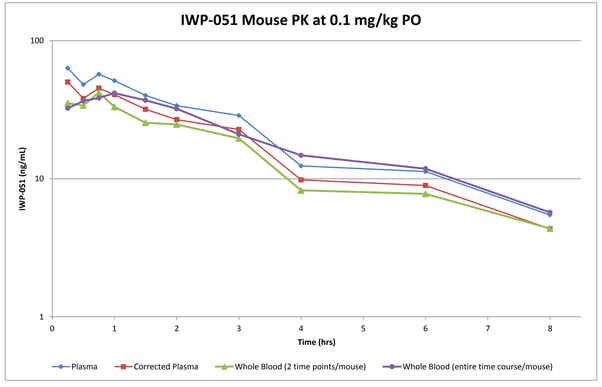
- Similar profiles observed between the traditional plasma sample collection (2 time points/mouse; with RBC partitioning correction) and the Mitra whole blood sampling (2 time points/mouse)

Figure 3: Mitra microsamples (2 time points/mouse) vs. Mitra microsamples (entire time course/mouse)



- Similar profiles indicate that collecting the entire PK time course from a single mouse provides comparable results

Figure 4: Comparison of PK parameters for IWP-051



Group	Matrix	Sampling	AUC_{0-t} (hr*ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)
1	plasma	2 time points/mouse	158	2.2	0.25	50
1	whole blood (Mitra)	2 time points/mouse	139	2.4	0.75	42
2	whole blood (Mitra)	entire time course/mouse	182	2.8	0.92	42

Conclusions

- Confirmed that the traditional plasma sample collection and the Mitra whole blood sampling show similar PK profiles and parameter results
- Collecting the entire PK time course from a single mouse provides comparable results
- The Mitra tip removal prototype provided consistent results and was easy to utilize
- The ability to conduct an entire PK study using a single mouse provides a cost savings by reducing labor, animal use and test article requirements

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