Evaluation of Automated Blood Sampler (ABS) with Dried Blood Spot (DBS) Technology for Routine Rat PK Studies in Discovery DMPK

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ABSTRACT 2613 POSTER 309

INTRODUCTION

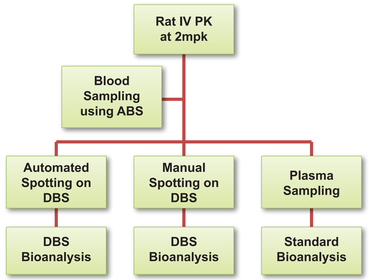
Conventional plasma sampling has been used for determining drug exposure because it is generally considered to be easier to store and handle. However, collection of plasma requires a relatively large volume (100-500 µL) of whole blood to obtain enough plasma for bio-analysis. In mouse PK, it becomes a bigger issue as the total blood collection volume is limited. As a result, PK studies in mice generally required different sets of animals and at a larger quantity which could add inter animal variability and physiological differences. Moreover, the method of manually drawing blood from the animal requires the animal to be anesthetized or restrained with some device, both of which cause stress to the animal. Such stress could affect PK results by reducing absorption or altering metabolism. Furthermore, traditional plasma sampling method is time-consuming; it requires pre-labeling multiple tubes, spinning down whole blood to separate the plasma component and capping and freezing the samples post-collection.

Dried blood spot (DBS) sampling is the collection of whole blood onto filter paper and because of its advantages; it has appealed to many pharmaceutical companies. The DBS technique allow the use of low blood sample volumes (15-25 µL), making it particularly attractive for use in rodent PK studies while applying the principle of the 3Rs (reduce, refine and replace) to decrease animal use and minimize the amount of compound needed for the experiment. The low blood volume micro-sampling is also advantageous to TK studies where the duration of the studies is longer and additional time points are required. Moreover, by using dried blood sampling one can achieve actual blood PK rather than plasma and report on systemic rather than plasma CL. In addition, by simplifying the sample storage and transport procedures, it adds additional cost benefit as well as improve the quality of the data by minimizing human errors. Coupled with the automated blood sampling (ABS) system, the process of conducting rodent PK studies has been further streamlined by eliminating the labor-intensive manual withdrawals while increasing the efficiency and throughput of *in vivo* PK studies.

OBJECTIVE

We evaluated the feasibility of using ABS incorporating the DBS technology for routine rat PK screening. Four internal compounds from different project and chemical series were administered intravenously at 2 mg/kg in DMA/PEG/D5W (15/50/35). These compounds had pKa values ranging from 4-12, cLogP ranging from 1-4 and blood to plasma (BP) ratios ranging from 0.7-3. Full PK profiles from three different groups of animals (n=3) were obtained over a 24 hr period. The study design can be seen on **Figure 1**. The groups were as follows: 1) Blood samples spotted directly on the DBS disks by the ABS, 2) Blood samples collected by ABS but manually spotted on the DBS disks, and 3) Blood samples collected by ABS, spun down and plasma component collected and processed using the conventional technique.

Figure 1. Study Design



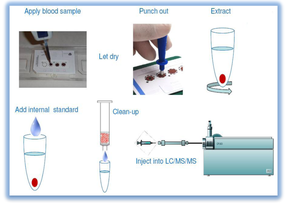
METHODS

PK studies were carried out in male JVC CD-IGS rats weighing 250-300 g (10-12 weeks of age). All samples were collected using the Instech ABS units at the following time points: 0.083, 0.25, 0.5, 1,2,4,6,8, 24 hr post-dose with the exception of 0.0167 hr post-dose which was collected manually. See **Figure 2** for the automated blood sampler. Blood was automatically sampled from the rats through a catheter implanted in the jugular vein and connected to a refrigerated fraction collector (4ºC).

METHODS

Figure 2. Automated Blood Sampler (ABS)

Figure 3. DBS Extraction Procedure



Sample Preparation

DBS analyses:

- 25 µL aliquot of each sample was spotted on the DMPK-C card and air dried for >2 hr
- 3-mm diameter disk was punched from the center of the DBS card into a 96-well plate
- 200 µL aliquot of the IS was added, mixed for 5 mins on a multi-tube vortexer and sonicated for 20 mins at RT

Plasma analyses:

- Blood samples were collected into a tube containing Lithium Heparin as an anticoagulant and kept at 4ºC.
- The tube was mixed well and centrifuged at approximately 3000 rpm for 10 mins to separate the plasma.
- 50 µL of each plasma samples was transferred to 96-well extraction plate
- 150 µL of IS was added and mixed for 5 mins on a multi-tube vortexer
- All samples were filtered on a vacuum apparatus and supernatant was collected and analyzed via LC-MS/MS analysis.
- Refer to **Figure 3** for a visual representation of DBS extraction procedure.

In Vivo Determination of B/P Ratio

- BP ratio was determined at 2 hr post dose following IV dosing of test compounds.
  - 25 µL blood sample was mixed with 25 µL of DI water and 25 µL counterpart control plasma
  - At the same time, 25 µL plasma sample was mixed with 25 µL of DI water and 25 µL counterpart control blood
  - Mixture was vortexed and sonicated for 10 mins
  - 300 µL of IS was added to both sample mixtures and vortexed
  - All samples were subjected to the same filtration procedure as mentioned in sample preparation

Analytical Instrumentation

- Column: Thermo Hypersil Gold RP C18 column 1.9 µm (50 x 2.1 mm)
- Elution: Gradient flow, Mobile Phase A: Water with 0.1% FA, Mobile Phase B: Acetonitrile with 0.1% FA
- Detector: TSQ Quantum Ultra Triple Quadrupole Mass Spectrometer utilizing MRM mode and ESI ionization coupled to the Accela Binary Pump and the HTC Pal Leap Autosampler.

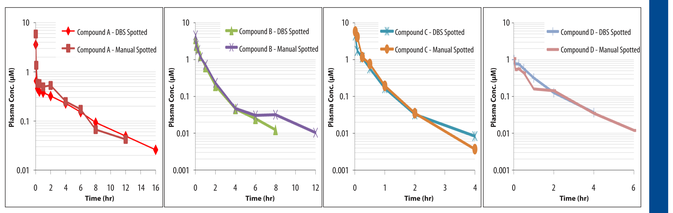
RESULTS

Calibration curves were made by plotting peak area ratios of analyte and internal standard against nominal concentration of analyte. The calibration curve parameters were calculated using weighted ($1/x^2$) linear regression method. All calibration standards were quantified within 20% of their nominal concentration and as such the data were deemed accurate and precise within a non-regulatory environment. The calibration curve was linear over the concentration range of 1-2500 nM for all analytes. The correlation coefficient *r* was above 0.99 in both DBS blood and plasma.

RESULTS

The time-concentration profiles of all four compounds for both spotting methods can be seen in **Figure 4**. This was designed to compare the time-concentration profile collected from the automated DBS sampling system to that collected from a standard ABS system from which DBS samples were then manually spotted. The indistinguishable plots demonstrated that the quality of DBS spots collected by the ABS system is comparable to that of DBS prepared manually and that the system is reliable and effective for the use of routine rat PK studies collecting low volume (25 µL) of DBS samples.

Figure 4. Concentration-Time Profiles for ABS Spotting vs. Manual Spotting



The PK parameters were calculated using non-compartmental analysis in Phoenix WinNonlin system. **Table 1** shows the area under the curve (AUC) of all test compounds in plasma and DBS blood along with the *in vivo* BP ratios. The BP ratio calculated from the DBS data (blood concentrations) and the plasma data (plasma concentrations) was in good agreement with the one obtained from the *in vivo* assessment using conventional methodology with the exception of compound D that had a mean BP ratio of 1.3 and 2.7 for BP ratio calculated through the AUC method and BP ratio calculated through the standard method, respectively.

The other key PK parameters were tabulated in **Table 2**. Average PK parameters of all analytes obtained from automated DBS sampling and manual DBS sampling were similar to that of plasma sampling after correcting for their corresponding BP ratios. The notable difference was observed in the same compound (Compound D) with high BP ratio that exhibited ~2X lower CL. It is also important to note that this compound had varying degree of BP ratios calculated from both methods that could explain this discrepancy. Factors such as differential binding to specific component in the blood and concentration dependence partitioning could explain this variation in BP ratio. Thus, careful evaluation must be considered in order to avoid erroneous comparison of DBS and plasma data.

Table 1. Comparison of BP ratio from AUC with *In Vivo* BP ratio

Compound	Matrix	AUC 0-t (µM·hr)	AUC (BP) ratio	*In Vivo* BP ratio
Compound A	Blood ABS-DBS	2.34	0.67	
	Blood Manual DBS	2.54	0.73	
	Plasma	3.49		0.76 ± 0.06
Compound B	Blood ABS-DBS	1.08	0.52	
	Blood Manual DBS	1.51	0.73	
	Plasma	2.07		0.70 ± NA
Compound C	Blood ABS-DBS	2.78	3.51	
	Blood Manual DBS	3.33	4.21	
	Plasma	0.791		3.3 ± 0.20
Compound D	Blood ABS-DBS	1.03	1.50	
	Blood Manual DBS	0.830	1.21	
	Plasma	0.688		2.7 ± 0.71

Note: *In vivo* BP ratio values are mean ± standard deviation of three replicates

Table 2. PK Parameters of Test Compounds in Plasma or Blood (n=3)

Compound	Matrix	CL (mL/min/kg)	Vss (L/kg)	$T_{1/2}$ (hr)
Compound A	Blood ABS-DBS	38.1 ± NA	2.83 ± NA	2.2 ± NA
	Blood Manual DBS	36.2 ± 9.2	3.44 ± 0.50	3.0 ± 0.18
	Plasma	26.1 ± 6.0	1.31 ± 0.32	1.8 ± 0.06
Compound B	Blood ABS-DBS	89.4 ± 13.3	2.40 ± 0.04	0.78 ± 0.23
	Blood Manual DBS	64.5 ± 13.9	1.49 ± 0.29	1.3 ± 1.3
	Plasma	46.0 ± NA	1.49 ± NA	1.2 ± NA
Compound C	Blood ABS-DBS	28.9 ± 2.9	9.68 ± 2.94	4.0 ± 1.16
	Blood Manual DBS	24.0 ± 1.4	5.46 ± 0.63	3.7 ± 1.25
	Plasma	101 ± 2	25.6 ± 1.4	2.9 ± 0.51
Compound D	Blood ABS-DBS	107 ± NA	7.17 ± NA	0.85 ± NA
	Blood Manual DBS	131 ± 8.9	10.4 ± 1.46	1.1 ± 0.20
	Plasma	164 ± 37	36.0 ± 4.2	3.7 ± 0.91

All data are mean ± standard deviation

CONCLUSIONS

- The Instech ABS system has been successfully demonstrated to perform automated whole blood sample collection for DBS analysis in exploratory rat PK studies for four internal test compounds.
- Although the automated blood sampling requires the animals to be cannulated, the reduction of handling and removal of anesthesia during the sampling process decreases animal stress and improves the quality and reproducibility of the samples.
- PK exposure profiles were comparable when measured in ABS-spotted DBS, manually-spotted DBS and plasma analysis indicating good agreement between DBS analysis and traditional plasma analysis.
- It is important to note that using automated DBS in lieu of plasma samples did not change our placement category of low or high clearance values based on our internal guidelines for progressing compounds into subsequent *in vivo* PK studies.
- Based on these results, we can conclude that the ABS system coupled with DBS collection is promising to obtain reliable full PK profiles with increased efficiency and throughput while gaining significant synergies from the advantages of both techniques.

REFERENCES

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