

# Application of ESI-LC-MS/MS to mouse pharmacokinetic studies using serial and parallel blood sampling techniques



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## OVERVIEW

Serial versus parallel blood sampling techniques were evaluated in mouse PK studies. The development of sensitive LC-MS/MS methods for the test analytes was a prerequisite for effective quantification of low ng/mL concentrations in the small volumes of blood obtained from the serial technique. Our results show that serial sampling in mouse PK studies is a valid alternative to conserve valuable compound, limit animal usage, and compare interanimal variability.

## INTRODUCTION

- Mice are commonly used for preclinical efficacy and PK assessment in early drug discovery stage. Due to their small size, parallel blood sampling is generally used, i.e. each mouse is subject to only one blood draw through cardiac puncture.
- With parallel sampling, the number of animals required can be large, depending on the number of time points and replications. Consequently, a large amount of test compound is needed for administration.
- Another disadvantage is that PK parameters obtained from the parallel sampling can not evaluate individual difference among animals.
- To address the above mentioned issues, we have developed serial blood sampling technique for mouse PK studies, i.e. the blood samples for the whole time course are collected from a single animal.

## 1 – STANDARD CURVE STATISTICS ON MULTIPLE VALIDATION RUNS IN MOUSE BLOOD (n=3)

Compound (SRM transition)	Curve range (ng/mL)	r <sup>2</sup> (y=ax <sup>2</sup> +bx+c)	Level (ng/mL)	Inter-day precision and accuracy (mean conc. ± % RSD)
ANTIPYRINE (188.9 → 77.2 m/z)	1 - 1000	> 0.9929	. 1 (LLQ) . 50 . 1000	. 0.98 ± 15% . 48.4 ± 13% . 997.4 ± 6%
CAFFEINE (195.6 → 179.2 m/z)	10 - 5000	> 0.9971	. 10 (LLQ) . 250 . 1000	. 9.75 ± 18% . 238.2 ± 11% . 991.9 ± 3%
CIPROFLOXACIN (331.9 → 314.1 m/z)	5 - 1000	> 0.9934	. 5 (LLQ) . 50 . 1000	. 4.67 ± 18% . 56.0 ± 4% . 1070.6 ± 6%
DEXTROMETHORPHAN (272.0 → 147.0 m/z)	1 - 1000	> 0.9959	. 1 (LLQ) . 50 . 1000	. 0.99 ± 4% . 49.9 ± 6% . 984.2 ± 3%
ERYTHROMYCIN (734.4 → 158.1 m/z)	2.5 - 5000	> 0.9983	. 2.5 (LLQ) . 50 . 1000	. 2.47 ± 18% . 53.6 ± 6% . 996.5 ± 7%

## MATERIALS AND METHODS

### IN VIVO

- **Animals, chemicals and materials**
  - Male CD-1 mice [CrI:CD1 (ICR)], weighing 20-30 g, were purchased from Charles River Laboratories (Wilmington, MA).
  - Antipyrine, caffeine, ciprofloxacin, dextromethorphan and erythromycin were purchased from Sigma (St. Louis, MO).
  - Microvette Lithium-Heparin coated capillary tubes and vials were purchased from Sarstedt (Germany).

- **Animal work and analysis**
  - Each mouse was administered with test compound via tail vein at 5 mg/kg (dosing volume 5 mL/kg)
  - Total of 3 animals were used for each independent serial sampling experiment. Blood samples from whole time course (5, 15, 30, 60, 180, 360, and 1440 min) were collected from each animal via saphenous vein; 20-30 µL withdrawn at each time point
  - Total of 21 animals were used for each independent parallel sampling experiment. Blood sample at each time point (5, 15, 30, 60, 180, 360, or 1440 min) was collected from one animal via cardiac puncture (typically 300 µL is collected).
  - Fifteen microliters of blood sample were then submitted for quantitative bioanalysis by HPLC-MS/MS.
  - The fundamental pharmacokinetic parameters (half-life, clearance, volume of distribution and AUC) were obtained from the non-compartmental analysis using WinNonlin.

### BIOANALYTICAL

- **Sample preparation**
  - Blood sample proteins precipitated by addition of equal volume of acetonitrile (15 µL of blood)
  - Next, samples were centrifuged and then directly injected onto LC-MS

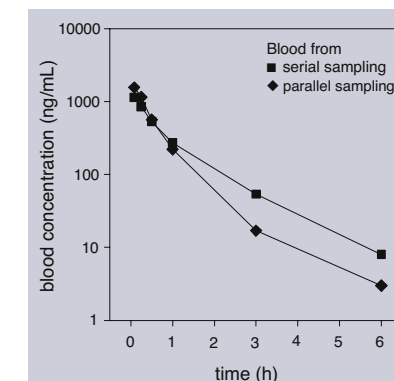
### LC-MS

- Phenomenex Synergi Max-RP 80 column, 2 x 50 mm, 4 micron
- Shimadzu binary LC system (LC-10AD) and SIL-5000 autosampler
- Mobile phase A: 13.3 mM ammonium formate/6.7 mM formic acid in water
- Mobile phase B: 6 mM ammonium formate /3 mM formic acid in water/acetonitrile (1/9, v/v)
- Thermo Electron TSQ Quantum Discovery: ESI+ and SRM

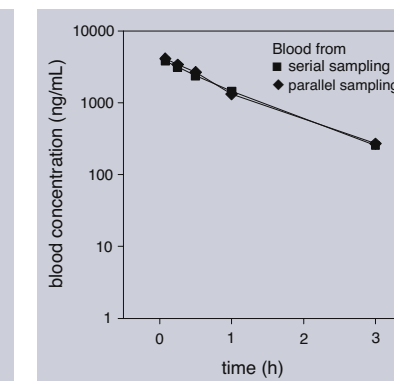
## 2 – BLOOD CONCENTRATION-TIME PROFILES

CONCENTRATION-TIME PROFILE AFTER IV ADMINISTRATION WITH:

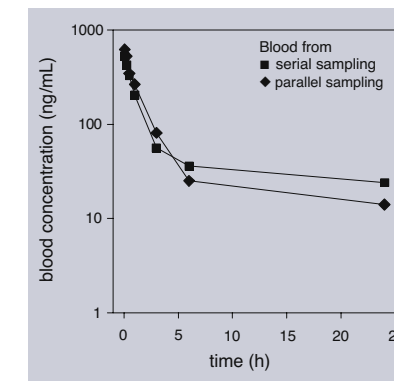
### ANTIPYRINE



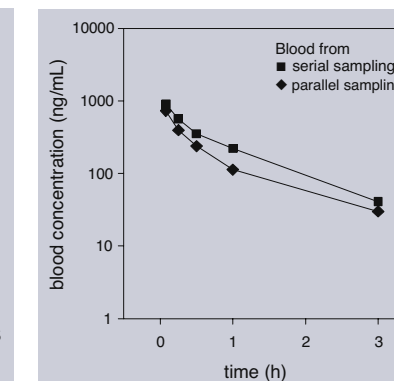
### CAFFEINE



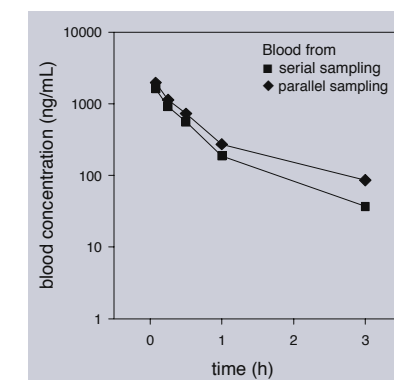
### CIPROFLOXACIN



### DEXTROMETHORPHAN



### ERYTHROMYCIN



## 3 – COMPARISON OF SERIAL AND PARALLEL SAMPLING METHODS

	Advantages	Disadvantages
<b>SERIAL SAMPLING</b>	<ul style="list-style-type: none"> <li>• Requires fewer animals</li> <li>• Requires less test compound</li> <li>• Able to compare individual difference</li> </ul>	<ul style="list-style-type: none"> <li>• Complicated technique</li> <li>• Small sample size</li> <li>• Unable to harvest plasma</li> <li>• Potential excessive blood loss</li> </ul>
<b>PARALLEL SAMPLING</b>	<ul style="list-style-type: none"> <li>• Simple technique</li> <li>• Large sample size</li> <li>• Able to harvest plasma</li> </ul>	<ul style="list-style-type: none"> <li>• Requires more animals</li> <li>• Requires more test compound</li> <li>• Unable to compare individual difference</li> </ul>

## RESULTS

- Sensitive, accurate, and reproducible analytical methods were successfully developed for quantifying reference compounds in small volumes of mouse blood (1).
- The validation results obtained from serial sampling were compared to those from parallel sampling.
- Blood drug concentration-time profiles are presented (2).
- Overall the profiles generated from these two sampling methods are relatively close, especially for caffeine.

## CONCLUSION

- We have established a technique for serial sampling from one mouse, which generated the same quality of data as traditional parallel sampling.
- The animals did not show any observed adverse effect, suggesting the amount of blood loss is tolerable to the animals.
- Although not significant, there are differences in some PK parameters generated from these two methods for some compounds. Further studies are needed to understand the factors that caused those differences.
- Each sampling method has its own advantages and disadvantages (3). If the number of animals and the amount of test compound are an issue, the serial sampling method provides an alternative way to conduct mouse PK at early drug discovery stage.