Comparison of Blood Microsampling Techniques for Discovery PK Studies in Rats: Capillary Microsampling (CMS) and a Dried Matrix Microsampling Device

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1 Overview

The capillary microsampling (CMS) method is described and shown to be useful for rat drug discovery PK studies. An alternative microsampling procedure using Mitra, a dried blood sampling device is also shown for comparison. Both procedures were based on rat tail vein sampling and were found to work well for rat discovery PK sampling.

The described CMS procedure has multiple advantages:

- Only 8 µL blood sample required for each timepoint
- Addition of dilution solution (25% ACN in water) at time of sampling stabilizes the sample quickly
- Sample can be frozen and more than one aliquot can be taken from each sample

The described Mitra procedure would be useful when collecting dried blood samples is important. The results from the Mitra samples were very similar to those obtained from blood CMS.

2 Introduction

In an effort to reduce the number of laboratory animals needed for a discovery pharmacokinetic (PK) study, the pharmaceutical industry has started to utilize microsampling methods for rodent studies [1-4]. Blood microsampling can be defined as a blood sample with a volume of less than 50 µL. Capillary microsampling (CMS) has emerged as one of the simplest ways to collect blood microsamples. Recently, Mitra™, an alternative device for collecting dried blood samples, has become available. The Mitra device can be used to collect 10 µL of blood as a dried blood sample [5-7]. This study reports the results obtained from a comparison of these devices to determine the utility of these two different modes of blood microsampling in rats.

3 Methods : *In Vivo*

Two rat studies were performed; in each study, four male rats were dosed orally at 10 mg/kg with fluoxetine. Eleven serial blood samples were taken from each rat starting at 0.5 h and continuing to 48 h. All samples were assayed for both fluoxetine and a metabolite, norfluoxetine by HPLC-MS/MS.

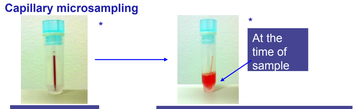
Study 1: blood was sampled from the tail vein of each rat in two ways: (1) using an 8-µL capillary and (2) collecting a standard whole blood sample.

Study 2: blood was sampled from the tail vein of each rat in two ways: (1) using an 8-µL capillary and (2) using a Mitra blood sampling device.

The CMS sample was diluted with 72 µL of a dilution solution (25% acetonitrile in water) as part of sample collection. The Mitra sample was air dried for two hours as part of sample collection.

4 Capillary Microsampling (CMS) Method

- **Capillary microsampling**



- **Blood sample analysis**

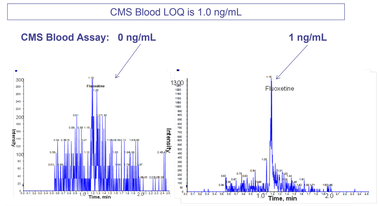
25.0 µL diluted blood sample protein precipitation → Vortex; → Assay by LC-

* Photo selected from "Capillary Microsampling of Liquid Matrices" poster by Ove Jonsson, Asta Zeneca R&D, Sweden.

5 Methods: CMS Assay

The CMS sample was vortexed in the sample tube to allow the blood to mix with the dilution solution at the time of sampling. The CMS samples were then frozen until assayed. The assay utilized standard protein precipitation of a 25-µL aliquot of the diluted blood samples. The blood calibration curve and QC samples were prepared in the range of 1.00 to 10,000 ng/mL by spiking the appropriate working solutions into control rat blood. For the analysis of the CMS samples, the prepared rat blood calibration curve and QCs were diluted 1:10 with 25% ACN in water to yield a calibration curve and QCs in diluted blood in the range of 0.10 to 1,000 ng/mL. The analysis was performed via HPLC-MS/MS procedures using a Sciex API 6500 mass spectrometer.

6 Fluoxetine Bioanalysis: LOQ for Diluted Rat Blood by CMS Assay

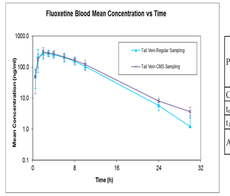


Note: LOQ assay based on the analysis of a 0.1 ng/mL diluted blood standard.

7 Study 1: Comparison of Whole Blood and CMS Sampling *In Vivo* Study Design

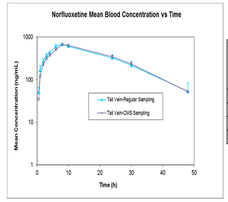
- **Test Compound:** Fluoxetine
- **Animal:** SD Rats, Male (N=4)
- **Route/Dose:** PO/ 10 mg/kg of Fluoxetine
- **Sample Time:** 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 30, 48 hours
- **Sampling Method:** (a) 10 µL whole blood from tail vein
  (b) 8 µL CMS sample from tail vein

8 Comparison of Whole Blood and CMS Exposure from Rat Tail Vein for Fluoxetine



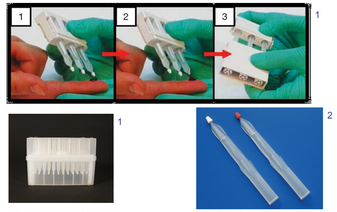
Parameter	Unit	Fluoxetine	
		8 µL CMS	10 µL Whole Blood
C_{max}	ng/mL	344	306
t_{max}	hours	2.50	3.00
$t_{1/2}$	hours	4.10	3.48
AUC_{last}	ng*hours/mL	2980	2740

9 Comparison of Whole Blood and CMS Exposure from Rat Tail Vein for Norfluoxetine



Parameter	Unit	Norfluoxetine	
		8 µL CMS	10 µL Whole Blood
C_{max}	ng/mL	690	682
t_{max}	hours	8.50	8.00
$t_{1/2}$	hours	8.60	9.05
AUC_{last}	ng*hours/mL	15400	15200

10 Mitra Microsampling Device



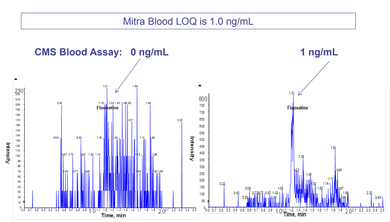
1: photos selected from Phenomenex poster: http://blob.phenomenex.com/webdocument/nextervx_mitra_asms_poster.pdf
2: Photo selected from Spooner et al. [5]

Before (left) and after (right) being filled with blood.

11 Mitra Dry Blood Sample Assay

- Prepare STDs and QCs in fresh control rat blood
- Use Mitra device to sample STDs and QCs (touch tip to sample)
- Dry Mitra device for 2 hours at room temperature
- Extract Mitra tips with 200 µL of IS solution in 50%ACN-50%MeOH for 1 hour while vortex-shaking at 1750 rpm
- Transfer 50 µL supernatant and dilute with 100 µL of water
- Inject 5 µL for LC/MS/MS analysis

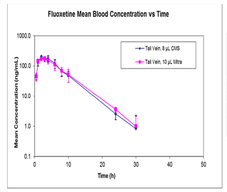
12 Fluoxetine Bioanalysis: LOQ for Rat Blood by Mitra Assay



13 Study 2: Comparison of CMS and Mitra Sampling *In Vivo* Study Design

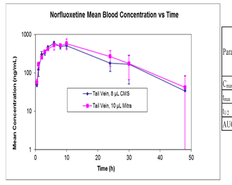
- **Test Compound:** Fluoxetine
- **Animal:** SD Rats, Male (N=4)
- **Route/Dose:** PO/ 10 mg/kg of Fluoxetine
- **Sample Time:** 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 30, 48 hours
- **Sampling Method:** (a) 8 µL CMS sample from tail vein
  (b) 10 µL Mitra sample from tail vein

14 Comparison of CMS and Mitra Exposure from Rat Tail Vein for Fluoxetine



Parameter	Unit	Fluoxetine	
		8 µL CMS, Diluted at Sample time	10 µL Mitra
C_{max}	ng/mL	209	181
t_{max}	hours	2.25	2.50
$t_{1/2}$	hours	4.44	3.49
AUC_{last}	ng*hours/mL	1560	1530

15 Comparison of CMS and Mitra Exposure from Rat Tail Vein for Norfluoxetine



Parameter	Unit	Norfluoxetine	
		8 µL CMS, Diluted at Sample time	10 µL Mitra
C_{max}	ng/mL	615	640
t_{max}	hours	7.00	8.50
$t_{1/2}$	hours	8.31	8.63
AUC_{last}	ng*hours/mL	11800	13200

16 Discussion

Overall, these studies provide additional data that blood CMS is a valuable approach for serial blood sampling from discovery rat PK studies. The first fluoxetine study used the 8-µL CMS blood sampling method with dilution at the time of sample collection because this was the sampling methodology that we demonstrated previously to be useful for discovery PK studies in the mouse [4]. These data also support recent studies reported by Jonsson et al. [1] and Dillen et al. [3] that successfully demonstrated the application of blood CMS to support rat PK or TK studies. Using this method as the reference method, allowed us to compare it in the second study with the PK profile results that were obtained from the Mitra dried blood sampling method. The PK profile and PK results obtained from the two sampling methods were very similar for both fluoxetine and norfluoxetine.

17 Conclusions

- The results obtained for each sample set (CMS and Mitra) were compared for both fluoxetine and norfluoxetine. There was very good agreement in the PK results from the CMS samples and the Mitra samples for both fluoxetine and norfluoxetine. For fluoxetine and norfluoxetine, the AUC and Cmax values based on CMS were within 15% of the AUC and Cmax values based on the Mitra sampling. Both methods were easy to implement for the sample collection step.
- CMS of blood works well and can be used for serial bleeding in discovery rat PK studies and should be applicable as well to rat PK/PD and TK studies.
- Mitra sampling could also be used for serial sampling in discovery rat PK studies when collecting dried blood samples is needed.

18 References

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