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Original article

# Utility of capillary microsampling for rat pharmacokinetic studies: Comparison of tail-vein bleed to jugular vein cannula sampling

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ABSTRACT

*Introduction:* Serial sampling methods have been used for rat pharmacokinetic (PK) studies for over 20 years. Currently, it is still common to take 200–250 μL of blood at each timepoint when performing a PK study in rats and using serial sampling. While several techniques have been employed for collecting blood samples from rats, there is only limited published data to compare these methods. Recently, microsampling (≤50 μL) techniques have been reported as an alternative process for collecting blood samples from rats.

*Methods:* In this report, five compounds were dosed orally into rats. For three proprietary compounds, jugular vein cannula (JVC) sampling was used to collect whole blood and plasma samples and capillary microsampling (CMS) was used to collect blood samples from the tail vein of the same animal. For the two other compounds, marketed drugs fluoxetine and glipizide, JVC sampling was used to collect both whole blood and blood CMS samples while tail-vein sampling from the same rats was also used to collect both whole blood and blood CMS samples.

*Results:* For the three proprietary compounds, the blood AUC as well as the blood concentration-time profile that were obtained from the tail vein were different from those obtained via JVC sampling. For fluoxetine, the blood total exposure (AUC) was not statistically different when comparing tail-vein sampling to JVC sampling, however the blood concentration-time profile that was obtained from the tail vein was different than the one obtained from JVC sampling. For glipizide, the blood AUC and concentration-time profile were not statistically different when comparing the tail-vein sampling to the JVC sampling. For both fluoxetine and glipizide, the blood concentration profiles obtained from CMS were equivalent to the blood concentration profiles obtained from the standard whole blood sampling, collected at the same sampling site.

*Discussion:* The data in this report provide strong evidence that blood CMS is a valuable small volume blood sampling approach for rats and that it provides results for test compound concentrations that are equivalent to those obtained from traditional whole blood sampling. The data also suggest that for some compounds, the concentration-time profile that is obtained for a test compound based on sampling from a rat tail vein may be different from that obtained from rat JVC sampling. In some cases, this shift in the concentration-time profile will result in different PK parameters for the test compound.

Based on these observations, it is recommended that a consistent blood sampling method should be used for serial microsampling in discovery rat PK studies when testing multiple new chemical entities. If the rat tail vein sampling method is selected for PK screening, then conducting a bridging study on the lead compound is recommended to confirm that the rat PK obtained from JVC sampling is comparable to the tail-vein sampling.



## 1. Introduction

While serial sampling in rats has been utilized routinely for the last 20 years, the introduction of microsampling is a more recent option that has become possible as high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) systems have become more sensitive. The improved sensitivity of the HPLC–MS/MS systems means that one can assay 5–10 μL aliquots of rat plasma or blood samples rather than having to use the 25–50 μL aliquots of sample that were needed with the previous HPLC–MS/MS systems. This new capability has led to an interest in microsampling as a way to collect serial samples in rodent pharmacokinetic (PK) studies.

Currently, it is still common to take 200–250 μL of blood at each timepoint using serial sampling when performing a PK study in rats. Blood microsampling can be defined as taking a blood sample that is

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no more than 50 μL total volume (Chapman et al., 2014). Recent examples of microsampling in rodent PK or toxicokinetic (TK) studies have been published by Jonsson et al. (Jonsson et al., 2012a; Jonsson, Villar, Nilsson, Eriksson, & Konigsson, 2012b; Jonsson et al., 2013; Nilsson, Ahnoff, & Jonsson, 2013), Dillen et al. (Dillen et al., 2014) and Korfmacher et al. (Korfmacher et al., 2015). The advantage of microsampling for rat PK studies is that the smaller volume can allow one to take more samples from each rat, so that serial bleeding could still be done even in a multi-day PK study. One could also use microsampling in rats when collecting samples for TK in the main study group in a regulated safety study. As pointed out by Chapman et al. (Chapman et al., 2014), microsampling can provide a reduction of up to 50% in the number of rats needed in a regulated good laboratory practice (GLP) rat safety study. Using fewer rats in a safety study not only saves money, but also supports the goal of the pharmaceutical industry to reduce the number of laboratory animals needed to test new chemical entities.

The initial goal of the studies described in this report was to demonstrate that microsampling of rats could be performed by sampling from the tail vein using a glass capillary microsampling (CMS) tube. For this effort, a single blood CMS sample was obtained from the tail vein and compared to both a whole blood sample obtained from the same rat via jugular vein cannula (JVC) sampling as well as plasma from the JVC sampling. The results from these studies indicated a potential difference in test compound blood concentration depending on the sampling site.

We found only limited literature reports on the impact of sampling location in the rat. In a report by Hui et al. (Hui et al., 2007), only minor differences in PK parameters were observed for several compounds evaluated when sampling in the rat was done by using tail vein or femoral artery cannula or retro-orbital bleeding methods. In this study fluoxetine after oral administration exhibited minor, but statistically significant lower $C_{max}$ and AUC values with tail-vein sampling than with retro-orbital bleeding. The additional observation that the AUC values obtained after intravenous administration of fluoxetine were not statistically different among the three bleeding techniques supported the authors' conclusion that the bleeding technique (sampling site) utilized does not greatly influence the observed PK behavior of fluoxetine. The statistically significant, yet minor, oral exposure differences noted for fluoxetine were hypothesized to be due to its effect on thermoregulatory responses which have been linked to reduced tail blood flow.

In another study, Johannessen et al. (Johannessen, Tyssebotn, & Aarbakke, 1982) evaluated the PK of antipyrine and acetaminophen from data obtained by simultaneous blood sampling from a tail cut and a cannulated femoral artery in the rat. Significant differences in the concentrations and associated kinetics for both drugs were found by comparison of the results obtained from the two sampling sites. The hypothesis the authors proposed and investigated was that the differences were due to a low tail blood flow. Increasing tail blood flow through temperature elevation was demonstrated to increase concentrations of antipyrine in the tail blood.

An advantage of blood CMS sampling is that one can take simultaneous samples from multiple sites from one rat for comparison of the test compound levels. This capability was used in the rat to compare JVC sampling to tail vein sampling for selected compounds. This report is a summary of the findings from these studies.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Compounds A, B, C and RP107 (internal standard used in some of the bioanalytical assays) were obtained from the Sanofi chemical library. Acetonitrile (ACN) (HPLC solvent grade) was obtained from Honeywell Burdick & Jackson. Ammonium acetate (AR grade) was obtained from Sigma-Aldrich. Fluoxetine hydrochloride (AR grade) powder was purchased from Fluka and glipizide powder (AR grade) was purchased from Sigma. Norfluoxetine and internal standards (fluoxetine-D6, norfluoxetine-D6 and glipizide-D11) were purchased from Cerlliant® as 1.00 mg/mL or 0.100 mg/mL solutions. Water (in-house deionized water grade) was prepared using a Millipore Water Purification System.

The glass capillaries used for the CMS blood sampling were coated with EDTA and were designed to hold 8 μL; the capillaries (REF 173313) were purchased from Vitrex Medical A/S (Herlev, Denmark). The capillary tube holders were obtained from Sarstedt (Ref 95.1048). The sample tubes and caps (cat # MP32033 and MP53101) were obtained from Nova Biostorage Plus, LLC.

### 2.2. Animals and compound formulation and dosing

Cannulated male Sprague Dawley rats were obtained from Harlan Laboratories, Inc. (Dublin, VA) and were approximately 10 to 11 weeks old (approximately 240 to 320 g) at the time of dosing. Cannulated male Sprague Dawley rats were received with jugular vein catheter (JVC) implantations to facilitate blood collections. Animals were assigned to study groups (either $n = 3$ or $n = 4$ per group) based on acceptable health and JVC patency. Experimental procedures were approved by the Agilux CRO (Worcester, MA) Institutional Animal Care and Use Committee (IACUC) and were in accordance with the Animal Welfare Act (Animal Welfare Standard, 1990) and NIH guidelines (National Research Council NIH Guide for the Care and Use of Laboratory Animals, 2011).

Compound A was administered orally at 10 mg/kg (parent) and was formulated in 40% w/v sulfobutylether-beta-cyclodextrin (SBE-β-CD) in 30 mM citrate buffer in water, pH 3, at a target concentration of 1 mg/mL. Compound B was administered orally at 10 mg/kg (parent) and was formulated in 20% w/v hydroxypropyl-beta-cyclodextrin (HP-β-CD) in 30 mM citrate buffered normal saline, pH 3, at a target concentration of 1 mg/mL. Compound C was administered orally at 10 mg/kg (parent) and was formulated in 30 mM citrate buffered normal saline, pH 3, at a target concentration of 1 mg/mL. Fluoxetine (HCl) was administered orally at 10 mg/kg (freebase) and was formulated in 10% w/v hydroxypropyl-beta-cyclodextrin (HP-β-CD) in water at a target concentration of 1 mg/mL. Glipizide was administered orally at 1 mg/kg and was formulated in 18% w/v hydroxypropyl-beta-cyclodextrin (HP-β-CD) in 10 mM phosphate buffer in water, pH 6.0, at a target concentration of 0.1 mg/mL.

### 2.3. PK sampling procedures

For compounds A, B, and C, rat blood samples (approximately 50 μL via JVC and 8 μL for CMS) were collected into $K_2$EDTA coated tubes at 0.0833, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose. Blood samples were collected serially from both the JVC and tail vein at each timepoint. Each rat was manually restrained for JVC blood collection and then immediately placed into a whole body restrainer for tail-vein CMS collections. Once restrained, the tail was swabbed with alcohol and the microsample was collected from a tail vein either by a tail nick (small slice with a surgical blade made over the tail vein) or by a butterfly catheter placed directly into a tail vein. For both methods, the tail was "milked" by gently applying even pressure from the base to the end so that 8 μL of blood was collected into a $K_2$EDTA coated CMS tube. The 8-μL CMS sample tube filled with blood was then placed into a screw cap sample tube containing 72 μL of dilution solution (25% acetonitrile in water, accurately measured). The sample tube was recapped and vortexed until the blood in the capillary tube was mixed in the dilution solution. Following collection of the final serial samples at 24 h post-dose, a terminal blood sample was collected from animals under deep anesthesia via cardiac puncture. The JVC samples were processed to provide both a 10-μL (measured) whole blood sample and a 10-μL (measured) plasma sample, while the tail-vein samples were collected using 8-μL glass CMS tubes to provide the blood CMS sample. In this