Altasciences CLINICAL RESEARCH
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Large Molecule Application of Volumetric Absorptive Microsampling for the Determination of a Single-Rodent PK Profile for Exenatide by LC-MS/MS

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OVERVIEW

PURPOSE

To demonstrate the applicability of Volumetric Absorptive Microsampling (VAMs) technology for the generation of a PK-profile derived from a single rodent for exenatide, a 4.2 kDa GLP-1 agonist biotherapeutic used in the treatment of type 2 diabetes.

METHOD

Rat blood at each time-point was sampled onto a Mitra™ microsampling device (10 µL), dried for 24 hours at RT in the presence of desiccant and extracted using 70% acetonitrile. Data were acquired using a SCIEX Triple Quad 6500+ operated in positive ESI-MRM mode.

RESULTS

The VAMs method for exenatide demonstrated a linear range between 2.0 -1000 ng/mL with intra-assay precision and accuracy ≤ 8.8% and ≤ 2.6%, respectively. Average recovery was 75%, obtained without hematocrit effect. Individual PK-profiles successfully derived from three subjects were in excellent agreement.

INTRODUCTION

Microsampling strategies in pre-clinical research allows consolidation of satellite TK and main study groups in an effort to replace, refine and reduce animal numbers. Additionally, toxicological effects can be correlated with exposure in the same individual. Capillary microsampling techniques circumvent the hematocrit (HCT) effect often reported for DBS analysis, however processing is tedious and drugs exhibiting non-specific binding or requiring matrix stabilization are problematic. A recent alternative is Volumetric Absorptive Microsampling (VAMs), wherein an accurate volume of blood is absorbed onto a hydrophilic polymeric tip, thereby simplifying sample collection. The applicability of VAMs for the generation of a PK profile derived from a single rodent sampling only ten microlitres of blood per time-point is examined for exenatide.

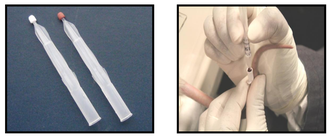

Figure 1. Neoteryx Mitra™ 10 µL microsampling tips used to collect blood from rat tail vein using an abbocath.

METHODS

PK STUDY IN SPRAGUE DAWLEY® RATS

- Exenatide dose: 210 µg subcutaneous
- 10 µl Mitra™ blood samples collected from tail vein
- Subjects: 3
- Total blood volume = 300 µL/rat (10 time-points collected in triplicate)

VAMS EXTRACTION PROCEDURE

Mitra™ Tip (10 µL), $^{13}C_9{}^{15}N$-exenatide (10 µL), 150 µL ACN:H_2O (70:30)

↓

Aggressive agitation/ultrasonication followed by centrifugation

↓

Aliquot 80 µL of supernatant, dilute with H_2O (100 µL)

LC-MS/MS DETECTION

- Shimadzu Nexera X2 UHPLC system
- Waters XBridge C_{18} BEH Peptide column
- Gradient elution with 0.2% CH_3OOH in H_2O and ACN:MeOH (8:2)
- SCIEX Triple Quad API 6500+ operated in (+) ESI-MRM

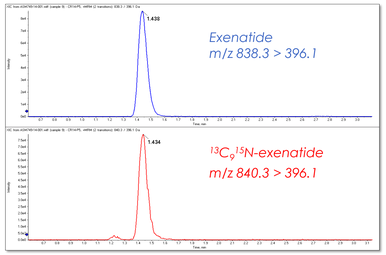

Figure 2. Extracted ion chromatograms of exenatide and $^{13}C_9{}^{15}N$-exenatide (IS) with corresponding MRM transitions.

RESULTS

ADVANTAGES OF VAMS MICROSAMPLING

Sampling Volume vs. Blood Hematocrit

Precision and accuracy data for Mitra™ sampling volume, determined gravimetrically as a function of blood hematocrit level, revealed an absence of hematocrit effect as noted in Table 1 below.

Table 1. Hematocrit effect on blood sampling volume using VAMS

Stats	HCT = 0	HCT = 30	HCT = 44	HCT = 55	HCT = 66
Average Volume (µL)	10.56	10.40	10.58	10.69	10.74
N	12	12	12	12	12
% CV	2.7	3.0	3.1	3.3	3.5
Difference from HCT 44	-0.1%	-1.7%	0.0%	1.1%	1.6%

Effect of Hematocrit Level on Recovery

Exenatide at high QC concentration extracted from blood with hematocrit levels between 20% and 66% referenced against plasma (0% HCT) indicated minimal recovery bias between average blood yields and plasma. Further, recovery differences within the normal HCT range of 34% - 48% were largely insignificant (Figure 3).

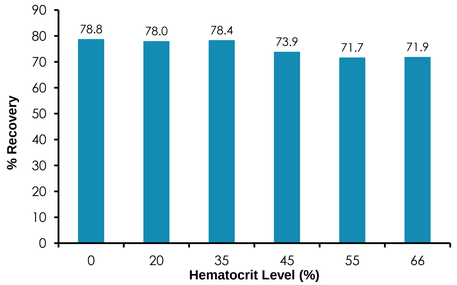

Figure 3. Exenatide recovery at different hematocrit levels.

SENSITIVITY, LINEARITY, ACCURACY AND PRECISION

A major challenge in the analysis of biotherapeutics is low sensitivity due to multiple charging and variable fragmentation. For exenatide MS analysis (+5 charge-state monitored), sensitivity was notably improved using the SCIEX 6500+ when compared to the API 5500 (Figure 4).

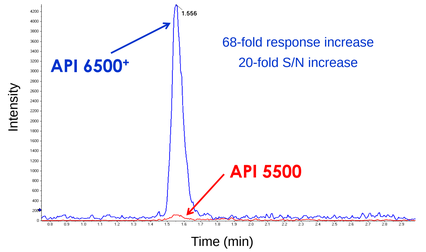

Figure 4. Sensitivity comparison of SCIEX API 5500 vs. 6500+ for an extracted LOQ sample of exenatide (2.0 ng/mL, 540 fg on-column).

As illustrated in Figure 5, calibrants extracted from Mitra™ demonstrated excellent linearity and precision (r^2 = 0.9966) within a 500-fold analytical range. Selectivity, matrix effect and matrix factor all met acceptance criteria. Further, low and high QCs prepared in blood at HCT levels between 20% and 66% demonstrated back-calculated concentrations within ±15% when determined against calibrants prepared in blood at a HCT level of 44%.

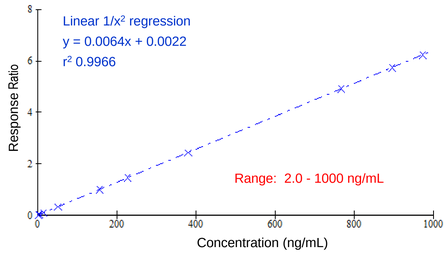

Figure 5. Exenatide calibration curve extracted from Mitra™.

Extraction of QC samples in three different batches demonstrated excellent between-run precision and accuracy as highlighted in Table 2.

Table 2. Exenatide between-run precision and accuracy.

Stats	Concentration (ng/mL)			
	QC LOQ 2.00 ng/mL	QC1 6.00 ng/mL	QC2 200.00 ng/mL	QC3 750.00 ng/mL
Mean	2.05	6.06	204.89	769.32
S.D.	0.12	0.40	12.99	67.60
N	18	18	18	18
% C.V.	5.7	6.6	6.3	8.8
% Nominal	**102.5**	**100.9**	**102.4**	**102.6**

PK APPLICATION

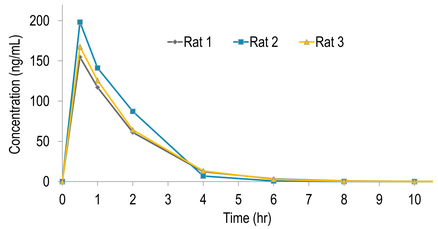

Figure 6. PK profiles of exenatide in Sprague Dawley® rats (*n*=3) following a single subcutaneous dose of 210 µg.

CONCLUSION

In this first reported application of VAMs technology for the quantitation of a biotherapeutic, the technique demonstrated excellent linearity, precision and accuracy for exenatide extracted from rat blood whilst negating hematocrit effect. Further, sample collection using Mitra™ was significantly simplified when compared to alternative techniques such as capillary microsampling.