Validation of an MFLC-MS/MS Assay for the Quantitative Analysis of a Novel Peptide, AT-01, Extracted from K_2EDTA Plasma

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INTRODUCTION

Since the synthesis of the first therapeutic peptide, insulin, in 1921, our understanding of proteins and peptides and their therapeutic potential has increased significantly. As our understanding of this potential expands, targeted biopharmaceutical candidates are being identified and explored at an increasing rate. As these treatments become more mainstream and effective, the need for robust, GLP-compliant quantitative assays is growing. Previously, the majority of analysis has been done utilizing ELISA assays or similarly intensive methods. While robust, these methods do not offer the ease and high throughput potential that can be achieved with a solid MFLC-MS/MS method. In support of two GLP studies featuring a proprietary novel peptide candidate, GLP-compliant methods were developed in both rat and dog K_2EDTA plasma utilizing a straightforward MFLC-MS/MS method.

OVERVIEW

Purpose:
- Validate an MFLC-MS/MS method for the quantitative bioanalysis of a novel peptide, AT-01, from rat and dog plasma.

Methods:
- SPE micro-elution and extraction using a Phenomenex Strata XPro plate and MFLC-MS/MS analysis.

Results:
- Validated assays suitable for regulated analysis
- Accuracy and precision better than ±15% (±20% at LLOQ)

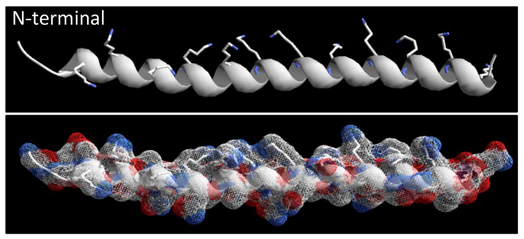

Figure 1: AT-01, $C_{202}H_{351}N_{71}O_{62}$, ~5kD

METHODS

Extraction
- Samples loaded onto Strata XPro micro-elution plate
- Peptide extracted using 1:74:24 TFA:ACN:H_2O and diluted with H2O + 0.1% FA
- Dynamic range: 30-5000 ng/mL

HPLC Parameters
- MPA: H_2O + 0.1% FA; MPB: ACN + 0.1% FA
- Flow rate: 40 µL/min
- Kinetics 1.7 µm Biphenyl 50 x 1.0 mm

Mass Spectrometry
- Sciex API6500
- Positive ion mode
- MRM Transitions:
  AT-01: 530.6 → 582.5
  AT-01 Stable Label: 533.2 → 585.3

Methodology	MFLC-MS/MS	
Analyte	AT-01	
Matrix	Rat K_2EDTA Plasma	Dog K_2EDTA Plasma
Internal Standard (IS)	AT-01 Stable Label	
Extraction Method	Solid Phase Extration (SPE) using Strata XPro micro-elution plates	
Validated Range	30.0 - 5000 ng/mL	
Calibration Model	Linear ($1/x^2$)	
Precision (% CV) **Intrabatch:** **Interbatch:**	 3.2% – 9.7% 3.2% – 10.7 %	 3.5% - 9.0% 3.2% - 6.0%
Accuracy (% Bias) **Intrabatch:** **Interbatch:**	 -5.4% - 15.7% (LLOQ) -5.4% - 9.2%	 -1.6% - 9.0% 1.5% - 10.5%
Bench Top Stability	Up to 6.2 hours at ambient	Up to 3 hours on wet ice
Freeze-Thaw Cycles	4 cycles	3 cycles
Processed Sample Stability	96.5 hrs post preparative stability	170.7 hrs post preparative stability
Long Term Stability	139 days -70˚C	65 days -70˚C

Table 1: Validation Summary

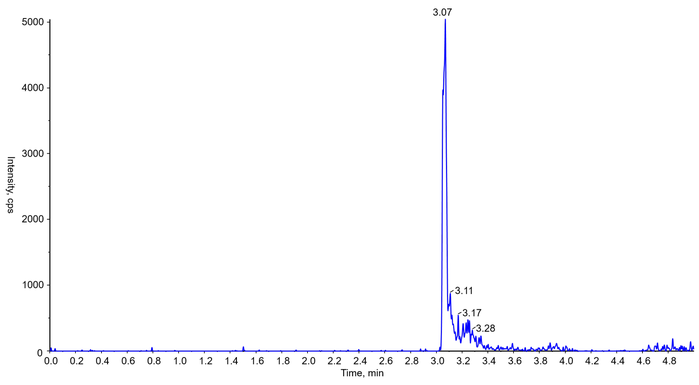

Figure 2: MFLC-MS/MS Chromatogram of Extracted AT-01 From Rat Plasma (30.0 ng/mL)

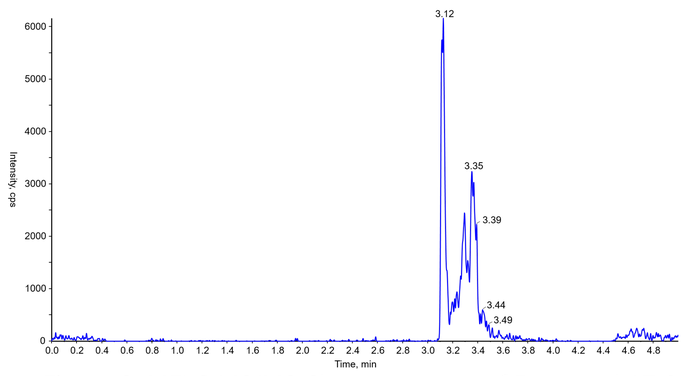

Figure 3: MFLC-MS/MS Chromatogram of Extracted AT-01 From Dog Plasma (30.0 ng/mL)

CONCLUSION

- Methods were developed and validated for analysis of novel peptide, AT-01, in both rat and dog plasma.
- Methods are simple, selective, and robust.
- Methods were successfully applied to analyze 526 pre-clinical pharmacokinetic samples.
- Demonstrated reproducibility upon incurred sample repeat (ISR) analysis.