Stabilization of a Peptide for Validation of HPLC/MS/MS Methods for the Quantitative Analysis of Peptides from Blood and Plasma

Chad Christianson and Shane Needham, Alturas Analytics, Inc. 1324 Alturas Drive, Moscow, Idaho 83843
Sarah Walter and Felix Karim, KAI Pharmaceuticals, Inc. 270 Littlefield Ave., South San Francisco, California 94080

Overview

- **Purpose** - Develop and validate a method to stabilize a prototypical peptide in plasma and blood for quantitative bioanalysis by HPLC/MS/MS
- **Methods** – Solid-phase extraction and HPLC/MS/MS
- **Results** – Using chemical and enzymatic inhibitors to stabilize and recover the peptide, a method was developed and validated for the HPLC/MS/MS quantitative determination of peptide concentrations from plasma and blood

Introduction

Increasingly, peptides are being used as therapeutic agents. Stabilization and recovery of a peptide drug from blood or plasma is critical to establishing a validated bioanalytical assay. As an example, we outline several processes that were evaluated to stabilize and recover a prototype peptide from plasma or blood. Stabilization entailed the rapid inhibition of proteolytic degradation of the peptide to allow for adequate sample collection and processing times from the biological fluid of interest. Once stabilized in these matrices, we were able to validate an HPLC/MS/MS method for the quantitative analysis of this peptide. The optimized stabilization and recovery method provided for a lower limit of quantitation that was <10 ng/mL. This method was shown to be accurate (107%) and precise (7%). To date, this HPLC/MS/MS assay has been used to analyze >1000 samples for the determination of the peptide levels in rat, dog, rabbit and human studies.

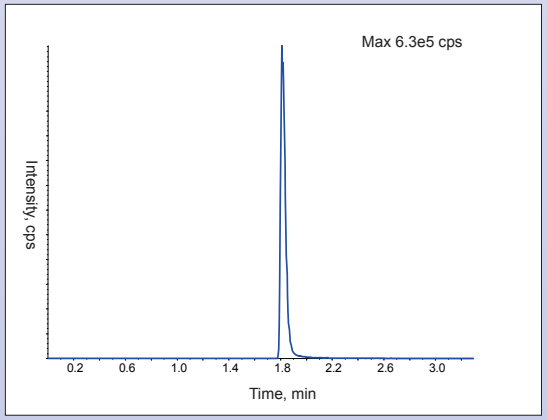
Method

- 96 well plate solid phase extraction was mixed mode reversed-phase and weak cation exchange
- Gradient reversed phase HPLC was used with acetonitrile and water (1% formic acid)
- HPLC/MS/MS was used in the positive ion mode with the TurboIonspray™ source (API4000)

Stabilization Methods Attempted in Plasma and Blood

- Type A (NaF) and Type B (Dichlorvos, Phenylmethanesulfonyl Fluoride) esterase inhibitors
- Addition of acid to inhibit chemical degradation
- Addition of protease inhibitors
- Performing sample preparation on ice

HPLC/MS/MS Chromatogram from the Analysis of Peptide A from Human Plasma



Summary of Validation Parameters Assessed for the HPLC/MS/MS Bioanalysis ofPeptide A Extracted from Human Plasma

QC Level (ng/mL) (%)	Intra-assay Accuracy (%)	Inter-assay Accuracy (%)	Freeze Thaw Stability (% Difference from Nominal)	Benchtop Stability (Hours)	Extract Stability (Hours)	Long Term Stability @ -70 C (Days)
4.00 (LLOQ)	81.9	81.9	—	—	—	—
12.0	104	107	0.8	1.5	>62	>544
500	105	104	-1.1	1.5	>62	>544
2400	101	100	-4.4	1.5	>62	>544
3000 (ULOQ)	96.9	—	—	—	—	—

Peptide A Degradation In Human Blood and Plasma

Matrix	Treatment	Temperature	Time (hours)	% Degradation
Plasma	NaF	RT	3	79
Plasma	Protease Inhibitor Cocktail	RT	3	-0.5
Whole Blood	No additive	RT	2	78
Whole Blood	Protease Inhibitor Cocktail	RT	2	49
Whole Blood	Protease Inhibitor Cocktail	Ice	1.5	9.5

Conclusion

- Several methods of stabilization and recovery of a prototypical peptide in blood and plasma were evaluated, including chemical and enzymatic methods.
- Adequate stabilization allowed for the development of an HPLC/MS/MS method that was then validated for the quantitative measurement of the peptide in blood and plasma from multiple species.
- Various methods were successfully employed to inhibit degradation of the peptide in blood or plasma with differing degrees of success and quantitative robustness. Based on the presented data, systematic experiments can be used to evaluate and optimize methods of stabilization and recovery for peptide quantitation in various biologic samples.