Poster Number 14R0930

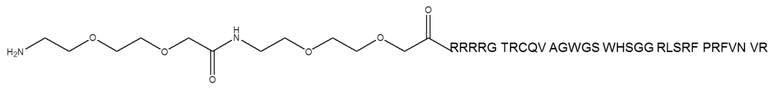
Stabilization of a Therapeutic Peptide, MPCAP 120-146WH5RMP, during and after Blood Collection for a Quantitative LC-MS/MS Assay

Qiang Liu[1], Douglas Bailey[1], Nicole Vu[1], Thomas Kupiec[1] and H. Anne Pereira[2]
[1]ARL Bio Pharma, Inc., Oklahoma City, OK 73104, USA.
[2]Department of Pharmaceutical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73117, USA.

2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER

Purpose

- Therapeutic peptides are often subject to protease-mediated degradation and chemical modification. Therefore, stabilization of peptides in biometrics is essential for the accurate quantification of peptides during toxicokinetic and pharmacokinetic studies.
- MPCAP 120-146WH5RMP, a multifunctional peptide based on the cationic antimicrobial protein CAP37, demonstrates strong bactericidal activity, chemotactic activity, and significant corneal wound healing properties.
- The purpose of current study is to develop and validate a method stabilizing MPCAP 120-146WH5RMP against protease-mediated degradation and chemical modification during and after blood sample collection for a quantitative LC-MS/MS assay.



Method

- Pooled and mixed gender rat blood was utilized during the evaluation of reducing reagent and different inhibitors. The protease inhibitors were evaluated including Halt Protease & Phosphatase Inhibitor Cocktail with and without EDTA at varied concentrations, Pepstatin A, and alpha-2-Macroglobulin. Briefly, 120 µl of selected inhibitors were added into 860 µl of rat blood. The sample was mixed and set 10 minute prior adding peptide standard stock solution. After mixing, the spiked rat blood was allowed to rest for 30 minutes before plasma preparation.
- The chemical modification through disulfide bond formation of cysteine residual was also evaluated. The peptide recovery was compared between rat plasma with and without the reducing reagent Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP).
- The stable isotope labeled MPCAP 120-146WH5RMP - 13C2415N16 were used as internal standard and was added into plasma samples prior the methanol extraction and protein precipitation. 2% of formic acid was also added to plasma for overcoming the non-specific binding and facilitate the extraction.
- The sample was separated from the matrix using a Restek Raptor ARC-C18, 2.7 um, 2.1 x 30 mm column. The HPLC mobile phases consists of 0.5% Formic Acid in 1% ACN/Water (A) and 0.5% Formic Acid in ACN (B). Extracted peptide was then analyzed by ABSciex API-2000 triple quadruple mass spectrometer in positive ion mode, equipped with Agilent 1100 HPLC system.

Method Time	% Mobile Phase - B
0 – 0.5 min	2% isocratic
0.5 –2.0 min	2% – 90%
2.0 – 3.5 min	90% isocratic
3.5 – 4.0 min	90% – 2%

Result

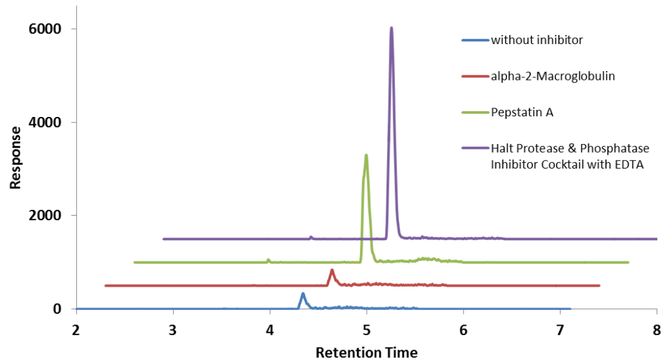


Fig. 1 The recovery of MPCAP 120-146WH5RMP after 30-minute incubation in rat blood using Halt Protease & Phosphatase Inhibitor Cocktail with EDTA as protease inhibitor was 1.4, 8.4 and 10.6 folds higher in comparison to Pepstatin A, alpha-2-Macroglobulin and without inhibitor, respectively.

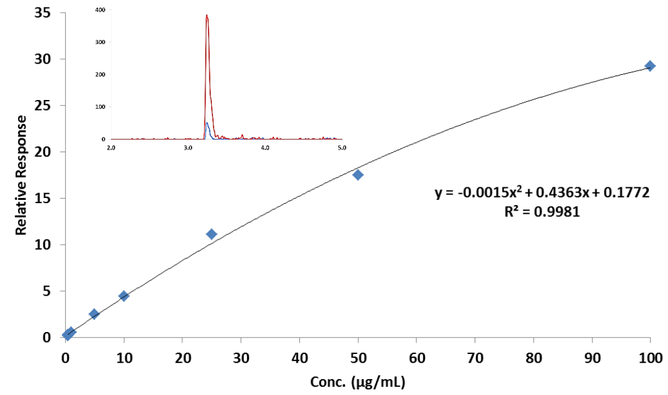


Fig. 2 The optimized LC-MS/MS assay provided LOD as 0.1 µg/ml and quantification range from 0.5 to 100 µg/mL for MPCAP 120-146WH5RMP in rat plasma.

Sample Name	Concentration (µg/mL)	Average of Three Preparations (µg/mL)	%RSD of Three Preparations	% Change from Immediate Extraction
Immediate Extraction	30.1	27.00 (± 2.70)	9.99%	NA
	25.2			
	25.7			
No Inhibitors + 4 Hours	2.28	NA	NA	**8.44%**
Inhibitors + 4 hours	26.8	28.27 (± 0.71)	2.50%	**104.69%**
	27.8			
	30.2			
Inhibitors + 1x Freeze/Thaw	26.5	26.97 (± 1.88)	6.96%	**99.88%**
	27.8			
	26.6			
Inhibitors + 3x Freeze/Thaw	24.6	26.30 (± 1.41)	5.38%	**97.41%**
	25.7			
	28.6			

Table. 1 By combining Halt Protease & Phosphatase Inhibitor Cocktail, EDTA and TCEP, the recovery of peptide was 104.69%, 99.88% and 97.41% for spiked blood sample at refrigerator for 4 hours, one frozen thaw, and three frozen thaws in comparison to the immediately extracted blood samples.

Accuracy Sample	Calculated Concentration (µg/mL)	Percent Relative Error	Average Concentration (µg/mL)	Percent Relative Error
1.429 µg/mL Prep. A	1.51	**5.7%**	**1.57**	**9.63%**
1.429 µg/mL Prep. B	1.67	**16.9%**		
1.429 µg/mL Prep. C	1.52	**6.4%**		
38.10 µg/mL Prep. A	36.6	**-3.9%**	**38.8**	**1.92%**
38.10 µg/mL Prep. B	38.7	**1.6%**		
38.10 µg/mL Prep. C	41.2	**8.1%**		
71.43 µg/mL Prep. A	70	**-2.0%**	**69.3**	**-3.02%**
71.43 µg/mL Prep. B	67.3	**-5.8%**		
71.43 µg/mL Prep. C	70.7	**-1.0%**		

Table. 2 The accuracy of QC samples at 1.4, 38.1, and 71.4µg/mL were 109.63%, 101.92% and 96.98%. The average precision (%RSD) of QC samples was 6.92%. .

Assay Concentration	Robustness Condition	Average Result (µg/mL)	Average Initial Conditions (µg/mL)	% of Normal Conditions	%RSD
1.5 µg/mL	+ Source Voltage 5V	1.74	1.71	2.15%	5.77%
	- Source Voltage 5V - Source Gas 5 psi	1.89		10.74%	14.62%
	Mobile Phase Organic + 10%	1.75		2.73%	5.63%
	Mobile Phase Organic - 10%	1.87		9.37%	8.68%
40 µg/mL	+ Source Voltage 5V	40.63	43.77	-7.16%	1.64%
	- Source Voltage 5V - Source Gas 5 psi	42.00		-4.04%	7.17%
	Mobile Phase Organic + 10%	48.70		11.26%	2.90%
	Mobile Phase Organic - 10%	47.13		7.69%	6.86%
75 µg/mL	+ Source Voltage 5V	89.03	83.25	6.95%	3.48%
	- Source Voltage 5V - Source Gas 5 psi	72.63		-12.75%	0.21%
	Mobile Phase Organic + 10%	81.77		-1.78%	3.79%
	Mobile Phase Organic - 10%	80.50		-3.84%	NA*

Table. 3 The same three samples prepared for Ruggedness above were analyzed after minor changes were made to the method. The average sample result of triplicate injection is NMT ± 15% of the average results obtained under normal conditions of the method at the same concentration.

Conclusion

Addition of protease inhibitors and reducing reagent during and after blood collection significantly improved the stability and recovery of MPCAP 120-146WH5RMP. The optimized LC-MS/MS method is robust, rugged, sensitive and suitable for monitoring the therapeutic peptide with the desired precision and accuracy.

Acknowledgements

This research was supported by NIH 5U01AI075391.