DEVELOPMENT OF A SPE LC-MS/MS METHOD FOR THE BIOANALYTICAL QUANTIFICATION OF PRAMLINTIDE FROM SERUM

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INTRODUCTION

- Pramlintide acetate (SYMLIN™) is a synthetic analogue of the human hormone amylin developed as an adjunctive therapy for patients with type 1 and 2 diabetes.
- With recent research indicating a role for amylin in Alzheimer's Disease models, interest in amylin and amylin agonists is rising.
- Hydrophobic peptides such as pramlintide often suffer from non-specific binding (adsorption) to any labware they come into contact with (plates, pipette tips, etc…) making method development difficult as it can lead to poor recovery, loss of analyte, and poor limits of detection.
- This work describes optimization and development of a selective sample preparation strategy and LC-MS/MS analysis to achieve LLOQs of 25 pg/mL from 100 µL of serum.

METHODS

Sample Preparation

Pramlintide was spiked into rat or human serum (100 µL), diluted with water, and extracted using an OASIS WCX (weak cation exchange), 96-well, µElution SPE device according to the protocol detailed in Figure 1. Samples were eluted in 25 µL of elution solvent, collected in a QuanRecovery 96-well plate with MaxPeak High Performance Surfaces (HPS), and then diluted with 25 µL of water for a final sample volume of 50 µL (Figure 2).

Pramlintide WCX SPE

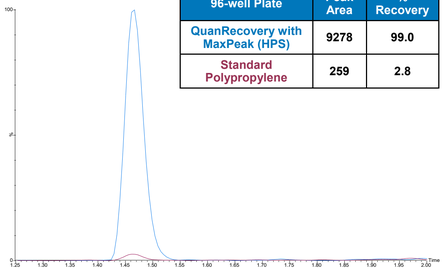
Load: Dilute serum with water

Wash 1: Water

Wash 2: 20% ACN

Elute: 1% TFA in 75:25 ACN:H_2O

Figure 1. Optimized WCX SPE protocol for the extraction of pramlintide from serum



96-well Plate	Peak Area	% Recovery
QuanRecovery with MaxPeak (HPS)	9278	99.0
Standard Polypropylene	259	2.8

Figure 2. Peak area and recovery of 10 ng/mL pramlintide stored in standard polypropylene and QuanRecovery with MaxPeak (HPS) 96-well plates

***LC System:* ACQUITY UPLC I-Class PLUS (Fixed Loop)**

- Column: ACQUITY UPLC Peptide CSH C_{18}, 130Å, 1.7 µm, 2.1 x 50 mm
- Column Temperature: 60°C
- Mobile Phases: A: 0.1% formic acid in water
  B: 0.1% formic acid in acetonitrile
- Cycle Time: 7.0 minutes
- Injection Volume: 10 µL

***MS System:* Xevo TQ-XS Mass Spectrometer**

- Capillary Voltage: 1.0 kV
- Cone Voltage: 15 V
- Source Temperature: 150°C
- Desolvation Temperature: 600°C
- System Calibration: Low Resolution (1.0 Da FWHM)

Precursor (*m/z*)	Product (*m/z*)	Collision Energy (eV)	Product Ion Identification
988.36	968.11	20	[3H+]3 / b27
988.36	930.78	26	[4H+]4 / y35

Table 1. Mass spectrometry conditions for pramlintide, including precursor and fragment ions

RESULTS

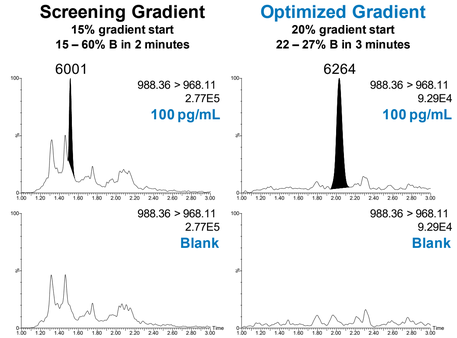


Figure 3. Matrix suppression and chromatographic interferences were significantly decreased by adjusting the chromatographic gradient

- *Gradient start was increased from 15 to 20% acetonitrile (mobile phase B) which decreased matrix interferences*
- *Gradient was shallowed from 15—60% B over 2 minutes, to 22—27% B in 3 minutes to separate pramlintide from remaining matrix interferences*

A Human Serum QC Statistics				
QC Level	QC Concentration (pg/mL)	Mean (N=3) calculated QC concentration (pg/mL)	Mean (N=3) % accuracy	Mean (N=3) % RSD
LLOQ	25	24.0	96.1	3.5
LQC	75	77.4	103.3	5.0
MQC	2500	2619.1	104.8	1.1
HQC	40000	39309.7	98.3	2.8

B Rat Serum QC Statistics				
QC Level	QC Concentration (pg/mL)	Mean (N=3) calculated QC concentration (pg/mL)	Mean (N=3) % accuracy	Mean (N=3) % RSD
LLOQ	25	23.5	93.9	3.7
LQC	75	72.2	96.2	3.1
MQC	2500	2512.6	100.5	5.2
HQC	40000	36628.5	91.6	1.7

Table 2. QC sample statistics for pramlintide extracted from 100 µL human (A) and rat (B) serum. Accuracies between 92—105 % were achieved, with single digit RSDs (< 5%)

Calibration Curve Statistics				
Species	Curve (pg/mL)	Weighting	Linear fit (r^2)	% Accuracy
Human	25 – 50,000	$1/X^2$	0.995	91.3 – 111.0
Rat	25 – 50,000		0.996	92.3 – 105.9

Table 3. Calibration performance of pramlintide extracted from human and rat serum. Curves were linear (r^2 > 0.99) with accuracies ranging 91—111 %

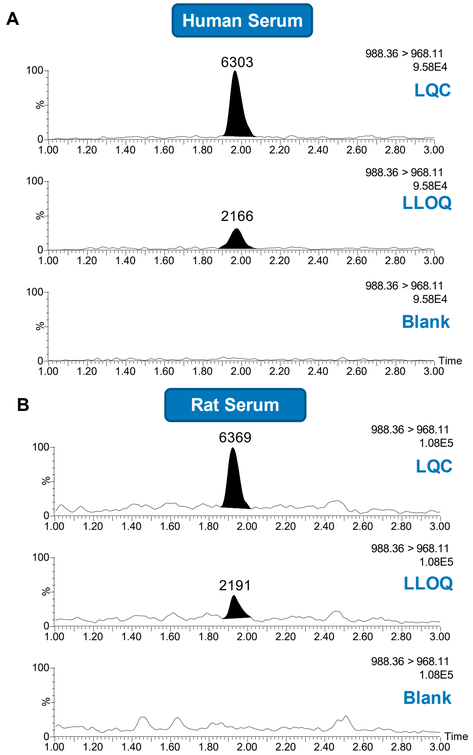


Figure 4. Representative blank, LLOQ, and LQC chromatograms for pramlintide extracted from 100 µL of human and rat serum

DISCUSSION

- An optimized weak cation exchange (WCX) SPE protocol **improved recovery** of the highly hydrophobic peptide, pramlintide to ~ 75% *(Figure 1)*
- **QuanRecovery** 96-well plates with MaxPeak (HPS) **mitigated non-specific binding** and provided a 36-fold increase in analyte peak area in near solution *(Figure 2)*
- Matrix suppression of the assay was significantly decreased due to the use of **selective column chemistry** and **optimized chromatography gradients** *(Figure 3)*
- Quantitative performance was excellent, with a dynamic range of 25—50,000 pg/mL *(Table 3)*, and QC accuracies from 92—105% with RSDs < 5% *(Table 2)*
- Chromatographic performance highlighting the **sensitivity and selectivity** of pramlintide extracted from human and rat serum is illustrated in *Figure 4*

CONCLUSIONS

A SPE-LC-MS/MS method was successfully developed for the pg/mL quantification of pramlintide from rat and human serum:

- To date, first published method for the quantification of pramlintide acetate from serum
- This work employs a simple sample preparation strategy using WCX SPE and QuanRecovery sample plates with MaxPeak (HPS) to deal with hydrophobic and challenging peptides
- Combined with UPLC separation and a tandem quadrupole MS, high sensitivity quantification of pramlintide, with LLOQs of 25 pg/mL, was achieved from human and rat serum

REFERENCES

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