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

Caitlin M. Dunning, Mary E. Lame, Mark D. Wrona, Kim Haynes, and Ian Edwards
Waters Corporation, Milford, MA, USA

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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 nearing patent expiry dates, and 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…). This can make 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.

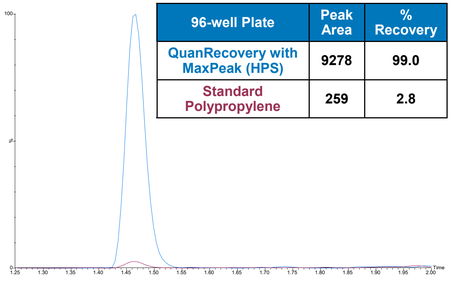
METHODS

Sample Preparation

Pramlintide was spiked into rat or human serum (100 µL) and diluted with water (100 µL). Wells of a weak cation exchange, 96-well, µElution SPE device were conditioned with methanol (200 µL) and then equilibrated with water (200 µL). The diluted serum samples (200 µL) were loaded onto the SPE device, then washed with water (200 µL), followed by 20% acetonitrile in water (200 µL). Pramlintide was eluted from the sorbent using a 1 x 25 µL aliquot of the elution solvent containing 1% trifluoroacetic acid in 75:25 (v/v) acetonitrile: water. Eluates were 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 (Figures 1 and 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-MS Conditions

LC-MS/MS quantification of the intact peptide was performed using a Waters Xevo TQ-XS tandem quadrupole MS (ESI+). Chromatographic separation was achieved using an ACQUITY I-Class UPLC PLUS system with an ACQUITY UPLC Peptide CSH C_{18}, 130Å , 1.7 µm, 2.1 mm x 50 mm column, at a flow rate of 0.4 mL/min using a linear gradient with 0.1% formic acid in water and acetonitrile. Final injection volume was 10µL. MRM transitions used for quantification and MS conditions are summarized in Table 1.

Precursor (*m/z*)	Product (*m/z*)	Cone Voltage (V)	Collision Energy (eV)	Product Ion Identification
988.36	968.11	15	20	[M+3H]3+ / b27
988.36	930.78	15	26	[M+ 4H]4+ / y35

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

RESULTS

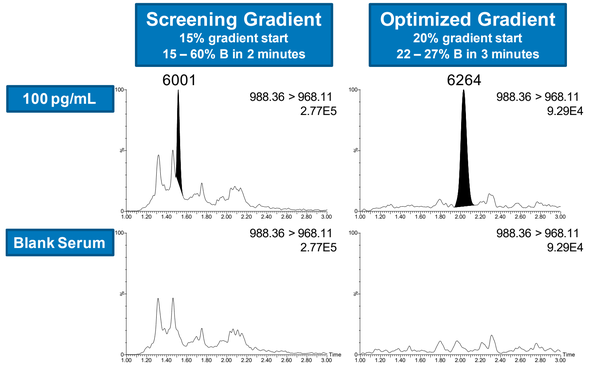


Figure 3. Matrix suppression and chromatographic interferences were significantly decreased by adjusting the chromatographic gradient.

- *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 %.

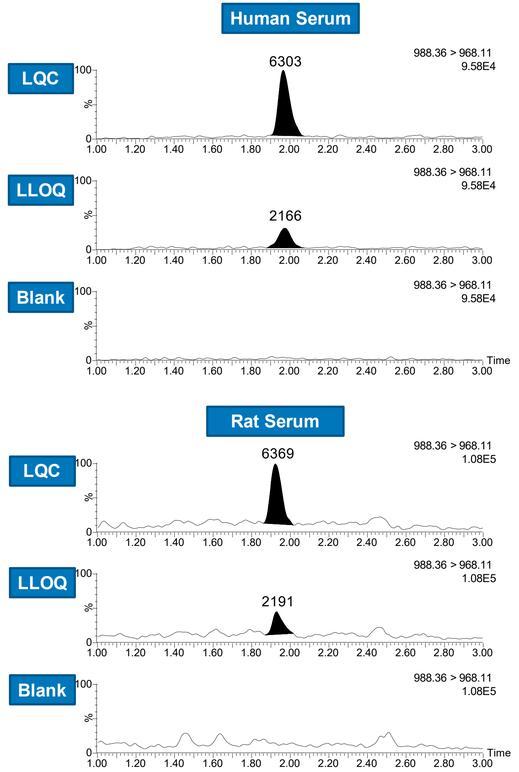


Figure 4. Representative blank, LLOQ, and LQC chromatograms for pramlintide extracted from 100 µL of human and rat serum.

DISCUSSION

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

- An optimized weak cation exchange (WCX) SPE protocol improved the 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 pramlintide peak area in neat solution (Figure 2)
- Use of a sub-2-µm column and optimized chromatography gradients provided improved analyte selectivity and a significant decrease in matrix suppression of the assay (Figure 3)
- Quantitative performance was excellent, with a dynamic range 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

CONCLUSION

To date, this is the first published sample preparation and LC-MS/MS method for the quantification of pramlintide acetate from serum. The work described here employs a simple sample preparation strategy using weak cation exchange SPE and QuanRecovery sample plates with MaxPeak High Performance Surfaces to deal with hydrophobic and challenging peptides. Combining this approach with UPLC separation and a tandem quadrupole MS resulted in high sensitivity quantification of pramlintide from 100 µL of human and rat serum, achieving LLOQs of 25 pg/mL.

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