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

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. Hydrophilic 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 select sample preparation strategy and LC-MS/MS analysis to achieve LLOQs of 25 pg/mL from 100 µL of serum.

MATERIALS AND 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 LC-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 1 x 25 µL acid in 75:25 (v/v) aqueous/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).

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 slowed from 16–90% B over 2 minutes, to 23–27% B in 3 minutes to separate pramlintide from remaining matrix interferences

Figure 4. Representative blank, LLOQ, and LOC 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 (WCE) SPE protocol improved the recovery of the highly hydrophobic peptide, pramlintide, to ~ 75% (Figure 1).

Quantitative recovery of 96-well plates with MaxPeak (HPS) mitigated non-specific binding and provided a 36-fold increase in pramlintide peak area in method solution (Figure 3).

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.

Table 3. Calibration performance of pramlintide extracted from human and rat serum. Curves were linear (r² > 0.99) with accuracies ranging 91–111%.

Table 2. QC sample statistics for pramlintide extracted from 100 µL human (A) and rat (B) serum. Mean interferences between 95–105% were achieved, with single digit RSDs (< 5%).

Table 1. Sample preparation and LC-MS/MS conditions for pramlintide, including precursor and fragment ions.

REFERENCE

