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Development of Improved Chromatographic Methods for the Separation of Synthetic Peptides Medications and Associated Impurities

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PURPOSE

Glucagon-Like Peptide-1 agonists (GLP-1s) are a class of synthetic peptide medications that are prescribed for the treatment and management of obesity and type-II diabetes[1]. Recently, drugs such as semaglutide, have boomed in popularity as a weight management treatment after success in clinical trials[2]. Given the prevalence of GLP-1s, it is important that the quality control for this class of pharmaceuticals is supported by versatile, sensitive, and reproducible chromatography methods. While there are chromatographic methods for some of the common GLP-1s, to our knowledge, there is not a single method to separate an updated panel of this class of synthetic peptides[3-6]. Further, the U.S. Food and Drug Administration (FDA) recently released Product Specific Guidelines (PSG) for some of the synthetic peptides on the market[7]. In this presentation, the FDA states the importance for impurity analysis of synthetic peptides.

METHOD(S)

In the work shown here a single HLPC-UV/MS method was developed for the analysis of a variety of GLP-1s, glucagon and associated impurities utilizing design of experiment fundamentals and a systematic protocol in combination with a novel surface modification technology based on hybrid organic/inorganic surface based on an ethylene-bridged siloxane chemistry. First, screening methods were employed using two unique column stationary phases (4.6 x 150 mm 2.5 µm particle size) and mobile phase additives (formic acid and trifluoracetic acid) in a step-by-step manner. The mobile phases consisted of A (water) and B (acetonitrile) throughout the method development process. Once the best screening method had been identified. The separation method was further optimized by modification of gradient duration, column temperature, injection volume and flow rate. Sample stocks solutions were optimized specific for each of the GLP-1s, and further additives and diluents where also optimized during the creation of the panel samples.

RESULT(S)

The systematic protocol approach for method development suggests screening a variety of columns and reversed phase mobile phase combinations to determine the most appropriate conditions to optimize chromatographic separations. Four different columns and mobile phase eluent combinations were investigated in this study. Based on our screening experiments we selected the XSelect Premier Peptide CSH C_{18} column with the formic acid mobile phases for our method due to the overall speed and resolution this combination of method conditions produced (Figure 1). We further chose these conditions based on the maximized chromatographic peak height performance of glucagon and the separation of the glucagon desamido impurities when compared to the other column and mobile phase combinations. Optimization of this method produced baseline separation of glucagon and it's four common desamido impurities, that are a result of deamination of the peptide's amino acid residues[4] (Figure 2). We further investigated the use a novel surface modification technology based on hybrid organic/inorganic surface based on an ethylene-bridged siloxane chemistry This technology has previously been shown to mitigate undesirable metal and peptide interactions leading to improvements in chromatographic separation parameters such as chromatographic peak area count, tailing and retention time reproducibility. [8,9]. An example of the improved chromatographic peak area this is shown in Figure 3 for both liraglutide and semaglutide.

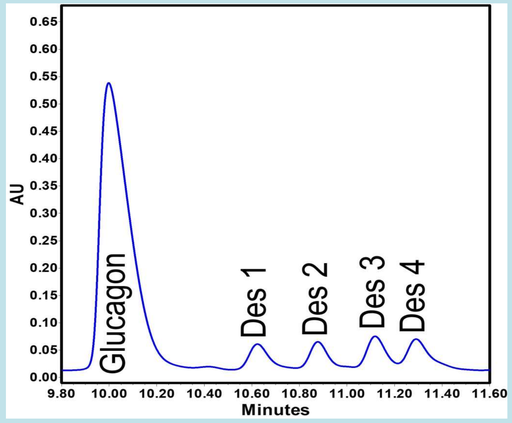

Figure 2. Representative chromatogram showing the results using the focus gradient conditions to separate the glucagon individual standard (750ug/mL) and associated desamido impurities

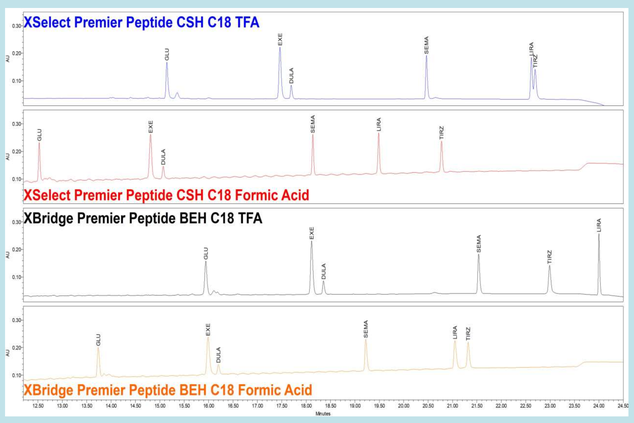

Figure 1. Stacked chromatogram demonstrating the differences of retentivity and selectivity of the GLP-1 standard mix from each of the column and mobile phase combinations.

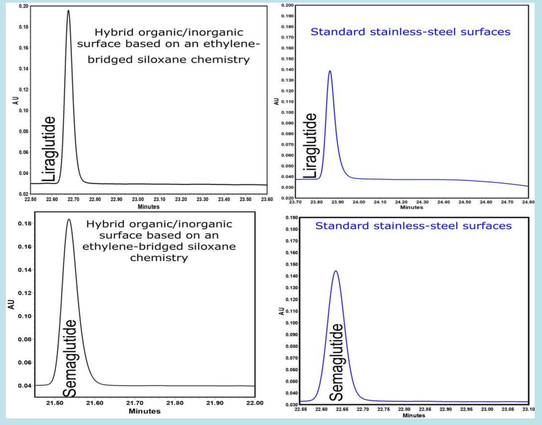

Figure 3. Representative chromatogram of method performance for both the MaxPeak™ HPS Technology and traditional stainless-steel systems for liraglutide and Semaglutidee.

CONCLUSION(S)

The data shown here demonstrated the utilization of a systematic method development protocol was able to produce quality separations of glucagon like peptides agonists (GLP- 1s), glucagon and associated impurities. Glucagon and desamido impurities were base line separated and detected by both UV and MS detection. The purity of the chromatographic peaks was determined both by PDA and MS spectral data and further providing information on desamido associated impurity relatedness to glucagon. The use of a novel surface modification technology based on hybrid organic/inorganic surface based on an ethylene-bridged siloxane chemistry. provided improvements in chromatographic peak area of up to 28 %, peak height of up to 57 %, and reduction in peak tailing of 13 % for a variety of GLP-1s tested.

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