Development of Separation Methods for GLP-1 Synthetic Peptides Utilizing a Systematic Protocol and MaxPeak™ High Performance Surface Technology

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Introduction

Peptides offer higher specificity than small molecules, and low immunogenicity which makes them excellent candidates for new medications. Specifically synthetic peptides produced through solid phase peptide synthesis allow for more control with more predictable impurities with a relatively low manufacturing cost.

An excellent example of upcoming synthetic peptide drugs is Glucagon-Like Peptide-1 Agonists (GLP-1s) which are prescribed for the treatment and management of obesity and type-II diabetes1. Recently, GLP-1 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.

Here we address these needs and developed a single HLPC-UV/MS method for the analysis of a variety of GLP-1s. The experiment was based around a systematic protocol which was key in helping to identify high-risk factors and to shorten the method development time. Beyond this MaxPeak high performance (HPS) technology was used to improve chromatographic performance.

Systematic Protocol

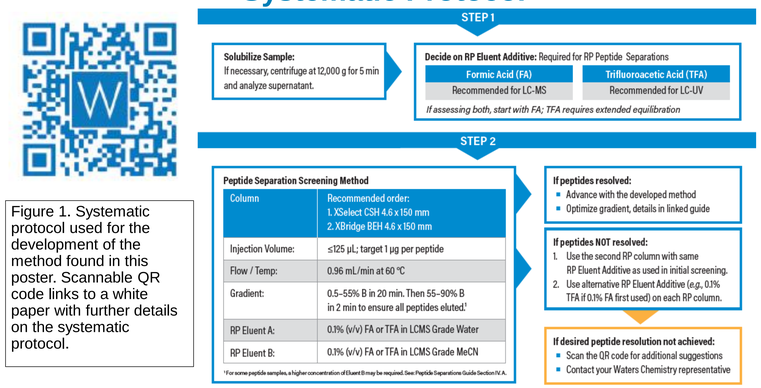
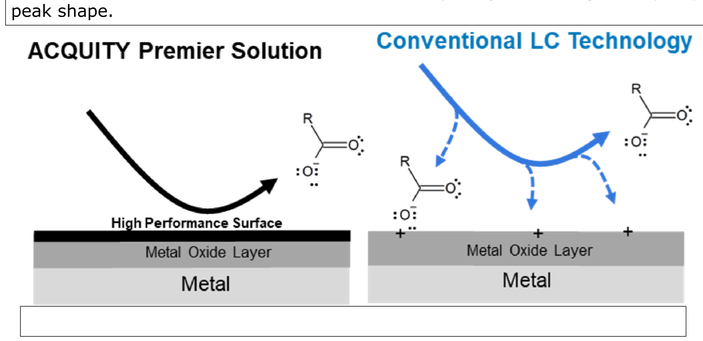


Figure 1. Systematic protocol used for the development of the method found in this poster. Scannable QR code links to a white paper with further details on the systematic protocol.

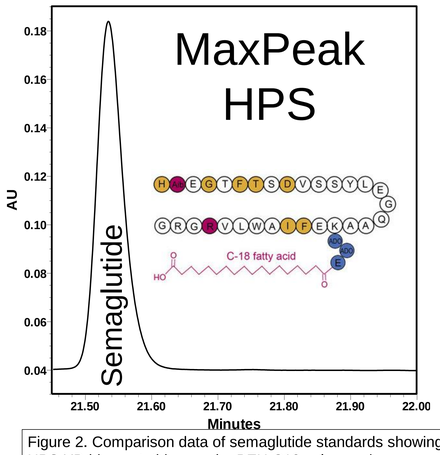
A common yet seldomly talked about problem that is a high-risk variable in HPLC analysis is metal oxide analyte interactions. These interactions can adsorb analytes in a varying amount through several injections. This can often be seen as a high relative standard deviation in peak area or height, worsened limits of detection, and poor chromatographic peak shape. Resolving these issues is possible with time consuming passivation protocols and sacrificial analyte injections. These additional steps before an analysis introduce a significant amount of risk in the method.

Based on molecular structure some classes of compounds can be more prone to this interaction including: peptides, oligonucleotides, phosphorylated compounds and many more. Peptides are particularly vulnerable because they act as Lewis bases, allowing them to donate electrons and chelate to the metal oxide found within standard stainless-steel high-performance liquid chromatography (HPLC) systems.

Waters has created a solution to this issue, by coating internal metal surfaces with MaxPeak High performance surface technology these detrimental interactions can be eliminated. Eliminating this interaction leads to increased peak area, peak height, with reduced relative standard deviation of multiple injects, and generally improved peak shape.



Results



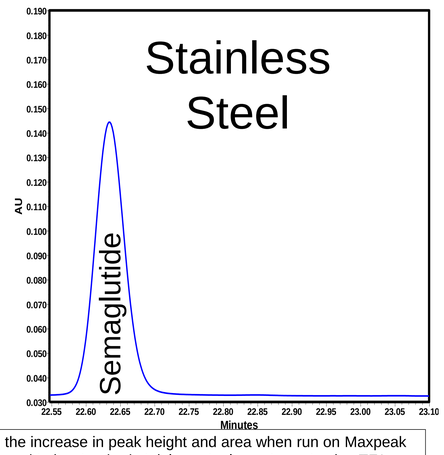
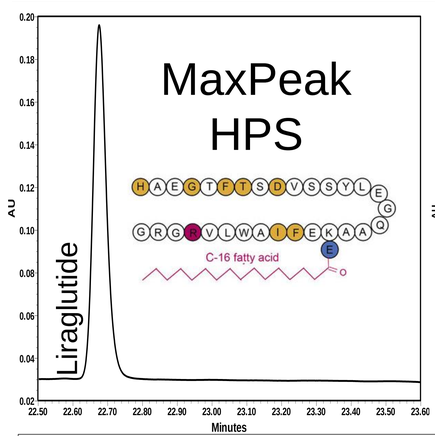


Figure 2. Comparison data of semaglutide standards showing the increase in peak height and area when run on Maxpeak HPS XBridge petptide premier BEH C18 column whgen compared to its standard stainless steel counterpart using TFA as a eluent additive.

MaxPeak HPS technology was used to reduce the risk associated with metal-oxide interaction that is known to occure with peptides. Peptides and oligonucleotides tend to chelatate with the chromium oxide layer found on stailess steel hardware. Traditionally this issue was resolved with passivation methods where acids or other compounds are run through the instreument followed by sacrificial injections of the analyte to fill any active site on the surface of the metal. Before this lengthy procedure is followed inconsistencies due to the analyte adsorbtion can significantly affect the reproducibility of a method. Beyond this these methods introduce risk to the quality of the method due to the issues that arise qwhne they are not followed properly or done for a sufficient amount of time.

MaxPeak HPS Technology elimates these interactions. This elimation leads to better general chromatographic results with the majority of comppounds. The analysis of thes GLP1 compounds specifically saw aproxamately 20% increase in height when analyzing semaglutide and aproxamately a 40% increase in peak heght whgen analyzing Liraglutide. Beyuond this the reproducibility of the method increased all without difficult passivation steps before the analysis. Overall HPS technology leads to a better separation that is less risky when compared to standard stainless steel. Additionally the life of MaxPeak HPS columns tends to be higher as it does not require the sacrificial injections to get reliable results



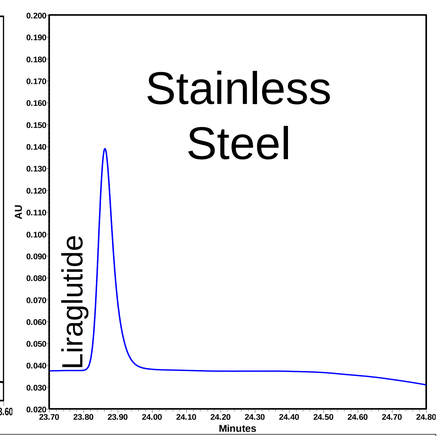
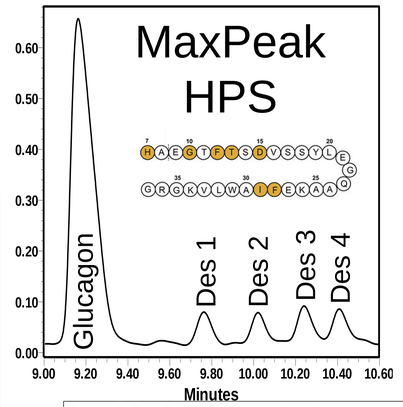


Figure 3. Comparison data of liraglutide standards showing the increase in peak height and area when run on MaxPeak HPS Xselect petptide premier CSH C18 column when compared to its standard stainless-steel counterpart using TFA as a eluent additive.



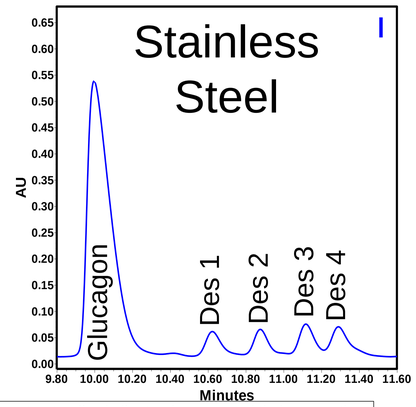
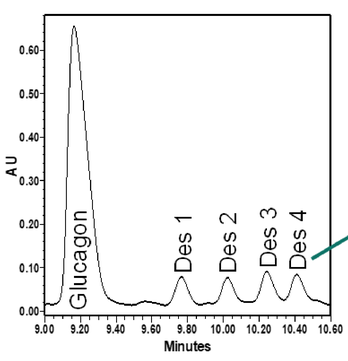
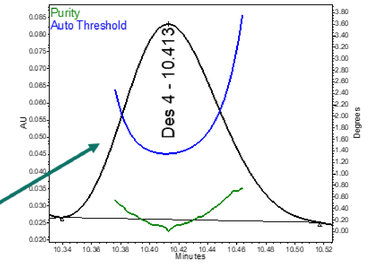


Figure 3. The Desamino impurities of glucagon are structurally similar to glucagon and their separation shows the versatility of this protocol.

Peak Purity

Being able to show evidence that a peak is pure is a useful tool when looking at impurities. With glucagon desamino impurities are not individually available. When this is the case using spectroscopic analysis can provide reasonable confidence in the purity of a peak. Using the empower peak purity tools to test for sprectal homogeinity the purity angle less than the purity threshold suggesting that the peaks were pure with no detectable coelutions. Data from both the QDA mass detector and the PDA Uv detector were used for this analyisis.





	Name	Migration Time (min)	Purity1 Angle	Purity1 Threshold	Area (µV*sec)	Height (µV)	Purity1 Flag
1	Glucagon	9.168	0.120	0.368	4785486	630739	
2	Des 1	9.767	0.336	1.454	307275	62482	
3	Des 2	10.025	0.395	1.540	256412	56356	
4	Des 3	10.245	0.402	1.258	299411	64590	
5	Des 4	10.413	0.270	1.350	258058	56956	

Conclusion

The thoughought assessment of the risk before conduction the development of a separation method allowed for the creation of a more quality method while reducing time. Asessing the high risk variables showed ther benefit in MaxPeak HPS technology when working with these compounds. MaxPeak HPS technology

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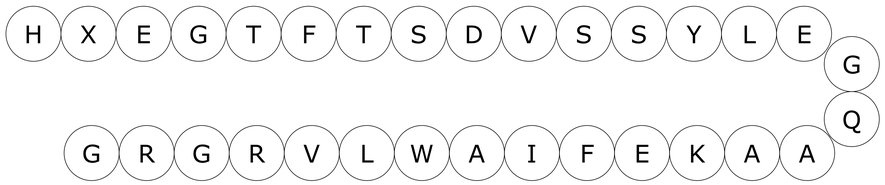
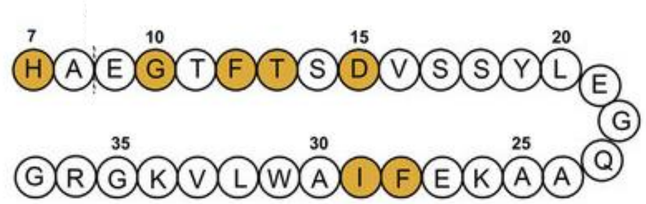
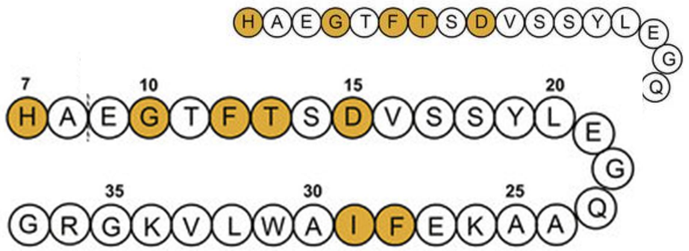
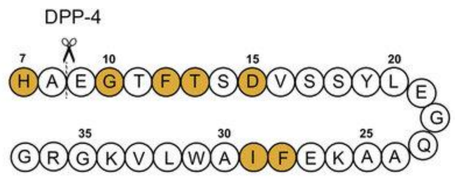
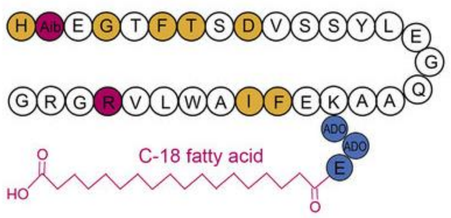








GLP-1



Semaglutide

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