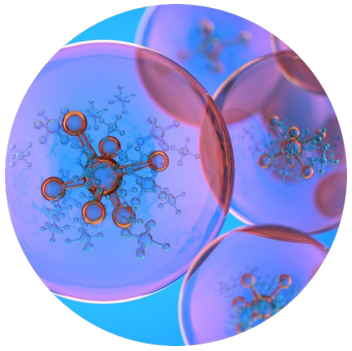

TN-1394

Aggregate Analysis of Liraglutide Using Biozen™ dSEC-1

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Introduction

Liraglutide is an agonist of the glucagon-like peptide-1 (GLP-1) receptor, utilized in the management of type 2 diabetes and obesity. It functions by increasing insulin secretion, lowering glucagon levels, and delaying gastric emptying. These actions result in better blood sugar regulation and facilitate weight loss. It emulates the effects of the endogenous hormone GLP-1, which is responsible for appetite control and glucose metabolism[1]. As a synthetic peptide therapeutic, Liraglutide is prone to aggregation, which can compromise its safety, efficacy, and shelf-life.

Size Exclusion Chromatography (SEC) is a key analytical technique employed to monitor aggregation by separating molecular species based on size. However, SEC of peptides and small proteins can be challenging due to their marked susceptibility to non-specific interactions. Hydrophobic and/or ionic interactions may compromise chromatographic performance, leading to issues such as peak tailing or analyte adsorption. Due to Liraglutide's hydrophobic nature, SEC analysis often requires organic solvents and acidic conditions to ensure accurate resolution.

In this technical note, we demonstrate the successful performance of Biozen dSEC-1 SEC columns for aggregate analysis of Liraglutide. The hydrophilic nature of the stationary phase imparts inertness to the media which is reflected in the reduced need for organic solvents in the mobile phase, thereby improving chromatographic results and assist in robust and reproducible method development.

Sample Preparation

Liraglutide: Liraglutide Injection (source: Teva Pharmaceuticals)

18 mg in 3 mL contains the following 30 amino acids, glucagon-like peptide-1 analogue drug.

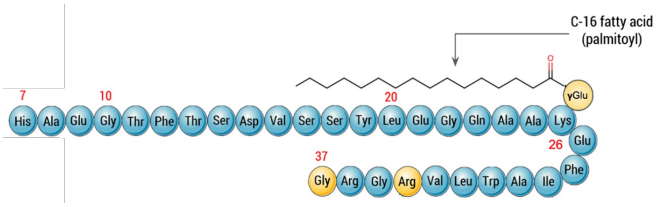


Image modified from https://pdb101.rcsb.org/global-health/diabetes-mellitus/drugs/incretins/drug/liraglutide/liraglutide

Sample aggregation was further induced by exposure to ambient light for 3 days. All samples were injected before and after light exposure, injecting the commercial solution directly at volumes of 3.4 µL to achieve a load of 20 µg of the Liraglutide on column. All analyses were done in triplicate.

LC Conditions

Column: Biozen 1.6 µm dSEC-1, 90 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4801-E0
Mobile Phase: (60:40, v/v) 1X PBS*:Acetonitrile
Isocratic: 12 minutes
Flow Rate: 0.2 mL/min
Injection Volume: Injection volume selected to introduce 20 µg on column
Temperature: 40 °C
LC System: Waters ACQUITY® UPLC H-Class
Detection: UV @ 280 nm

*1X PBS (Phosphate Buffered Saline) contains 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 1.8 mM KH2PO4.

Results and Discussion

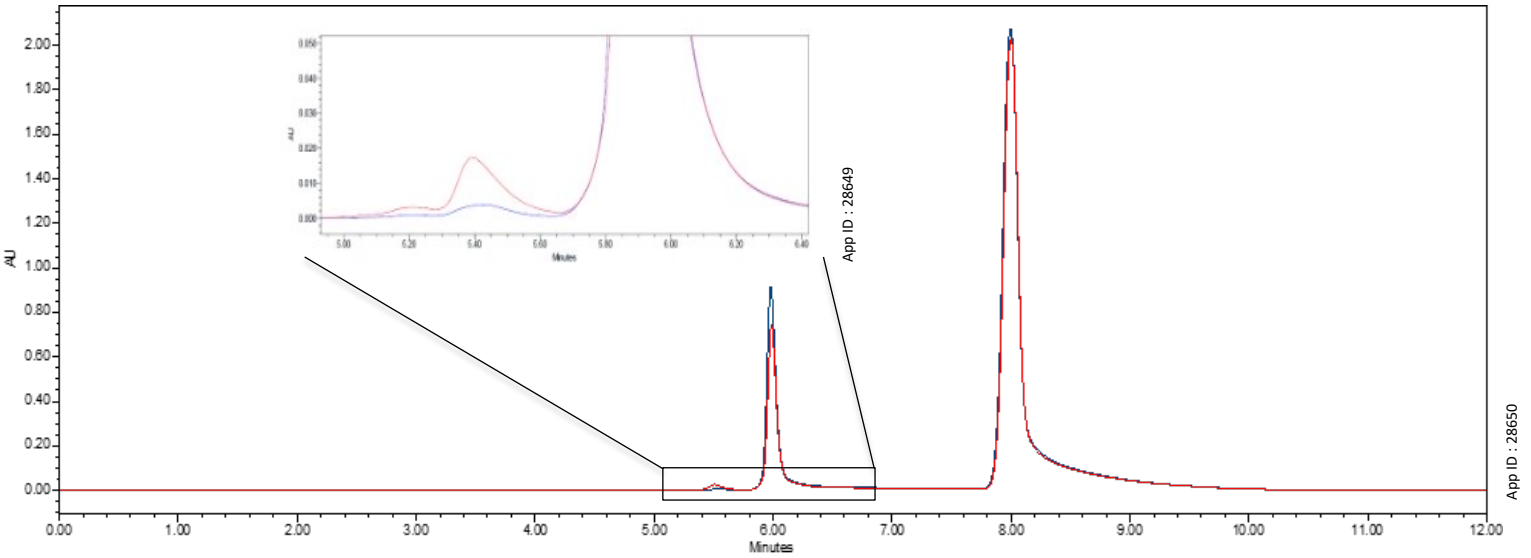
In this study, we evaluated key chromatographic performance parameters for both the monomer and aggregates components in a commercial Liraglutide injection solution using Biozen dSEC-1 SEC columns. Analyses were conducted on both the original product and a light-stressed sample exposed for three days. Effective separation of the monomer and aggregate peaks was achieved. A summary of the main chromatographic results is presented in **Table 1**.

Table 1. Summary of observed retention times, peak areas, % of the species present, resolution between dimer and monomer and peak asymmetry for Liraglutide before and after photoinduced aggregation (n=3).

Liraglutide		Biozen dSEC-1 (150 x 4.6 mm, 1.6 µm)		
		Aggregate 1	Aggregate 2	Monomer
Before exposure to light	Retention Time (min)	5.21	5.43	5.90
	Area (%)	0.105	0.899	98.996
	Resolution	-	1.1	1.96
	Peak asymmetry	-	-	1.53
After exposure to light	Retention Time (min)	5.24	5.39	5.91
	Area (%)	0.778	4.567	94.656
	Resolution	-	0.8	1.56
	Peak asymmetry	-	-	1.47

Using Biozen dSEC-1 column (150 × 4.6 mm, 1.6 µm) three peaks corresponding to the monomer and 2 aggregates were successfully separated in a 12-minute run. Resolutions of 1.96 and 1.56 were achieved between the monomer and closest eluting aggregate, for samples before and after photolytic stress, respectively (see Figure 1). For both samples, good retention time and peak areas reproducibility are observed with RSD of no more than 0.08% and 5.4%, respectively. The method demonstrates acceptable sensitivity, enabling reliable detection and quantification of two aggregates in the final product, with signal-to-noise (S/N) ratios of 13 for aggregate 1, 75 for aggregate 2, and 10,917 for the monomer. The S/N ratios are even higher for the stressed sample reaching 54, 313 and 7,523, respectively.

Figure 1. Chromatogram of Liraglutide analyzed using the Biozen dSEC-1 SEC column (150 x 4.6 mm, 1.6 µm). Traces of a representative injection were made for samples before (blue) and after (red) exposure to light.



Conclusion

We demonstrated that Biozen dSEC-1 SEC column is a suitable size exclusion column for Liraglutide aggregation studies, showing good resolution between aggregate and monomer and excellent retention time and peak area reproducibility. This was achieved without the need of exposing the sample to mobile phases with high organic content or added acidic modifiers which are known to influence peptide aggregation behavior.

References

1 Mannucci, E., & Lamanna, C. (2010). Incretins and the specific mechanism of action of liraglutide, the first applicable human glucagon-like peptide 1 analog in the treatment of type 2 diabetes. Journal of Receptor, Ligand and Channel Research, 105-112. https://doi.org/10.2147/JRLCR.S6345

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