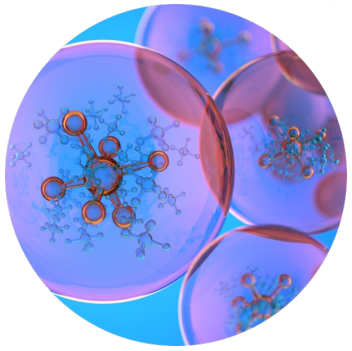

TN-1395

Aggregate Analysis of Semaglutide Using a Biozen™ dSEC-1 SEC Column

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Introduction

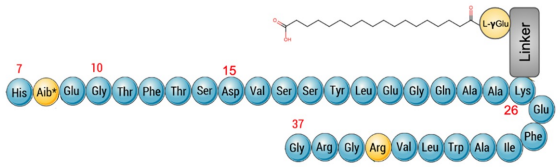
Semaglutide is an agonist of the glucagon-like peptide-1 (GLP-1) receptor, utilized in the management of type 2 diabetes and obesity. Semaglutide functions by increasing insulin secretion, lowering glucagon levels, and delaying gastric emptying. These actions result in better blood sugar regulation and 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, Semaglutide 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 Semaglutide'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 Semaglutide. The hydrophilic nature of the stationary phase imparts inertness to the media, reducing the need for organic solvents in the mobile phase. Thus, improving chromatographic results and assisting in robust and reproducible method development.

Sample Preparation

Semaglutide: OZEMPIC® 8 mg in 3 mL contains the following 30 amino acids, glucagon-like peptide-1 analogue drug.



*Aib = α-aminoisobutyric acid

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

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 7.4 µL to achieve a load of 20 µg of the Semaglutide 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: 0.40 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 Semaglutide injection solution, using a Biozen dSEC-1 SEC column. 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 for Semaglutide before and after photoinduced aggregation (n=3).

Semaglutide		Biozen dSEC-1 (150 x 4.6 mm, 1.6 µm)		
		Aggregate 1	Aggregate 2	Monomer
Before exposure to light	Retention Time (min)	-	2.71	3.03
	Area (%)	-	0.18	99.8
	Resolution	-	-	3.16
After exposure to light	Retention Time (min)	2.61	2.71	3.03
	Area (%)	0.07	0.88	99.0
	Resolution	-	1.21	3.36

Using a Biozen dSEC-1 SEC 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. Resolution of 3.16 and 3.36 was achieved between the monomer and closest eluting aggregate for samples before and after photolytic stress, respectively (**Figure 1**). For both samples, retention time and peak area reproducibility are observed with RSD of no more than 0.28 % and 4.02 %, respectively. Good sensitivity is clearly achieved, being able to detect and quantify the aggregate in final product with S/N of 26, 26126 for aggregate 2 and monomer, respectively. The S/N ratios are even higher for the stressed sample, reaching 14, 99 and 19212 for aggregate 1, aggregate 2 and monomer, respectively.

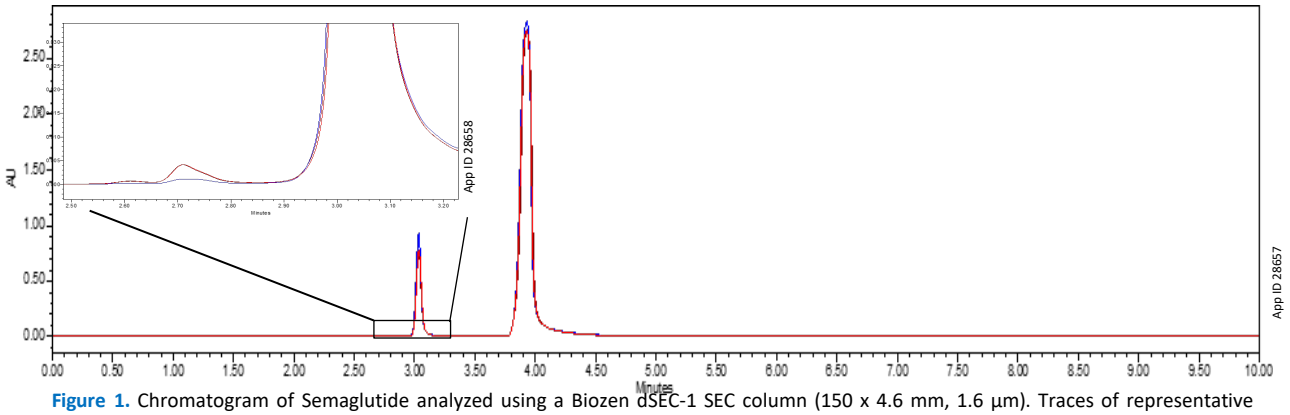


Figure 1. Chromatogram of Semaglutide analyzed using a Biozen dSEC-1 SEC column (150 x 4.6 mm, 1.6 µm). Traces of representative injections were made for samples before (blue) and after (red) exposure to light.

Conclusion

We demonstrated that Biozen dSEC-1 SEC columns are suitable size exclusion columns for Semaglutide 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.

References

1 Sorli, C., Harashima, S. I., Tsoukas, G. M., Unger, J., Karsbøl, J. D., Hansen, T., & Bain, S. C. (2017). Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in patients with type 2 diabetes (SUSTAIN 1): a double-blind, randomised, placebo-controlled, parallel-group, multinational, multicentre phase 3a trial. *The lancet Diabetes & endocrinology*, *5*(4), 251-260. https://doi.org/10.1016/S2213-8587(17)30013-X

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