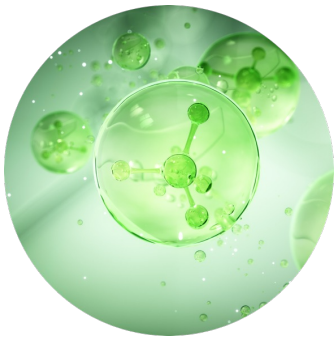

TN-1392

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

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Introduction

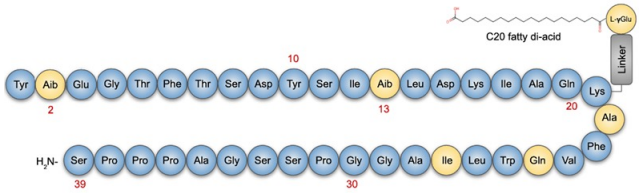
Tirzepatide is a synthetic peptide therapeutic that acts as a dual agonist of the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors[1]. It is used in the management of type 2 diabetes and obesity, functioning by enhancing insulin secretion, suppressing glucagon release, and delaying gastric emptying. These actions contribute to improved glycemic control and significant weight loss[2]. As a large and hydrophobic peptide (molecular weight ~4813 Da), Tirzepatide is prone to aggregation, particularly under stress conditions such as pH shifts, oxidation, and elevated temperatures. Aggregation can compromise product safety, efficacy, and shelf-life, making its detection and quantification a critical aspect of formulation development and quality control.

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 Tirzepatide'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 Tirzepatide. 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 assisting in robust and reproducible method development.

Sample Preparation

Tirzepatide: Mounjaro® 2.5 mg in 0.5 mL contains the following 39 amino acids, glucagon-like peptide-1 analogue drug.



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 4.00 µL to achieve a load of 20 µg of the Tirzepatide on column. All analyses were done in triplicate.

LC Conditions

Column:	A: Biozen 1.6 µm dSEC-1, 90 Å B: Biozen 3.0 µm dSEC-1, 90 Å
Dimensions:	A: 150 x 4.6 mm B: 300 x 4.6 mm
Part No.:	A: 00F-4801-E0 B: 00H-4802-E0
Mobile Phase:	(70:30, v/v) 1X PBS*:Acetonitrile
Isocratic:	A: 10 minutes B: 18 minutes
Flow Rate:	0.40 mL/min
Injection Volume:	Injection volume selected to introduce 20 µg on column
Temperature:	Ambient
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

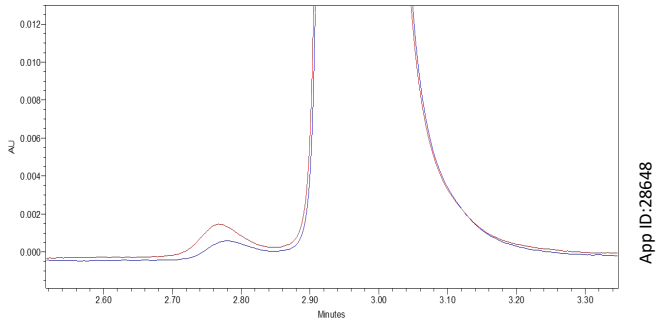
In this study, we measured several chromatographic performance features (retention time, peak area, RSD, and resolution peak asymmetry) for both the monomer and aggregate, in a commercial Tirzepatide injection solution using Biozen dSEC-1 SEC columns. Samples tested include the final product as received and the stressed product after 3-day exposure to light. Successful separation between the monomer and aggregate peak was obtained for both column dimensions tested, with the main chromatographic features measured summarized in **Table 1**.

Separation was achieved using a Biozen dSEC-1 SEC column (150 × 4.6 mm, 1.6 µm) in a 10-minute run with resolutions of 1.33 and 1.56, for samples before and after photolytic stress, respectively (**Figure 1**). For both samples, reproducible retention time and peak areas are observed with RSD of ≤0.06 % and ≤4.35 %, respectively. The method exhibits sensitivity well over acceptable values with signal-to-noise ratios of 7.8 and 23, respectively, enabling reliable detection and quantification of the aggregate in both the final product and the stressed sample.

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

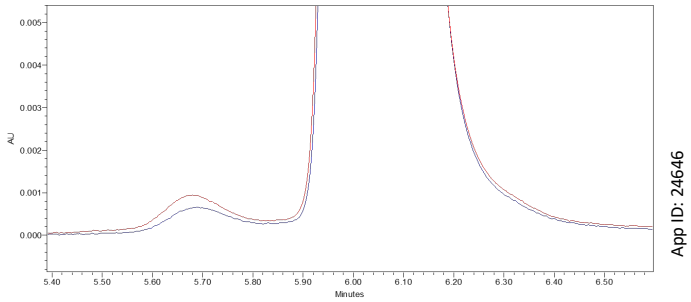
Tirzepatide		Biozen dSEC-1 (150 x 4.6 mm, 1.6 µm)		Biozen dSEC-1 (300 x 4.6 mm, 3 µm)	
		Aggregate	Monomer	Aggregate	Monomer
Before exposure to light	Retention Time (min)	2.78	2.95	5.69	6.02
	Area (%)	0.17	99.8	0.17	99.8
	Area RSD (%)	3.82	0.15	0.18	0.04
	Resolution	1.33		1.64	
	Peak asymmetry	n.a.	1.57	n.a.	1.78
After exposure to light	Retention Time (min)	2.77	2.95	5.68	6.02
	Area (%)	0.30	99.6	0.22	99.7
	Area RSD (%)	4.35	0.22	3.29	0.05
	Resolution	1.56		1.73	
	Peak asymmetry	n.a.	1.56	n.a.	1.77

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



When evaluating the Biozen dSEC-1 SEC column (300 × 4.6 mm, 3 µm) baseline resolved peaks were observed, achieving values of 1.64 and 1.73, for samples before and after photolytic stress, respectively. A representative chromatogram of these samples is shown in **Figure 2.** in this case, good retention time and peak areas reproducibility are observed for both sample types with RSD of ≤0.08 % and ≤3.29 %, respectively.

Figure 2. Chromatogram of Tirzepatide analyzed with the Biozen dSEC-1SEC column (300 x 4. 6mm, 3 µm). Traces of a representative injections were made for samples before (blue) and after (red) exposure to light.



Notably, this excellent performance was achieved using a widely used buffer with a moderate addition of organic solvent (30 % acetonitrile). This outcome further demonstrates that Biozen dSEC-1 SEC column offers a highly hydrophilic stationary phase, making it suitably inert to secondary hydrophobic interactions with therapeutic peptides.

Conclusion

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

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