



Determination of Tirzepatide High MW Aggregation Using Phosphate Buffer

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Introduction

Tirzepatide is a glucose-dependent insulinotropic polypeptide (GIP)/Glucagon-like peptide-1 (GLP-1) dual receptor agonist and a new drug for the treatment of Type 2 Diabetes. Compared to traditional GLP-1 receptor agonists, Tirzepatide has more significant hypoglycemic and weight loss effects.

This application note attempts to establish chromatographic conditions for the detection of Tirzepatide aggregates using a biologically inert size exclusion chromatographic (SEC) column. The Biozen dSEC-2 column contains highly hydrophilic particles packed into titanium alloy column hardware, thereby reducing any potential interaction between protein or peptide molecules and the column packing and column hardware. Due to the high inertness and sharp peak shape provided by the dSEC-2 column, the sensitivity for detecting trace amounts of aggregates in preparations can be improved.

The results shown in **Figures 1 and 2** suggest that the aggregate and main peaks have symmetrical and sharp peak shapes, good separation, and high detection sensitivity. Therefore, the use of the Biozen 3 µm dSEC-2 SEC column under these method conditions is suitable for the detection of Tirzepatide aggregates.

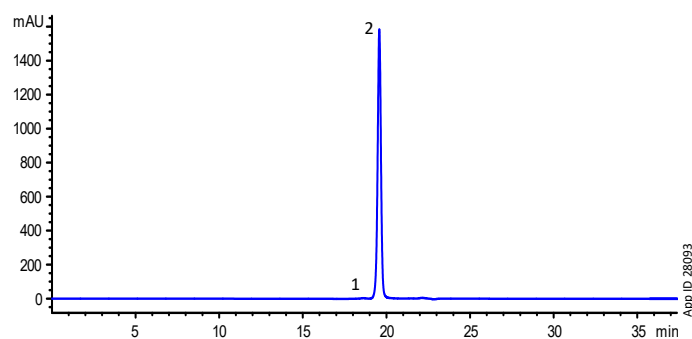
LC Conditions

Column: Biozen™ 3 µm dSEC-2
Dimensions: 300 x 7.8 mm
Part No.: [00H-4788-K0](#)
Mobile Phase: 100 mM Sodium Chloride + 50 mM Phosphate Buffer (pH 6.8) / Acetonitrile (70:30, v/v)
Flow Rate: 0.5 mL/min (Isocratic)
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 214 nm
System: Agilent® 1260 Infinity II

Table 1. Tirzepatide Structural Information.

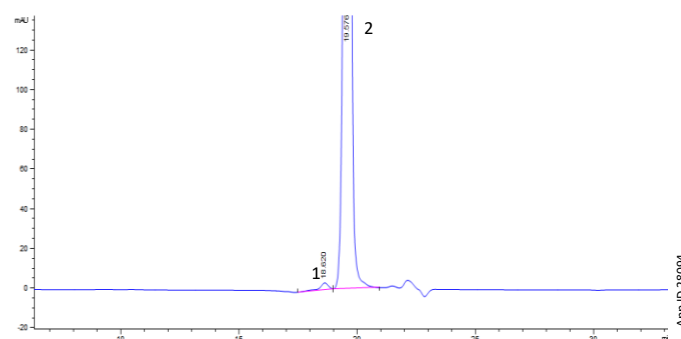
| Name | Molecular Weight (Da) | Structural Formula |
|-------------|-----------------------|------------------------------|
| Tirzepatide | 4813.45 | $C_{225}H_{348}N_{48}O_{68}$ |

Figure 1. Representative Chromatogram of Tirzepatide and Aggregate.



| Peak No. | Analyte | Retention Time (min) | % Area | Asymmetry | Resolution |
|----------|---------------|----------------------|--------|-----------|------------|
| 1 | HMW Aggregate | 18.62 | 0.38 | 1.65 | - |
| 2 | Tirzepatide | 19.58 | 99.62 | 1.01 | 2.11 |

Figure 2. Zoomed-In Representative Chromatogram of Tirzepatide and Aggregate.



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