Millennial Scientific

APPLICATION NOTE

HPLC Separation of Tirzepatide on NanoPak-C All Carbon HPLC columns

Background. Tirzepatide is a long-acting peptide drug used to treat type 2 diabetes and obesity. It exerts **insulinotropic** effects by enhancing the incretin response, where glucose exposure stimulates insulin secretion through gut-derived hormones. The two primary incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are secreted after nutriend ingestion, including glucose, and act on their respective receptors to promote insulin release. Tirzepatide functions as a dual agonist of the GLP-1 and GIP receptors, binding to these targets and triggering pharmacological responses similar to the endogenous incretins.

Optimizing Tirzepatide separation and purification requires careful selection of stationary phase, mobile phase composition, pH, flow rate, and temperature. NanoPak-C all-carbon columns offer high pH and temperature stability along with tunable properties such as selectivity, pore size, and surface area. These features improve peptide separation performance and help reduce operational and maintenance costs in development and manufacturing workflows. This application note summarizes optimized methods for HPLC separation of Tirzepatide on NanoPak-C all carbon columns under both acidic and alkaline conditions.

Probe Analytes

Tirzepatide: 1 mg/mL in DI water

Acidic condition: *Sample prepared at 1 mg/mL in DI water, adjusted to pH 2.5.*

Alkaline condition: *Sample prepared at 1 mg/mL in DI water, adjusted to pH 8.5.*

Instrumentation

HPLC Conditions	
Methods	
Column	Nanopak-C All Carbon 150 x 4.6 mm, 6 µm
Mobile phase (Acidic)	Mobile Phase A: 0.1% TFA in water (pH 2.5) Mobile Phase B: 0.1% TFA in Acetonitrile Gradient: Time %B 0 20 10 65
Mobile phase (Alkaline)	Mobile Phase A: Ammonium Acetate Buffer (pH 8.5) Mobile Phase B: Acetonitrile Gradient: Time %B 0 20 10 50
Injection volume	10ul
UV detection	220nm
Oven temperature	25deg C

Results. Figure 1a and **b** show representative chromatograms of Tirzepatide under acidic and alkaline conditions on the NanoPak-C all-carbon column. Under acidic conditions (sample in DI water adjusted to pH 2.5), Tirzepatide elutes at a retention time of 5.3 minutes. Under alkaline conditions (sample in DI water adjusted to pH 8.5), Tirzepatide elutes at 6.8 minutes. These results demonstrate that mobile phase pH and sample environment significantly influence Tirzepatide retention on the all-carbon stationary phase.

These data show that NanoPak-C all-carbon columns support robust Tirzepatide separation across acidic and alkaline conditions, enabling flexible method development for peptide purification and QC.

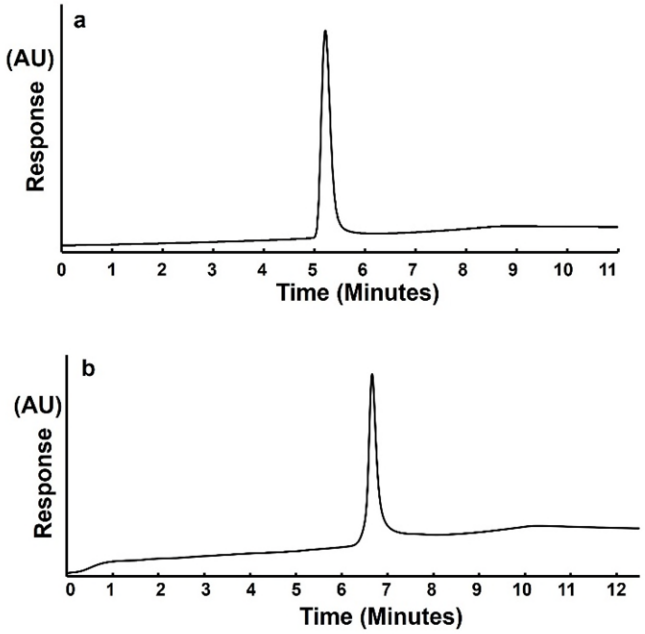

Figure 1. Representative chromatogram of Tirzepatide under (a) acidic and (b) alkaline conditions.