Application Brief

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Analysis of GLP-1 Agonists Using the Agilent 1290 Infinity II Bio LC System and Altura Ultra Inert HPLC Column

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Abstract

This application brief describes analysis of glucagon-like peptide-1 (GLP-1) receptor agonists using the Agilent 1290 Infinity II Bio LC and an Altura Peptide Plus column with Ultra Inert technology.

Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists, a class of synthetic peptides with significant therapeutic potential, mimic the GLP-1 hormone and regulate blood sugar.[1] Impurities associated with these synthetic peptides may affect the safety and efficacy of final therapeutic products. These peptides, typically composed of approximately 30 amino acids with chemically linked fatty acid chains, exhibit significant changes in chemical properties even with small structural differences. GLP-1 agonists are inherently hydrophobic, and reversed-phase liquid chromatography (RPLC) is primarily employed for their impurity separation. A major challenge in RPLC analysis of GLP-1 agonists is nonspecific interaction, as these peptides readily bind to metallic surfaces in standard LC systems. This leads to poor chromatographic performance, resulting in tailed peaks and reduced analyte recovery. Therefore, selecting the right system requires a flow path that is fully optimized, specifically utilizing a biocompatible LC system coupled with inert column hardware, is critical for accurate and reliable analysis. The Agilent 1290 Infinity II Bio LC System, when paired with Altura Ultra Inert HPLC columns, provides an optimal platform for synthetic peptide analysis, effectively minimizing analyte-surface interactions. This application brief demonstrates the LC/UV analysis of tirzepatide and retatrutide peptides utilizing the Agilent bio-separation workflow solution, showcasing its capabilities in GLP-1 agonist analysis.

Experimental

Table 1. Instrument and sample conditions.

Liquid Chromatography	**Agilent 1290 Infinity II Bio LC System**
Column	Altura Peptide Plus, 2.1 × 150 mm, 2.7 µm (p/n 227215-903)
Samples	Tirzepatide and Retatrutide (1 mg/mL) Thermal stress: 85 °C for 3 hours
Mobile Phase A	0.1% FA
Mobile Phase B	0.1% FA in ACN
Gradient	Time (min) %A %B 0.00 80 20 1.00 80 20 20.00 40 60 25.00 10 90 25.10 80 20 30.00 80 20
Column Temperature	55 °C
Flow Rate	0.4 mL/min
UV	214 nm, 220 nm, 280 nm
Mass Spectrometry	**Agilent 6545XT AdvanceBio LC/Q-TOF**
Ion Mode	Positive ion mode, dual AJS ESI
Drying Gas Temperature	325 °C
Drying Gas Flow	13 L/min
Sheath Gas Temperature	350 °C
Sheath Gas Flow	12 L/min
Nebulizer	35 psi
Capillary Voltage	4000 V
Nozzle Voltage	1000 V
Fragmentor Voltage	175 V
Skimmer Voltage	65 V
Oct RF Vpp	750 V
Reference Mass	922.009798
MS Range	100–1700 *m/z*
MS Acquisition Rate	2 spectra/sec

Results and discussion

An LC instrument configuration optimized for biomolecule analysis, including the column, is a critical factor in the analysis of synthetic peptides. The Agilent 1290 Infinity II Bio LC System consists of biocompatible flow paths, ensuring the integrity of peptides during analysis. Altura columns are engineered with inert-coated stainless steel hardware to prevent nonspecific interactions. Figure 1 shows the chromatograms of tirzepatide and retatrutide peptides on an Altura Peptide Plus 2.7 µm column. High-efficiency separation was achieved, with the column yielding well-defined and narrow peaks. The RSD of peak area and tailing factor were 0.69–1.22% and 0.9–0.83%, respectively (n = 5).

Chromatograms of control and thermally stressed tirzepatide and retatrutide samples are shown in Figure 2. The Altura Peptide Plus column demonstrated exceptional separation efficiency and resolution, clearly resolving multiple impurity peaks from the main API peak, with excellent peak shape. Upon thermal stress, both peptides exhibited notable degradation, as indicated by the well-resolved, lower-intensity degradation product peaks, highlighting the column's high sensitivity and discriminating power for detecting subtle compositional changes.

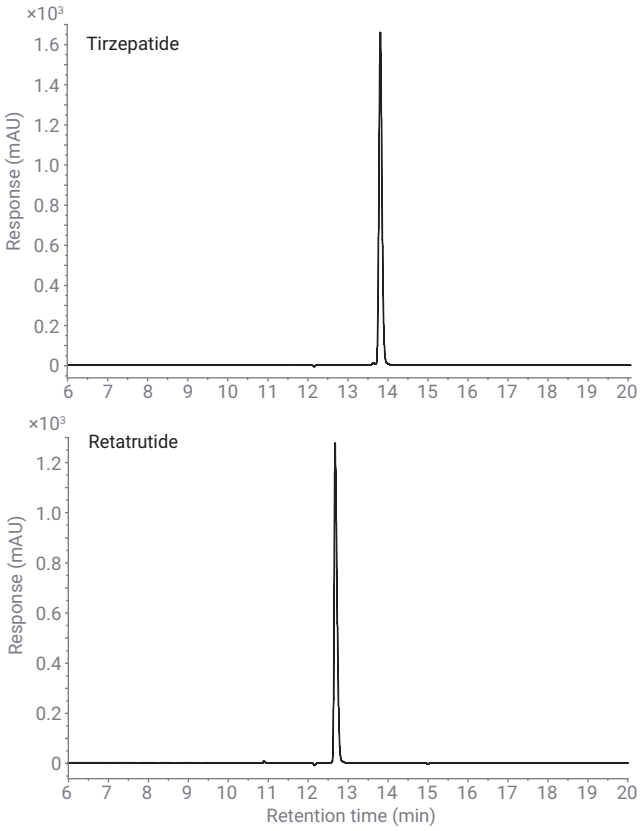

Figure 1. LC-UV chromatograms of tirzepatide and retatrutide.

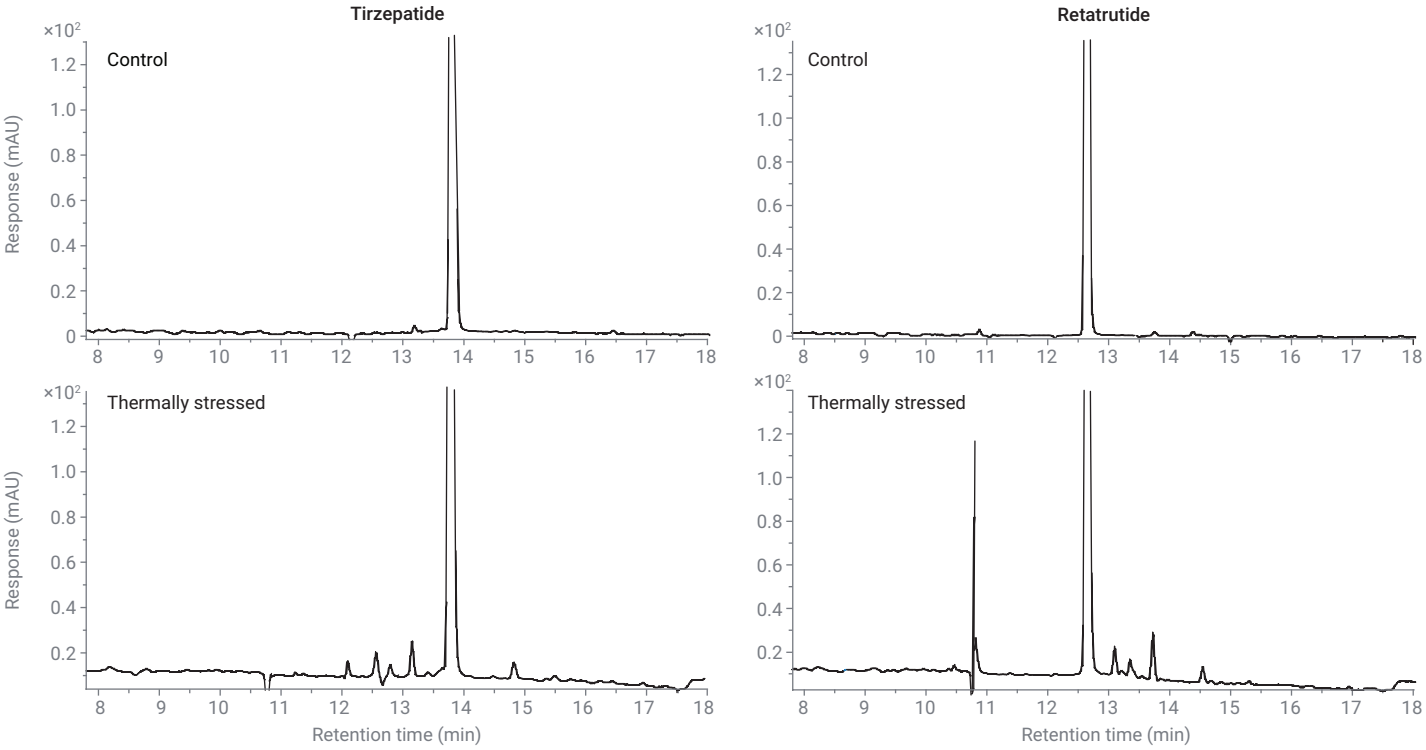

Figure 2. LC-UV chromatogram comparison of control and thermally stressed tirzepatide and retatrutide.

LC/MS analysis of tirzepatide and retatrutide was performed on an Altura Peptide Plus column to confirm its identity. The TIC chromatograms of tirzepatide and retatrutide exhibit well-defined main peaks with distinct signals corresponding to minor impurity products. The column delivered sharp, high-intensity peaks (4813.68 Da for tirzepatide and 4731.54 Da for retatrutide), indicating excellent column efficiency and resolution. The low-intensity peaks are attributed to impurities and isomeric variants of the peptide.

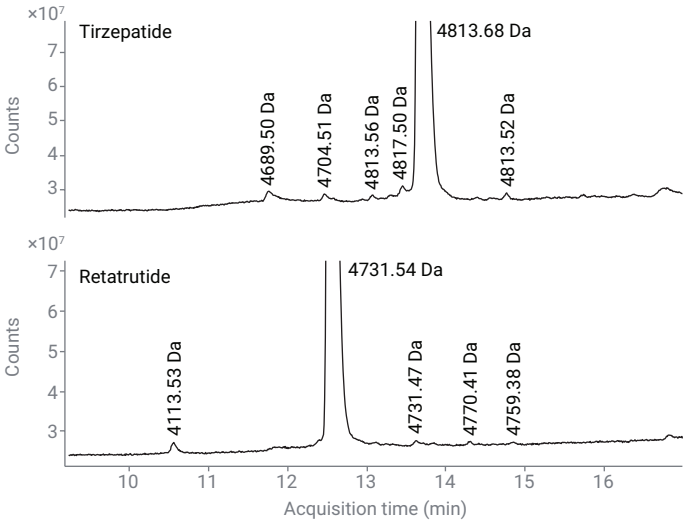

Figure 3. TIC of tirzepatide and retatrutide with deconvoluted masses.

Conclusion

These results demonstrate that the Agilent 1290 Infinity II Bio LC System and Altura Peptide Plus column provide a powerful tool for the analysis of synthetic peptides. The LC system biocompatible flow path and the Altura column with Ultra Inert technology ensure optimal chromatographic performance. The effective separation of impurities and degradation products from the main peptide peak makes it an excellent choice for quality control and forced degradation studies of tirzepatide and retatrutide.

Reference

1. Müller, T. D.; *et al.* Glucagon-Like Peptide 1 (GLP-1). *Mol. Metab.* **2019**, *(30)*, 72–130.

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