Poster Reprint

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Integrated LC/UV and LC/MS Workflows for Impurity and Degradation Characterization of GLP-1 Agonists

David L. Wong[1] , Suresh Babu C.V.[2]

[1]Agilent Technologies, Inc., Santa Clara, CA, USA

[2]Agilent Technologies, Singapore

Introduction

Synthetic peptide therapeutics are a rapidly growing and technically demanding area of biopharmaceutical development, requiring precise control of molecular identity, purity, and stability. Minor structural variations, including truncations, deletions, or chemical modifications, can significantly influence peptide behavior and consistency, making comprehensive analytical characterization essential.

Glucagon-like peptide-1 (GLP-1) receptor agonists are a key class of peptide therapeutics, typically consisting of 30–40 amino acids and often incorporating fatty acid moieties to extend half-life. These modifications increase molecular complexity and hydrophobicity, presenting challenges for analytical characterization and requiring highly selective separation methods.

This work demonstrates the application of a biocompatible LC and single quadrupole and quadrupole time-of-flight mass spectrometry system, coupled with an ultra-inert LC column, for the analysis of GLP-1 peptides. The platform delivers efficient separation, strong impurity resolution, and effective MS compatibility, enabling accurate molecular weight confirmation for complex peptides. Analysis of GLP-1 agonists such as tirzepatide and retatrutide from multiple suppliers highlights the utility of this workflow for peptide identity verification, impurity profiling, and comparative quality evaluation.

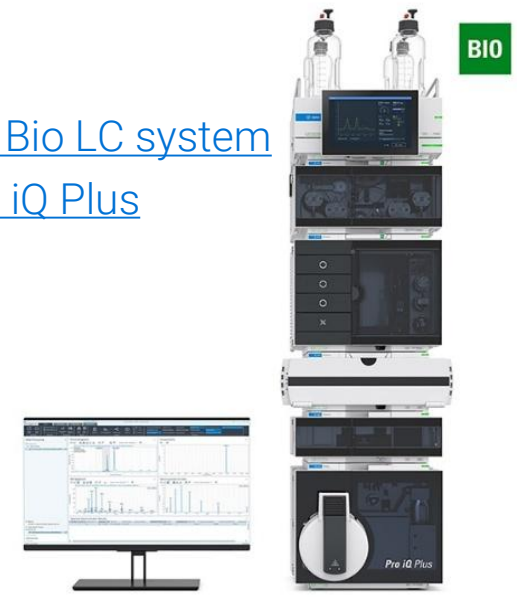
GLP-1 agonist sequence	
Liraglutide	HAEGTFTSDVSSYLEGQAA-{Lys-N6-[N-(1-oxohexadecyl)-L-g-glutamyl]}-EFIAWLVRGRG
Semaglutide	H-{Aib}-EGTFTSDVSSYLEGQAA-{C18 diacid-γ-Glu-(AEEA)2-Lys}-EFIAWLVRGRG
Tirzepatide	Y-{Aib}-EGTFTSDYSIXLDKIAQ-{C20 diacid-gamma-Glu-(AEEA)2-Lys}-AFVQWLIAGGPSSGAPPPS
Retatrutide	Y-{Aib}-QGTFTSDYSI-{α-Me-Leu}-LDK-{Lys(AEEA-γGlu-C20 diacid)}AQ-{Aib}-AFIEYLLEGGPSSGAPPPS-NH2

Refer to Application notes (5994-8951EN) and (5994-8837EN) for LC and MS conditions

Experimental

Peptide Analysis: Single Quadrupole LC/MS system

Agilent 1290 Infinity II Bio LC system
Agilent InfinityLab Pro iQ Plus

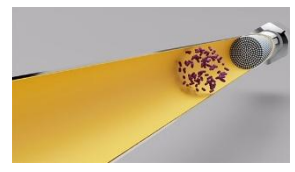


Characterization: LC/Q-TOF MS

Agilent 1290 Infinity II Bio LC system
Agilent 6545XT AdvanceBio LC/Q-TOF MS

Ultra Inert HPLC Columns

Agilent Altura Ultra Inert HPLC Columns



Innovative Ultra Inert technology

- Reduced nonspecific binding
- Better peak shape
- Enhanced sensitivity
- Long lifetime
- Rapid equilibration

Results and Discussion

LC/UV of GLP-1 agonists

- LC configuration optimized for biomolecule analysis, including column selection, is critical for synthetic peptide analysis
- Agilent 1290 Infinity II Bio LC System features biocompatible flow paths to maintain peptide integrity
- Altura Peptide Plus 2.7 µm column uses inert-coated stainless steel to minimize nonspecific interactions
- High-efficiency separation achieved for tirzepatide and retatrutide peptides
- Well-defined, narrow peaks observed in chromatograms
- Excellent reproducibility:
  - Peak area RSD: 0.69–1.22% (n = 5)
  - Tailing factor: 0.83–0.90

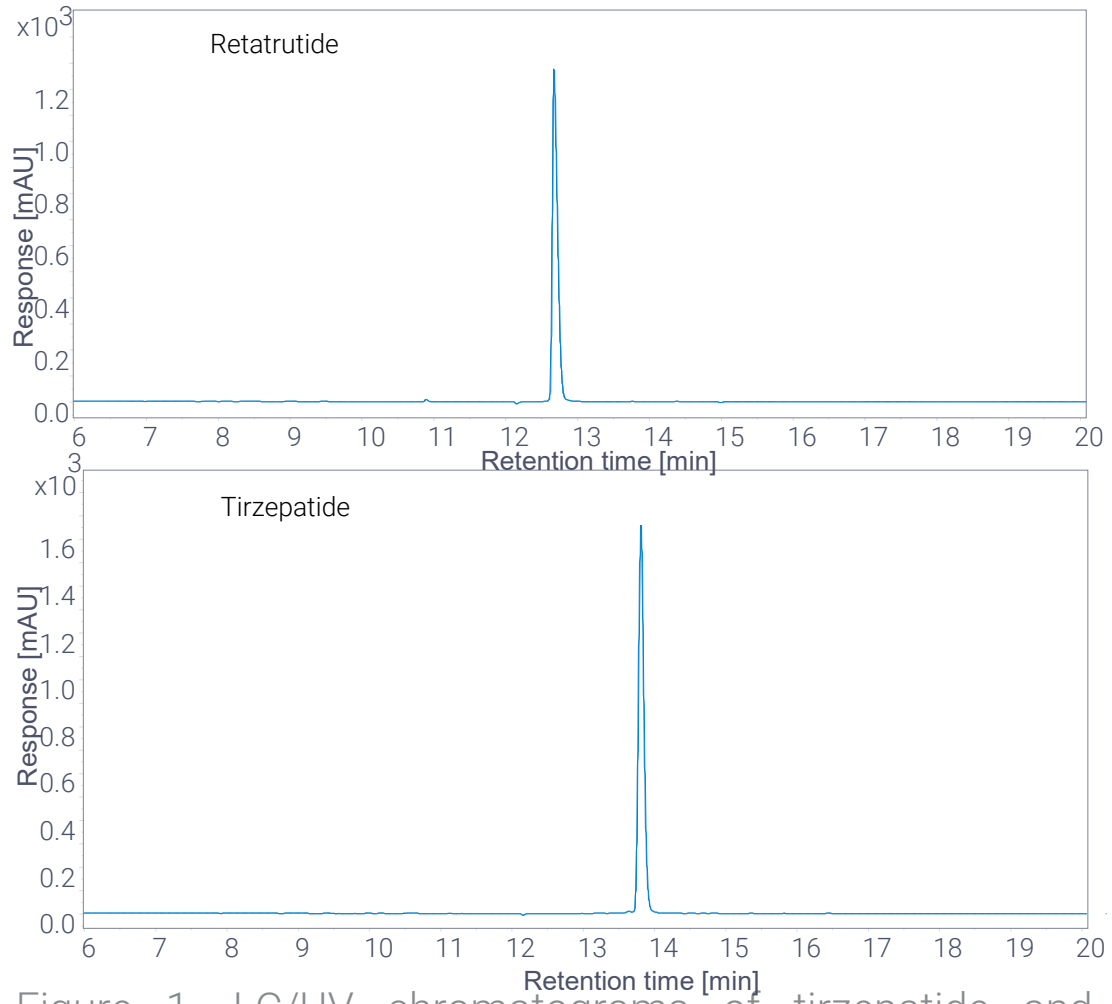


Figure 1. LC/UV chromatograms of tirzepatide and retatrutide.

LC/UV of thermally stressed GLP-1 agonists

- Chromatograms of control and thermally stressed tirzepatide and retatrutide samples
- The Altura Peptide Plus column exhibited exceptional separation efficiency and resolution
- Multiple impurity peaks were clearly resolved from the main API peak, with excellent peak shape
- Thermal stress led to noticeable degradation in both peptides
- These results highlight the column's strong discriminating power for detecting subtle compositional changes.

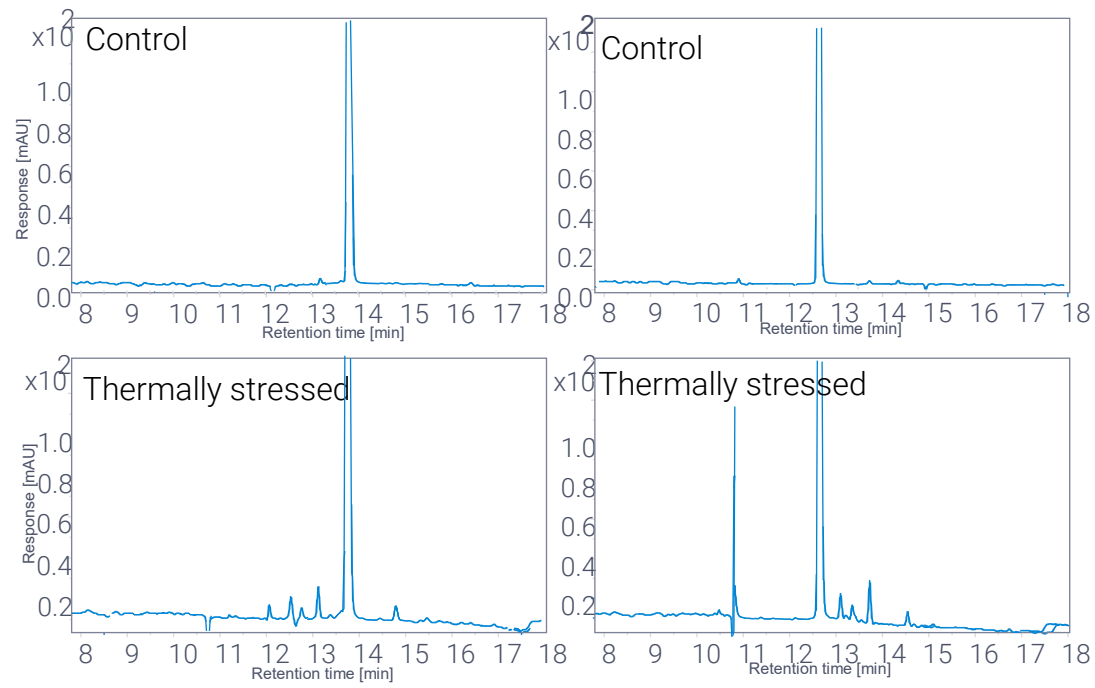


Figure 2. LC/UV chromatogram comparison of control and thermally stressed tirzepatide and retatrutide.

LC/Q-TOF MS of GLP-1 agonists

- LC/MS analysis of tirzepatide and retatrutide.
- TIC chromatograms for both peptides showed well-defined main peaks.
- Distinct signals corresponding to minor impurity products were observed.
- The column produced sharp, high-intensity peaks at 4813.68 Da for tirzepatide and 4731.54 Da for retatrutide.
- Low-intensity peaks were attributed to impurities and isomeric variants of the peptides.

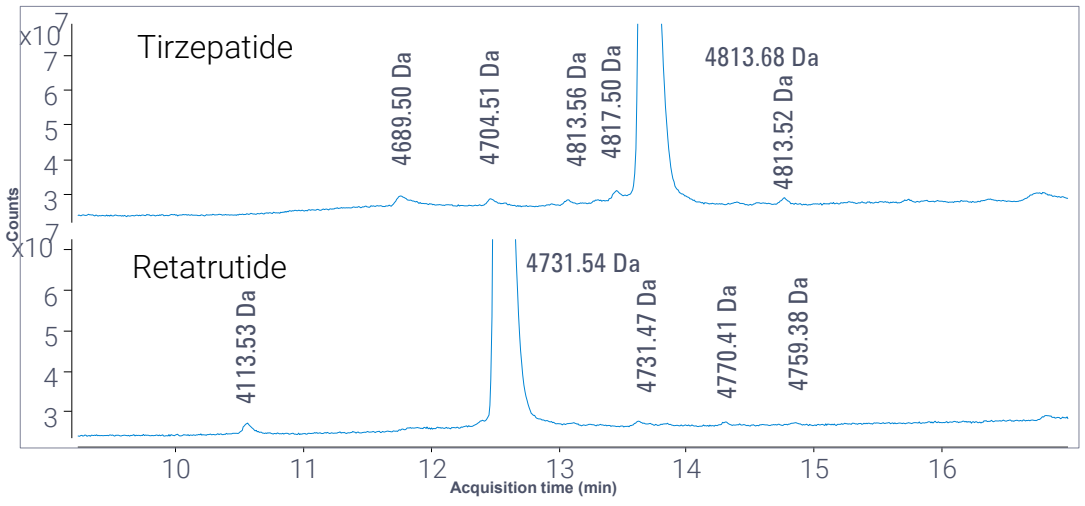


Figure 3. total ion chromatogram with deconvoluted masses, acquired using the 6545XT LC/Q-TOF.

Results and Discussion

LC/MS of GLP-1 agonists

Analysis shows distinct purity profiles across GLP-1 agonists, with semaglutide exhibiting high purity, while liraglutide, tirzepatide, and retatrutide display varying levels of chromatographically resolved or coeluting impurities.

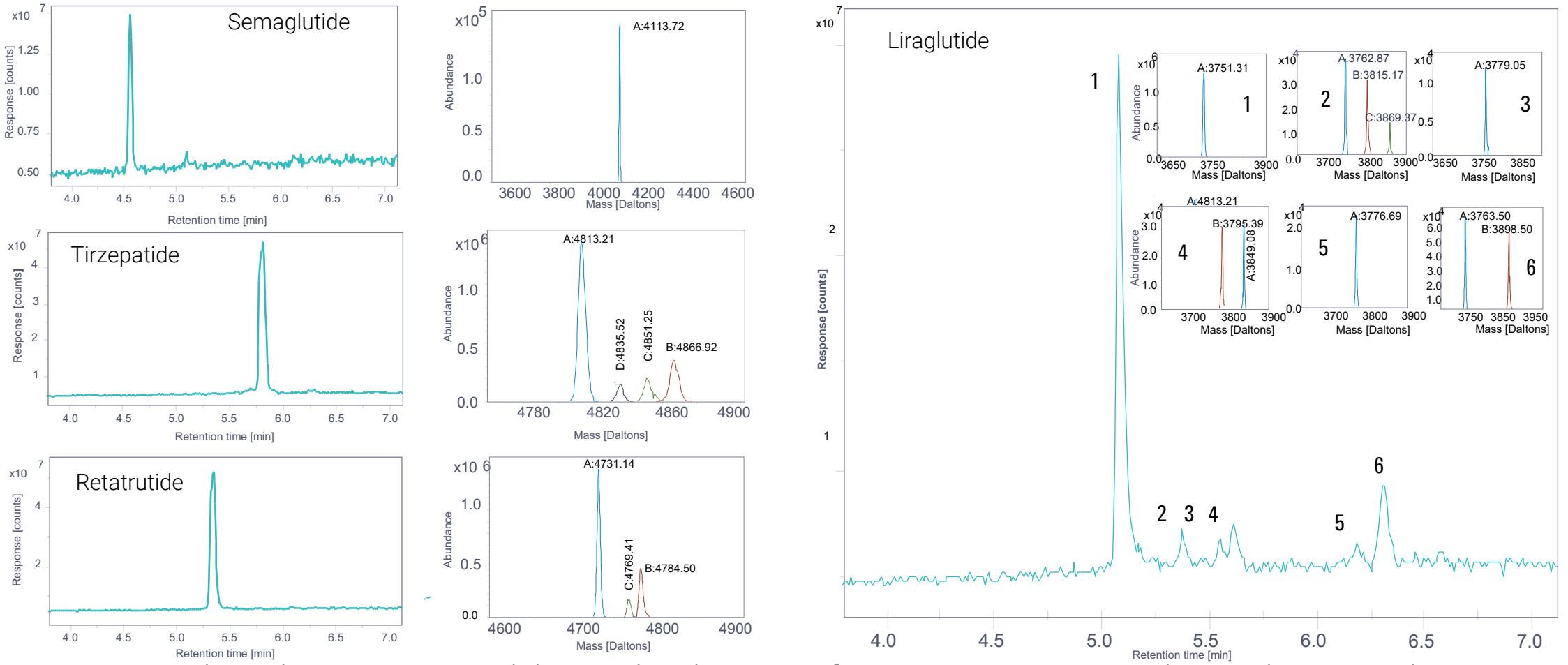


Figure 4. Total ion chromatogram and deconvoluted spectra of GLP-1 agonists, acquired using the Pro iQ Plus.

LC/MS analysis of GLP-1 from different sources

- Comparative analysis of peptides from different sources is essential to provide evidence for supplier qualification.
- Liraglutide and Tirzepatide samples revealed clear differences in impurity profiles across the three suppliers.
- This comparison demonstrates the effectiveness of the Altura Peptide Plus column

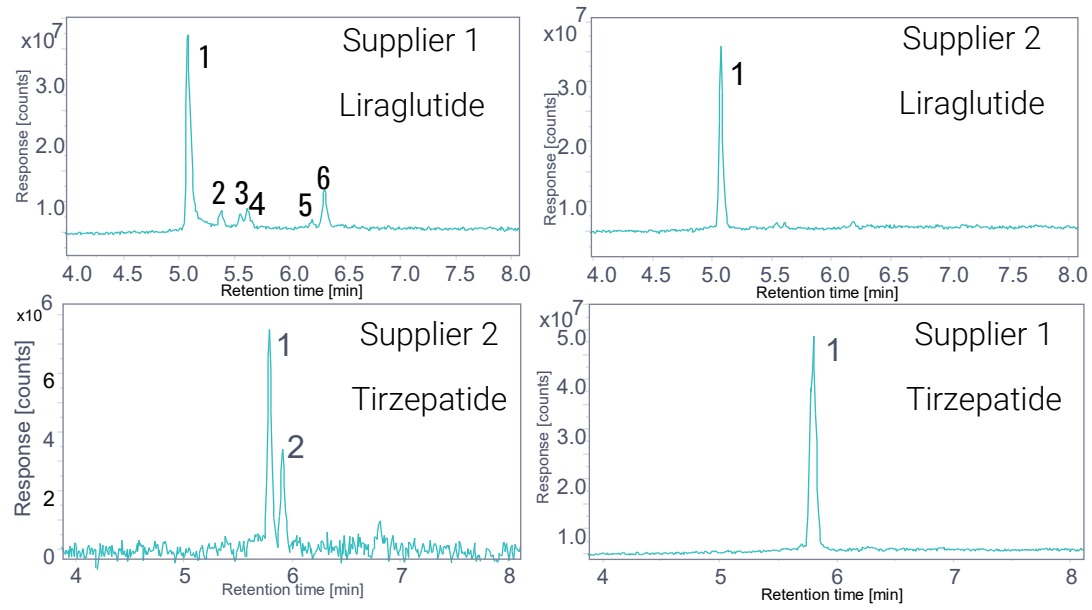


Figure 5. Total ion chromatogram of liraglutide and tirzepatide from different suppliers, , acquired using the Pro iQ Plus.

Conclusions

- The Altura Peptide Plus column provides efficient separation of closely related peptide species and impurities
- The method successfully differentiates impurity patterns across multiple suppliers of GLP-1 peptides, highlighting its value for supplier qualification
- LC/MS and LC/UV workflows enable high-confidence analysis of complex peptide therapeutics, including impurity and degradation profiling

References

1. Suresh Babu C V. Analysis of GLP-1 Agonists Using the Agilent 1290 Infinity II Bio LC System and Altura Ultra Inert HPLC Column. Agilent Technologies application note, publication number 5994-8837EN, 2025
2. Suresh |Babu C V. Therapeutic Peptide Analysis of GLP-1 Agonists. Agilent Technologies application note, publication number 5994-8951EN, 2026

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