Application Note

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Therapeutic Peptide Characterization of GLP-1 Agonists

Using an Agilent Altura Peptide Plus column and an Agilent InfinityLab Pro iQ Plus

Suitable for Agilent
1290 Infinity III LC

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Abstract

The biopharmaceutical industry requires analytical control of synthetic peptide therapeutics, particularly for identity and purity. Liquid chromatography coupled with mass spectrometry (LC/MS) is the method of choice for this analysis, providing high-resolution separation and precise mass identification, especially when sourcing peptides from multiple manufacturers with varying synthesis procedures. This study demonstrates the suitability of the Agilent Altura Peptide Plus column coupled with the InfinityLab Pro iQ Plus for characterizing therapeutic peptides.

Introduction

The integrity of synthetic peptide therapeutics is critical in the biopharmaceutical industry, demanding strict analytical control to ensure the safety and effectiveness of the final product. Confirming peptide identity through molecular weight analysis and assessing purity to detect impurities are basic to this process. For consistent manufacture, regulatory approval, and predictable biological performance, these analyses ensure molecular structure and the absence of impurities resulting from synthesis or degradation.[1] Liquid chromatography coupled with mass spectrometry (LC/MS) is an indispensable technique for this analysis, providing simultaneous high-resolution separation and precise mass identification.

When obtaining peptides from several contract manufacturers, each with different synthesis and purification procedures, thorough examination becomes even more crucial. Therapeutic peptides such as Glucagon-Like Peptide-1 (GLP-1) analogs are often produced by various CMOs or suppliers, each employing distinct solid-phase synthesis methods, protecting group strategies, and purification processes. These variations can lead to a diverse set of product-related impurities, including truncated species and postsynthetic modifications. Therefore, a comparative analysis of peptides from different suppliers is a basic requirement. It ensures uniform quality and confirms that every batch meets the standard specifications for identity, purity, and stability while facilitating supplier qualification.

This study demonstrates the suitability of the Agilent Altura Peptide Plus column for characterizing therapeutic peptides like GLP-1 from various suppliers, with emphasis on identity confirmation and impurity analysis. The column provided high-resolution separations of the main peptide API from its impurities and excellent mass spectrometry compatibility for accurate molecular weight confirmation.

Experimental

Reagents and chemicals

Semaglutide, liraglutide, retatrutide, and tirzepatide peptides were purchased from three different suppliers and stored according to the manufacturer's instructions. LC/MS-grade acetonitrile (ACN) was obtained from Agilent Technologies. Ultrapure water was collected from an in-house Millipore Sigma Milli-Q system.

Analytical equipment

- Agilent 1290 Infinity II bio LC system, including:
  - Agilent 1290 Infinity II bio high-speed pump (G7120A)
  - Agilent 1290 Infinity II bio multisampler (G7167B)
  - Agilent 1290 Infinity II thermostatted column compartment (G7116B)
- Agilent InfinityLab Pro iQ Plus (G6170A)

Software

Agilent OpenLab CDS (version 2.8)

Sample preparation

For molecular weight confirmation experiments, liraglutide, semaglutide, retatrutide, and tirzepatide peptides from Supplier 1 were dissolved to 1.0 mg/mL in DMSO. For comparative analysis, liraglutide and tirzepatide were dissolved to 1.0 mg/mL in 30% ACN.

LC/MS analysis

LC separation was performed on an Altura Peptide Plus column (p/n 227215-903) using a 15-minute gradient (Table 1). The raw data were acquired by an InfinityLab Pro iQ Plus (Table 2) and data analysis was performed in OpenLab CDS (version 2.8).

Table 1. Liquid chromatography parameters.

Parameter	Agilent 1290 Infinity II Bio LC System
Column	Agilent Altura Peptide Plus, 2.1 × 150 mm, 2.7 µm (p/n 227215-903)
Sample Thermostat	10 °C
Mobile Phase A	0.1% FA in water
Mobile Phase B	0.1% FA in ACN
Gradient	Time (min) %A %B 0.00 80 20 5.00 52 48 10.00 42 58 11.00 40 60 12.00 20 80 14.00 20 80 14.10 80 20 15.00 80 20
Column Temperature	60 °C
Injection Volume	5 µL
Flow Rate	0.4 mL/min

Table 2. MS data acquisition parameters.

Parameter	Agilent InfinityLab Pro iQ Plus
Ion Source	Agilent Jet Stream ESI source
Polarity	Positive
Gas Temperature	300 °C
Drying Gas Flow	11 L/min
Nebulizer	30 psi
Sheath Gas Temperature	250 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	3000 V
Nozzle Voltage	0
Fragmentor	95 V
Scan Type	Scan
Scan Time	500 ms
Data Storage	Profile
MS Spectrum Range	*m/z* 200 to 3000

Results and discussion

Molecular weight and purity

The analysis uses the Altura Peptide Plus column and Pro iQ Plus to confirm the molecular weight and assess the purity profile of four GLP-1 agonists: semaglutide, liraglutide, tirzepatide, and retatrutide. The primary goal is to verify the identity of the target peptide and identify any impurity-related peaks. Figure 1 shows the total ion chromatogram (TIC) and deconvoluted spectra for each of the GLP-1 agonists. The analysis shows a distinct purity profile for each GLP-1 agonist. Semaglutide shows a single, distinct peak in the TIC and deconvoluted spectrum, confirming the identity and indicating a high degree of purity with no significant impurities detected. The TIC for liraglutide is more complex, showing the main liraglutide peak along with five small impurity peaks. While the main compound is confirmed, the sample contains several low-level impurities that separate chromatographically. Tirzepatide presents a single, distinct peak in the TIC, but is accompanied by three coeluting impurities visible in the deconvoluted spectrum. Retatrutide shows a single, distinct peak in the TIC, similar to semaglutide and tirzepatide, but the deconvoluted spectrum reveals two coeluting impurities. The results demonstrate that the Altura Peptide Plus column is well suited for impurity analysis.

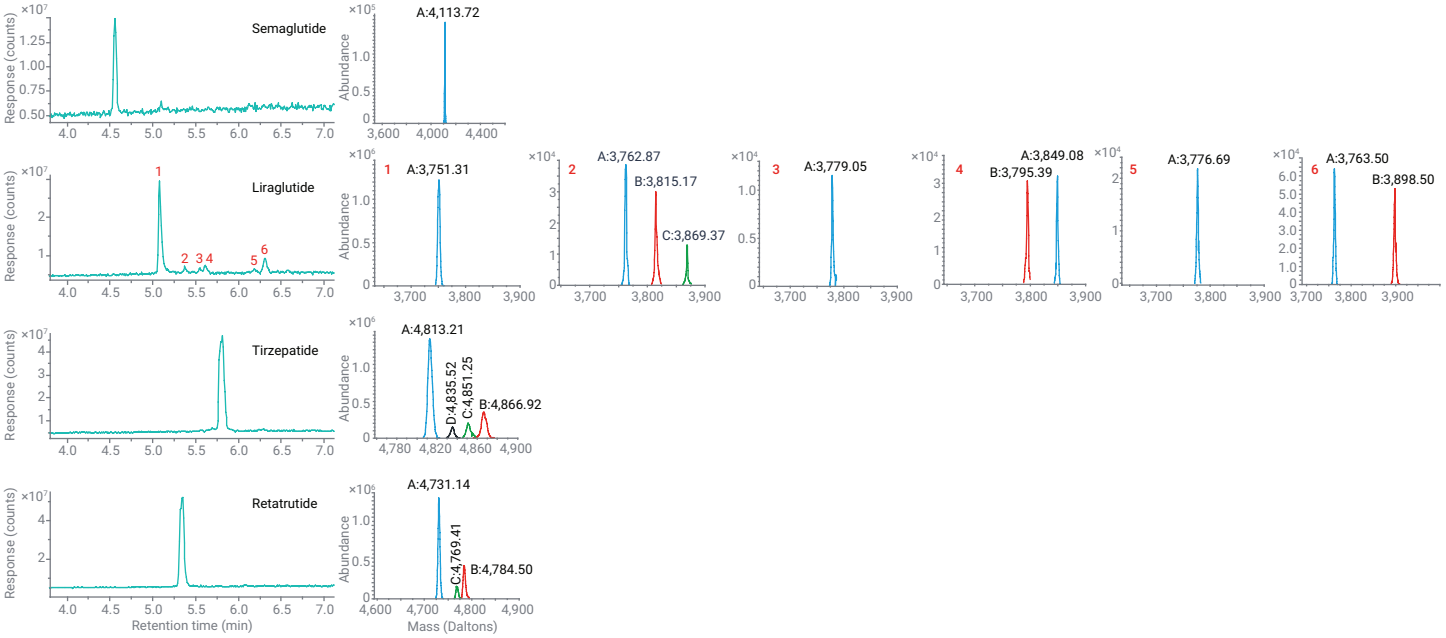
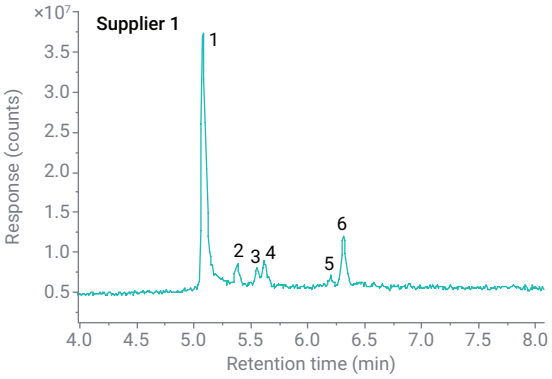


Figure 1. Total ion chromatogram and deconvoluted mass spectra of GLP-1 agonists.

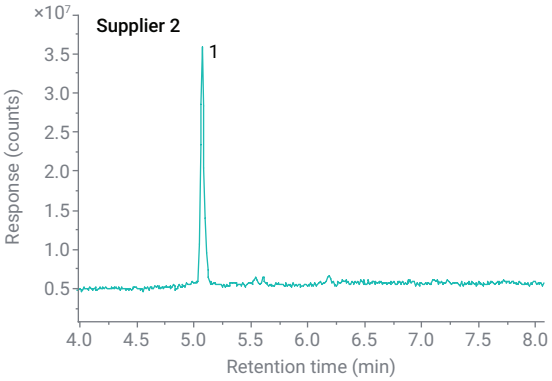
Analyzing GLP-1 from different sources

Peptide therapeutics like GLP-1 are frequently sourced from multiple contract manufacturers or suppliers. Each source may employ different solid-phase synthesis chemistries, protecting groups, and purification protocols, all of which can affect purity and stability. These variations can lead to a diverse set of product-related impurities, including truncated species and postsynthetic modifications. Therefore, a comparative analysis of peptides from different sources is essential to provide evidence for supplier qualification.

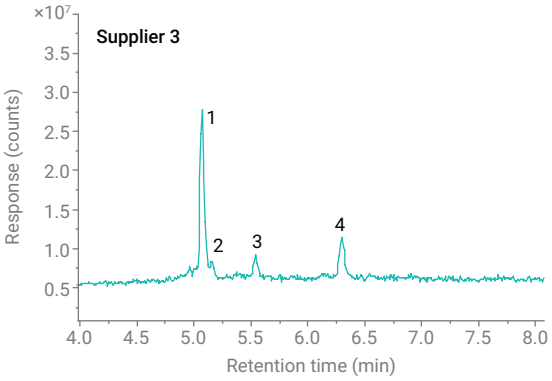
Figure 2 shows the TIC of liraglutide and tirzepatide from different suppliers. Analysis of liraglutide samples revealed clear differences in impurity profiles across the three suppliers. Supplier 2 exhibited the highest purity, featuring only the dominant liraglutide peak with no detectable impurities. Supplier 3 showed moderate purity, containing a few low-level impurity peaks with minor mass shifts. Conversely, Supplier 1 had the lowest purity, characterized by multiple impurity peaks showing significant mass shifts, suggesting higher levels of synthesis or degradation-related impurity.



Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
3751.31 (Liraglutide)	3762.87 (11.56) 3815.77 (64.46) 3869.37 (118.06)	3779.06 (27.75)	3795.39 (44.05) 3849.18 (97.87)	3776.69 (25.38)	3763.50 (12.19) 3898.50 (147.19)

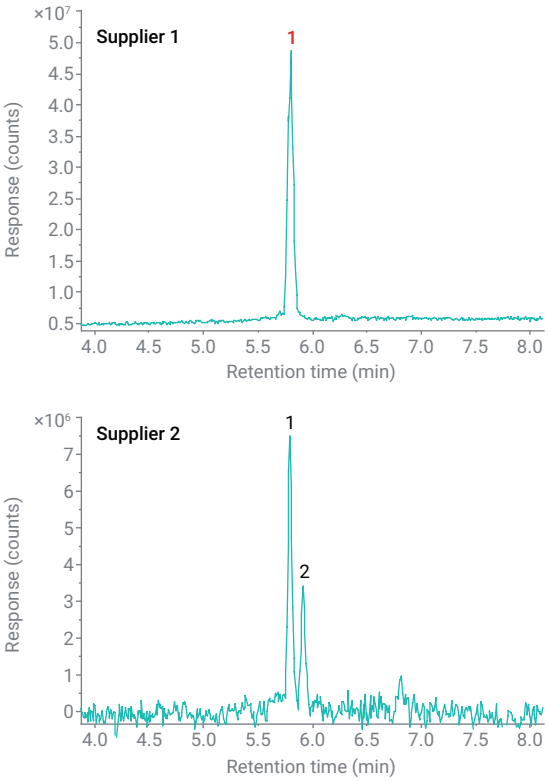


Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
3751.03 (Liraglutide) 3805.14 (54.11)	–	–	–	–



Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
3751.27 (Liraglutide)	3751.61 (0.34)	3779.53 (28.26) 3831.56 (80.29)	3763.55 (12.28)	–

Figure 2A. Total ion chromatogram of liraglutide from different suppliers. The mass difference (in Da) compared to the main peak (peak 1) is provided in parentheses. The impurity peak numbering across all chromatograms is independent of retention time.



Peak 1	Peak 2
4813.21 (Tirzepatide) 4835.52 (22.31) 4851.25 (38.04) 4866.92 (53.71)	–

Peak 1	Peak 2
4812.72 (Tirzepatide) 4866.30 (53.58)	4825.55 (12.83) 4878.91 (66.19)

Figure 2B. Total ion chromatogram of tirzepatide from different suppliers. The mass difference (in Da) compared to the main peak (peak 1) is provided in parentheses. The impurity peak numbering across all chromatograms is independent of retention time.

Analytical characterization of tirzepatide from two different suppliers revealed distinct differences in their purity profiles. Supplier 1 exhibited higher purity, dominated by the main tirzepatide peak. In contrast, Supplier 2 showed a lower purity profile with different mass species in addition to the main peak. This comparison demonstrates the effectiveness of the Altura Peptide Plus column and Pro iQ Plus for separating and sensitively detecting GLP-1 agonist impurities, facilitating rapid supplier quality assessment.

Conclusion

The study demonstrates that the Agilent Altura Peptide Plus column coupled with the Agilent InfinityLab Pro iQ Plus provides a robust platform for the analysis of GLP-1 agonists. The workflow effectively confirms molecular weight and identifies distinct impurity profiles for different peptides, highlighting variations in purity levels. Furthermore, the method successfully differentiates impurity patterns across multiple suppliers of liraglutide and tirzepatide, highlighting its value for supplier qualification and quality consistency assessments. This approach supports the rapid assessment of peptide therapeutics, contributing to the development and manufacture of safer and more effective biopharmaceuticals.

Reference

1. Colalto, C. Aspects of Complexity in Quality and Safety Assessment of Peptide Therapeutics and Peptide-Related Impurities: A Regulatory Perspective. *Regul. Toxicol. Pharmacol.* **2024**, *153*, 105699.

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