Poster Reprint

ASMS 2026
WP 604

LC/MS Characterization of GLP-1 Receptor Agonist Oligomers and Other Elusive Impurities

Rachel Franklin

Agilent Technologies, Inc., Corvallis, OR, US

Introduction

GLP-1 receptor agonists (GLP-1 RAs) are peptide therapeutics that can form impurities during synthesis, purification, and storage.[1]

Because impurities can affect product quality attributes, it is an important biotherapeutic development activity to characterize potential impurities that may arise. Detailed sequence information is needed to fully characterize impurities. Some impurities such as oligomerization and isomerization often push the limits of LC/MS workflows.

Here, we demonstrate a workflow for fast impurity screening without compromising the detailed impurity information by utilizing complementary LC/MS instruments:

- **Single Quadrupole MS:** Accessible and fast impurity screening using mixed-mode acquisition

- **Quadrupole Time-of-Flight MS:** Impurities flagged with the SQ are targeted for detailed sequence analysis with MS/MS

Impurities related to prolonged storage and forced oxidation were flagged for downstream characterization with a Q-TOF mass spectrometer.

For oligomerization analysis, an all-ions CID experiment was employed to reveal hidden differences between liraglutide isomers. (Figure 1)

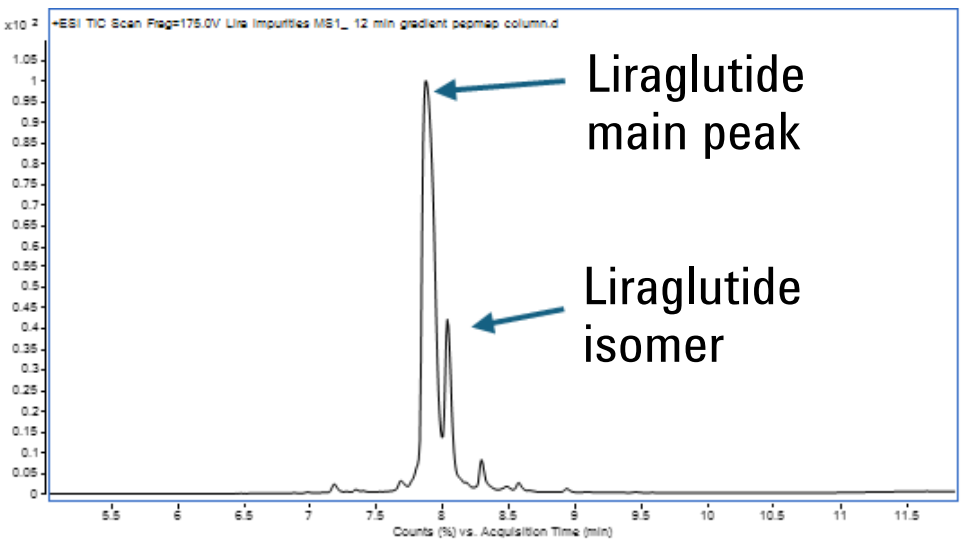

Figure 1. Total ion chromatogram (TIC) of liraglutide showing an abundant isomer peak and several low abundance impurities.

Experimental

Sample Preparation

Samples were diluted to 10µM in 15% acetonitrile and 0.1% formic acid. Liraglutide impurities were generated though forced oxidation with 0.75% hydrogen peroxide or through prolonged storage at 3°C for 15 months. For comparison, freshly prepared liraglutide samples were analyzed on the same day.

Instrumentation

The Altura PeptidePlus column was used for separation with the Agilent 1290 Infinity III Bio LC System. The InfinityLab Pro iQ Plus single quadrupole mass detector was used for oxidation impurity prescreening while detailed MS/MS investigation was accomplished using the 6545XT AdvanceBio LC/Q-TOF.

High *m/z* filter with all-ions CID for aggregate analysis

To study the dissociation pathways of high m/z oligomers, the quadrupole of the 6545XT Q-TOF was used as a filter to only transmit ions above 1,900 *m/z*. Then, monomer ejection patterns were monitored in the low *m/z* region using collision energy. (Figure 2)

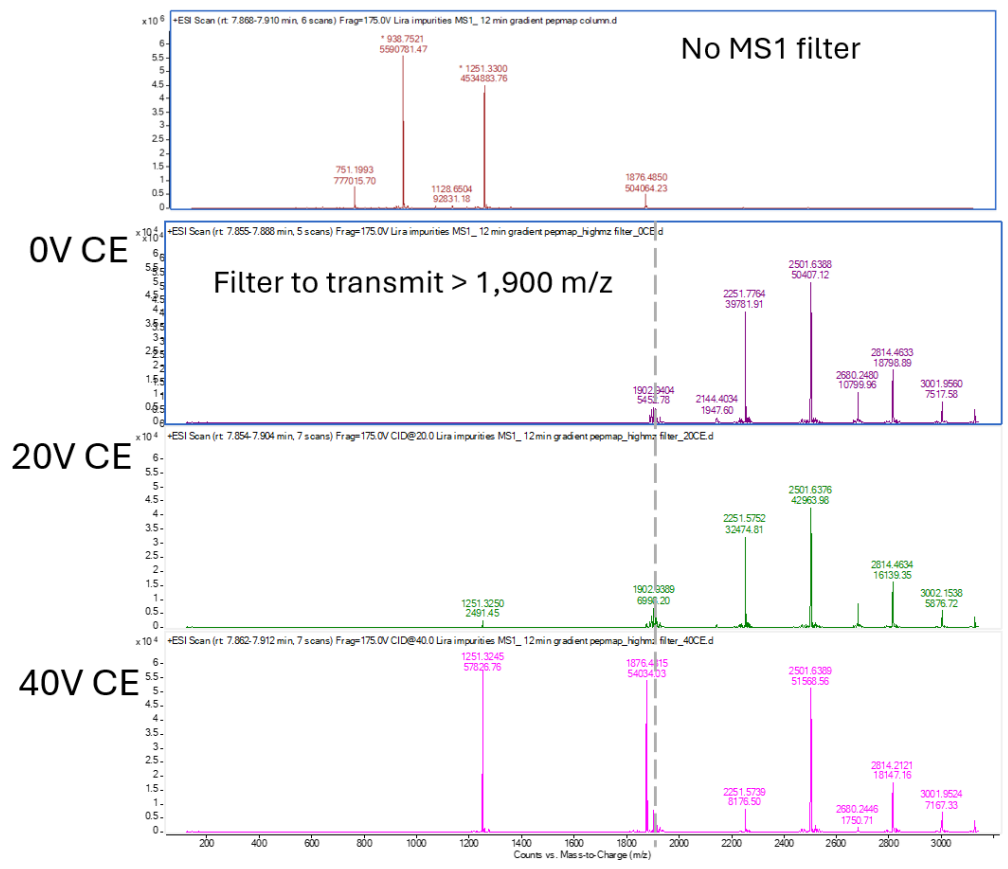

Figure 2. High *m/z* filter strategy for oligomer analysis.

Data Analysis

Data collection was performed using OpenLab Acquisition 2.8 and MassHunter Acquisition 11.0 . MassHunter Qualitative Analysis 12.0 was used for visualization of Q-TOF spectra. MassHunter BioConfirm 12.1 was used for sequence analysis while ExDViewer was used for sequencing impurities that were not identified by searching for predicted impurities.

Results and Discussion

Investigation of liraglutide oligomeric impurities

Comparison of liraglutide isomers showed distinct oligomerization behavior. Liraglutide rapidly formed oligomers in fresh samples but the isomer oligomerization was minimal. In aged samples, however, both isomers showed abundant oligomer formation. (Figure 3)

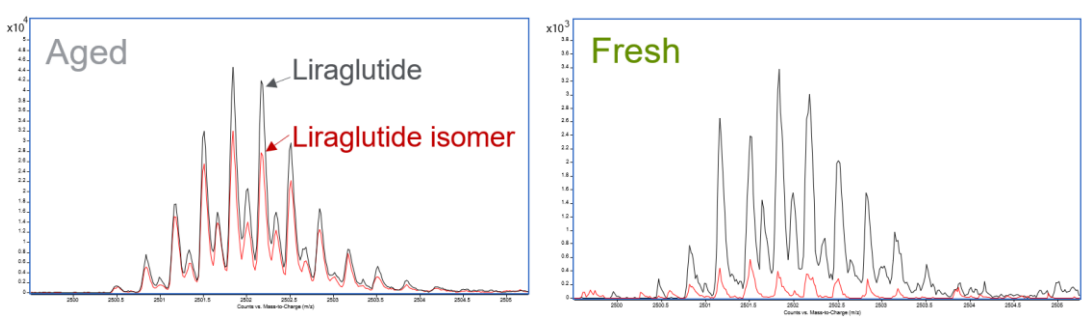

Figure 3. Overlapping dimer and tetramer signals at *m/z* 2501. The aged sample is on left and the freshly prepared sample is on the right.

Due to differences in oligomerization, CID was used to determine oligomer composition via controlled monomer dissociation. However, interpretation of dissociation pathways is complicated by overlapping isotope distributions from oligomer–oligomer and monomer–oligomer charge states. (Figure 3)

To overcome this issue, we explored a strategy which leverages the quadrupole as a high-pass *m/z* filter to remove non-oligomeric signals below 1,900 *m/z* . (Figure 2)

Monomer ejection rate was measured by recording the intensity of the 3^+ and 2^+ monomeric charge states as a function of collision energy (CE). This revealed that liraglutide isomers experience similar dissociation pathways for each charge state. (Figure 4)

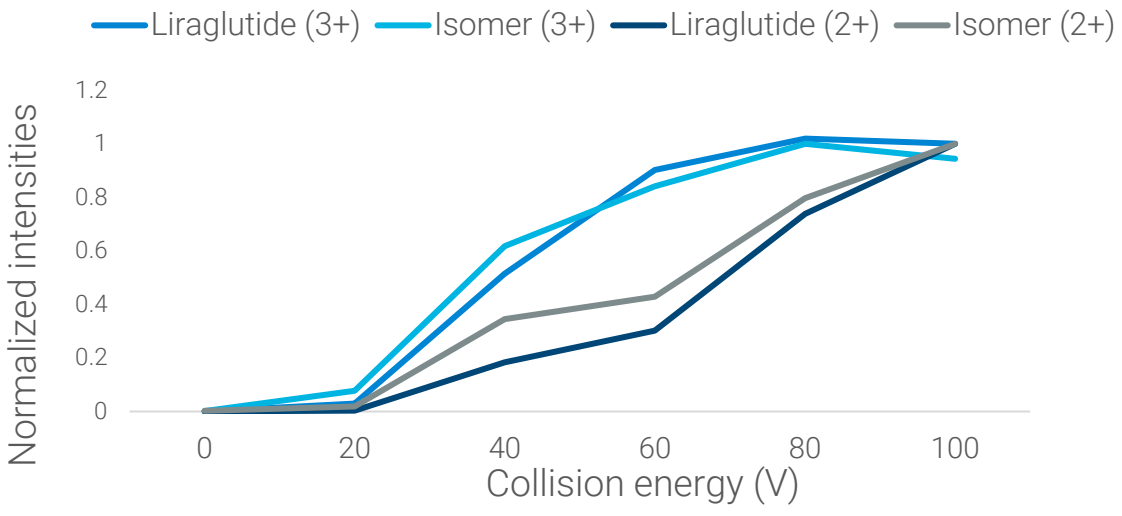

Figure 4. Intensities of monomer charge states for liraglutide and its isomeric form as a function of CE.

Although monomer ejection rates are similar between isomers, substantial differences are observed for the level of sodium adducts on ejected monomers.

For example, with 60V CE, only 1.8% of liraglutide monomers were sodiated while 49% of liraglutide isomers were sodiated, suggesting structural differences influence sodium adduction behavior. (Figure 5)

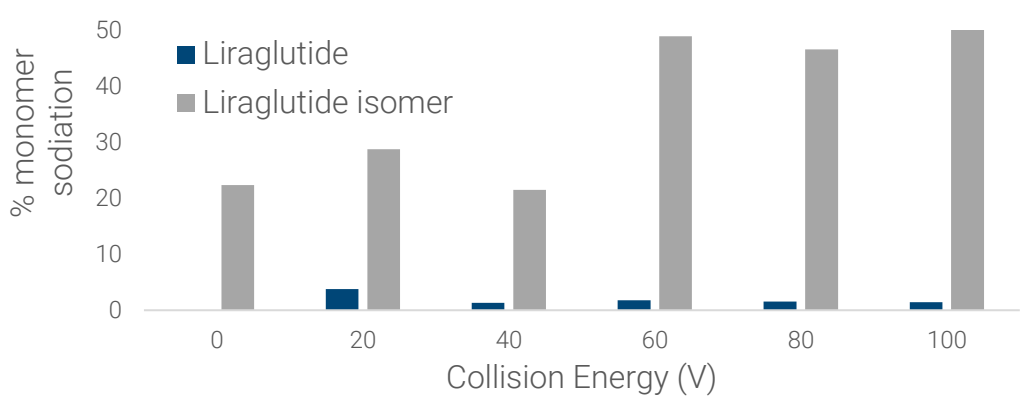

Figure 5. % sodiation relative to monomer 3^+ and 2^+ charge state intensities.

Guided de novo sequencing was used to localize the 12 Dalton addition to the N-terminus.

Impurity investigation of aged liraglutide revealed an abundant impurity 12 Da heavier than liraglutide which did not match the database. (Figure 6A)

Using non-targeted deconvolution and then generation of amino acid mass tags (Figure 6B), the 12 Da addition was localized to the N-terminus. This modification is consistent with reports of a carbon addition resulting from a reaction between excipient impurities and the N-terminal histidine. [2] (Figure 6C)

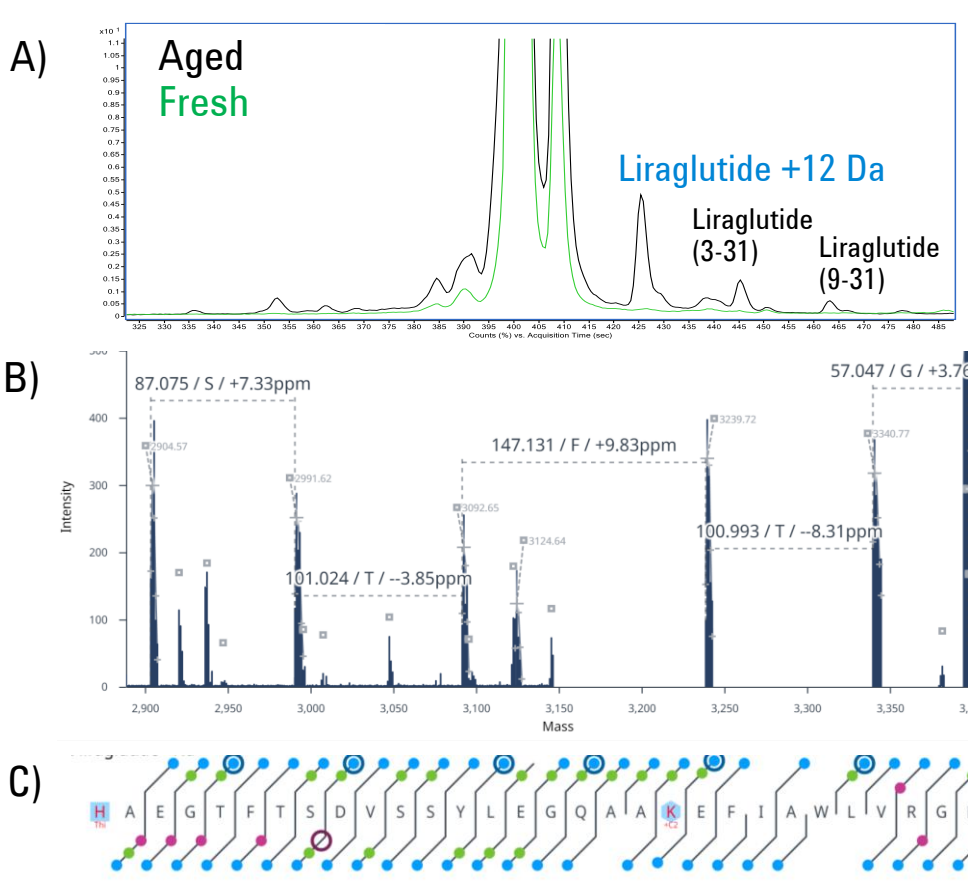

Figure 6. A) Liraglutide TIC with an abundant +12 Da impurity. B) De-charged spectrum with amino acid mass tags C) 12 Da modification localized to N-terminus.

Results and Discussion

Prescreening for impurities with the Pro iQ Plus SQ followed by detailed sequence analysis 6545XT Q-TOF

Forced oxidation experiments enable identification of oxidation hotspots within peptide sequences, supporting faster and more informed decision-making during development.

Using the Pro iQ Plus SQ, we rapidly screened liraglutide for the formation of oxidation impurities using the mixed-mode acquisition feature. (Figure 7)

Combining full scan and single ion monitoring (SIM) modes provides greater resolution and sensitivity for detecting impurities such as oxidation. Figure 7 highlights the complexity of oxidation patterns due to the presence of several isomers with unique retention times.

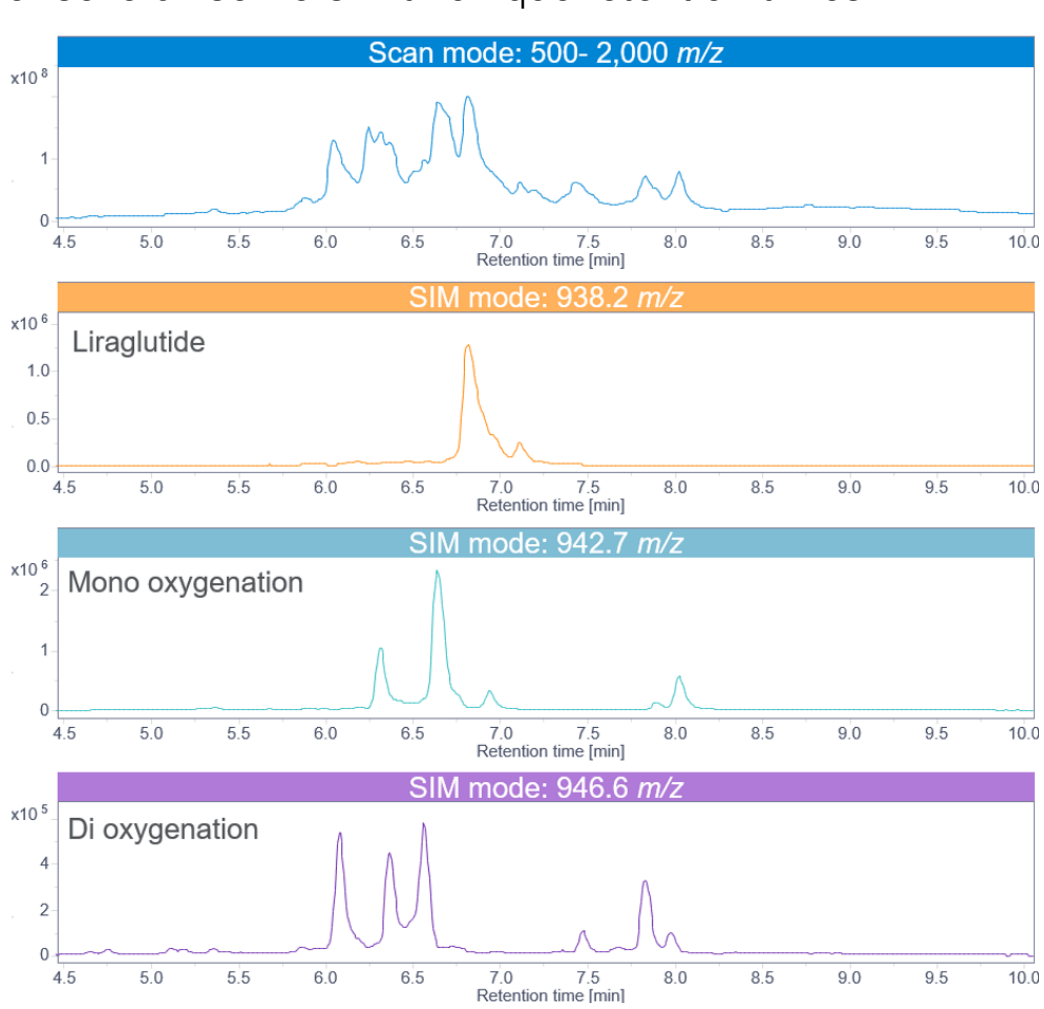

Figure 7. Mixed-mode acquisition using the Pro iQ Plus. The top spectrum contains the full scan range while the bottom spectra are targeting specific mass peaks representing unmodified liraglutide, mono oxidation, di oxidation.

Once impurities were flagged in the liraglutide samples, they were transferred to the 6545XT QTOF for downstream MS/MS analysis. CID was used to obtain sequence information and localize the oxidation.

Figure 8 shows the CID fragmentation pattern of di-oxygenated liraglutide. The oxidation modification was localized to the tryptophan in position 25.

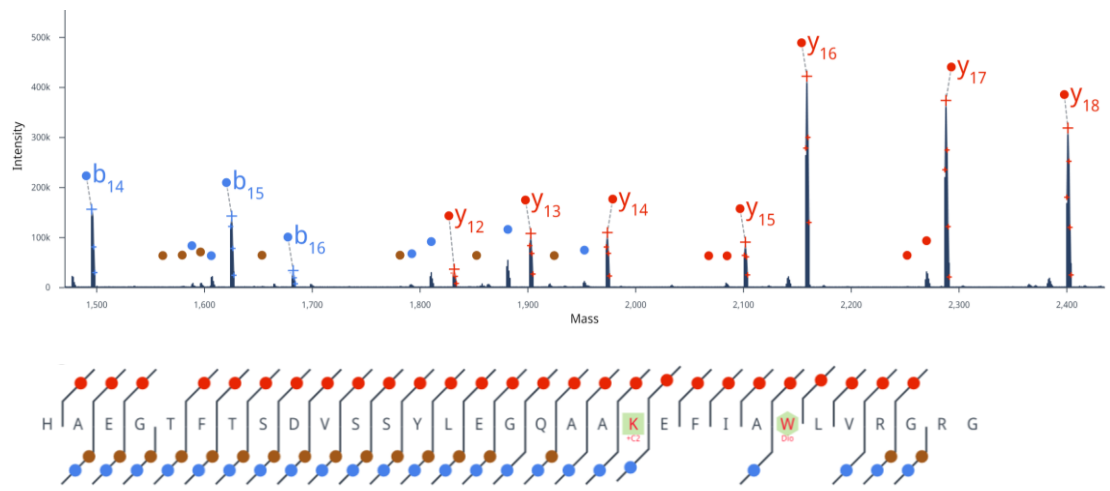

Figure 8. The top panel shows the b and y-type fragment ions in the deconvoluted mass spectrum. Each fragment corresponds to a circle in the sequence coverage map.

Conclusions

- Complementary LC/MS workflows enable fast screening and deep characterization of impurities, reducing time to confident impurity assignment.
- Mixed-mode acquisition on the InfinityLab Pro iQ Plus mass detector enables rapid screening of multiple oxidation products, supporting identification of oxidation hotspots in development.
- High m/z filtering with all-ions CID reveals distinct sodium adduct patterns between liraglutide isomers which may impact oligomerization pathways.
- Guided de novo sequencing enables the localization of unexpected impurities such as the addition of 12 Da at the N-terminus.

References

[1]Přáda Brichtová, E.; Edu, I. A.; Li, X.; Becher, F.; Gomes dos Santos, A. L.; Jackson, S. E.Effect of lipidation on the structure, oligomerization, and aggregation of glucagon-like peptide-1. Bioconjugate Chem. 2025, 36, 401−414.

[2]Sheikh, A. R.; Vitore, J. G.; Bhalekar, V. S.; Jain, S.; Kukreja, D.; Giri, T.; Sharma, N.; Benival, D.; Shah, R. P.Reactivity of N-terminal histidine of peptides toward excipients and excipient-related impurities: A case study of liraglutide excipient compatibility. J. Pharm. Sci. 2024, 113, 3246−3254

Conflict of Interest Statement:

The author is an employee of Agilent Technologies, which sells the instrumentation used in this analysis. As such, the author discloses a conflict of interest, in respect to their commercial involvement.

https://www.agilent.com/en/promotions/asms

DE-014611

Published in USA, June 30, ,2026

Agilent
Trusted Answers