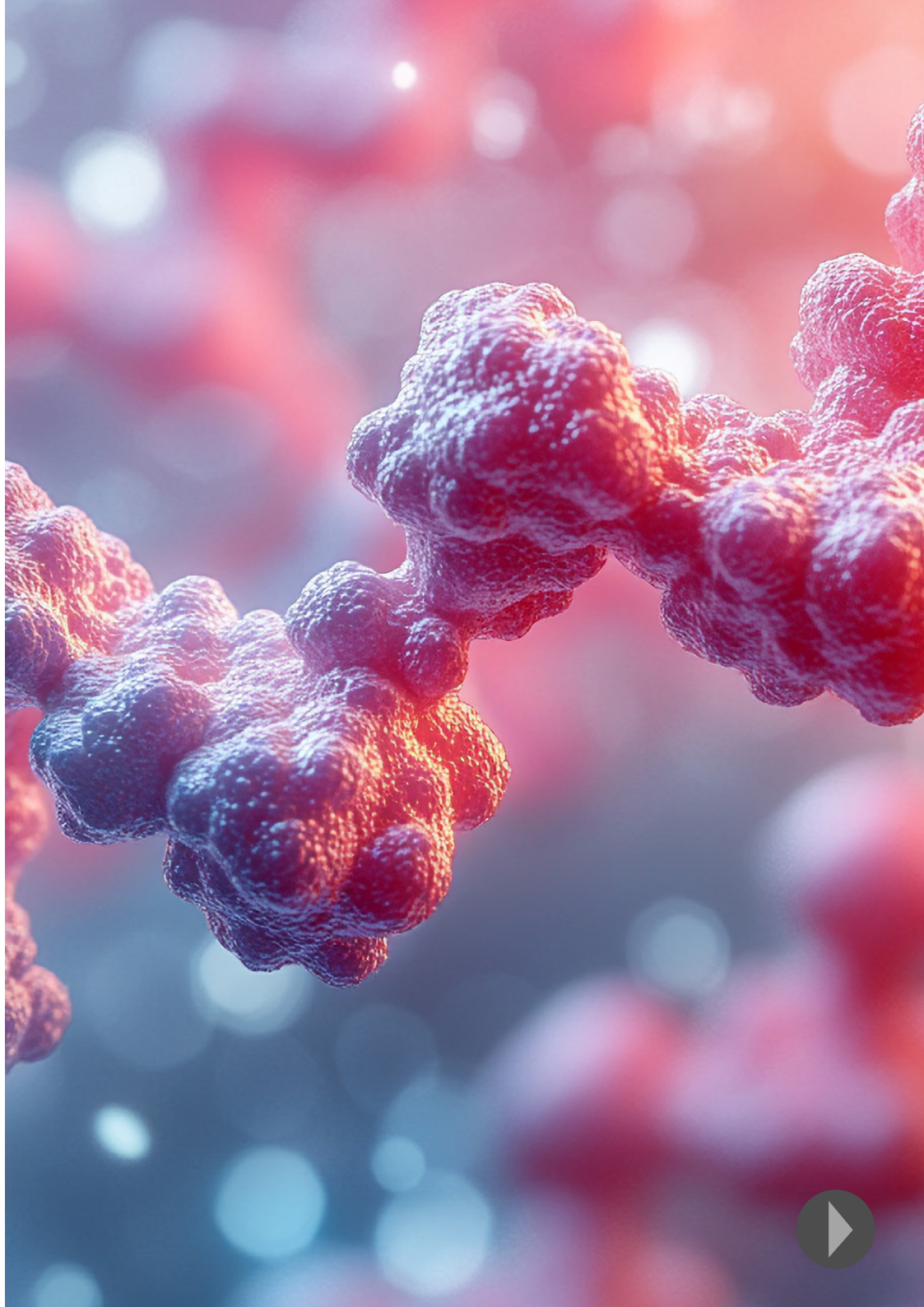
Waters™

Analytical Tools and Methodologies to Address the Challenges in the Development and Production of GLP-1 Receptor Agonists

APPLICATION NOTEBOOK



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INTRODUCTION

Glucagon-like peptide 1 receptor agonists (GLP-1RA) have rapidly emerged as a transformative class of therapeutics—initially developed for managing diabetes, they are now gaining widespread attention for their effectiveness in promoting weight loss as well as many other indications including obstructive sleep apnea, kidney disease, and reducing dementia risk. As their clinical applications expand, the need for robust purification, characterization, and monitoring strategies becomes increasingly critical to ensure safety, efficacy, and regulatory compliance.

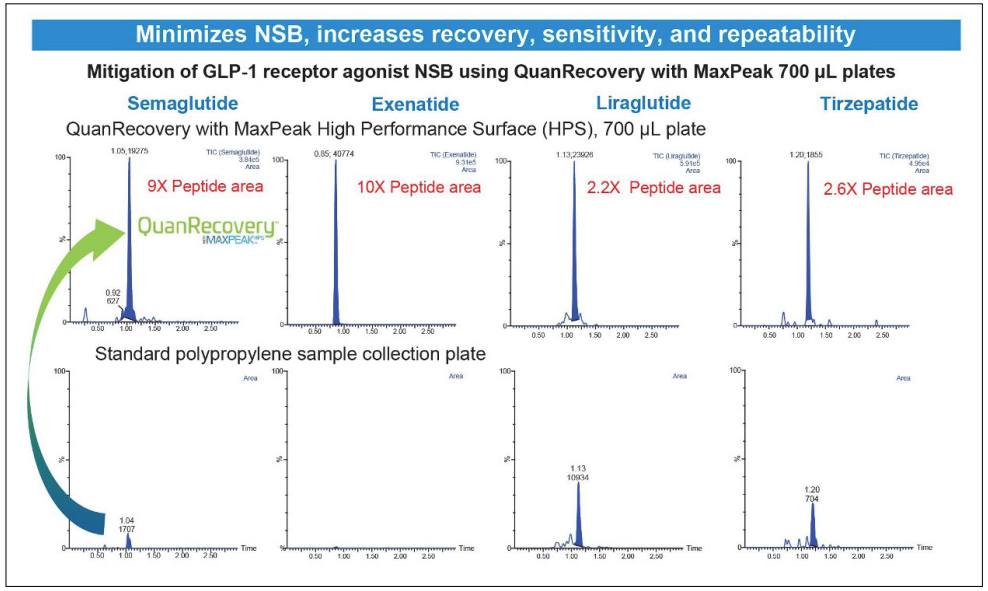
This application notebook focuses on the analytical challenges and solutions associated with GLP-1RA. Therapeutic peptides continue to be one of the fastest-growing segments in the pharmaceutical industry, offering advantages in efficacy, safety, and tolerability over many small molecule drugs. However, their complexity demands precise control over structure and impurity profiles to meet stringent quality standards.

Within these pages, you'll find a collection of workflow-based solutions from Waters, designed to support the analysis, characterization and purification of GLP-1RA. These user-friendly tools are intended to streamline your processes and accelerate development timelines.

We've created this notebook as a practical resource to support your research and development efforts. Whether you're working directly with GLP-1RA or exploring other peptide-based therapeutics, we hope you find the insights and applications presented here both valuable and actionable.

Improved GLP-1 Receptor Agonist Peptide Recovery Using a QuanRecovery with MaxPeak High Performance Surfaces (HPS) Collection Plate

Read the full application note.



Representative LC-MRM-MS chromatograms demonstrating mitigation of non-specific binding to collection plates for the GLP-1 receptor agonist therapeutic peptides, semaglutide, exenatide, liraglutide, and tirzepatide (1 ng/mL prepared in a solution of 80/20/1 water, acetonitrile, and formic acid) prepared in a QuanRecovery with MaxPeak HPS, 700 µL Collection Plate vs standard polypropylene sample collection plates.

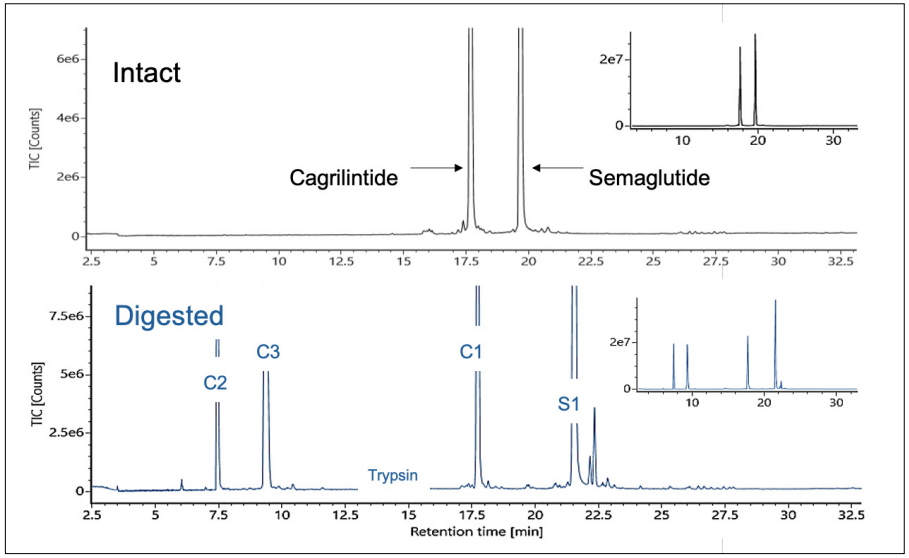
APPLICATION BENEFITS

- Use of QuanRecovery with MaxPeak HPS Collection Plates effectively mitigate NSB of the GLP-1 receptor agonist therapeutic peptides, significantly improving LC-MS detection and robustness of the assay.

GLP-1 (glucagon-like peptide-1) receptor agonist therapeutics are used to treat metabolic diseases like type 2 diabetes and obesity. However, analyzing them with liquid chromatography-mass spectrometry (LC-MS) can be challenging due to their large size and the hydrophobicity of their fatty acid conjugate moieties. A major issue with these peptides is non-specific binding (NSB), where the peptides interact with and adhere to surfaces or materials used during sample preparation, resulting in peptide loss and unreliable results. In this study, we demonstrate that the use of a Waters QuanRecovery with MaxPeak HPS 700 µL Collection Plate effectively reduces non-specific binding and improves recovery of GLP-1 peptides such as semaglutide, exenatide, liraglutide, and tirzepatide. Ultimately, this is an improvement in analyte sample handling that can help improve assay sensitivity and reliability.

Characterization and Impurity Profiling of Combined Amylin and GLP-1 Analogs with RapiZyme Trypsin

Read the full application note.



Total ion chromatograms of intact cagrilintide/semaglutide (top) and RapiZyme Trypsin-digested cagrilintide/semaglutide (bottom). Sequence and mass information for the intact and tryptic peptides are listed in the table.

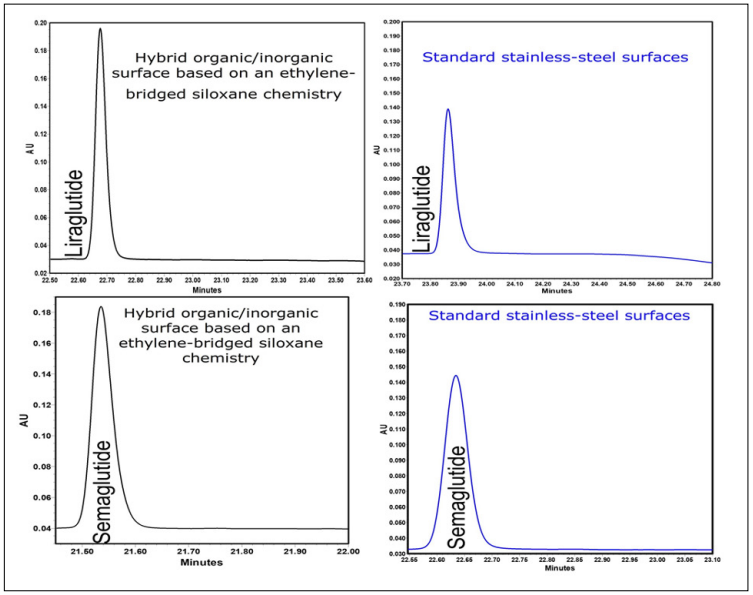
APPLICATION BENEFITS

- RapiZyme Trypsin delivers a comprehensive, bottom-up analysis of the combined therapeutic with more than 99.5% digestion completion, thereby aiding in the characterization of peptide residue modifications. Benefits RapiZyme Trypsin delivers a comprehensive peptide map of combined cagrilintide and semaglutide with >99.5% digestion completion
- Digestion improves chromatographic separation of peptides with minor amino acid residue modifications
- Liquid chromatography-mass spectrometry (LC-MS) analysis of digests facilitates peak identification and characterization of peptide residue modifications

Glucagon-like peptide 1 receptor agonists (GLP-1RAs) are a class of biotherapeutic peptide-like drugs currently undergoing a rapid increase in demand and use. These peptide-based therapeutics are often fatty acid modified to improve their potency, complicating their reversed phase (RP) impurity analysis. Combined lipopeptide therapeutics that target more than one receptor are in development for improved treatment of Type 2 diabetes and weight loss. Here, Waters™ RapiZyme Trypsin is used for the characterization and impurity analysis of combined amylin and GLP-1 analogs, cagrilintide and semaglutide.

Development of Separation Methods for GLP-1 Synthetic Peptides Utilizing a Systematic Protocol and MaxPeak™ High Performance Surface Technology

Read the full application note.



Representative chromatogram of method performance comparing MaxPeak™ HPS Technology and traditional stainless-steel systems for liraglutide and semaglutide.

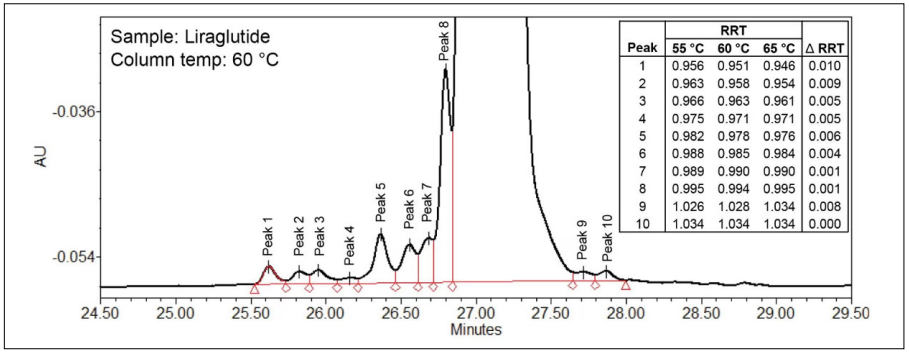
APPLICATION BENEFITS

- Successful separation methods for synthetic peptides can be generated with a systematic protocol as outlined in the MaxPeak Premier Reversed-phase Column Screening Kit.
- Empower tools such as PDA peak purity processing and mass spectra data from the ACQUITY™ QDa™ can provide confidence in impurity identification.
- MaxPeak HPS Technology was shown to improve the chromatographic peak area, height, and tailing of synthetic peptides and impurities compared to traditional stainless-steel systems.

GLP-1s are synthetic peptide drugs used to treat type-II diabetes and obesity. Recently, GLP-1s, such as semaglutide, have risen in demand. Currently, there is not a single method capable of separating and identifying a full panel of the GLP-1s being prescribed today. In this application, we developed a reproducible HPLC-UV/MS method that covers a variety of GLP-1s currently on the market. We also show the capability of this method to separate and identify related impurities. Finally, we demonstrated the benefits of using MaxPeak™ High Performance Surface (HPS) Technology, when compared to traditional stainless-steel systems, for synthetic peptide analysis.

Temperature Dependence on Reversed-Phase Separations of Fatty Acid Modified GLP-1 Receptor Agonists and Their Impurities

Read the full application note.



Peak	RRT 55 °C	RRT 60 °C	RRT 65 °C	Δ RRT
1	0.956	0.951	0.946	0.010
2	0.963	0.958	0.954	0.009
3	0.966	0.963	0.961	0.005
4	0.975	0.971	0.971	0.005
5	0.982	0.978	0.976	0.006
6	0.988	0.985	0.984	0.004
7	0.989	0.990	0.990	0.001
8	0.995	0.994	0.995	0.001
9	1.026	1.028	1.034	0.008
10	1.034	1.034	1.034	0.000

Integrated and annotated RPLC-UV results for liraglutide on the ACQUITY Premier Peptide CSH C_{18}, 1.7 µm Column at 60 °C. Relative retention times (RRT, calculated relative to the parent peak) at each column temperature and ΔRRTs are listed in the inset table. ΔRRTs represent the difference between the maximum and minimum RRTs across the reported temperature range.

APPLICATION BENEFITS

- A method to modulate the resolution of GLP-1 RA impurity species during reversed phase separations
- Insights into the importance of tightly controlling column and mobile phase temperatures for the reversed-phase analysis of fatty-acid modified GLP-1 Ras

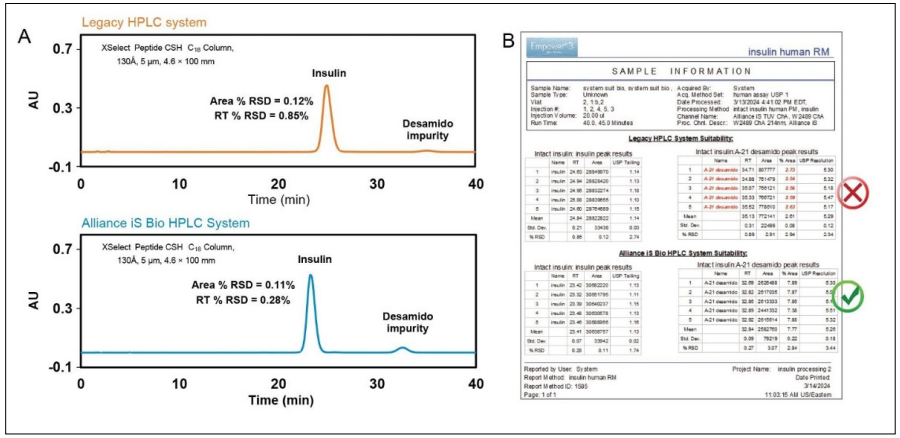
Here, we show that the reversed-phase separation of fatty acid modified GLP-1 RAs and their impurities is highly dependent on column temperature. The temperature dependence of these separations can be used to modulate and optimize resolution of critical impurity species. These results also emphasize that strict temperature control for these separations is essential, and capability should be considered when selecting instrumentation for reversed phase separation of fatty acid modified GLP1-RAs.

Leveraging the Alliance™ iS Bio HPLC System as a Modern HPLC for Peptide Drug Substances Analysis in QC Environments

Read the full application note.

APPLICATION BENEFITS

- The Alliance iS Bio HPLC System delivers consistent performance for QC environments enabled by low dispersion volume and improved mixing behavior
- Modernization of compendial methods reduces operating costs and analysis time



Intact insulin identification using compendial method. Both the legacy HPLC system and Alliance iS Bio HPLC System are able to (A) resolve the insulin human peak from the A-21 desamido impurity and (B) meet the three acceptance criteria of monographs. The Alliance iS Bio HPLC System was able to deliver a higher degree of accuracy in the composition of the mobile phase. In Empower report, results that failed to meet monograph acceptance criteria are flagged in red.

The Alliance iS Bio HPLC System with MaxPeak™ High Performance Surfaces (HPS) Technology is a bio-inert liquid chromatography (LC) system designed to reduce surface/analyte interaction for biopharmaceutical manufacturing environments. As a modern HPLC instrument, the Alliance iS Bio HPLC System is engineered with features including Waters innovative MaxPeak HPS, lower dispersion volume, and a large volume mixer. Together with MaxPeak Premier columns, these features enable manufacturing environments to modernize methods, save resources, and increase throughput for biopharmaceuticals. In this study, we evaluate the Alliance iS Bio HPLC System in a routine QC testing environment for peptide-size biopharmaceuticals.

Insulin, insulin analogs, glucagon, and GLP-1 peptides were chosen as test cases given their relevance as classic and new treatment of diabetes with benefits of weight management. The study results demonstrate the Alliance iS Bio HPLC System is capable of running legacy compendial methods while allowing users the flexibility to take advantage of modern column chemistries to reduce operating costs. When compared against a legacy HPLC system, results demonstrate the Alliance iS Bio HPLC System is well suited for QC environments in the analysis and routine testing of biopharmaceuticals.

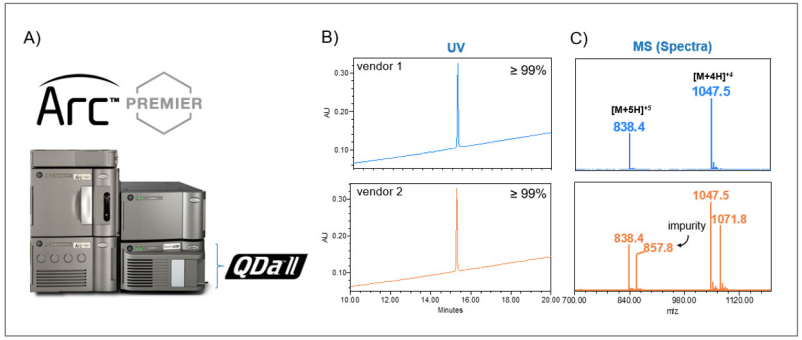
Accelerating Method Development and Manufacturing of GLP-1 Analogs with LC-UV/MS

Read the full application note.

Glucagon-like-peptide-1 (GLP-1) receptor agonists have recently gained significant attention as a metabolic regulator for treating type 2 diabetes and obesity resulting in unprecedented demand. In response, manufacturers are actively reassessing their production processes to enhance efficiency and meet growing market needs. Solutions that can utilize information more efficiently to expedite decisions in a timely manner are required to ensure that manufacturers can meet regulatory standards and drug product safety while improving productivity.

In this study, we present a QC-friendly LC-UV/MS workflow designed to alleviate the workload on analytical laboratories. Access to orthogonal mass data allows supporting labs to make quicker and more informed decisions during method development, reducing errors, and increasing overall productivity.

This solution integrates the ACQUITY™ QDa™ II Mass Detector with Empower™ Chromatography Data System (CDS) as a compliance-ready, scalable software solution for instrument control, data acquisition, review, and reporting with full audit trail capabilities coupled with the Arc™ Premier System. The capabilities of this unique LC-UV/MS platform were demonstrated through a real-world use case involving the rapid detection and putative identification of a process-related impurity in a GLP-1 analog.



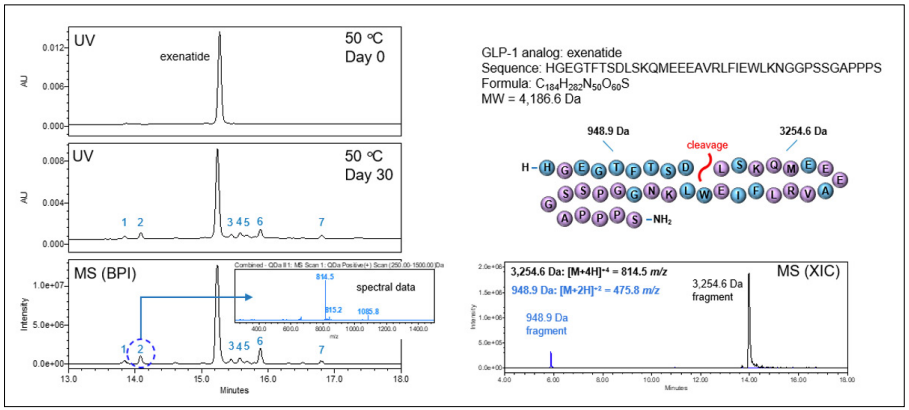
The ACQUITY QDa II Mass Detector configured as an in-line detector with the ACQUITY Premier UPLC System. Exenatide from two vendors were compared for purity via an ACQUITY QDa II Mass Detector. (B) UV chromatograph. (C) Spectra of the main species peak.

APPLICATION BENEFITS

- Orthogonal mass information enables detection of impurities that may be overlooked using traditional UV-based detection, increasing confidence in results.
- ACQUITY QDa II Mass Detector enhances analytical labs' capabilities to more effectively investigate and resolve out-of-specification results.
- The integrated LC-UV/MS workflow helps manufacturers meet regulatory requirements while ensuring both product safety and operational productivity.

Application of LC-UV/MS Workflows to Increase Efficiency in Impurity Profiling of GLP-1 Analogs

Read the full application note.



An Exenatide control sample (top panel) was exposed to thermal stress for a 30-day period (middle panel) with 7 "new" peaks observed in the stressed sample. Spectral data was used to calculate the observed mass of each peak for putative identity of degradants (bottom panel). B) As an example, peak 2 was determined to have an observed mass of 3,254.1 Da. which correlates to a non-enzymatic cleavage occurring between aspartic acid and leucine residues based on GLP-1 sequence information and was confirmed with retention time and mass using extracted ion chromatograms.

APPLICATION BENEFITS

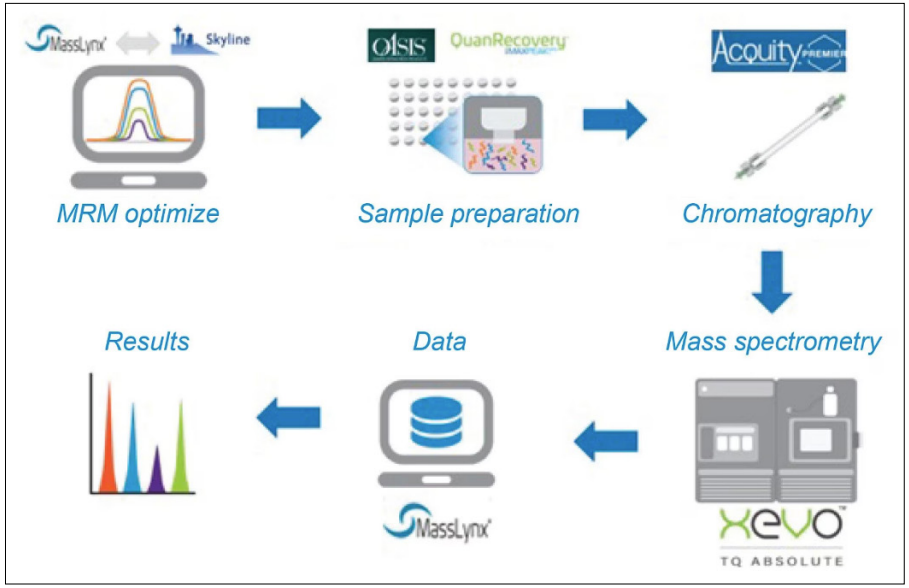
- Orthogonal mass information provides data-driven insights on forced degradation impurities generated via oxidation, pH and thermal stress.
- ACQUITY QDa II Mass Detector enhances analytical labs capabilities in raw material screening, process control, lot release, and stability monitoring.
- LC-UV/MS workflow expedites risk-based decision making and increases overall productivity in the lab.

Glucagon-like-peptide-1 receptor agonists have gained significant attention recently as a metabolic regulator for treating type 2 diabetes and obesity. The increased visibility as a weight-loss treatment has increased demand and propelled research into new analogs and delivery methods. The surge in demand has introduced analytical challenges from peptide synthesis, impurity profiling, and stability monitoring, as manufacturers look to drive efficiency and scale-up production. LC-UV/MS platforms offer the potential to alleviate the burden on analytical laboratories during late-stage method development and early manufacturing phases of GLP-1 analogs. Access to orthogonal mass information allows support labs to make informed decisions more efficiently to reduce errors and increase overall productivity.

In this study, the unique capabilities of a LC-UV/MS workflow to speed-up analysis are demonstrated in the identification of impurities related to GLP-1 analogs that underwent chemical and thermal stress conditions.

Comprehensive Workflow for the Quantification of Peptides and Proteins in Plasma: Semaglutide a Case Study

Read the full application note.



Schematic of the Optimized Comprehensive workflow for the bioanalytical analysis of peptides by LC-MS/MS.

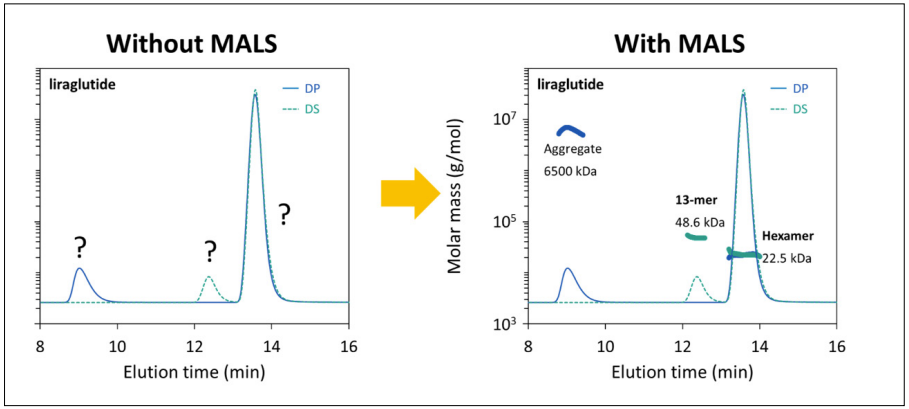
APPLICATION BENEFITS

- Xevo TQ Absolute Mass Spectrometer enabled high sensitivity quantification of semaglutide at sub ng/mL levels (LLOQ of 0.2 ng/mL) enabling accurate determination of PK elimination phase
- Oasis™ MAX µElution mixed-mode SPE plates delivers high selectivity for isolation of semaglutide from human plasma enabling analyte concentration, cleaner extracts and improved sample recovery
- MaxPeak™ High Performance Surfaces (HPS): Mitigates non-specific binding facilitating increased sensitivity through high peptide recovery and improved peak shapes with HPS in Waters QuanRecovery™ Plates, Premier columns, and ACQUITY Premier UPLC
- The integrated MassLynx-Skyline Interface (MSI) provides a workflow for simplifying MRM method development for peptides, eliminating the need to manually evaluate a large number of potential MRM transitions

Over the last two decades we have seen a significant increase in protein and peptide-based therapeutics, with “biologics” accounting for 21 of the 55 new medicines approved in 2023, most notably the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide. These protein and peptide therapies typically show high potency and specificity they also have a long pharmacokinetic (PK) half-life resulting in nmol/L or lower concentration levels in the circulatory system requiring a high sensitivity bioanalytical assay to accurately define the compound PK. The development of a suitable LC-MS/MS method for the quantification of proteins and peptides in biofluids is complicated by the formation of precursor ions with multiple charge states and the large number of possible product ions to be evaluated. Here we demonstrate the use of an automated workflow to select, optimize and compare peptide MRM transitions via MassLynx™-Skyline Interface for the high sensitivity quantification of semaglutide in human plasma using the Xevo™ TQ Absolute Mass Spectrometer coupled with an ACQUITY Premier UPLC.

Identification of GLP-1 Analog Oligomeric States Using SEC-MALS

Read the full application note.



UV chromatograms of liraglutide drug substance and generic drug product showing the native hexamer and aggregate species. The molar mass measured by MALS is overlaid on each peak.

APPLICATION BENEFITS

- The identification and quantification of oligomeric states, aggregates, and fibrils of GLP-1 receptor analogs is of vital importance to ensure the quality, safety, potency, and efficacy of these peptide therapeutics.
- SEC-MALS, both under native and non-native conditions, provides stable and reproducible quantitation of monomers, native oligomers and large aggregates as well as insights into more complex behavior, like reversible association.

Glucagon-like peptide-1 analogs (GLP-1a), like liraglutide and semaglutide, are among the highest-growing therapies with seven FDA-approved treatments and more than 50 in clinical trials as of 2024. A key challenge during their development, formulation, and manufacturing is the formation of complex structures that can span from oligomers to aggregates and fibrils. To ensure the safety and efficacy of GLP-1a based therapeutics, it is critical to characterize these complex structures to formulate the product properly to avoid the undesirable high order structures. This application note demonstrates the use of size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) under both native and denaturing conditions to identify, quantify, and characterize the monomer, oligomeric state and aggregates present in the commercial GLP-1a products and biosimilars.

SOLUTIONS

Learn more about the products, solutions, and services featured in this Application Notebook:

SYSTEMS

- ACQUITY Premier
- ACQUITY QDa II
- Alliance iS HPLC
- Alliance iS Bio
- Arc Premier
- BioAccord System
- Patrol UPLC
- Wyatt DAWN
- Xevo G3 QTof
- Xevo TQ Absolute

INFORMATICS & SOFTWARE

- Empower Software Solutions
- waters_connect

CHEMISTRIES & CONSUMABLES

- ACQUITY Premier Columns
- XBridge Premier Columns
- XSelect Premier Columns
- MaxPeak Premier SEC Columns
- MaxPeak Premier Solutions

SERVICE & SUPPORT

- Waters Column Coach
- Award Winning Services
- FlexUp Technology Replacement Program
- Leasing Financing

CONCLUSION

As the therapeutic landscape continues to evolve, particularly GLP-1 receptor agonists—are playing an increasingly vital role in addressing complex health challenges. Ensuring their quality, safety, and efficacy requires analytical tools that are not only powerful but also practical for everyday use in the lab. Through the workflows and examples presented in this notebook, we hope to have provided valuable insights and actionable guidance to support your efforts in peptide purification, characterization, and impurity monitoring. Whether you're advancing early-stage research or refining manufacturing processes, we trust this resource will serve as a helpful companion in your journey toward developing safe and effective peptide-based therapeutics.

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