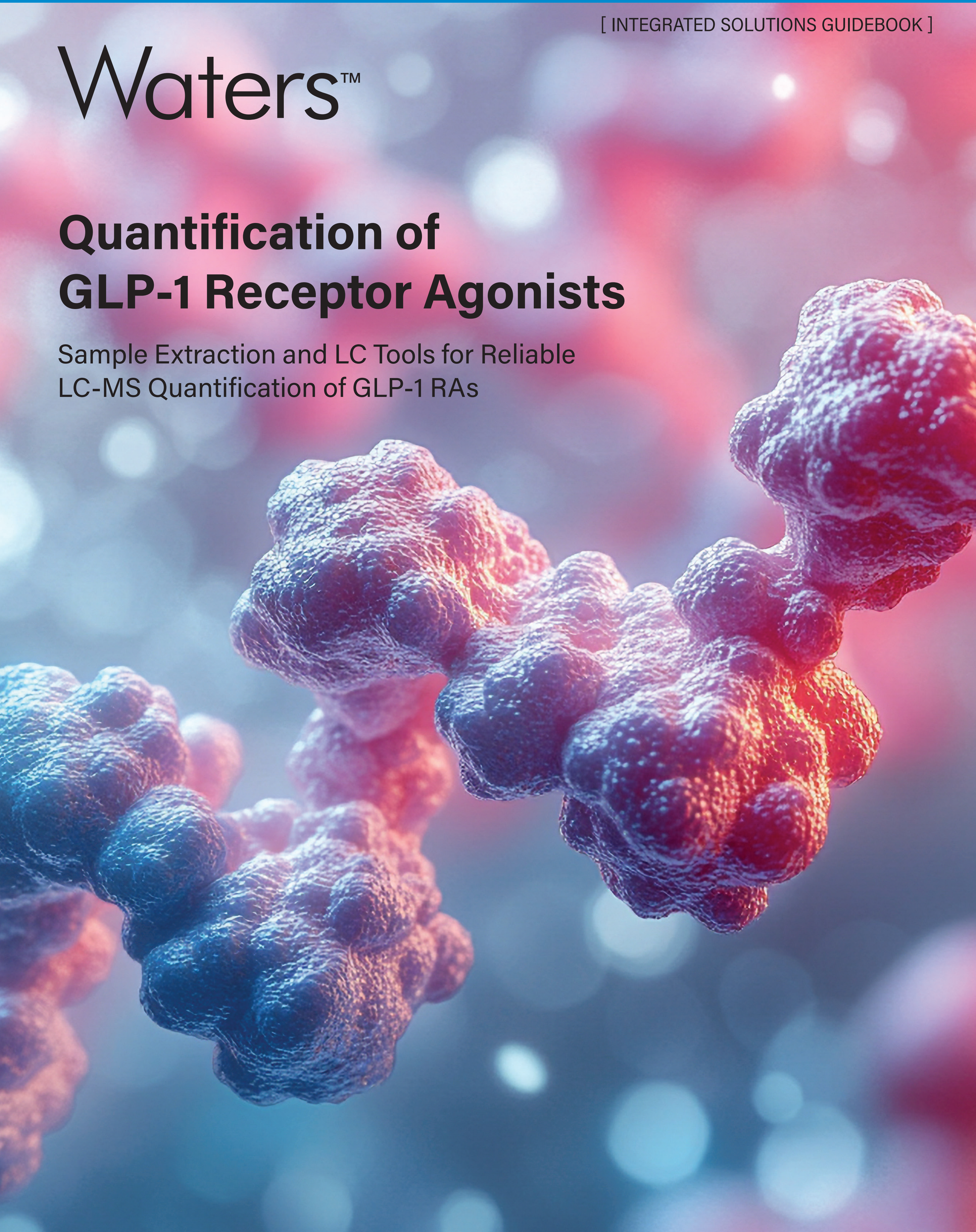
[INTEGRATED SOLUTIONS GUIDEBOOK]

Waters™



Quantification of GLP-1 Receptor Agonists

Sample Extraction and LC Tools for Reliable LC-MS Quantification of GLP-1 RAs

Introduction

Quantification of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) is increasingly critical as these therapies for type II diabetes and obesity are projected to exceed $200 billion in annual sales by 2031. With rapid growth in both innovator and generic programs, reliable bioanalysis is essential for discovery, development, and bioequivalence assessment.

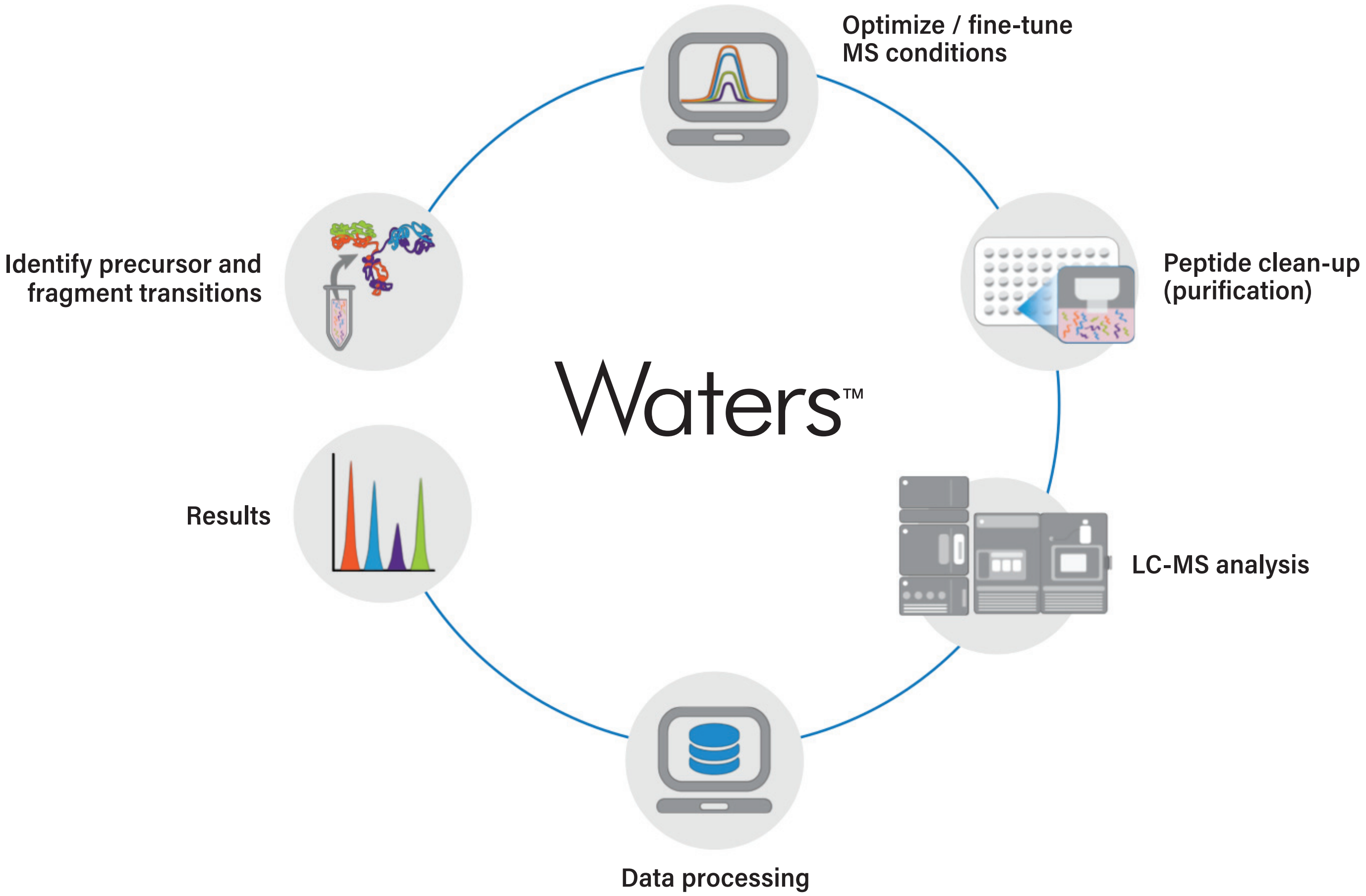
Unlike small molecules or monoclonal antibodies, peptides present unique bioanalytical challenges such as chemical instability, rapid enzymatic degradation, and non-specific binding (NSB). Directly applying small or large molecule workflows often fails, making purpose-built methods essential. As more labs adopt peptide testing, workflows must balance robustness with simplicity to ensure reproducibility and efficiency.

This guidebook highlights solutions for GLP-1 receptor agonist quantification, including:

- Best practices for sample preparation
- Strategies to reduce non-specific binding (NSB)
- Column selection for improved peptide separations
- MRM optimization for sensitivity and selectivity
- Purpose-designed sample prep and LC-MS to improve assay robustness

Whether you're supporting drug discovery, clinical development or bioequivalence testing, the approaches outlined in this guide enable confident, streamlined quantification of GLP-1 receptor agonists.

Peptide Quantification Workflow for GLP-1 RAs



Sample Preparation Challenges and Considerations

Low Recovery

GLP-1 receptor agonists are known to be highly protein bound, particularly to albumin in plasma. Failure to disrupt protein binding will cause these peptides to pass through the solid-phase extraction (SPE) sorbent unretained due to size exclusion mechanisms.

Non-Specific Binding (NSB)

Non-specific binding is defined as analyte losses that occur due to binding to any surface to which the analyte is exposed. This is common with peptides, which are notoriously sticky and tend to bind (absorb) to glass, polypropylene, and metal surfaces.

Solubility

Maintaining solubility throughout sample preparation and analysis is critical, starting from initial solubilization from powder, through sample extraction and LC-MS analysis. Ensuring or maintaining solubility is especially important in the SPE elution step and in chromatographic separations. Poor solubility can lead to poor recoveries as well as column fouling and carryover.

Assay Robustness

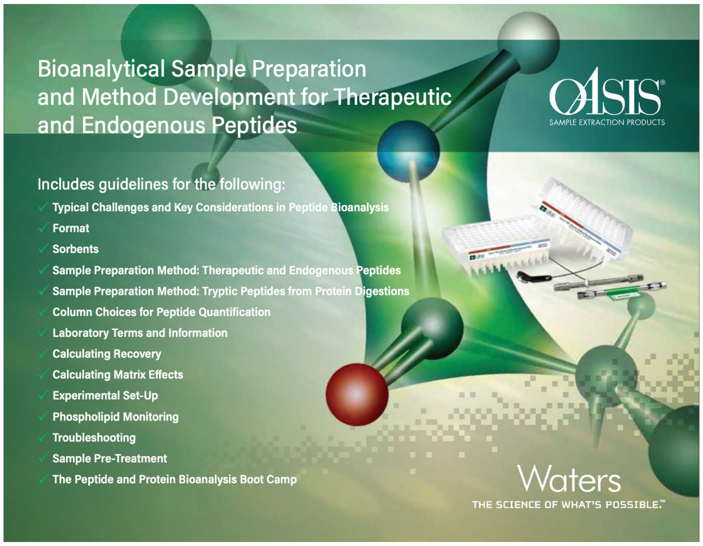
Robustness and reproducibility are critical to the success of every bioanalytical method. Complexity at each step of the analytical workflow can compromise robustness and lead to inconsistent results.

Plasma Pre-Treatment

The standard pre-treatment for plasma is a 1:1 dilution with 4% phosphoric acid. This dilutes the sample, decreasing viscosity and increasing the contact time with the sorbent. It also helps to disrupt protein binding. If the sample needs to be at a different pH for loading, try diluting 1:1 with 5% strong ammonia or with another appropriate buffer. If acid or base sample pre-treatment is not sufficient to disrupt protein binding, precipitation with an organic solvent may be necessary. Typical protein precipitation is not advised for peptides. A 1:1 protein precipitation is usually sufficient to ensure peptide recovery. Dilute the sample with aqueous solution such that the final concentration of organic is no more than 10–20%, or breakthrough during loading may occur.

Urine Pre-Treatment

Urine is a less complex matrix than plasma. Usually, it should be diluted 1:1 with an appropriate aqueous solution. Water is generally sufficient for reversed-phase SPE, or if using mixed-mode ion-exchange sorbent, good choices include 4% phosphoric acid or 5% strong ammonia. It is important to make sure your analyte and/or sorbent are in the correct ionization state for loading onto the sorbent. If buffering to a specific pH is required, make sure to use a high enough molarity solution to overcome the natural buffering capacity of urine. Also, be aware of sorbent capacity when using ion-exchange sorbents for urine extractions.



Bioanalytical Sample Preparation and Method Development for Therapeutic and Endogenous Peptides

Includes guidelines for the following:

- ✓ Typical Challenges and Key Considerations in Peptide Bioanalysis
- ✓ Format
- ✓ Sorbents
- ✓ Sample Preparation Method: Therapeutic and Endogenous Peptides
- ✓ Sample Preparation Method: Tryptic Peptides from Protein Digestions
- ✓ Column Choices for Peptide Quantification
- ✓ Laboratory Terms and Information
- ✓ Calculating Recovery
- ✓ Calculating Matrix Effects
- ✓ Experimental Set-Up
- ✓ Phospholipid Monitoring
- ✓ Troubleshooting
- ✓ Sample Pre-Treatment
- ✓ The Peptide and Protein Bioanalysis Boot Camp

For additional information, the Waters Bioanalytical Sample Prep and Method Development for Peptides Reference Card provides valuable information on how to create a sample prep method, how to choose the correct column for your peptide needs, and troubleshooting.

Solutions for Every Step of the GLP-1 RA Bioanalytical Workflow

Andrew+ Robot & Extraction+

Manual sample preparation can be laborious and lead to irreproducible results. The Andrew+™ Robot with Extraction+™ paired with OneLab™ Software offers a streamlined and intuitive solution to automate your GLP-1 RA sample preparation without compromising sample integrity.

Oasis HLB & Mixed Mode

Oasis™ HLB and mixed-mode 96-well µElution™ plates enable fast, selective solid-phase extraction (SPE) of peptides to achieve high recovery and low matrix effects for high sensitivity assays.

ACQUITY Premier Columns and Systems with High Performance Surfaces

MaxPeak™ Premier High-Performance Surface (HPS) Technology in Waters column hardware and SPE plates is also incorporated into Waters ACQUITY™ Premier and Arc™ Premier LC Systems, where it helps prevent non-specific adsorption of acidic analytes along the sample fluid path.

Xevo TQ Absolute XR Mass Spectrometer

The Xevo™ TQ Absolute XR mass spectrometer offers unparalleled levels of sensitivity and robustness. This means less unplanned maintenance and instrument downtime for laboratories that test high sample numbers, resulting in increased productivity while maintaining ultimate sensitivity.

waters_connect for Quantitation

waters_connect™ for Quantitation has an intuitive user interface that significantly reduces data review time. Built on a relational database, it provides robust method version control, traceability, and comprehensive audit trails, enabling deployment into regulated environments.

Selecting Optimal Consumables for Bioanalytical Sample Preparation

SPE Format

The 96-well plate µElution format was specifically chosen for the extraction of peptides from biological matrices for several reasons.

- Ideal for SPE clean-up and analyte enrichment of sample volumes ranging from 10 µL to 375 µL
- No evaporation and reconstitution necessary
- Up to 15x concentration without evaporation
- Elimination of dry-down step helps to minimize sample loss
- <15 µL residual volume
- Compatible with most liquid-handling robotic systems for automated, reliable, high-throughput SPE

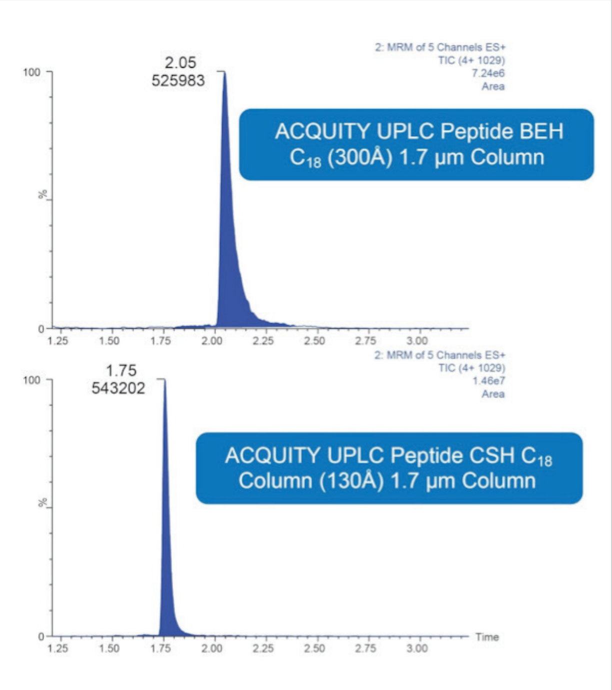
In addition, an entire 96-well plate can be processed manually in under 30 minutes, or less than 20 seconds per sample, significantly increasing throughput. Oasis sorbents are built on the unique, water-wettable Oasis HLB (Hydrophilic-Lipophilic Balance) copolymer and provide exceptional results when operating in either reversed-phase or mixed-mode protocols to support peptide method development.

Collection Plate

Traditional glass or polypropylene containers can adsorb biomolecules through either ionic or hydrophobic interactions. QuanRecovery™ Vials and Plates with MaxPeak HPS were designed by LC-MS scientists for LC-MS scientists to increase sensitivity, recovery, and reproducibility in biomolecule analysis. These QuanRecovery Vials and Plates with MaxPeak HPS reduce sample losses due to non-specific binding, allowing scientists to meet challenging requirements of highly sensitive LC-MS assays without concern for any losses during sample preparation. These vials and plates are provided in LC-MS autosampler-ready formats with low residual volumes that are designed to allow maximum injection volumes from low volume samples.

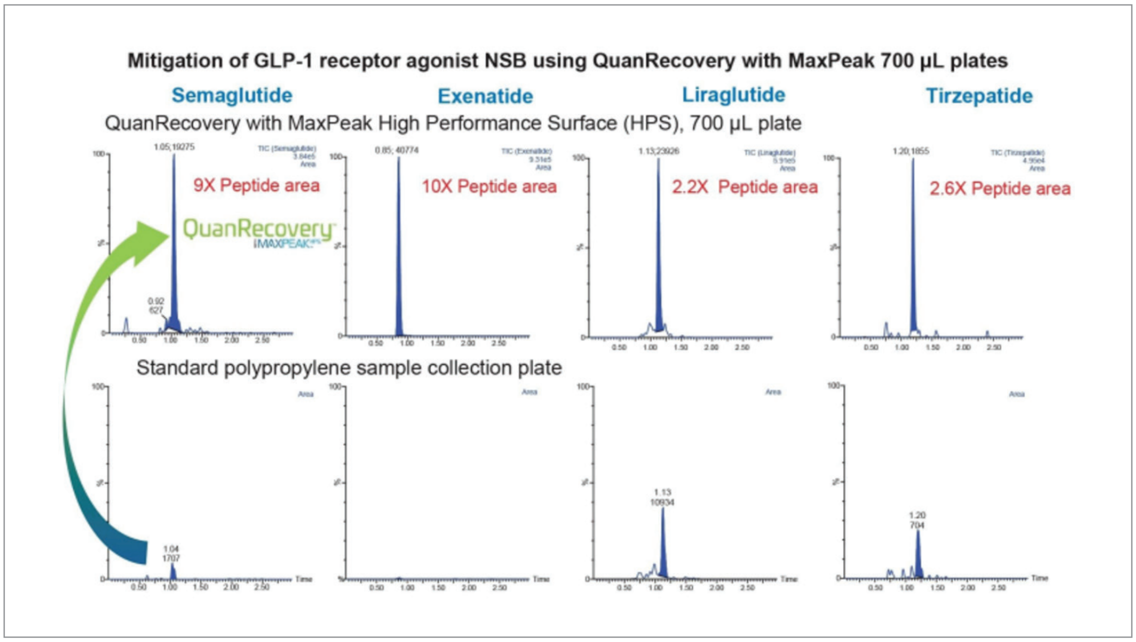
Recommended Reversed-Phase Columns

ACQUITY Premier Peptide BEH C_{18} (130 Å, 1.7 µm) and ACQUITY Premier Peptide CSH C_{18} (130 Å, 1.7 µm) Columns are both well-documented columns for excellent peptide separation performance. The CSH (Charged Surface Hybrid) C_{18} column is packed with high-pH-stable Ethylene Bridged Hybrid (BEH) particles containing a low-level positive surface charge, which help achieve improved chromatographic performance, reduced peak tailing, and better MS response for semaglutide, as described in this application note.



Semaglutide LC column evaluation, comparing the ACQUITY UPLC™ Peptide BEH C_{18} (300 Å) 1.7 µm Column and the ACQUITY UPLC Peptide CSH C_{18} (130 Å) 1.7 µm Column (0.4 mL/min @ 65°C), and demonstrating increased MS response and reduced peak tailing with the Peptide CSH C_{18} Column. 720008097.

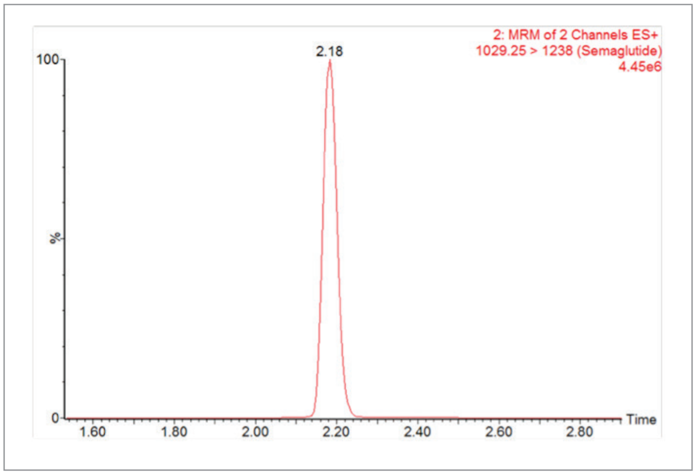
Addressing Non-Specific Binding with MaxPeak Premier High-Performance Surfaces



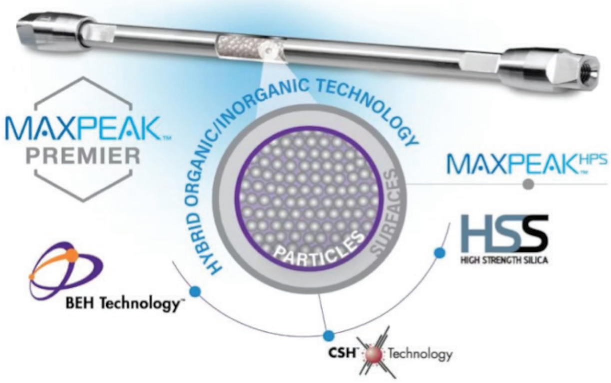
Representative LC-MRM-MS chromatograms demonstrating mitigation of non-specific binding to collection plates for the GLP-1 receptor agonist therapeutic peptides, semaglutide, exenatide, liraglutide, and tirzepatide (1 ng/mL prepared in a solution of 80/20/1 water, acetonitrile, and formic acid) prepared in a QuanRecovery with MaxPeak HPS, 700 µL Collection Plate vs standard polypropylene sample collection plates. 720008717.

One of the major issues with bioanalysis of GLP-1 RAs is non-specific binding (NSB), where the peptide interacts with and adheres to surfaces or materials used during sample preparation and analysis. This ultimately leads to peptide loss, low recovery, and unreliable results. Across four GLP-1 RA peptides tested, all showed improvements in recovery and sensitivity when using MaxPeak HPS Technology in sample collection plates.

Additionally, peptides can exhibit poor chromatographic peak shape when systems are not fully passivated. During bioanalytical method development of semaglutide, both ACQUITY Premier Systems and Columns with MaxPeak High Performance Surfaces were used to improve peak shape and overall recovery .



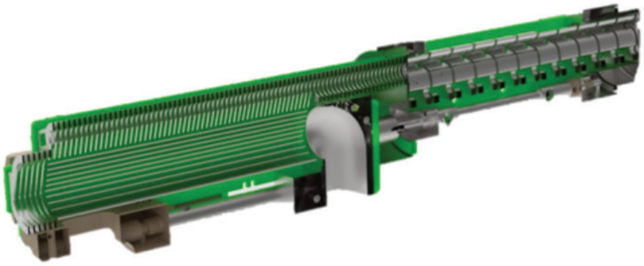
UPLC-MS/MS chromatogram showing semaglutide at 100 ng/m.



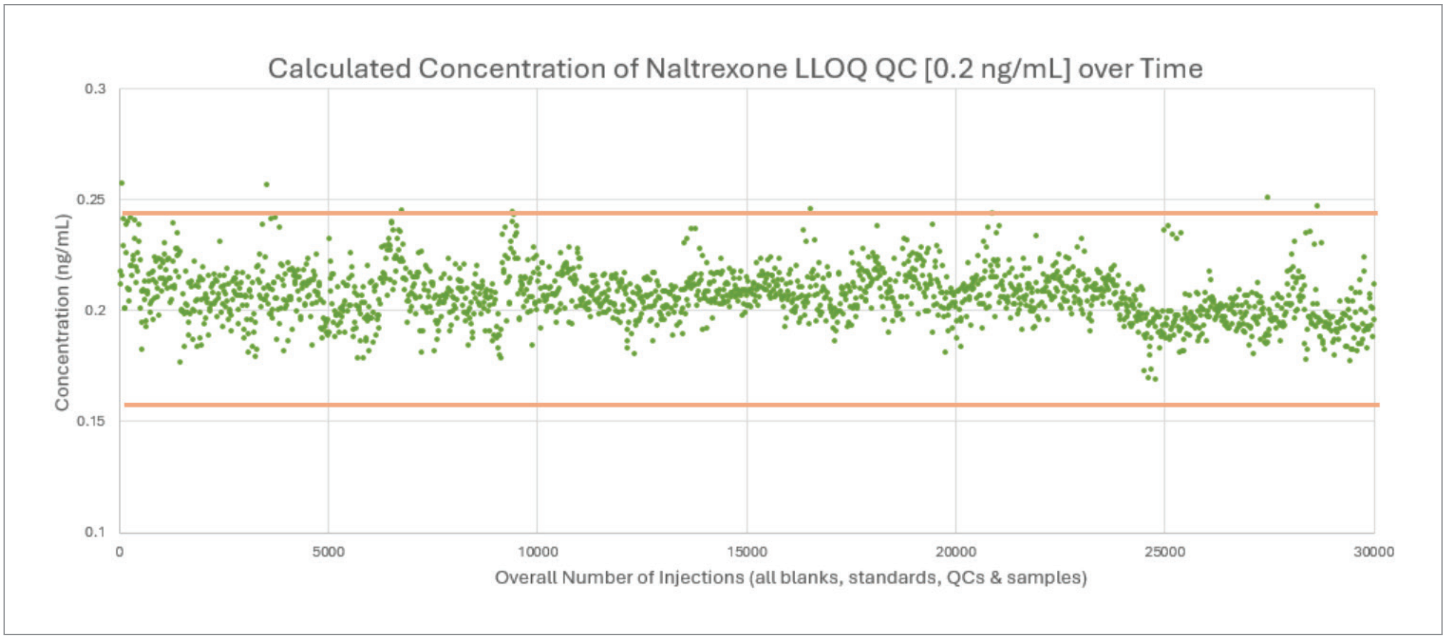
Unparalleled Sensitivity and Robustness with the Xevo TQ Absolute XR MS

Sensitivity and robustness are critical attributes of any bioanalytical method. Instruments used for analysis can suffer from deteriorating performance due to accumulation of matrix components on the ion guides and quadrupoles, depending on the level of sample clean up deployed. This often results in unplanned downtime for instrument maintenance.

The StepWave™ XR ion guide features a novel design that removes unwanted matrix components from the ion flight path, significantly improving platform robustness.



Data generated by scientists at Alderley Analytical (now a part of Synexa Life Sciences group) showed no compromise in instrument performance after 30,000 injections of protein precipitated plasma.



This figure shows data for LLOQ samples (0.2 ng/mL) of Naltrexone interspersed across 30,000 injections of plasma extracted samples. 99% of the LLOQ points were within +/- 20% of the expected concentration, thereby exceeding the acceptance criteria outlined in the ICH M10 method validation and study sample analysis guidelines.

Increased Productivity and Compliance with waters_connect for Quantitation

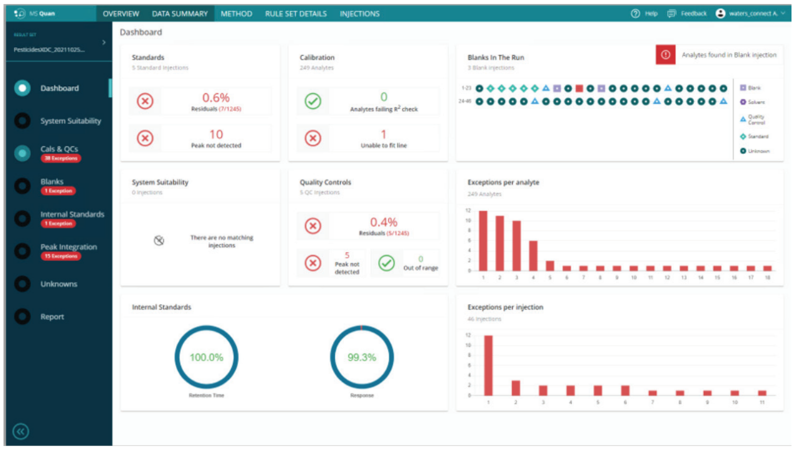
- waters_connect for Quantitation focuses your attention where it's needed most, providing a simple, insightful, and visual user experience that saves time and reduces error.
- It accelerates data review by using rulesets tailored to regulations, and it saves 50% of time spent on data review with Exception Focused Review (XFR).
- This compliance-enabled informatics platform enhances data integrity and traceability through comprehensive audit trails that meet regulatory guidelines such as 21 CFR Part 11.
- waters_connect for Quantitation is built on a relational database, providing robust method version control and traceability.
- Experience seamless and bidirectional connectivity with between waters_connect and LIMS platforms, ensuring your data and informatics pipelines can be upgraded, minimizing any disruptions to established processes.

Secure centralized storage

Privileges & restrictions on user-by-user basis

Qualification remotely from any client

Data & method sharing

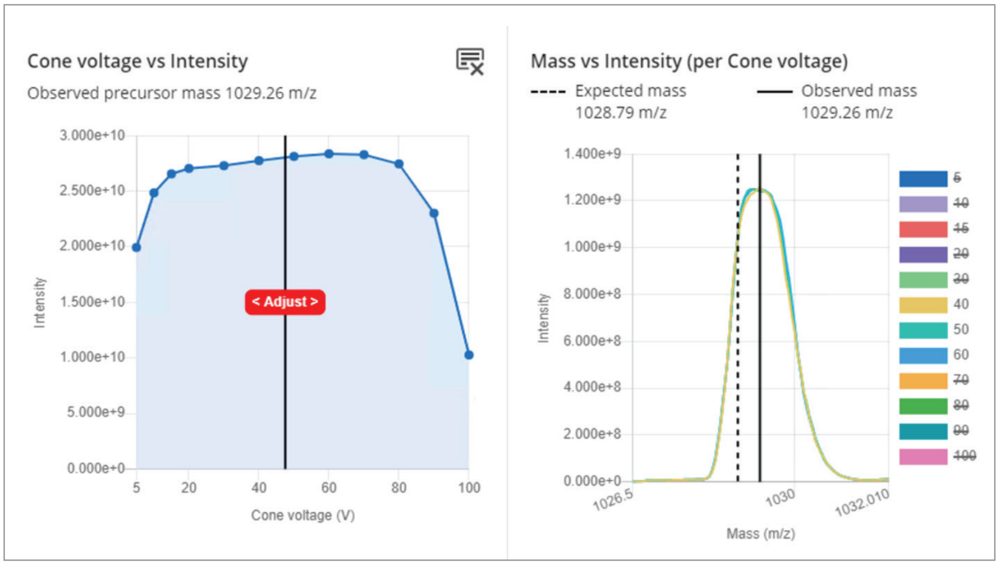


Automated MRM Method Development

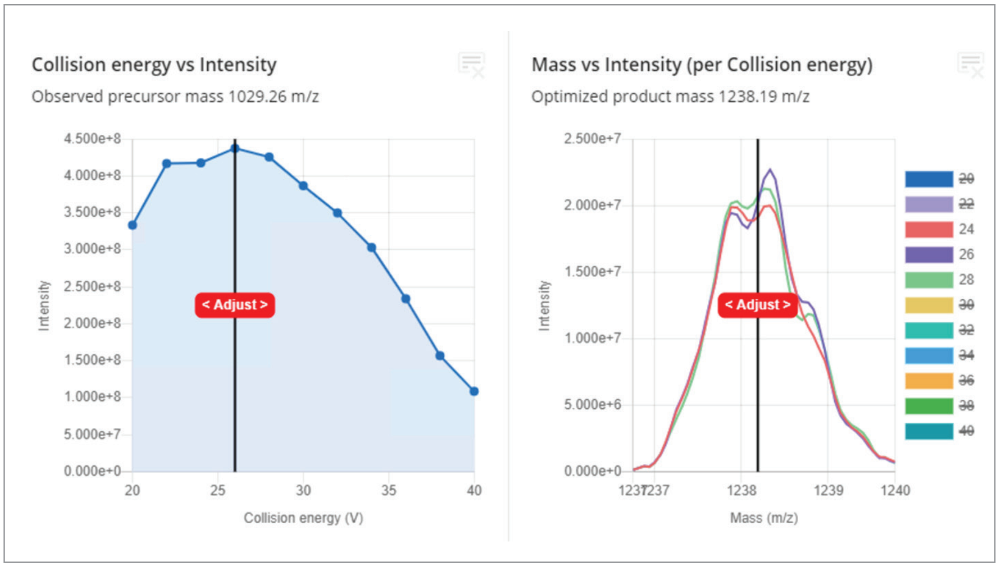
MRM method development, especially for large molecules like GLP-1 receptor agonists, can be challenging. Identifying the right precursor and product ions while optimizing parameters, including cone voltage and collision energy, is critical to maximizing assay sensitivity. Automated MRM optimization embedded within waters_connect for Quantitation systematically evaluates all variables to provide the most sensitive MRM transitions. Optimized parameters can be exported directly to the acquisition method to minimize transcription errors and maximize productivity.

For semaglutide, the MRM transition of 1029.25 > 1238 was identified to be the most sensitive transition and was used for quantitative analysis.

Cone Voltage Optimization



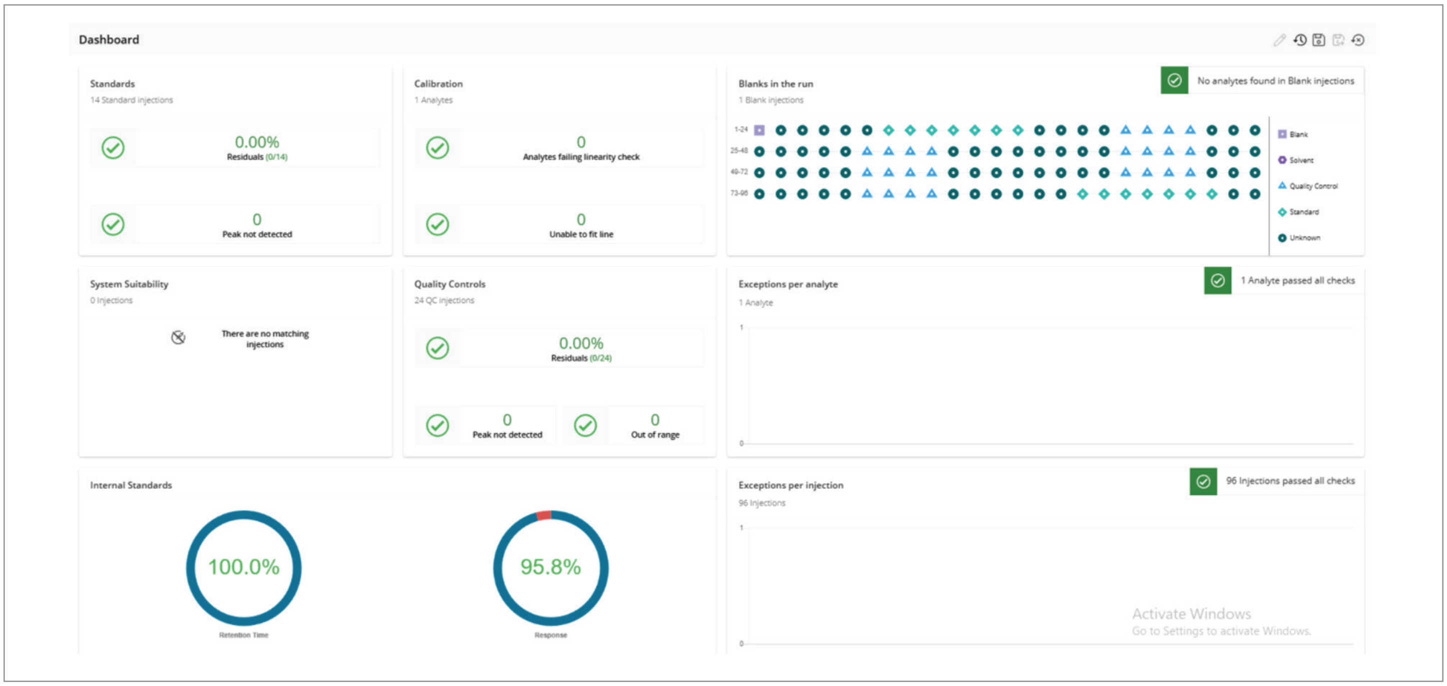
Collision Energy Optimization



Method development tool within waters_connect for quantitation systematically evaluates all aspects of MRM method development, including cone voltage and collision energy optimization to identify the best MRM transitions for any compound.

Efficient Batch Processing and Exception-Focused Review

The dashboard in waters_connect for Quantitation provides a quick overview of the entire batch, enabling the user to focus on injections that do not meet the user defined ruleset.



Dashboard

Standards
14 Standard injections
0.00% Residuals (0/14)
0 Peak not detected

Calibration
1 Analytes
0 Analytes failing linearity check
0 Unable to fit line

Blanks in the run
1 Blank injections
No analytes found in Blank injections

System Suitability
0 Injections
There are no matching injections

Quality Controls
24 QC injections
0.00% Residuals (0/24)
0 Peak not detected
0 Out of range

Exceptions per analyte
1 Analyte
1 Analyte passed all checks

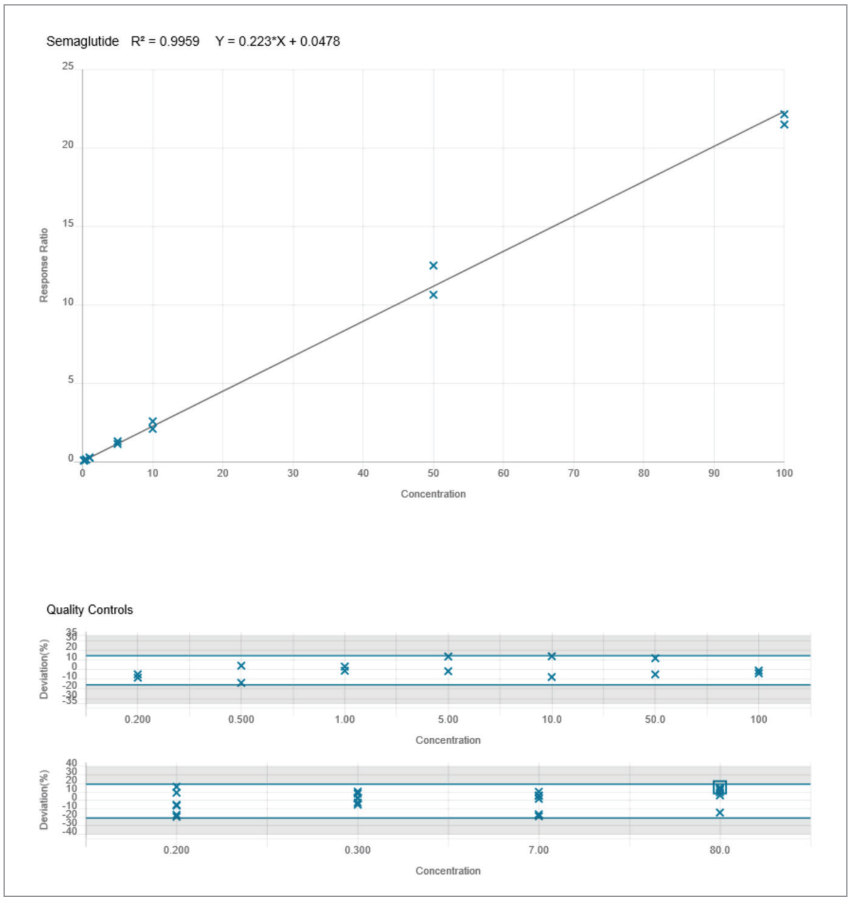
Internal Standards
100.0% Retention Time
95.8% Response

Exceptions per injection
96 Injections
96 Injections passed all checks

Activate Windows
Go to Settings to activate Windows.

A qualification batch for semaglutide extracted from plasma was analyzed using the optimal method parameters described so far. Using this method:

- Calibration curve for semaglutide was linear from 0.2 – 100.0 ng/mL with a $r^2 > 0.99$ using a 1/x weighting.
- All points on the calibration curve fell within the bioanalytical method validation criteria for +/- 15%.
- All replicates for all QC levels met the bioanalytical method validation criteria of +/-20% at the LLOQ and +/- 15% at all other levels.



GLP-1 RA Quantification Application Notes

Waters application notes are being published every day. Join us on the Resource tab of waters.com/GLP-1 to find helpful insights about GLP-1 RA quantification.

Application Notes

- Comprehensive Workflow for the Quantification of Peptides and Proteins in Plasma: Semaglutide a Case Study | Waters
- Improved GLP-1 Receptor Agonist Peptide Recovery Using a QuanRecovery™ With MaxPeak™ High Performance Surfaces (HPS) Collection Plate | Waters

Webinars and Reference Cards

- DMPK Webinar Series
- Oasis Peptide Bioanalysis Reference Card | Waters

Ordering Information

Columns for GLP-1 RA Bioanalysis

Diameter	1.7 µm
ACQUITY Premier CSH C_{18} Column 1.7 µm, 2.1 x 50 mm, 1/pk	186009460

Oasis HLB and Peptide Plates

	Part Number
Oasis HLB 96-well µElution Plate, 2 mg Sorbent per Well, 30 µm, 1/pk	186001828BA
Oasis HLB 96-well Plate, 5 mg Sorbent per Well, 30 µm, 1/pk	186000309
Oasis HLB 96-well Plate, 10 mg Sorbent per Well, 30 µm, 1/pk	186000128
Oasis HLB 96-well Plate, 30 mg Sorbent per Well, 30 µm, 1/pk	WAT058951
Oasis HLB 96-well Plate, 60 mg Sorbent per Well, 60 µm, 1/pk	186000679
Oasis Peptide Method Development 96 well µElution Plate, 2 mg, 30 µm, 1/pk	186004713

QuanRecovery Vials and Plates

	Part number
QuanRecovery with MaxPeak, 700 µL Plates, 25/pk	186009184
QuanRecovery with MaxPeak, 700 µL Plates, 5/pk	186009185
QuanRecovery with MaxPeak HPS 12 x 32 mm Screw Neck Vial, 300 µL, 100/pk	186009186
QuanRecovery with MaxPeak HPS Vial Package (186009186) with pre-slit PTFE silicone cap and septum, 100/pk	176004434

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