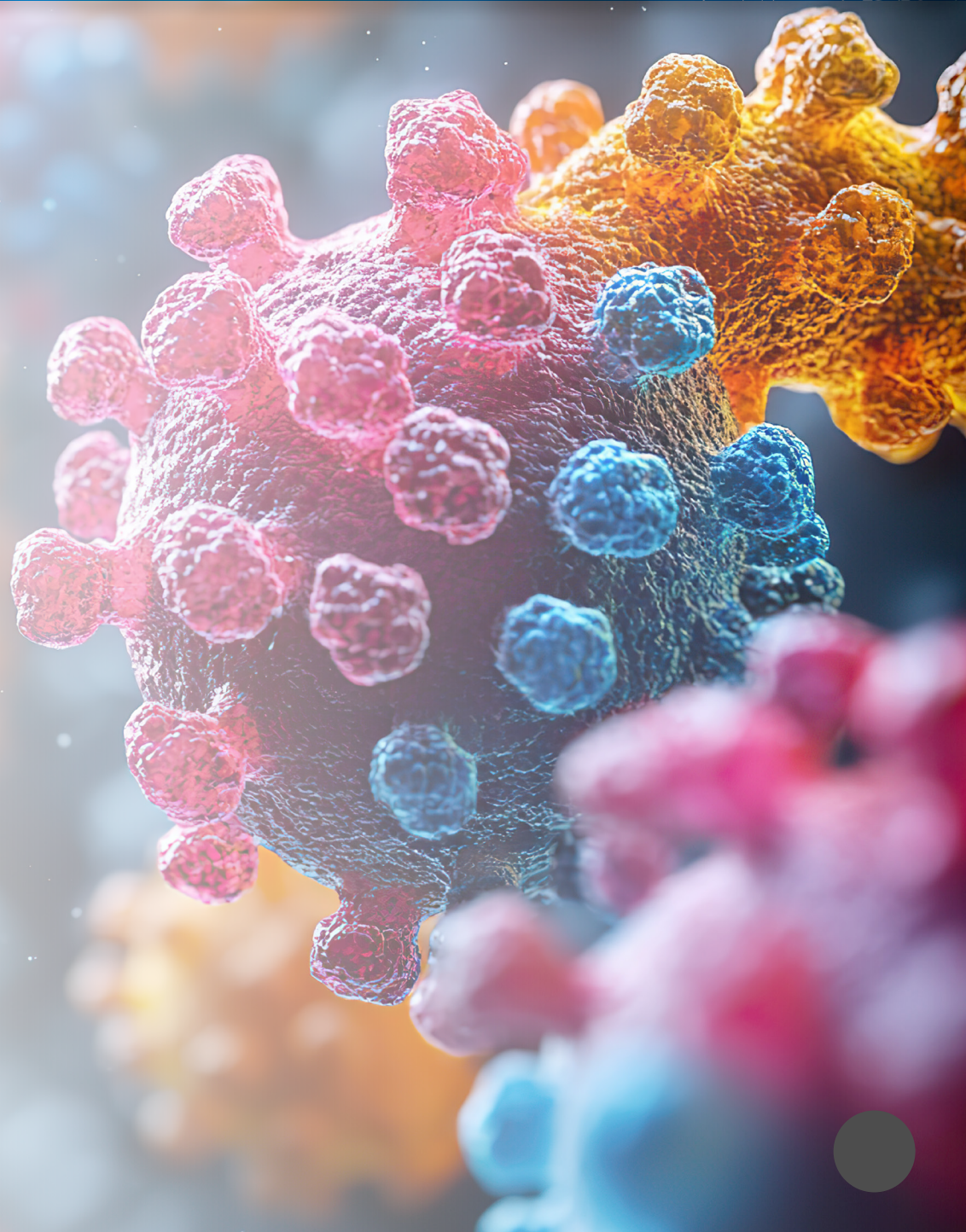


Waters™

From Molecule
to Medicine:
Exploring
PROTACs in
Drug Discovery
& Development



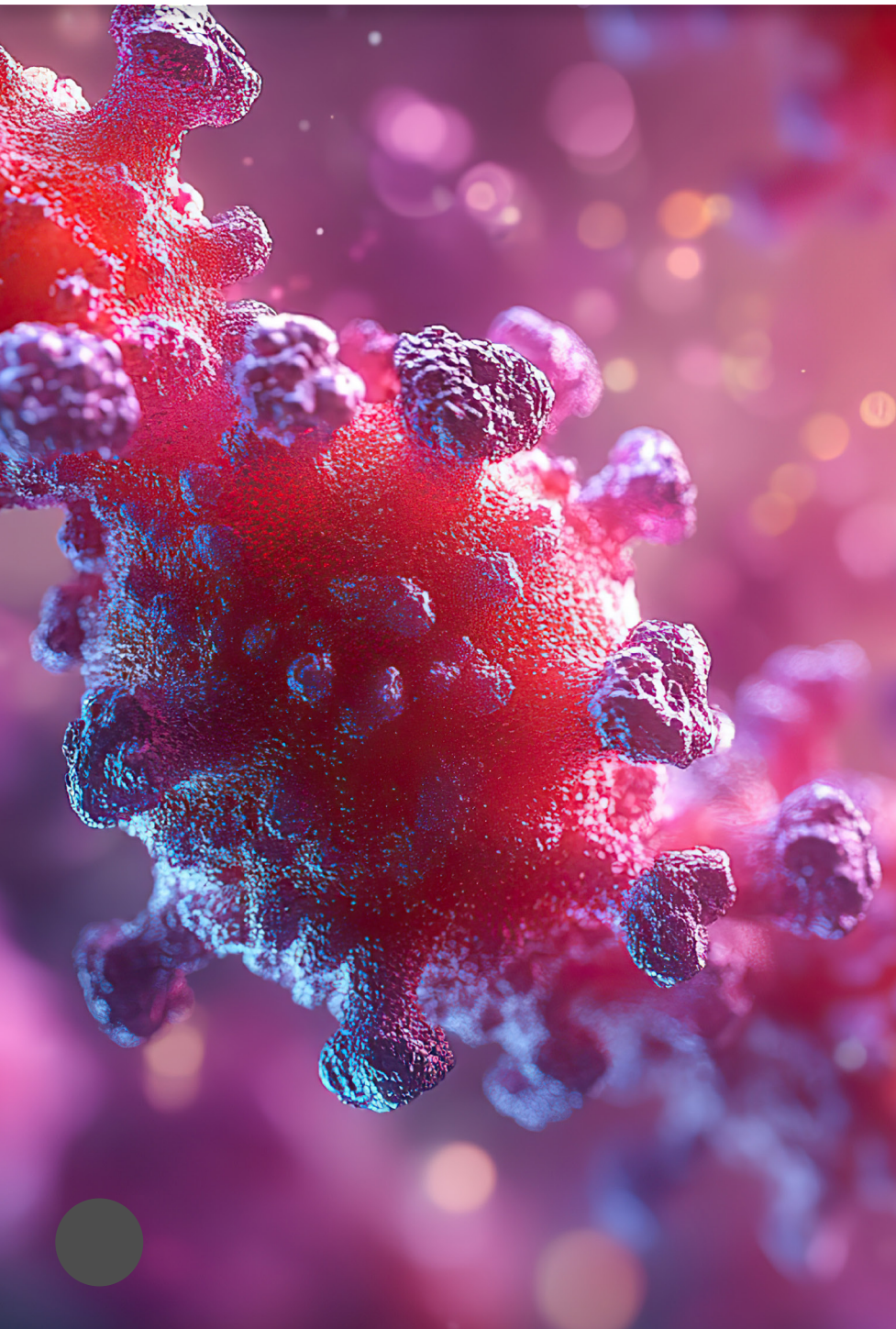


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Introduction

ADDRESSING THE ANALYTICAL CHALLENGES OF PROTACS DISCOVERY AND DEVELOPMENT



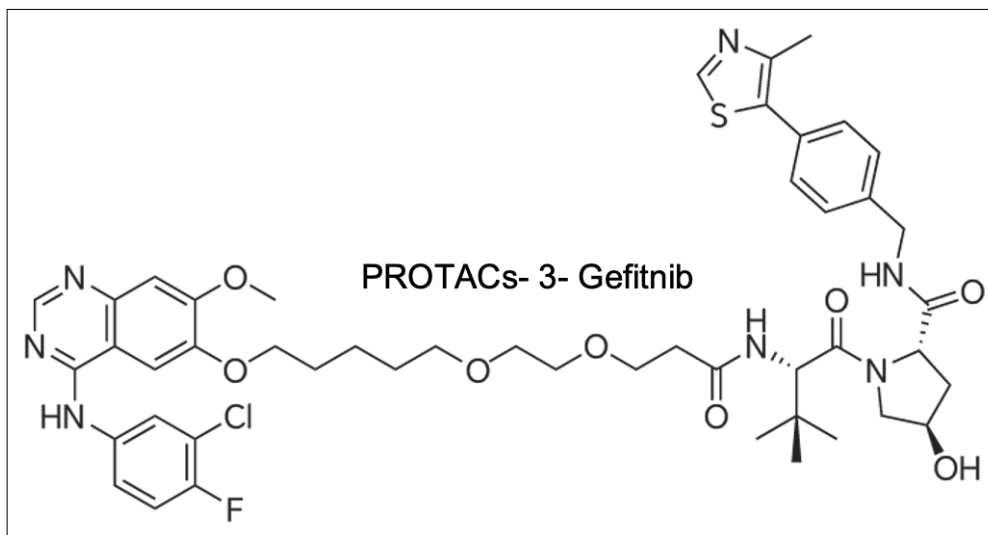
A New Era in Medicine: The Global Impact of PROTACs

Proteolysis Targeting Chimeras (PROTACs) are changing the rules of drug discovery. Instead of merely blocking harmful proteins, PROTACs harness the body's own system to remove them completely.

WHY THIS MATTERS:

- They expand therapeutic possibilities by targeting proteins once considered “undruggable.”
- They open new treatment options in oncology, neurology, autoimmune disease, and infectious disease—areas where existing therapies often fail.
- They promise faster progress from discovery to clinic, giving scientists and pharmaceutical companies a powerful new way to tackle complex diseases.

By unlocking access to previously unreachable proteins, PROTACs pave the way for transformative medicines that could reshape patient care worldwide.



PROteolysis Targeting Chimeras (PROTACs) are a class of bifunctional small molecules that catalytically degrade disease-causing proteins by hijacking the cell's own degradation system.

From Challenge to Solution – Enabling PROTAC Bioanalysis

Evaluating PROTACs in clinical pharmacokinetic (PK) samples presents notable challenges due to several interconnected factors:



1. Low Plasma Concentrations

Extremely low plasma concentrations
Only small amounts are needed to trigger protein degradation, making sensitivity a critical hurdle.



2. Complex and Unstable Structures

Their large, bifunctional makeup (comprising a target ligand, linker, and E3 ligase ligand) results in them being:

- Chemically unstable within biological matrices.
- Susceptible to degradation or metabolism, making accurate measurement more difficult.



3. Matrix Effects and Non-Specific Binding

Interactions with plasma proteins, plastics, or LC components often cause recovery loss and variability.



4. Lack of Standardized Assays

In contrast to small molecules, there are currently no standardized bioanalytical methods for PROTACs. Each compound frequently necessitates custom assay development, which can be both time-consuming and resource-intensive.

With Waters, you can unlock the full potential of PROTACs and bring breakthrough therapies to patients faster. Our advanced LC-MS bioanalytical solutions support the entire workflow—from complex sample preparation to compliant data reporting—giving pharmaceutical companies and CROs the sensitivity, selectivity, and reproducibility needed to confidently quantify emerging modalities like PROTACs.

This eBook will show you how Waters' end-to-end solutions accelerate development, streamline quantitation, and empower scientists to navigate the complexity of PROTAC bioanalysis with confidence.

From Molecule to Medicine: Supporting Every Step of PROTAC Bioanalysis

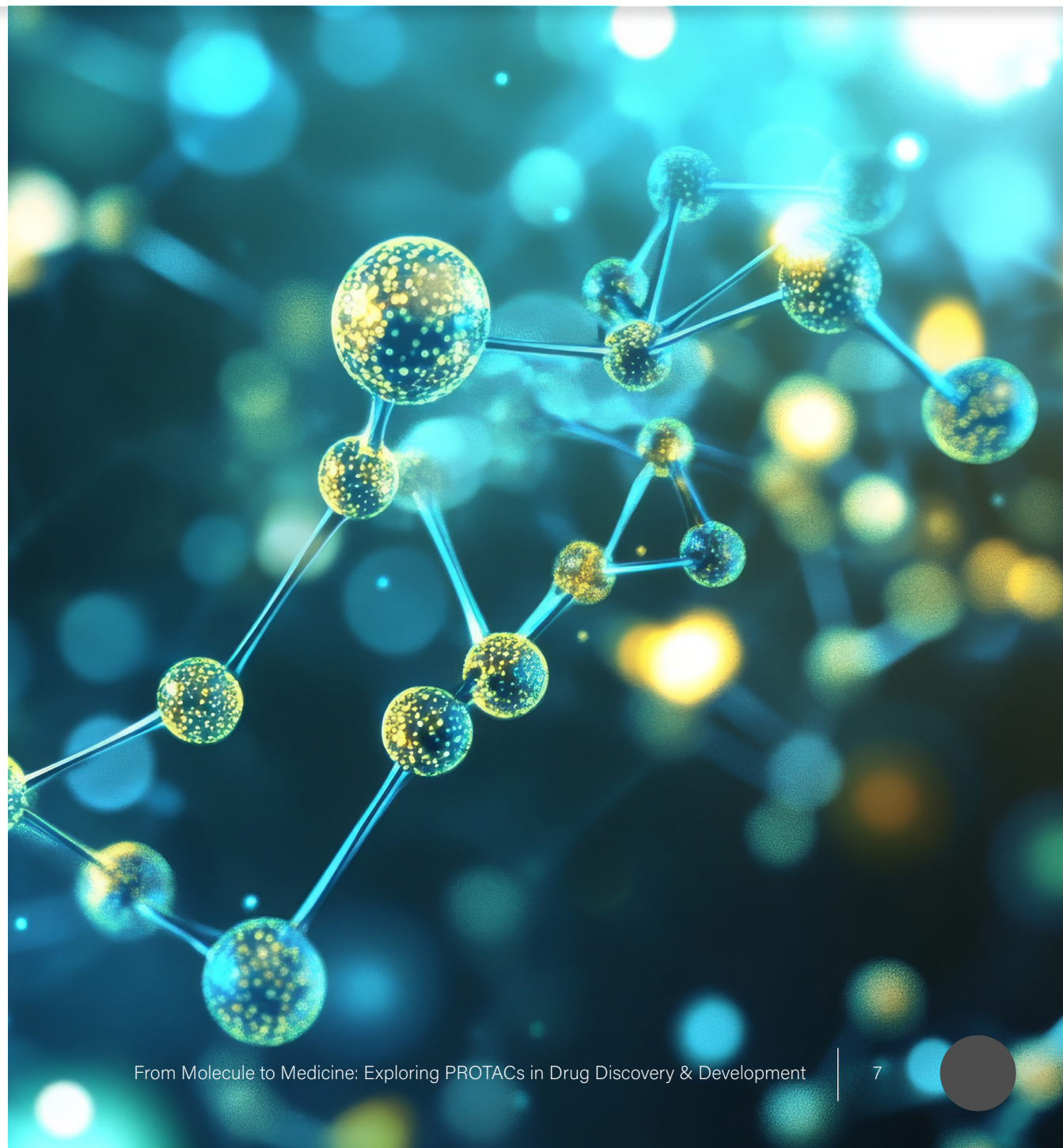
NAVIGATE THE COMPLEXITY OF PROTACS BIOANALYSIS WITH WATERS

In this eBook, discover how Waters advanced LC-MS bioanalytical solutions help pharmaceutical companies and CROs confidently quantify emerging modalities like PROTACs. With robust, sensitive, flexible tools across both discovery and regulated workflows, Waters supports the full bioanalysis journey - from complex sample preparation through compliant data reporting.

Explore how our end-to-end solutions accelerate development, streamline quantitation, and help scientists bring next-generation therapies to patients faster — making Waters a trusted partner in bioanalysis.



WATERS COMMITMENT TO THE EVOLVING THERAPEUTIC LANDSCAPE



Waters Commitment to the Evolving Landscape



[Learn more here.](#)



Leveraging decades of experience and substantial investment in analytical innovation, Waters brings comprehensive solutions to support PROTAC analysis across discovery, development, manufacturing, and QA/QC.

ADVANCED ANALYTICAL TOOLS

As complex therapeutic modalities, like PROTACs, move through the drug discovery-drug development pipeline, the need for advanced analytical tools continues to evolve.

At the heart of our quantitative bioanalysis solution is the advanced [Liquid Chromatography-Tandem Mass Spectrometry \(LC-MS/MS\) technology](#). LC-MS/MS delivers high sensitivity and selectivity, crucial for accurately quantifying PROTACs and their metabolites in complex biological matrices.



*waters_connect for
Quantitation Software*

Waters Commitment to the Evolving Landscape



[Read the full application note.](#)



Confidence in Compliance

Waters commitment to PROTAC bioanalysis goes beyond instrumentation. We support laboratories operating in regulated pharmaceutical environments, where data must meet strict standards for accuracy, integrity, and traceability.

waters_connect for Quantitation is a compliance-enabled informatics platform that enhances data integrity and traceability through comprehensive audit trails that meet regulatory guidelines such as 21 CFR Part 11.

Waters: Your Trusted Partner in Regulated Bioanalysis

Waters partners with you across every aspect of your regulated bioanalytical workflows, delivering deep product and scientific expertise that combines analytical precision with proven compliance support. We design our technologies to simplify complex challenges in emerging modality analysis, including the quantification of structurally diverse, low-abundance molecules like PROTACs.

With our global expertise, scientific partnerships, and commitment to innovation, Waters helps organizations confidently move from molecule to medicine - enabling regulatory success and improving patient outcomes.

SAMPLE PREPARATION SOLUTIONS FOR PROTAC ANALYSIS



Sample Preparation Solutions for PROTAC Analysis

Sample preparation is a critical first step in the quantitation of PROTACs, setting the foundation for accurate, sensitive, and reproducible results. This section outlines key techniques and technologies that improve analyte recovery, reduce matrix effects, and streamline LC-MS/MS performance.

FLEXIBLE APPROACHES FOR EVOLVING BIOANALYTICAL NEEDS

Upstream sample preparation strategies must align with both the complexity of the analyte and the goals of the program stage.

✓ **In early discovery:** simplified, broadly applicable extraction methods help support high-throughput bioanalysis without compromising robustness.

✓ **In development and clinical phases:** greater sensitivity and selectivity are often required—making more refined extraction strategies critical for confident decision-making.



Solid-Phase Extraction for PROTACs Analysis



[Read the full application note.](#)



Solid-phase extraction (SPE) plays a crucial role in PROTACs bioanalysis due to the complexity of both the molecules themselves and the biological matrices in which they are studied. With high molecular weights, diverse structural features, and potential stability challenges, PROTACs require carefully optimized cleanup workflows to ensure reliable quantitation.

SPE helps enrich analytes and remove interfering substances that may suppress ionization, improving sensitivity and reproducibility of LC-MS/MS methods. By tailoring sorbent selection and elution conditions, analysts can improve recovery of cleaved and intact PROTACs across a wide range of chemical profiles. This step is essential for maintaining method robustness in both discovery and regulated workflows.

Solid-Phase Extraction for PROTACs Analysis



[Read the full application note.](#)



OASIS™ PRiME HLB FOR PROTAC BIOANALYSIS

Oasis PRiME HLB Solid-Phase Extraction (SPE) Products are designed to simplify sample preparation by delivering rapid, high-throughput clean-up with minimal method development. In settings like discovery, where fast, generic methods are preferred, Oasis PRiME HLB provides quick and easy 2- and 3- step protocols that deliver acceptable recoveries while removing common biological matrix effects like phospholipids (>95%).

OASIS WAX FOR PROTAC BIOANALYSIS

Oasis WAX is a mixed-mode weak anion exchange sorbent that combines reversed-phase and ion-exchange retention mechanisms. This dual functionality is particularly beneficial for PROTACs that contain acidic functional groups or exhibit strong matrix interactions. By enabling greater analyte retention and enhanced selectivity, Oasis WAX helps improve the recovery of cleaved and intact PROTACs across complex biological matrices. This optimized clean-up minimizes ion suppression, enhances sensitivity, and supports reproducible quantitation in both early discovery and regulated bioanalytical environments.



Automated Sample Prep

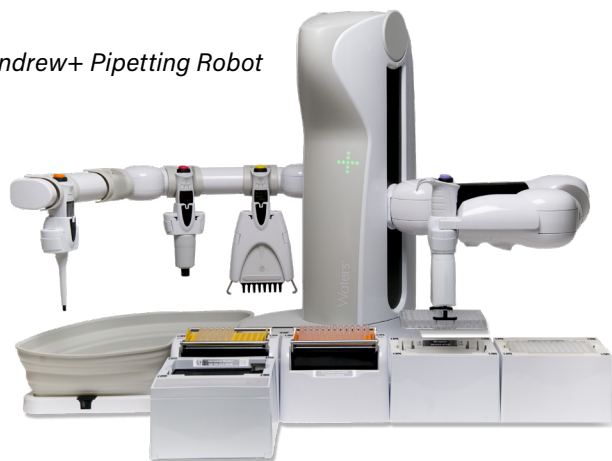


[*Read the latest news about the Andrew+ Pipetting Robot.*](#)

By pairing Waters solid-phase extraction (SPE) technologies with the, Andrew+™ Pipetting Robot and Extraction+™ Device, bioanalytical scientists can streamline workflows and meet the sensitivity, speed, and compliance demands of modern pharmaceutical development.



Andrew+ Pipetting Robot



- **Andrew+:** The Andrew+ Pipetting Robot offers fully automated liquid handling using electronic pipettes and labware holders called Dominos. By adding Device+, our ever-expanding portfolio of connected devices and tools for processes such as heating, shaking, or labware transportation, Andrew+ Robot evolves into a full walk-away automation solution.
- **Extraction+:** Real-time pressure profile control is a critical component of successful extraction methods. Automated pressure control is now possible thanks to a completely new vacuum pump with full connectivity to the built-in OneLab Software.
- **OneLab™ Software:** The intuitive design of the OneLab Software allows users to easily create protocols from scratch or download an existing validated protocol from the OneLab library to execute with the click of a button. This ease-of-use, combined with built in compliant ready tools, make Andrew+ an easy choice for bioanalytical labs looking to automate their sample preparation workflows
- **Advantages:** Automation with Andrew+ ensures consistent pipetting, reduced human error, and increased throughput — critical for managing high sample volumes and maintaining reproducibility in complex biological matrices.

Optimized LC-MS/MS Solutions for PROTACs

ADDRESSING KEY CHALLENGES FOR LABORATORIES



Advanced Liquid Chromatography (LC) for Reliable Separation



[Read the full brochure.](#)

The ACQUITY™ Premier System, featuring MaxPeak™ High Performance Surfaces (HPS) Technology minimizes analyte/surface interactions, improving analyte recovery, sensitivity, and reproducibility — essential for accurate quantitation in both discovery and regulated workflows.

When paired with MaxPeak Premier Columns, including Premier BEH™ C₁₈ and Premier HSS T3, the system delivers superior peak shape, low carryover, and enhanced retention stability, even when working with complex, low-abundance compounds like PROTACs.



Revolutionize your separations and enhance your lab efficiency with the ACQUITY Premier System.

Mass Spectrometry (MS) Detection With Absolute Sensitivity and Extreme Robustness



[Read the full application note.](#)

From pharmacokinetic profiling to metabolite ID, accurate quantitation at trace levels is essential to development, and in accelerating decision-making.

The [Waters Xevo™ TQ Absolute XR Mass Spectrometer](#) is the most sensitive and robust tandem quadrupole MS available. This instrument enables scientists to reach lower limits of quantitation across all therapeutic modalities and can detect PROTACs and their metabolites at picogram-per-milliliter levels.

EFFICIENT DATA MANAGEMENT AND PROCESSING WITH WATERS_CONNECT FOR QUANTITATION

Complement the unmatched sensitivity of the Xevo TQ Absolute XR with the most modern, intuitive, efficient quantitation software solution available on the market.

The [waters_connect for Quantitation Software](#) solution provides seamless workflows with one click — from acquisition to processing methods — and a radical commitment to ease of use and simplicity, enabling users of all experience levels to produce highly accurate, reliable, and sensitive results.

Accelerate data review by using rulesets tailored to regulations and save 50% of time spent on data review with Exception Focused Review (XFR).

KEY BENEFITS OF THE XEVO TQ ABSOLUTE XR FOR PROTAC ANALYSIS:

- Exceptional sensitivity for low-level quantification
- Fast polarity switching for capturing diverse metabolites
- Compact footprint for flexible lab integration
- Market-leading performance and uptime for routine workflows
- Compatible with MS imaging workflows



Enhancing PROTACs Bioanalysis With waters_connect System Monitoring Software



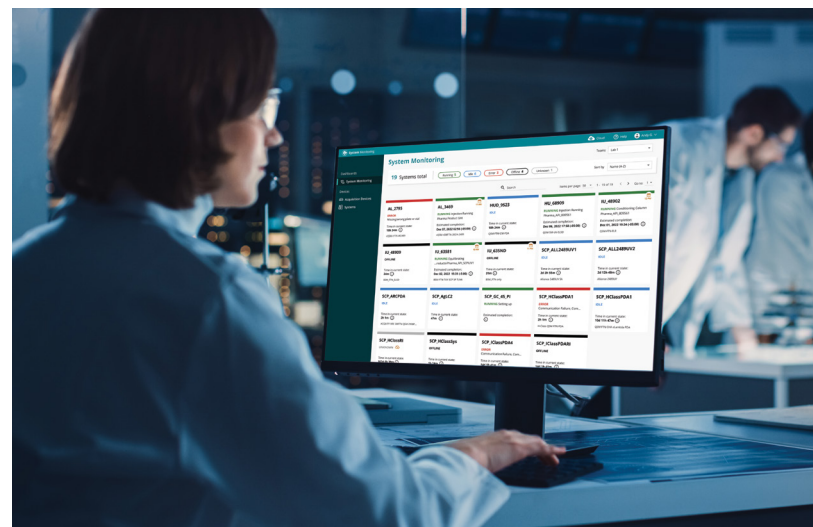
[Check out the full infographic.](#)

Managing instrument uptime and performance is essential for delivering reliable results at every stage of development.

Integrating System Monitoring Software with LC-MS platforms provides labs with real-time visibility and control across operations—helping to ensure robust, uninterrupted bioanalytical workflows.

- **Real-Time Instrument Monitoring:** waters_connect System Monitoring enables continuous, real-time monitoring of LC-MS systems. This functionality is vital for labs seeking to dynamically scale operations in response to evolving PROTACs analysis demands, ensuring optimal instrument usage and efficiency.
- **Rapid Troubleshooting and Issue Resolution:** The software promptly notifies lab personnel of system errors or status changes, enabling quick troubleshooting. This capability minimizes downtime and supports continuous operation, keeping PROTAC bioanalysis studies on track, especially during intensive study timelines.
- **Detailed Performance Insights:** By delivering a comprehensive view of instrument performance and historical data, waters_connect System Monitoring helps lab managers make informed decisions about system maintenance and management. This proactive approach prevents unexpected system failures and optimizes long-term operational efficiency.

By providing remote system status viewing, efficient system usage, rapid response to issues, detailed tracking of system history, and easy scheduling of instrument runs and maintenance, this software solution enhances laboratory efficiency, productivity, and data integrity.



ADVANCED SOLUTIONS FOR COMPREHENSIVE PROTACS CHARACTERIZATION



Powerful & Versatile HRMS Solutions



For laboratories exploring the complex biotransformation of novel therapeutic modalities like PROTACs, Waters high resolution mass spectrometry (HRMS) solutions offer the sensitivity, speed, and structural insights needed to characterize metabolites with confidence. When paired with streamlined UPLC separations and advanced informatics, HRMS becomes a powerful asset for metabolite identification studies—enabling the detection of known and unknown metabolites with precise localization of metabolic soft spots.

BENEFITS FOR YOUR LAB:

The advanced HRMS solutions address critical customer pain points like the need for higher selectivity, the ability to handle complex samples, and the requirement for robust data management and analysis tools. Laboratories can expect:

- Increased sensitivity and specificity in PROTAC detection
- Enhanced capability to identify and quantify PROTACs and their metabolites
- Streamlined workflows that reduce sample handling and increase throughput
- Sophisticated structural characterization tools that aid in distinguishing closely related metabolites

Structural Characterization and Biotransformation Profiling of PROTACs with Xevo G3 QTof



[Read the full application note.](#)

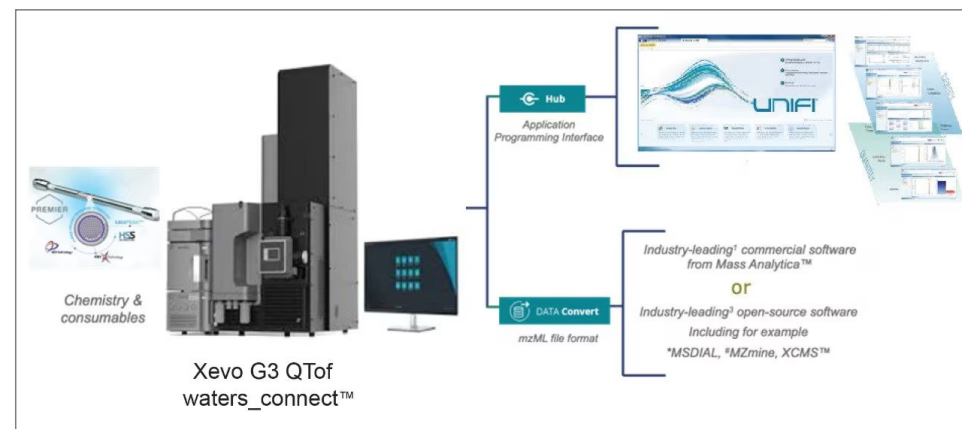
Understanding the **metabolic fate of PROTACs** is essential to progress the candidate from discovery through development and ultimately, to improve chances of clinical success.

In a recent in vivo study of a tyrosine kinase inhibitor PROTAC (PROTACs-3-gefitinib), this workflow identified **9 distinct metabolites** in rat plasma and urine, revealing major biotransformation routes such as **amide hydrolysis, N-dealkylation, and phase II conjugation** (sulfation, glucuronidation).

The combination of data-independent acquisition (DIA), rapid UPLC separations, and software-driven analysis enabled:

- Sub-3 ppm **mass accuracy** and >20 data points per peak
- Structural elucidation using **diagnostic fragment ions**
- Confidence in metabolite identification through **visualized time-course trends**
- Elimination of false positives using **control filtering and known transformations**

The integration of the powerful waters_connect Software Solution with the Xevo MRT and Xevo G3 QTof simplifies complex screening or discovery workflows. This solution allows scientists to **confidently characterize complex PROTAC metabolites**. By delivering **rich MS/MS fragmentation, rapid data acquisition, and chemically intelligent analysis**, these platforms enable scientists to move seamlessly from unknown screening to confident identification. From **early metabolic soft spot detection to quantitative time-course trend validation**, project teams can make faster, more informed decisions with **exceptional clarity and confidence**.



Spatial Localization of PROTACs with MS Imaging



[Read the
full poster.](#)

Mass spectrometry imaging (MSI) is emerging as a transformative tool in the analysis of PROTACs, offering spatially resolved insights into drug distribution, target engagement, and protein degradation within tissues.

In the context of PROTAC development, MSI can be used to track the localization of both the PROTAC molecule and its metabolites across different tissue regions. This is crucial for evaluating tissue penetration, off-target effects, and the efficacy of protein degradation *in situ*. For example, MSI can reveal whether a PROTAC reaches its intended site of action within a tumor, or whether it accumulates in non-target organs, which could inform safety and dosing strategies. Additionally, by combining MSI with immunohistochemistry or fluorescence imaging, researchers can correlate PROTAC-induced degradation with cellular markers, enhancing mechanistic understanding.

FROM DISCOVERY TO TARGETED MS IMAGING

1. Discovery MSI analysis for confident spatial localization

Waters HRMS suite of MS imaging solutions offers complementary DESI XS and MALDI technologies, enabling precise spatial localization of PROTACs within tissue samples.

2. Targeted MSI analysis

Waters targeted MSI solution with DESI XS on the Xevo TQ Absolute XR enables the most rapid and sensitive localization of drugs and their metabolites, providing crucial insights into biodistribution, tissue penetration, and target engagement.

Spatial Localization of PROTACs with MS Imaging (cont.)



[Read the full poster.](#)

DESI XS SUPPORTS CONFIDENT DECISION-MAKING THROUGH:

1. Label-free detection

Directly detects molecules and metabolites in tissue without the need for radioactive or fluorescent labelling

2. Minimal sample preparation

Analyzes tissue sections under ambient conditions with minimal preparation, simplifying workflows for faster decision making

4. High sensitivity and throughput

Optimized sprayer designs and high-performance mass spectrometers enable rapid imaging, reducing analysis time from hours to minutes while maintaining high mass resolution

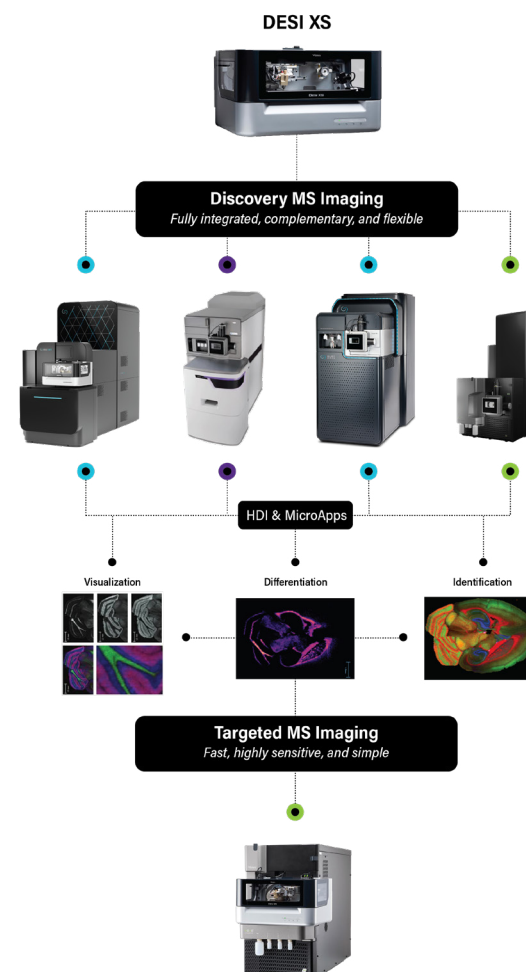
5. Compatibility with histological techniques

Non-destructive analysis allows the same tissue section to undergo further histological staining or immunohistochemistry after MSI analysis, enabling multimodal studies that correlate molecular data with cellular morphology

6. Simultaneous detection of parent drug and metabolites

Enables *in situ* detection of both the intact molecules and its metabolic products, providing a comprehensive view of drug metabolism and activity within the tissue

Discovery to Targeted MS Imaging



STREAMLINE YOUR LABORATORY OPERATIONS WITH WATERS SERVICES AND SUPPORT



Customized Training and Application Support

Waters is committed to enhancing your laboratory's proficiency. Our team of scientists, with deep technology and workflow expertise, is focused on supporting you throughout your journey. The dedicated Analytical Professional Services Team provides comprehensive training and application-specific support to ensure your lab personnel are well-equipped to operate sophisticated analytical tools and methodologies. Through our extensive training, dedicated support, and services, Waters helps your lab exceed regulatory requirements and achieve operational excellence.



With comprehensive offerings from the Analytical Professional Services team, you can:

- Optimize the value of your Waters Investment
- Reduce your total cost of ownership
- Facilitate adoption and system use among your critical users

OPTIMIZING INSTRUMENT PRODUCTIVITY AND ANALYTICAL SERVICES

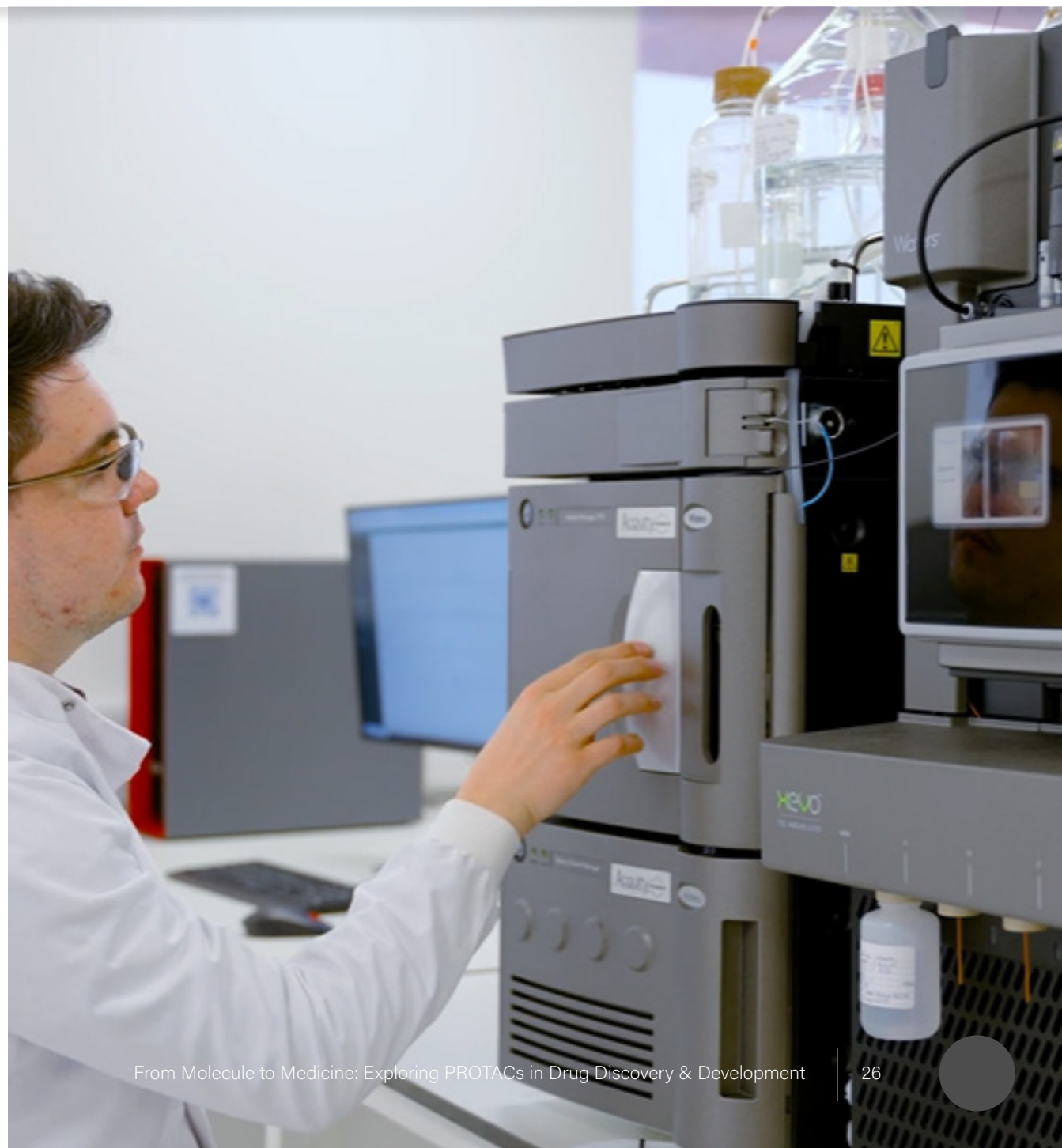
Waters helps maintain your instruments at peak productivity, minimizing downtime and enhancing lab performance. Our services include the latest technology upgrades, method transfer, software validation, and stringent compliance support to ensure your instruments deliver reliable and consistent results.


PROFESSIONAL AND COMPLIANCE SERVICES

Our Compliance Services team offers a comprehensive suite of services to ensure your laboratory meets all regulatory standards without disruption. From instrument to software qualification, we help you establish and maintain quality standards that meet regulatory requirements.

Our Software Compliance Services provide scalable, expert solutions to maintain control over your laboratory systems — from installation onwards, ensuring they are fit for intended use and aligned with regulatory demands.

CASE STUDY: ALDERLEY ANALYTICAL





“We’ve run over 30,000 dirty samples
on the [Xevo] TQ Absolute XR...
it’s proven to be very robust...and a
fantastic addition to the laboratory.”

Sally Hannam, Scientific Director, Scientific Strategy Team, Alderley Analytical

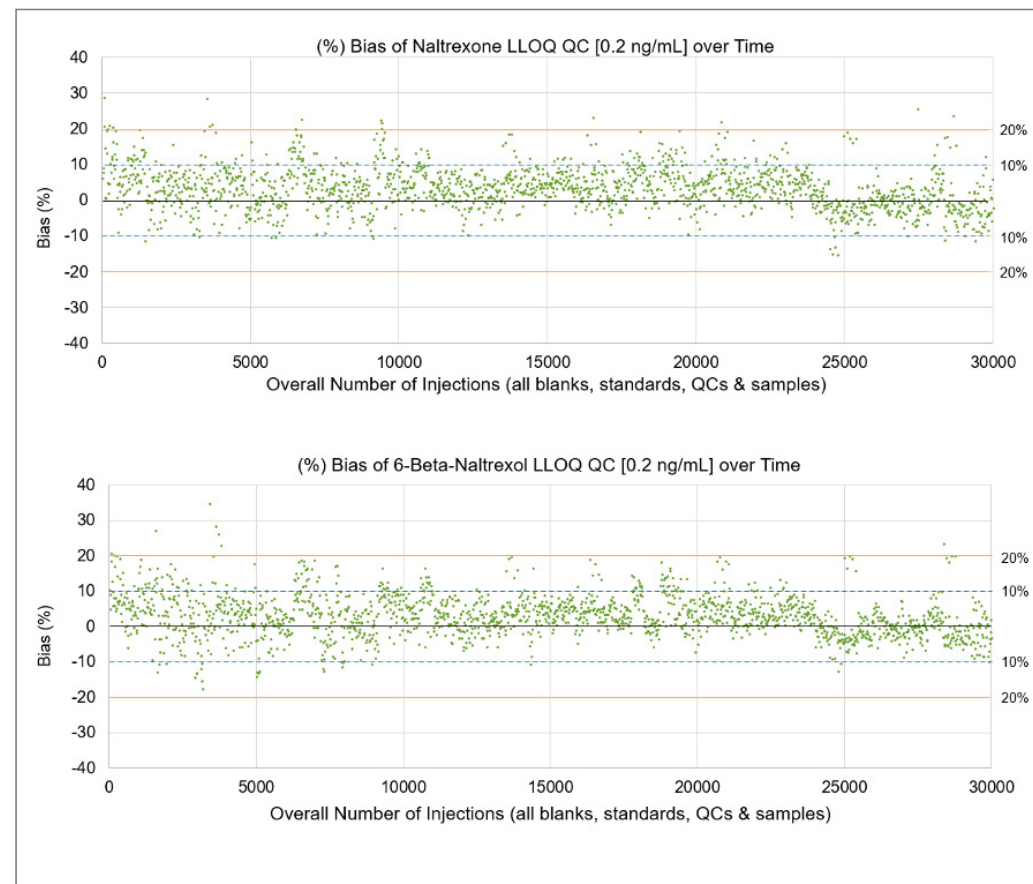


Delivering eXtreme Robustness in Bioanalysis

Alderley Analytical, now part of the Synexa Life Sciences Group, is a GLP- and GCP-accredited contract research organization (CRO) dedicated to supporting the development of new medicines. Known for high scientific standards and regulatory rigor, the team at Alderley Analytical requires robust, reliable instrumentation to validate diverse bioanalytical methods and deliver timely results to their customers.

KEY CHALLENGES AND SOLUTIONS

In bioanalysis, operational efficiency and minimal instrument downtime, even when the system has seen thousands of injections, are crucial. Using the Xevo TQ Absolute XR mass spectrometer, the team at Alderley Analytical were able to analyze over 30,000 samples and still achieve consistent, reliable performance even at the Lowest Limit of Quantification (LLOQ) level.



Support and Training



[Read the full poster.](#)

From the outset, Waters played a hands-on role in supporting Alderley Analytical — not only by providing advanced LC-MS technology, but also by helping the lab get up and running with confidence. The team at Alderley noted the value of an established relationship with Waters, which helped accelerate their operational readiness. Comprehensive training and responsive support enabled the lab's analysts to quickly adopt new workflows using waters_connect for Quantitation Software, fully leveraging its capabilities for regulated bioanalysis.

"The software is really intuitive and has great features for regulatory bioanalysis — especially for monitoring QC and calibration limits."



Sally Hannam

Scientific Director
Scientific Strategy Team, Alderley Analytical

BENEFITS AND IMPACT

The partnership with Waters has enabled Alderley Analytical to:

- ***Achieve reliability at scale***

The lab has run over 30,000 complex study samples of protein-precipitated human plasma on the Xevo TQ Absolute XR Mass Spectrometer, demonstrating exceptional robustness — even with dirty matrix — without performance drop-off.

- ***Ensure data integrity in regulated workflows***

With waters_connect Software, scientists can easily track calibration and QC limits, reducing the risk of compliance issues in GLP/GCP-accredited studies.

- ***Improve reproducibility for challenging assays***

Waters Premier technology helped resolve ion-metal interaction issues for problematic small molecules and metal chelators — achieving CVs <1% immediately after switching systems.

- ***Mitigate operational risk in clinical trials***

Reliable instrument uptime is essential when handling time-sensitive samples. By reducing downtime, Alderley maintained fast turnaround and protected valuable clinical trial data.

The Final Word: Collaboratively Advancing the Future of Innovation in PROTACs Bioanalysis



The journey from a Molecule to Medicine is a complex path that requires a wide range of analytical tools and workflows that can precisely and definitively answer critical questions at every stage. It requires a trusted partner who understands the evolving needs of drug discovery and development.

This eBook highlights the powerful tools and scientific insight Waters brings to the table, enabling your lab to navigate the challenges of PROTACs bioanalysis with precision, speed, and confidence.

From flexible and versatile HRMS solutions that streamline metabolite identification, to complete end-to-end bioanalysis workflows that ensure highest levels of sensitivity and reproducibility and imaging solutions that allow you to visualize tissue distribution, Waters offers the complete suite of technologies and workflows for all your PROTACs needs.

With Waters, you don't just get the right tools—you gain a committed ally in your mission to accelerate scientific discovery and deliver breakthrough therapies. Together, let's continue to push the boundaries of what's possible in bioanalysis.

Thank you for joining us on this journey.

Waters™



For your local sales
office, please visit
waters.com/contact

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