

[APPLICATION NOTEBOOK]

Versatile LC-MS/MS Solutions for the Evolving Bioanalysis Landscape

Waters™

LC-MS/MS Solutions to Support Reliable, Sensitive and Efficient Workflows for the Bioanalysis of Drug Modalities

Welcome to the Waters quantitative bioanalysis application compendium. Compiled here are abstracts and links to application notes covering a range of bioanalytical topics including:

- Small molecule therapeutics, including proteolysis targeted chimeras (PROTACs)
- Oligonucleotide therapeutics
- Peptide/protein therapeutics
- Efficiency enabling software tools
- Novel imaging approaches

Waters Tandem Quadrupole Mass Spectrometry solutions enable scientists to efficiently develop sensitive and robust quantitative methods, supported by novel chromatography technology that minimizes the risk of non specific binding for inherently susceptible compounds. Presenting optimized LC-MS/MS solutions for the evolving needs of Bioanalysis Laboratories.

Trust in Waters to support the challenges brought by new and varied drug modalities. Improve internal efficiencies with less re-analysis and reduced waste, ensuring data quality and workflow optimization.

Partnering with Waters ensures access to market leading application support and award-winning service. Waters supports successful bioanalytical laboratories achieve robust productivity day in, day out.



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Small Molecules



A Rapid UPLC-MS/MS Discovery Bioanalytical Method for the Quantification of Gefitinib and Four of Its Major Metabolites in Mouse Plasma

The large number of new molecules requiring evaluation that enter drug discovery each week means that methodologies need to be developed and implemented quickly, without the requirement for extensive method optimization. Therefore, rapid and simple Liquid Chromatography Triple Quadrupole (LC-MS/MS) methods are required to provide proper resolution of the target analyte from endogenous matrix and drug related metabolites. Ultra Performance Liquid Chromatography (UPLC) utilizing sub-2- μm particles, combined with tandem quadrupole mass spectrometry, is an enabling technology for this type of analysis. Due to the flat nature of the sub-2- μm particle LC van Deemter plot, these columns offer both high resolution and high throughput, thus significantly reducing the need for extensive method development.

Here we demonstrate the use of sub-2- μm UPLC and tandem quadrupole MS/MS for the quantification of gefitinib (Figure 1) in mouse plasma following both oral and intravenous (IV) administration.

Benefits and Highlights

- A simple and rapid UPLC-MS/MS methodology for the quantification of gefitinib and four of its metabolites
- Excellent separation between gefitinib and its major metabolites
- The assay demonstrated reliable and reproducible performance
- Good correlation between derived PK data and published literature

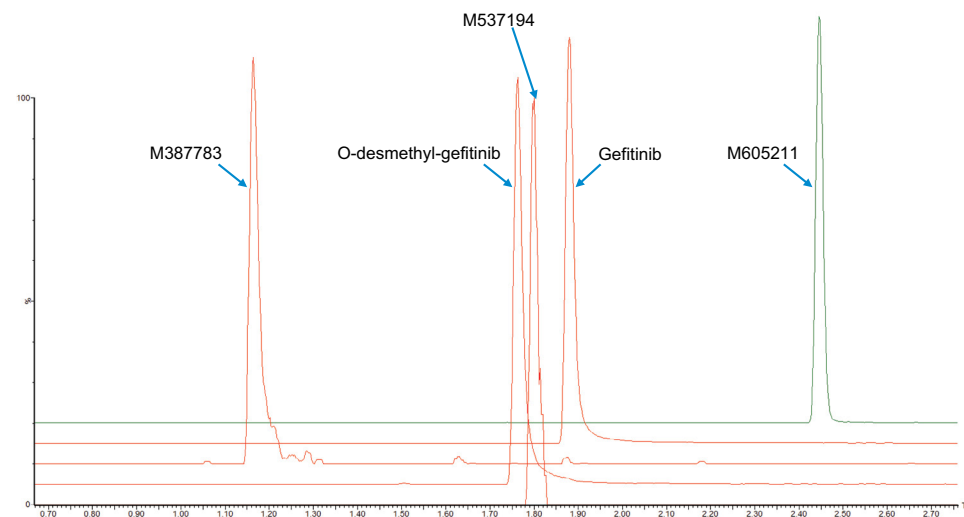


Figure 1. Typical chromatogram for gefitinib and four of its major metabolites.



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Quantification of Warfarin and Furosemide in Human and Rat Plasma for Discovery Bioanalysis

As the importance of quantifying drug candidates and biomarkers at lower concentrations increases, so does the need for more sensitive instrument platforms. In this application note, we describe a generic method for the extraction and quantification of two representative model compounds, Warfarin and Furosemide in human and rat plasma. Warfarin is an anticoagulant used in the treatment and prevention of thromboembolism. Careful and continuous monitoring of warfarin is essential to ensure that the drug concentration stays within its narrow therapeutic window. Furosemide is a diuretic used to treat edema in patients with heart failure, liver disease or a kidney disorder.

Using the ACQUITY™ Premier UPLC System and Xevo™ TQ Absolute Mass Spectrometer operated in negative ion electrospray ionization (ESI) mode, linear calibration curves from 0.025–100 ng/mL for Warfarin and 0.1–100 ng/mL for Furosemide from both rat and human plasma were generated. %CV for all points on the calibration curve and QCs were below 13% for both analytes in both matrices.

Benefits and Highlights

- Accurate, precise and sensitive quantification of Warfarin and Furosemide
- ACQUITY Premier UPLC System and Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer provide an ideal platform for discovery bioanalytical laboratories, providing excellent robustness and sensitivity across ionization polarity modes

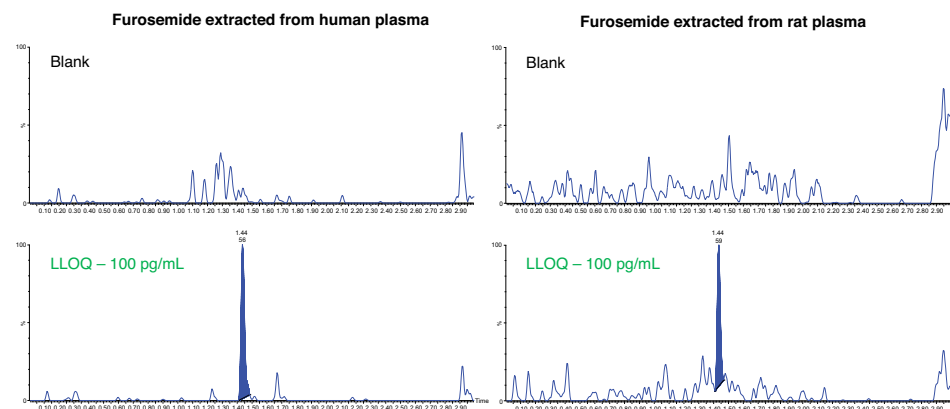


Figure 2. LLOQ for Furosemide extracted from human and rat plasma.



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High Sensitivity Bioanalysis of Midazolam and Imipramine in Rat Plasma Using the Xevo™ TQ-XS Tandem Quadrupole Mass Spectrometer

Bioanalysis forms a critical part of the drug discovery and development process, providing information on pharmacokinetics and dose response for new chemical entities. Lower doses of more potent drug candidates, along with reduced sample sizes from more ethically driven studies, 3R's (reduce, replace, refine), mean that liquid chromatography-Mass Spectrometry (LC-MS) method sensitivity is increasingly important. Concurrent with this requirement for increased sensitivity is the need to seamlessly transfer assays between laboratories, either within an organization or to a contract analysis organization. Thus, not only is MS sensitivity important, so too is instrument to instrument reproducibility.

In this application note we demonstrate the reproducible and robust sensitivity achievable using the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer as part of a UPLC-MS/MS system for bioanalytical studies. Sensitivity limits as low as 250 ag (0.25 femtograms) of midazolam and imipramine were detected on-column from a matrix sample (rat plasma) using a rudimentary, generic sample preparation technique (protein precipitation). This level of sensitivity was demonstrated to be reproducible and robust with limits of quantification determined to be 0.2 g/mL for both compounds.

Benefits and Highlights

- Robust, reproducible, and highly sensitive bioanalysis
- Simple sample preparation workflow

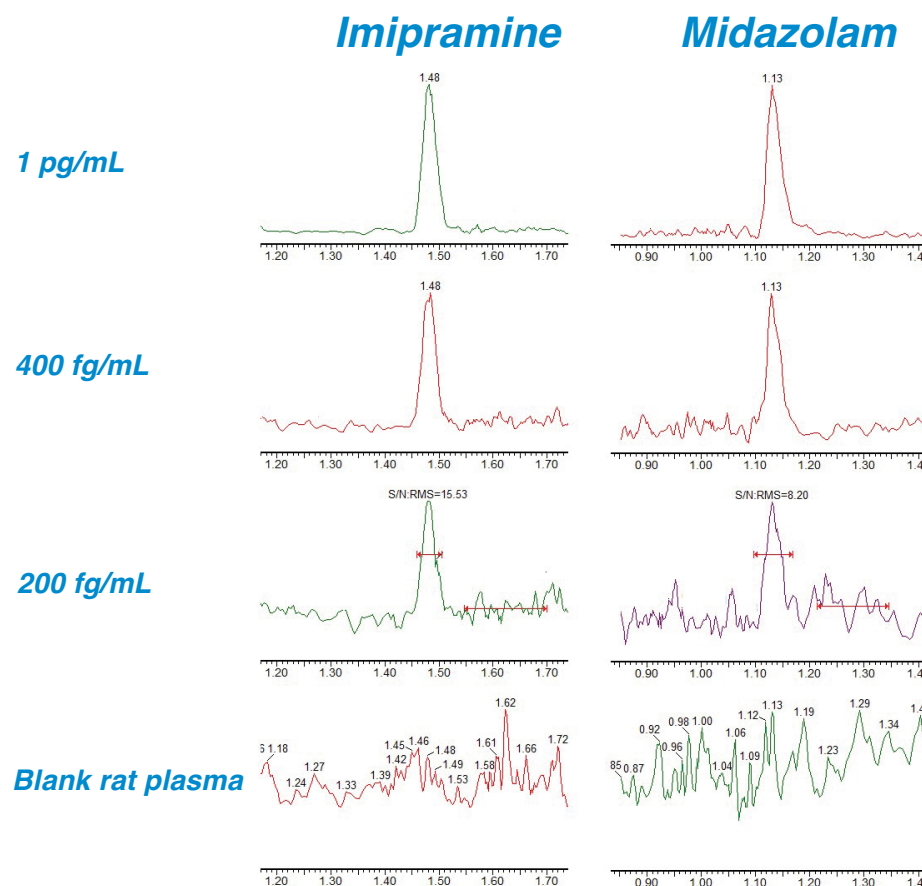


Figure 3. The limit of detection was determined to be 0.2 pg/mL for both analytes, with the lowest level calibrator and all four QC samples at that level being detectable in all cases



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Rapid High Sensitivity LC-MS/MS Bioanalytical Method for the Simultaneous Quantification of Gefitinib Based PROTACs

Targeted protein degraders such as Proteolysis Targeting Chimeras (PROTACs) represent a new approach to candidate drug design that can overcome the drug resistance experienced by many small molecule therapies. They can also increase access to previously undruggable proteins, whilst reducing the manufacturing costs, scale-up issues, shelf-life, stability, and storage issues associated with protein biotherapeutics. The accurate quantification of these PROTAC molecules in blood derived fluids is critical to support discovery and development, drug metabolism pharmacokinetic (DMPK) packages. A rapid (4-minute) LC-MS/MS bioanalytical assay for the quantification of PROTACs-3-gefitinib and gefitinib in rat plasma was developed. The limit of detection (LOD) was determined to be 20 pg/mL for gefitinib PROTACs-3 from 10 μ L sample, with a linear dynamic range of 20 pg/mL – 1,000 ng/mL. The assay was subjected to a 3-day validation with CV of 5% at the LOD.

Benefits and Highlights

- An LC-MS/MS bioanalytical method was developed and validated for the quantification of PROTACs-3-gefitinib in rat plasma
- Chromatographic separation was optimised to allow for the detection of the dosed compounds and the metabolites of gefitinib and gefitinib based PROTACs-3
- The method was successfully subjected to a three-day validation

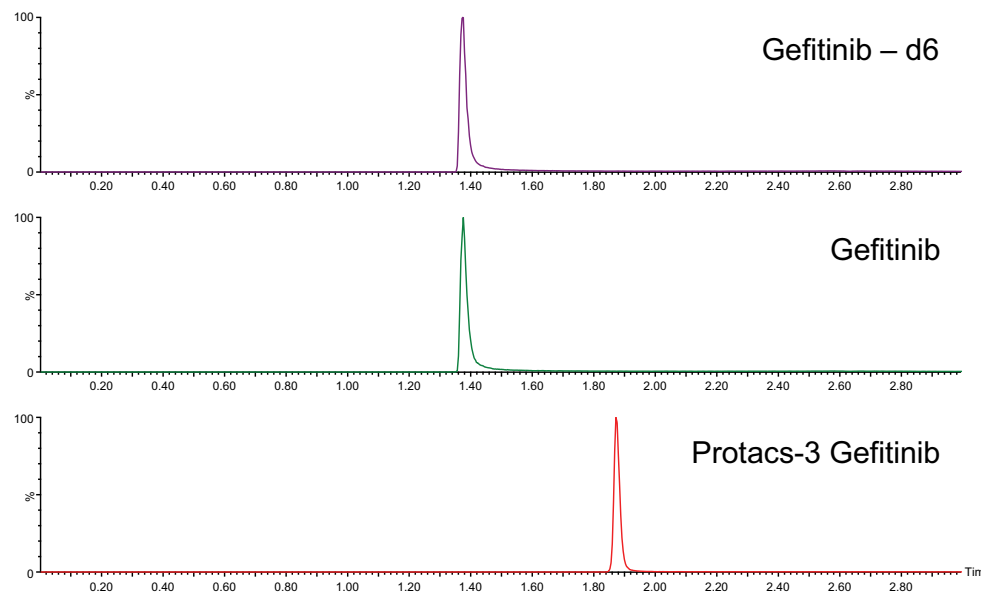


Figure 4. LC-MS/MS analysis of gefitinib based PROTACs-3, gefitinib-d6 and gefitinib



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Detection and Characterization of Drug Metabolites in Biofluids Using Survey Scan MS/MS Functionality on Waters Tandem Quadrupole Mass Spectrometers

The ability to rapidly screen biofluid samples for drug metabolites helps researchers identify metabolic soft spots, detect potentially toxic metabolites, and improve pharmacokinetic characteristics of candidate medicines. This can be a complicated and time-consuming process requiring the acquisition and analysis of mass spectrometry (MS) and MS/MS data. Tandem quadrupole mass spectrometers (TQ-MS) are used extensively for drug concentration determination due to their exquisite sensitivity in multiple reaction monitoring (MRM) mode, but they have other functionalities that can be applied to drug metabolism characterisation studies. Survey Scan acquisition mode on Waters tandem quadrupole mass spectrometers, allows for the rapid detection and characterization of drug biotransformation's using automatically generated diagnostic precursor and product fragment ions and constant neutral loss experiments. Survey Scan liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS) was applied to the analysis of urinary metabolites of methapyrilene. Over 30 drug related metabolites were detected using Survey Scan mode with precursor ion monitoring, by monitoring multiple common fragment ions of the dosed compound facilitated the localization of the site of metabolism.



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Benefits and Highlights

- Unbiased data dependent acquisition for nominal mass rapid metabolite screening
- Simple, easy to use MS/MS qualitative functionality
- Feature detection based on common fragment ion or constant neutral loss
- Precursor and product ion acquisition in a single analysis

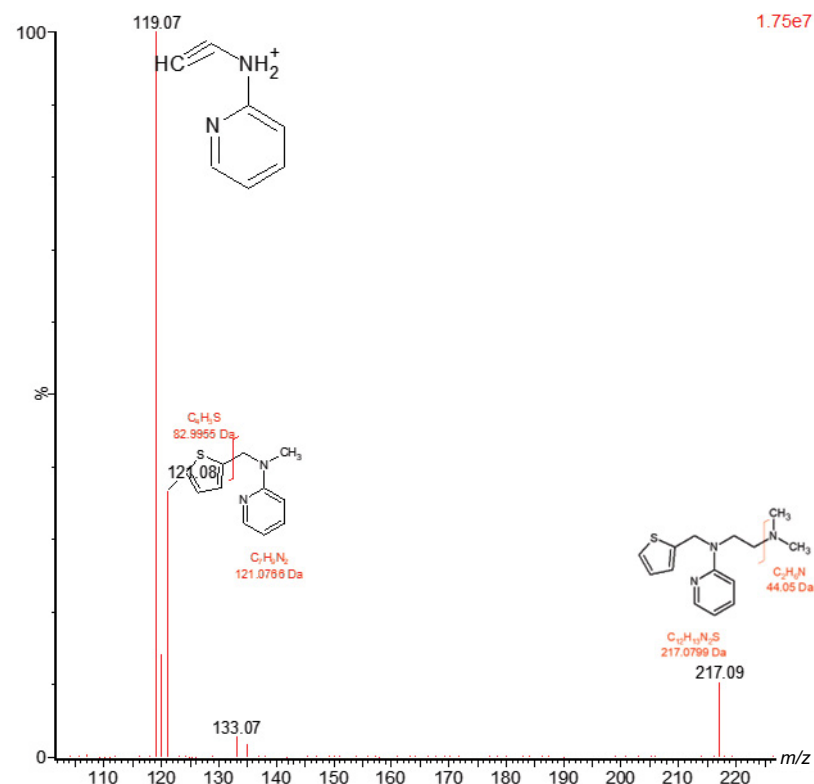


Figure 5. Product ion MS/MS analysis of methapyrilene authentic standard

Tandem Quadrupole Acquisition Modes: A Case Study for Discovery DMPK Studies

Tandem quadrupole mass spectrometers are extremely powerful and flexible instruments. They are the instruments of choice for high sensitivity quantitative analysis, with detection limits at the pg/mL level readily attainable and thus are extensively used to support drug metabolism pharmacokinetic (DMPK) studies such as first time in human (FTIH). The configuration of tandem quadrupole instruments along with modern fast data acquisition facilitates multiple other modes of data acquisition, such as neutral loss, precursor ion scanning and polarity switching, allowing the DMPK scientist to easily and quickly interrogate their samples to aid the drug discovery or regulator submission process.

Benefits and Highlights

- Powerful, flexible instrument with multiple acquisition modes.
- High sensitivity quantification and metabolite screening using a variety of acquisition modes
- Acquisition modes can be used to monitor for drug related material via diagnostic fragment ions, screen for classes of drug metabolites such as sulphates and glucuronides and for drug related impurities via diagnostic fragment ion

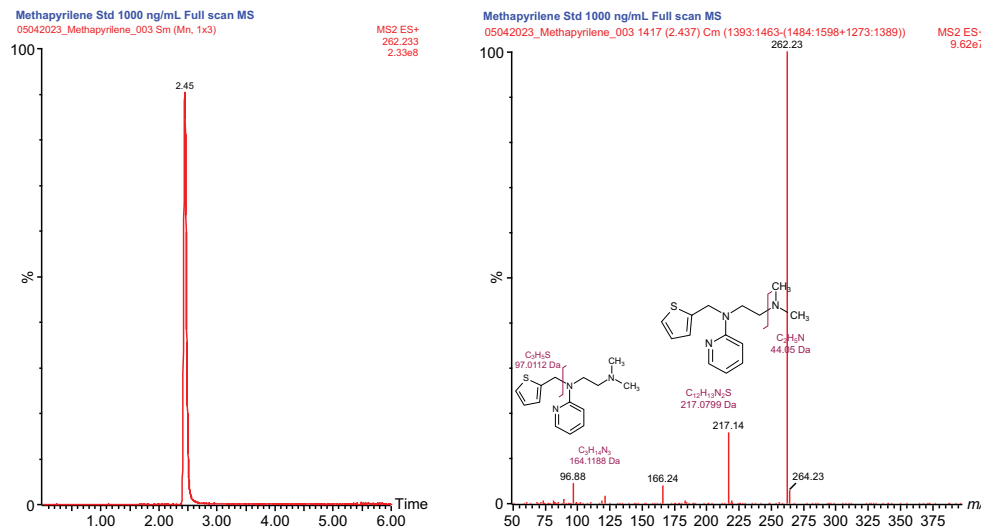


Figure 6. LC-MS chromatogram and full scan MS analysis of methapyrilene authentic standard



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Simple, Fast and Selective, Bioanalytical Sample Extraction for the Therapeutic Drug, Lenalidomide From Plasma Using Oasis™ MCX SPE

The following work demonstrates a simple, generic, broadly applicable, fully automated bioanalytical sample preparation strategy, requiring no method development, for the small-molecule therapeutic drug lenalidomide for several common bioanalytical extraction techniques including protein precipitation (PPT), liquid-liquid extraction (LLE), and solid phase extraction (SPE). LC-MS bioanalytical quantitation performance using mixed-mode SPE with Oasis WCX from plasma is highlighted using the Andrew+Pipetting Robot in combination with the Extraction+ Connected Device for fully automated liquid handling and solid phase extraction (SPE) sample preparation.

Benefits and Highlights

- Generic extraction method protocols, yielding high analyte recovery, and achieving reproducible results
- Fast bioanalytical extraction (<30 minutes) using the 96-well μ Elution extraction plate format
- Automated sample extraction, for “walk-away” method execution
- Excellent quantitative performance

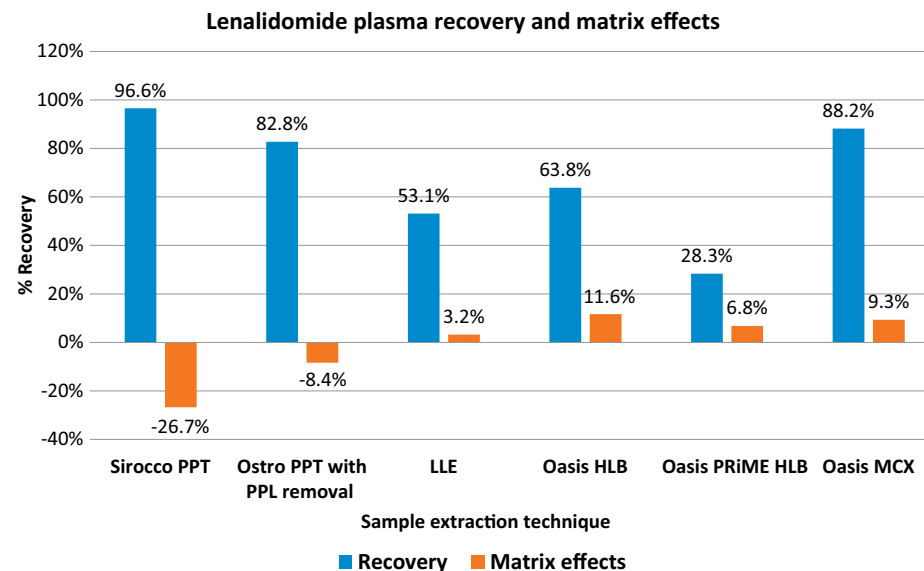


Figure 7. Representative recovery and matrix effects resulting from the extraction of lenalidomide from plasma using different techniques



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Fully Automated Bioanalytical Solid Phase Extraction Sample Preparation, using Extraction+ Connected Device with the Andrew+ Pipetting Robot

To achieve accurate, precise, and reproducible results, a skilled and experienced analyst is often required, as any errors or inconsistencies in the execution of the sample preparation protocol need to be minimized to avoid their propagation through the entire process. This can result in inaccurate quantification and potential loss of time and resources to re-extract out-of-control batches. These errors or inconsistencies can include pipetting errors when generating calibration curves and quality control (QC) samples, missed or incorrectly labelled samples, incorrect addition of reagents, analyst technique-dependent inconsistencies, and other variables that can compromise results. Introducing automation to the bioanalytical lab helps to minimize or eliminate some of these challenges, in addition to freeing up bioanalytical scientists for other tasks.

In this application note, the Andrew+ Pipetting Robot was used to generate calibration curves and QC samples using a diversity of small molecule pharmaceutical drugs in plasma. The Andrew+ Pipetting Robot (Andrew+) and the new Extraction+ connected device (Extraction+) were then used to perform solid phase extraction (SPE) of these prepared samples.

Benefits and Highlights

- Easy-to-use OneLab™ Software with data visualization for creating and transferring methods
- Automation compatibility with SPE plates and cartridge formats
- Reduced extraction performance variability through fully programmable vacuum pressure profiles with Extraction+ connected device
- Automated liquid handling and sample preparation increases efficiency, allowing the user to perform other tasks
- Full “walk-away” automation with no user intervention steps mitigates the risk of manual errors

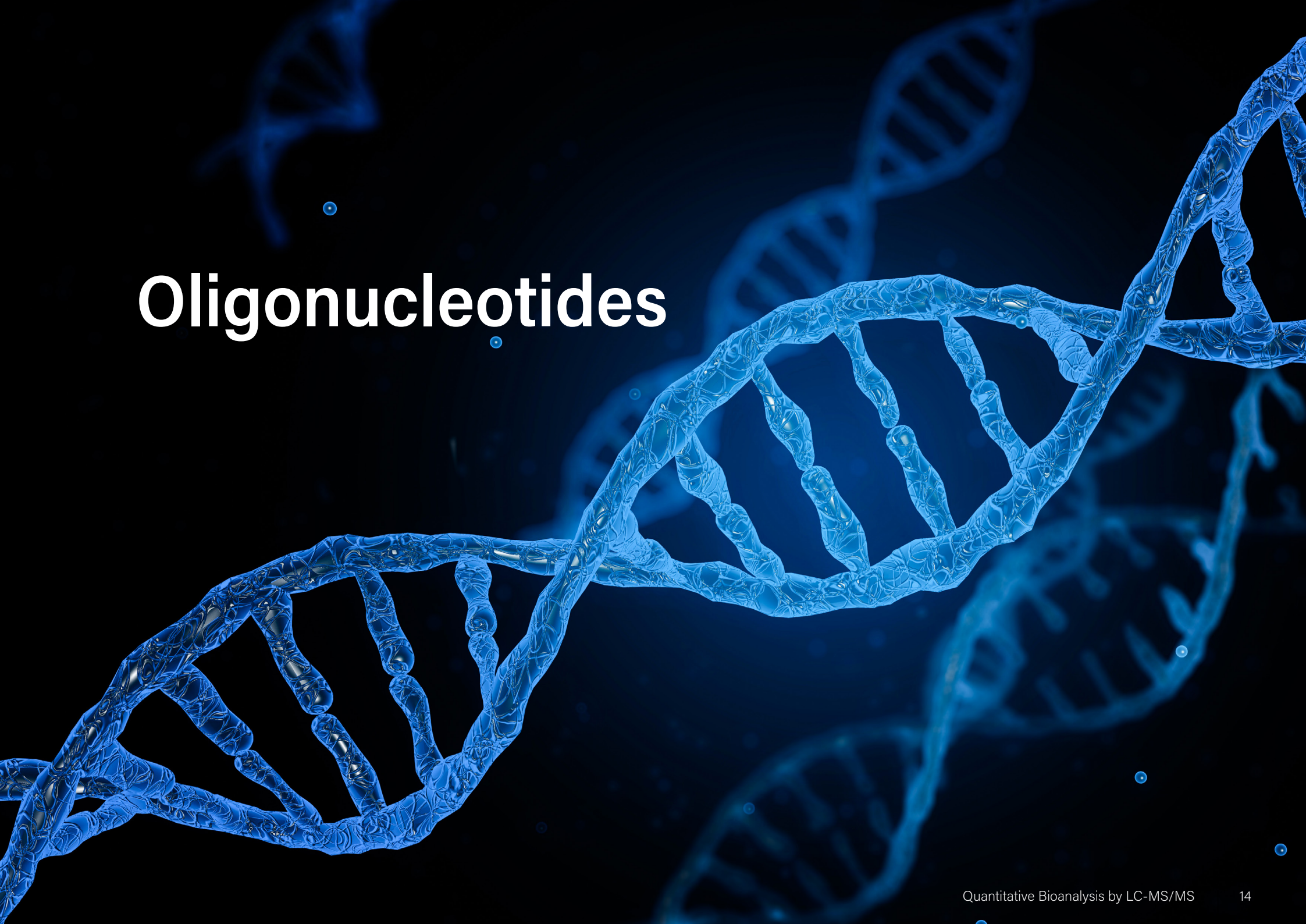


Figure 8. Andrew+ configured with the required Dominos



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Oligonucleotides



Sensitive LC-MS/MS Bioanalytical Quantitation of Antisense Oligonucleotides

Oligonucleotides are challenging substrates to measure due to many factors including but not limited to: requiring ion pairing or non-reversed phased chromatography; requiring careful handling and sample prep; and often suffering from non-specific binding issues from consumables and metal surface interactions often present in chromatographic systems.

In this application note, we demonstrate the performance capabilities of the high-performance Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer coupled to the Waters ACQUITY Premier System for the analysis of oligonucleotides in biological matrices. Quantification of oligodeoxythymidines standards (Waters MassPREP™ Oligonucleotide Separation Technology (OST) Standard) and GEM91, a fully phosphorothioated antisense oligonucleotide [d(P-Thio)(C-T-C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-T-C-T)-DNA] were investigated.

Benefits and Highlights

- Sensitive and routine quantitation of oligonucleotides in human plasma is demonstrated using the tandem quadrupole mass spectrometer
- The quantitation of a 25-mer fully phosphorothioated antisense oligonucleotide, Trecoverin (GEM91) and polyoligodeoxythymidine 15 to 30 mer oligonucleotide standards

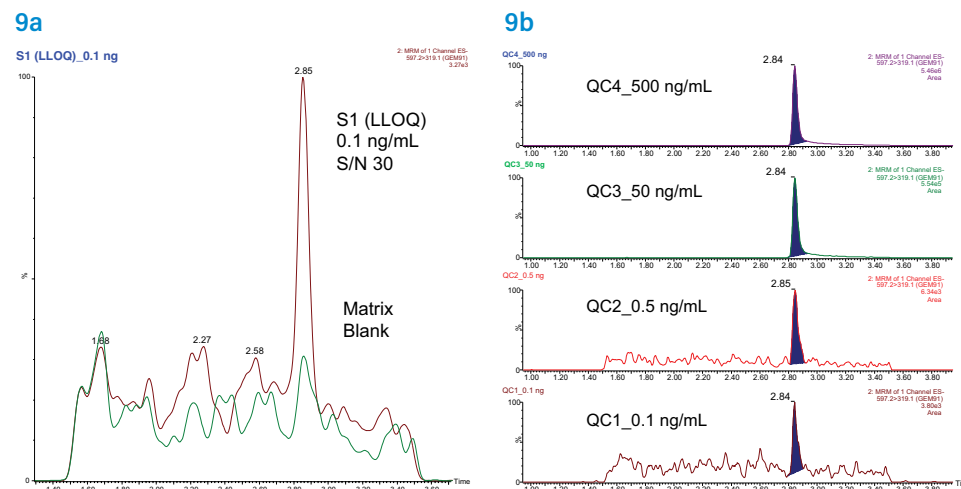


Figure 9a. GEM 91 (597.2>319.1) 0.1 ng/mL standard (LLOQ) overlaid with matrix blank (green trace).

Figure 9b. Representative QC traces for GEM91 (0.1–500 ng/mL)



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Sensitive Bioanalysis of Antisense Oligonucleotides of Various Lengths and Modifications

High sensitivity and five orders of dynamic range performance were described previously using GEM91/Trecovirsen. This application brief demonstrates the sensitivity and suitability of the Xevo™ TQ Absolute Triple Quadrupole MS for bioanalysis of oligonucleotides in human plasma matrix with varying lengths (18 to 33 nucleotides), linkers, and modifications.

Benefits and Highlights

- Sub ng/ml levels of sensitivity, with good dynamic range performance was observed in human plasma for antisense oligonucleotides
- Enhanced sensitivity for challenging negative ionization compounds for routine LC-MS/MS based quantitation of antisense oligonucleotides in biological matrices
- MaxPeak™ HPS technology reduces non-specific binding, improving chromatographic performance and recovery

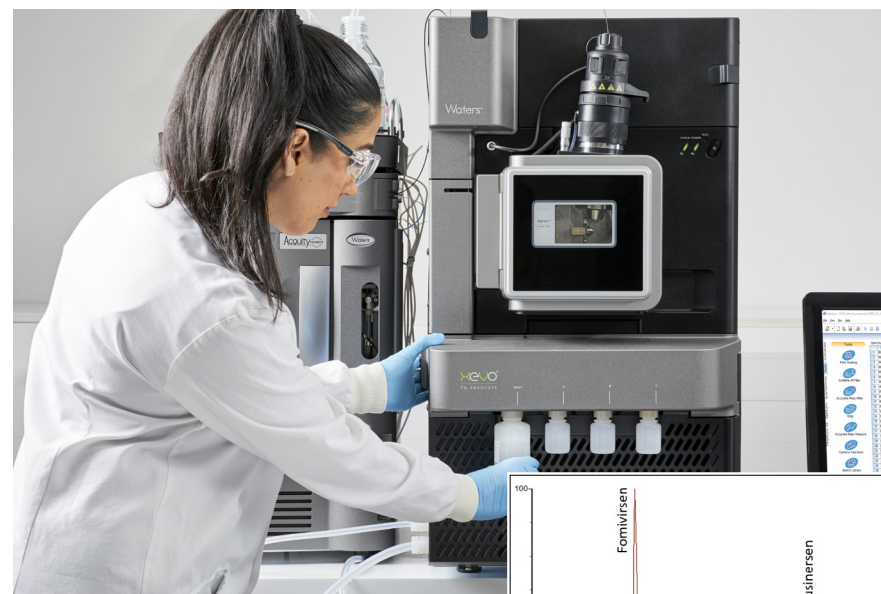
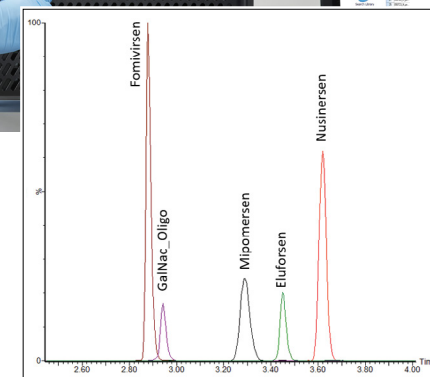


Figure 10. Waters Xevo TQ Absolute System with Waters ACQUITY Premier UPLC



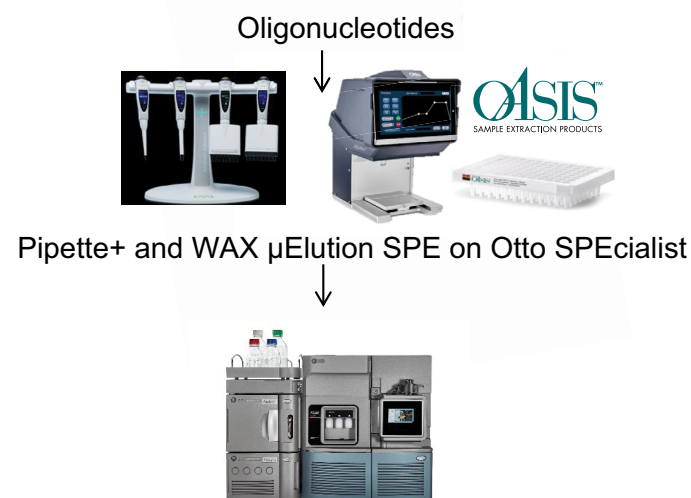
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LC-MS Bioanalytical Quantification of a GalNAc-siRNA Conjugate Oligonucleotide Using Semi-Automated Solid Phase Extraction

With a rise in research and development efforts related to oligonucleotide therapeutics (ONTs), there has been a growing need for more robust, sensitive, and selective sample preparation and LC-MS methods for evaluating these therapeutics. This can be challenging due to the complex and diverse characteristics of ONTs. They are poly-anionic in nature and are known to non-specifically adsorb to metal surfaces and proteins in biological samples. This can lead to analyte loss during biological sample preparation as well as binding to metal surfaces during LC analysis. Furthermore, when manually preparing samples, mistakes can be made by the scientist which can lead to even greater loss of the analyte of interest. All of these unwanted interactions can lead to poor, inconsistent chromatographic performance, and subsequent mass analysis. The following work demonstrates the capabilities of the Pipette+ in combination with the Otto SPEcialist for semi-automated liquid handling and solid phase extraction (SPE) of a GalNAc-siRNA conjugated oligonucleotide.

Benefits and Highlights

- Semi-automated sample preparation and extraction using Otto SPEcialist Positive Pressure Manifold and Pipette+ reduces variability between users and ensures accurate and reproducible results
- Transferrable methods on the easy-to-use Otto SPEcialist and OneLab Software allows for implementation of a sample preparation procedure across users and labs
- Improved method detection limits and reproducibility through mitigated metal adsorption of oligonucleotides
- Accurate and sensitive quantification of a GalNAc-siRNA conjugated oligonucleotide



LC-MS Analysis on Xevo TQ-XS and ACQUITY Premier

Figure 11. Bioanalytical sample preparation and LC-MS workflow of a GalNAc-siRNA conjugate oligonucleotide



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An Automated, Standardized, Kit-Based Sample Preparation Workflow for Bioanalytical Quantification of Therapeutic Oligonucleotides

Achieving reproducible performance with liquid chromatography mass spectrometry (LC-MS) based bioanalytical assays can be challenging. In general, the greatest source of variability for these assays arises from the sample preparation needed to extract the drug and its metabolites from biofluids, and this is especially true for oligonucleotide extractions.

A simple, broadly applicable sample preparation workflow for ONTs that reduces the need for method development and brings greater consistency and reproducibility to LC-MS bioanalytical results is therefore highly desired. The OligoWorks™ SPE Microplate Kit (OligoWorks Kit) from Waters has been designed with this in mind. It utilizes standardized, detergent free reagents, and a robust optimized protocol that works across a diverse range of ONTs with little to no method development needed. The automation friendly reagents and SPE devices provided in each kit make it easy to automate the sample preparation procedure on an automated liquid handler, like the Andrew+™ Pipetting Robot, which can further enhance analytical performance and productivity and reduce human error/variability.



[Read the Application Note](#)

Benefits and Highlights

- A standardized, detergent free, kit-based solution for the extraction and LC-MS quantification of therapeutic oligonucleotides from biomatrices requiring little to no method development
- Automation friendly workflow as demonstrated with the Andrew+ Pipetting Robot, with Click & Execute OneLab™ Software library methods
- Accurate, sensitive, and reproducible quantification across a diverse set of therapeutic oligonucleotides from extracted plasma samples

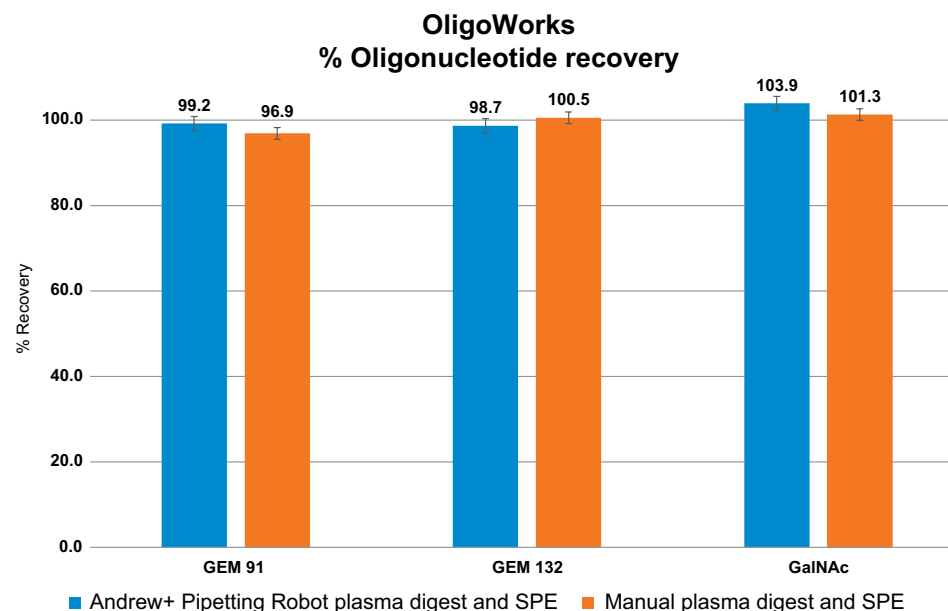
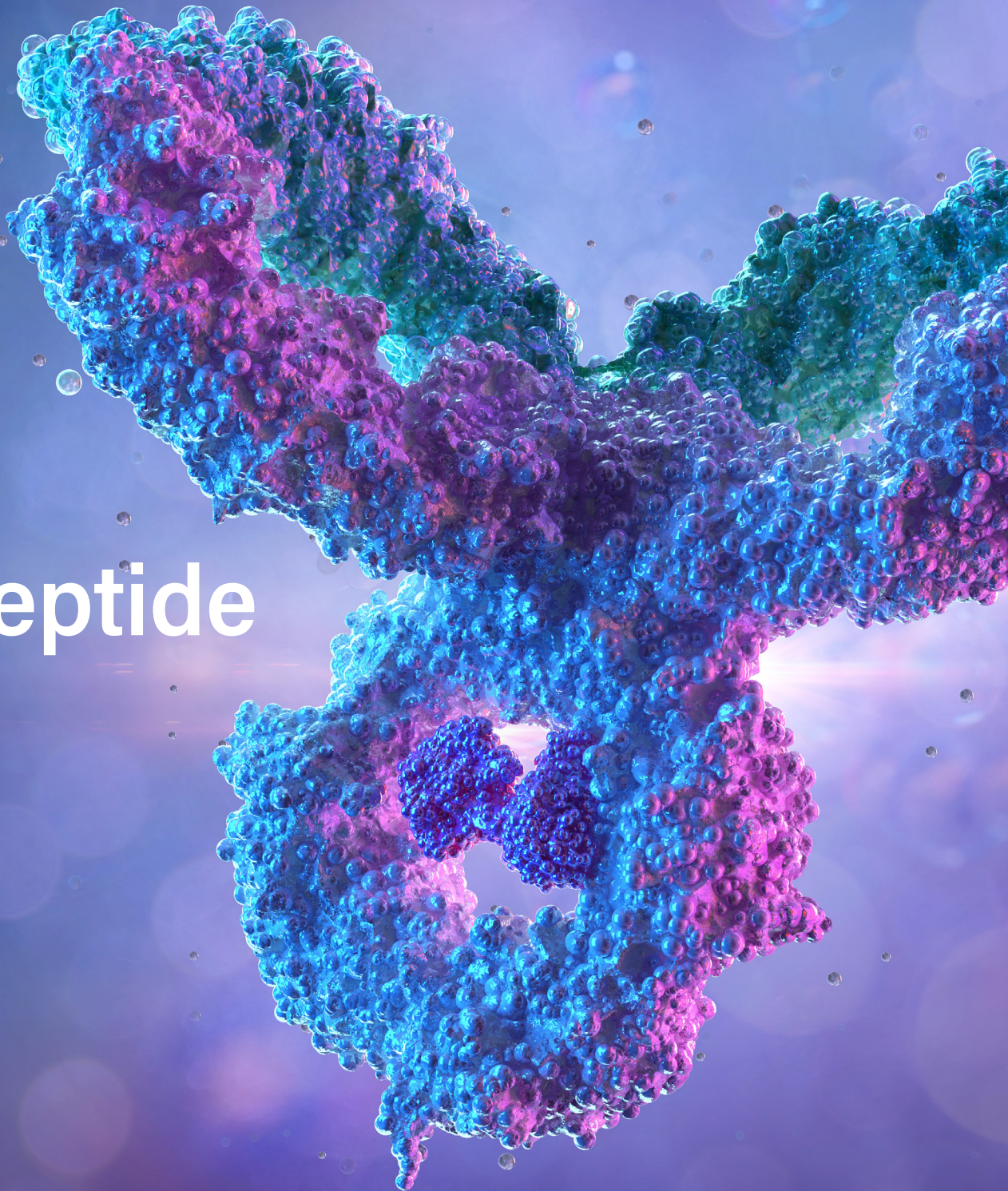


Figure 12. Comparable automated (Andrew+ Pipetting Robot) vs manual sample preparation and extraction performance using the OligoWorks Kits

Protein and Peptide Therapeutics



Automating Sample Preparation Workflows for Hybrid LC-MS/MS Bioanalysis of Protein Therapeutics

Automated liquid handlers in bioanalytical laboratories are routinely used to simplify and standardize sample preparation workflows, ultimately increasing throughput, reducing error, and improving assay performance. While used regularly for simple bioanalytical tasks, like serial dilution, protein precipitation, or solid-phase extraction, implementation of the liquid handlers for complex, multi-step workflows, like protein digestions, has not seen the same success. This could be attributed to the complicated method development and optimization of steps like immunoaffinity purification and protein digestion to achieve the high levels of sensitivity desired for accurate quantification from biological matrices. Successful automation implementation of the previously described protein quantification workflow requires assessment, verification, and potential re-optimization of the sub-steps contained within to ensure it meets the rigors of bioanalytical method development criteria.

This application note aims to describe the process of developing a successful, automated capture and digestion method for hybrid LC-MS/MS quantification of the fusion protein, etanercept (Enbrel).

Benefits and Highlights

- Highly sensitive, accurate, and reproducible hybrid LC-MS/MS method for protein quantification via the surrogate peptide approach
- Automating complex workflows, like protein quantification, can minimize human error, increase throughput and maximize productivity whilst achieving accurate and reproducible performance

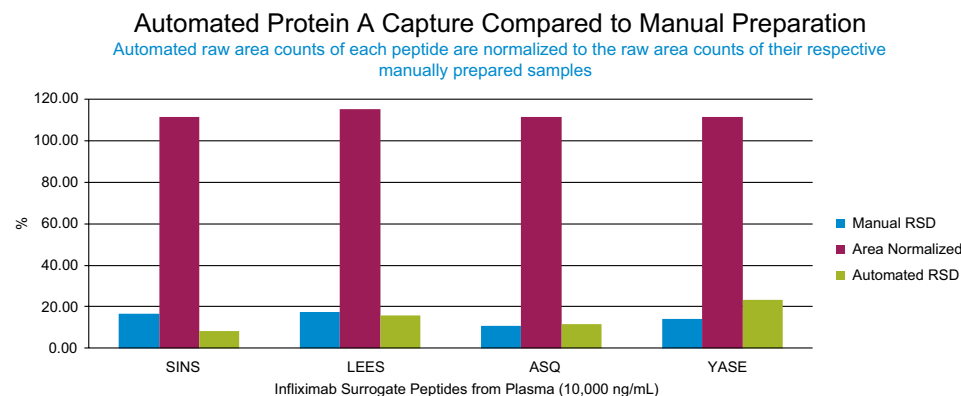


Figure 13. Comparable automated (STAR) vs. manual sample Protein A capture performance (peak areas and %RSDs) of surrogate peptides representing infliximab



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Development of a Hybrid Immunoaffinity-LC-MS/MS Method for the Quantification of Active Biotherapeutics Targeting TNF- α in Serum

Biotherapeutics have traditionally been quantified via immunoaffinity methods such as enzyme linked immunosorbent assay (ELISA), but these assays can suffer from cross-reactivity and a lack of specificity. However, when the immunoaffinity capture method is coupled to a high specificity detection method, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), the sensitivity and selectivity of the assay can be greatly improved. The most common strategy to prepare proteins for quantitative MS analysis is the surrogate peptide or bottom-up approach, employing enzymatic digestion and subsequent analysis of the resulting peptides. The method described here uses specific immunoaffinity enrichment with target tumor necrosis factor-alpha (TNF- α) followed by a standardized, kit-based approach to protein digestion, and LC-MS/MS detection to quantify 'free/active monoclonal antibodies (mAb') from ≤ 10 μ L of human serum.

This application note presents a highly sensitive and selective sample preparation strategy for the LC-MS/MS quantification of free/active biotherapeutic from human serum for the TNF- α targeting biotherapeutics: infliximab, adalimumab, and etanercept.

Benefits and Highlights

- High analytical sensitivity LC-MS/MS quantification of TNF- α targeting biotherapeutics
- Speed and reproducibility of a generic kit-based approach for protein quantification
- With the appropriate immunoaffinity capture reagents, this technique can be applied to other targets and biotherapeutics

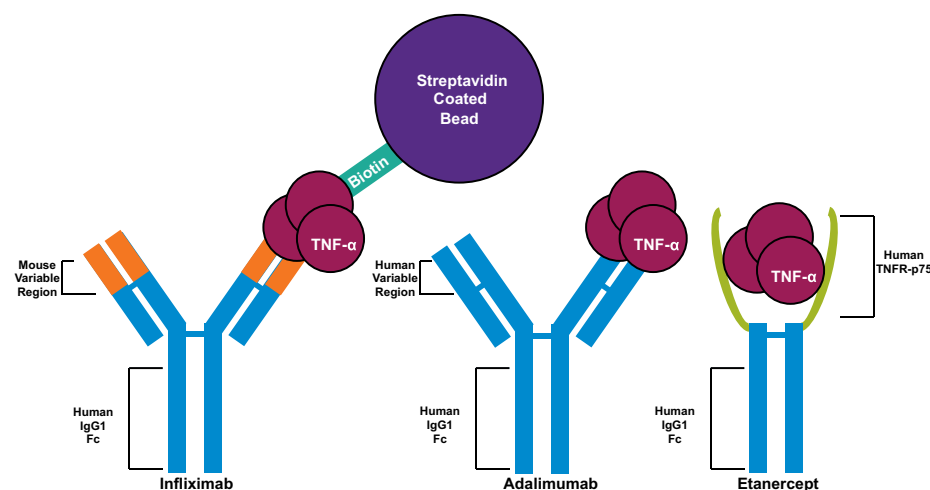


Figure 14. Tumor necrosis factor alpha (TNF- α) binds to the variable regions of infliximab and adalimumab, and to the TNFR-p75 region of etanercept. Biotinylated TNF- α is conjugated to a slurry of streptavidin coated magnetic beads.



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SPE-LC/MS Bioanalytical Quantification of the Biotherapeutic Peptide, Semaglutide From Plasma

The following work demonstrates the sensitive, selective, and robust liquid chromatography mass spectrometry (LC-MS) bioanalytical quantification of the peptide therapeutic, semaglutide from plasma. Using a combination of selective solid-phase extraction (SPE) sample preparation in the μ Elution 96-well format, QuanRecovery™ with MaxPeak™ High Performance Surfaces, 96-well plate for sample analysis to mitigate peptide non-specific binding, and LC-MS/MS analysis and quantification using the UPLC™ Peptide CSH™ C₁₈ Column, ACQUITY™ UPLC I-Class Plus System, and Xevo™ TQ-XS Mass Spectrometer, to achieve fast, sensitive, and highly reproducible and accurate quantification of semaglutide from plasma biomatrices.

Benefits and Highlights

- Selective, fast SPE extraction (<30 minutes)
- Fast UPLC analysis (4-minutes)
- High peptide recovery, and repeatability during analysis
- Accurate and sensitive quantification of semaglutide

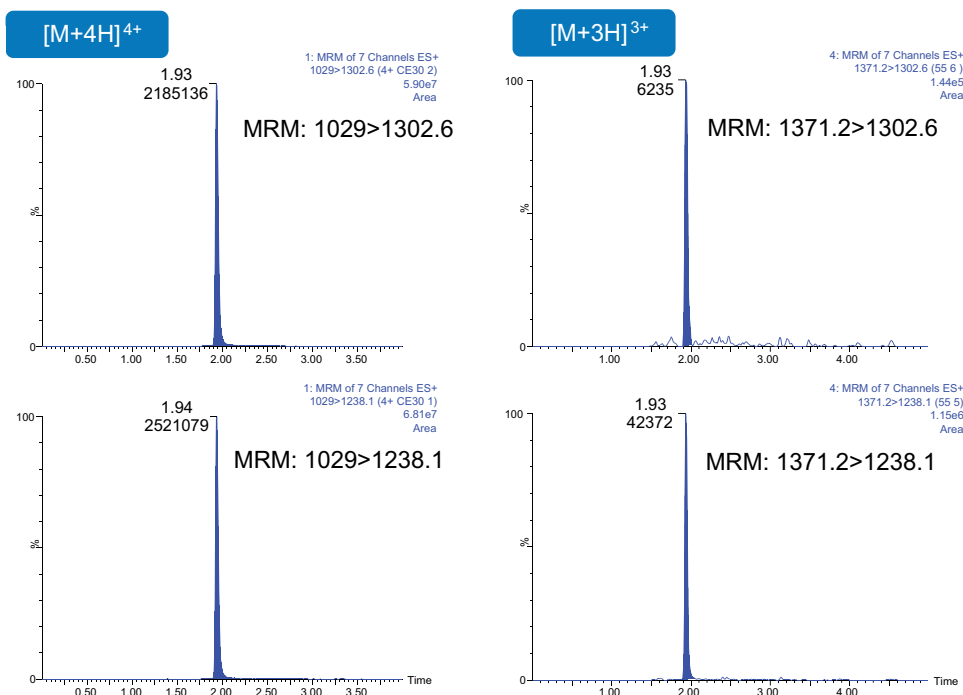
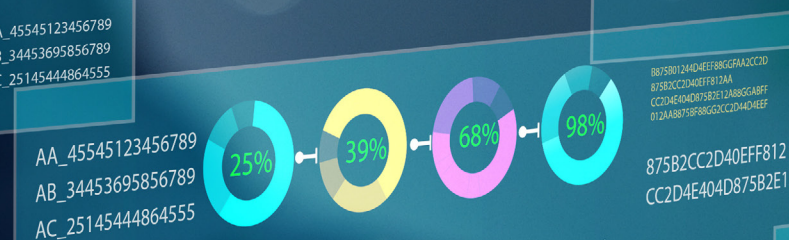


Figure 15. Representative semaglutide MS MRM chromatograms of the $[M+3H]3+$ and $[M+4H]4+$ multiply charged precursors



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Software Tools



QuanOptimize: A Software Tool that Enables Rapid, Consistent, and Accurate MRM Method Development for Large Numbers of Small Molecule Analytes

Discovery bioanalytical laboratories routinely develop methods for hundreds of compounds per week to support the various programs in their pipeline. Developing methods for each of these compounds individually can be time-consuming and tedious. Since the approach to small molecule method development is well understood, automation of this process is desirable. QuanOptimize automates the multiple reaction monitoring (MRM) method development for large sets of compounds. It can store the final method parameters to a database, automatically run sample lists using the optimized method, create data processing methods, and generate quantitative result with the click of a single button. QuanOptimize ensures consistent quality of methods across multiple users, reduces sample consumption and saves time by up to 5-fold.

Here, we discuss the use of QuanOptimize to develop methods for a set of 18 small molecule compounds using generic tune page and LC methods.

Benefits and Highlights

- Automated MRM method development for large number of analytes
- Automated generation of acquisition and data processing (TargetLynx) methods
- One-time, generic set-up for all compound types
- Consistent method quality across user experience levels
- Saves time and sample consumption

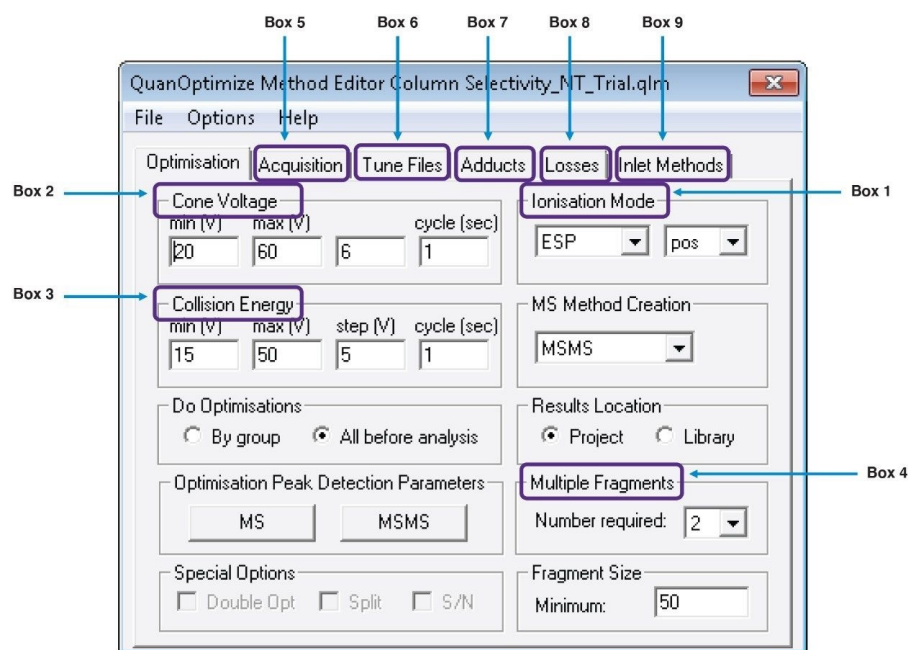


Figure 16. QuanOptimize Method Editor – Allows the user to set up parameters to be used during automated method development using QuanOptimize.



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MassLynx-Skyline Interface (MSI) – A New Automated Tool to Streamline MRM Method Development and Optimization for Large Molecule Quantification

Proteins and peptides are increasingly becoming routine analytes in laboratories performing quantification using LC-MS/MS. The fundamental differences between small molecules and peptides/proteins makes multiple reaction monitoring (MRM) method development and optimization for these larger molecules more challenging. Skyline is a freely available software tool created by the University of Washington (MacCoss Lab), and is widely used in targeted proteomics workflows, but is now increasingly used to aid method development of large molecule bioanalytical assays that support drug discovery and development. Current compatibility between MassLynx and Skyline enables the user to easily create acquisition methods, review data, and optimize collision energy to generate a final MRM method. Although this process is significantly simpler than trying to develop these methods manually without the aid of Skyline, it requires manual intervention to create and export acquisition methods (Skyline), acquire data (MassLynx), import, review and filter the data (Skyline), and generate a final acquisition method.



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Here, we describe a software tool, MassLynx Skyline Interface (MSI), which automates the workflow described above to make for a simpler user experience with minimal intervention.

Benefits and Highlights

- Automate and simplify multiple reaction monitoring (MRM) method development for peptides, digested proteins (therapeutics/biomarkers), or targeted proteomic LC-MS/MS assays using integrated Skyline interface within Waters MassLynx LC-MS/MS software

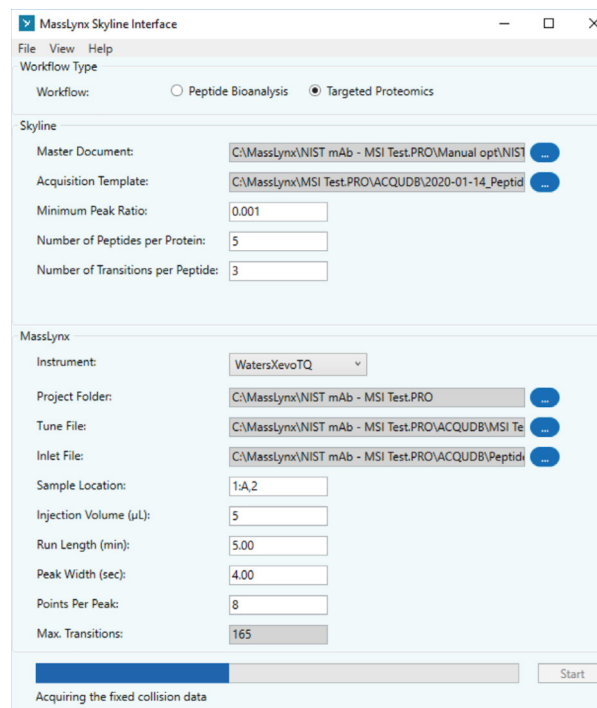


Figure 17. MassLynx Skyline interface.

Peptide Optimization Using Skyline and the Xevo TQ-XS

Moving from discovery to targeted proteomic experiments involves multiple reaction monitoring (MRM) transition optimization and refinement of many peptides from a protein. Compounding the process is the need to utilize peptides from a protein digest leading to a relatively complex sample, which can only be injected on-column to perform these optimization experiments. Similarly, quantification from a purified or synthetic peptide can have many of the same complexity challenges. Such iterative injection and review can be time consuming. The use of software to facilitate MRM assignment and visualization of the results can speed up this process to a few injections on-column. Skyline is one such, open source software that aids in the development of SRM /MRM transition optimization by on-column injection of sample. The software can perform in silico digestion of a protein using a variety of enzymes. Skyline works with MassLynx Mass Spectrometry Software to create MRM methods, then visualize the raw data, and finally, automatically refine MS methods for routine analysis.

This technology brief describes how to set up the software and perform such an experiment.

Benefits and Highlights

- The use of Skyline software via the Masslynx integrated Skyline interface streamlines optimization through to data processing and visualization

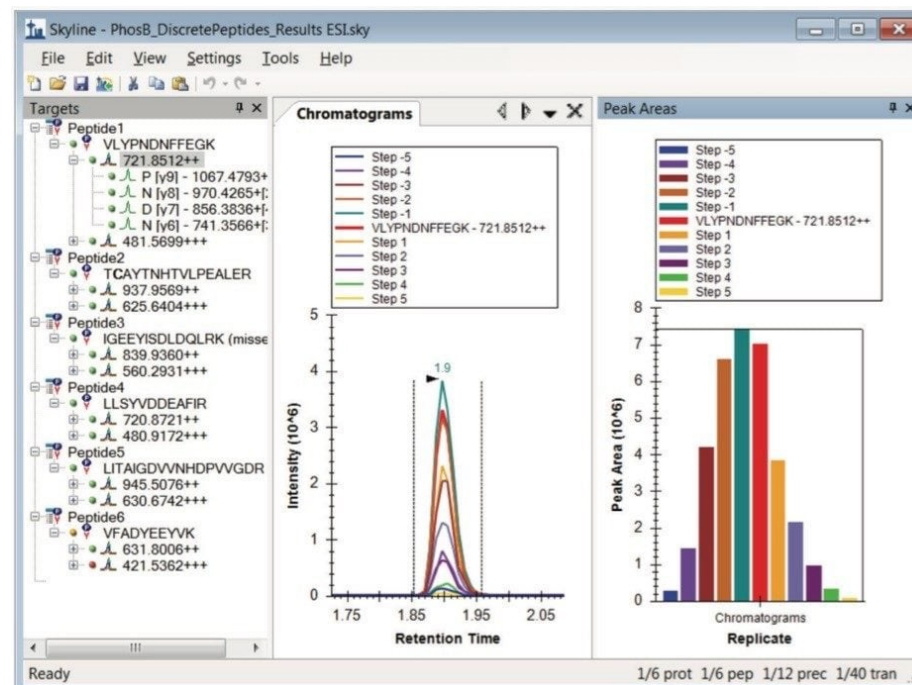


Figure 18. MRM optimization data acquired on the Xevo TQ-XS and imported into Skyline.



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Developing and Monitoring a High Sensitivity Bioanalysis MRM Method Using Intellistart™ and RADAR Technology

High sensitivity bioanalytical methods are used throughout the drug discovery and development process to provide accurate quantification data to support drug metabolism pharmacokinetic (DMPK), toxicokinetics (TK) studies, and randomized clinical trials. Over the last 25 years tandem quadrupole LC-MS/MS has become the technology of choice for quantitative bioanalysis. Developing reliable fit for purpose methodologies requires careful evaluation of LC and MS acquisition parameters. Waters™ Tandem Quadrupole MS systems are equipped with Intellistart Software, which facilitates the fully automated development of robust, high sensitivity Multiple Reaction Monitoring (MRM) methods. Intellistart optimizes ionization polarity, precursor ion, product ion(s), source voltages, and collision energies to identify the most optimal acquisition parameters. Xevo™ Tandem Quadrupole Mass Spectrometers are also equipped with RADAR Technology which allows the simultaneous monitoring of the LC background signal simplifying method development and assay monitoring.

Benefits and Highlights

- Automated optimization of MRM conditions using Intellistart Technology
- Rapid, efficient development of optimal SIR, and MRM conditions for non-expert and expert users alike
- Monitoring of background signals using RADAR technology, to simplify LC/MS method development, ensure method applicability, and troubleshooting

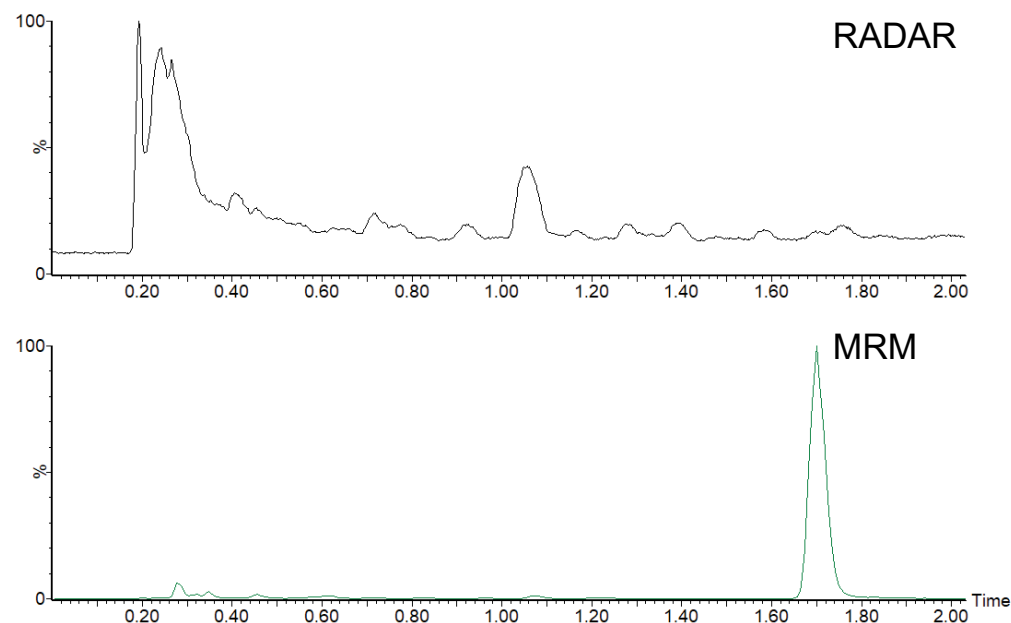
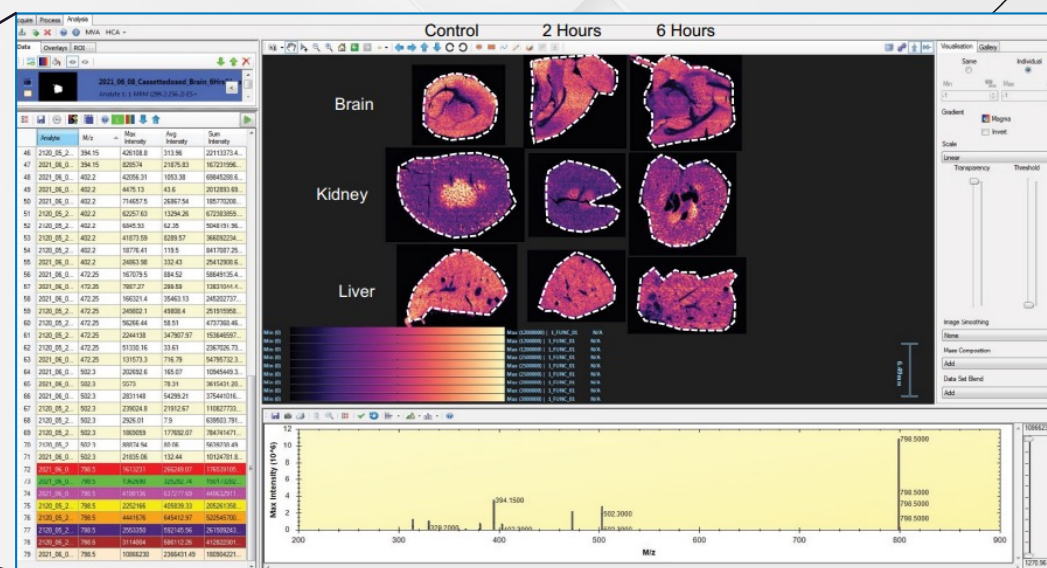


Figure 19. RADAR and MRM acquisition rat urine sample on D6 following oral administration of methapyrilene at 150 mg/Kg. Peak at $t_R=1.7$ min from the MRM transition methapyrilene 262.2→119.2 in positive ion ESI mode.



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Targeted Imaging



A High Sensitivity, High Throughput, Targeted MS Imaging Approach Enabling Pharmacokinetic And Biodistribution Analysis Of Cassette-Dosed Drugs

Cassette dosing of drugs for pre-clinical pharmacokinetic (PK) experimentation is a powerful way of increasing throughput at an early stage of the drug discovery workflow whilst at the same time minimizing animal usage in the pharmaceutical industry. Previous studies have shown limitations in using Matrix Assisted Laser Desorption Ionization (MALDI) in detecting all target compounds therefore, Desorption Electrospray Ionization (DESI) Mass Spectrometry Imaging (MSI) is a method of choice for cassette dosing experiments enabling the detection of the widest range of target compounds. Additionally, discovery MSI methodologies sometimes lack the sensitivity required to detect all drugs and metabolites especially in the brain where their concentration is expected to be a lower level.

Benefits and Highlights

- Application of a sensitive and selective, targeted mass spectrometry imaging workflow to cassette dosing for label free tissue distribution studies from 0-6 hours
- The study visualized the spatial distribution of 4 non-proprietary drugs and some of their known metabolites in multiple tissues (Brain, Liver and Kidney) after oral cassette dosing, surpassing the reach of traditional discovery MS Imaging techniques

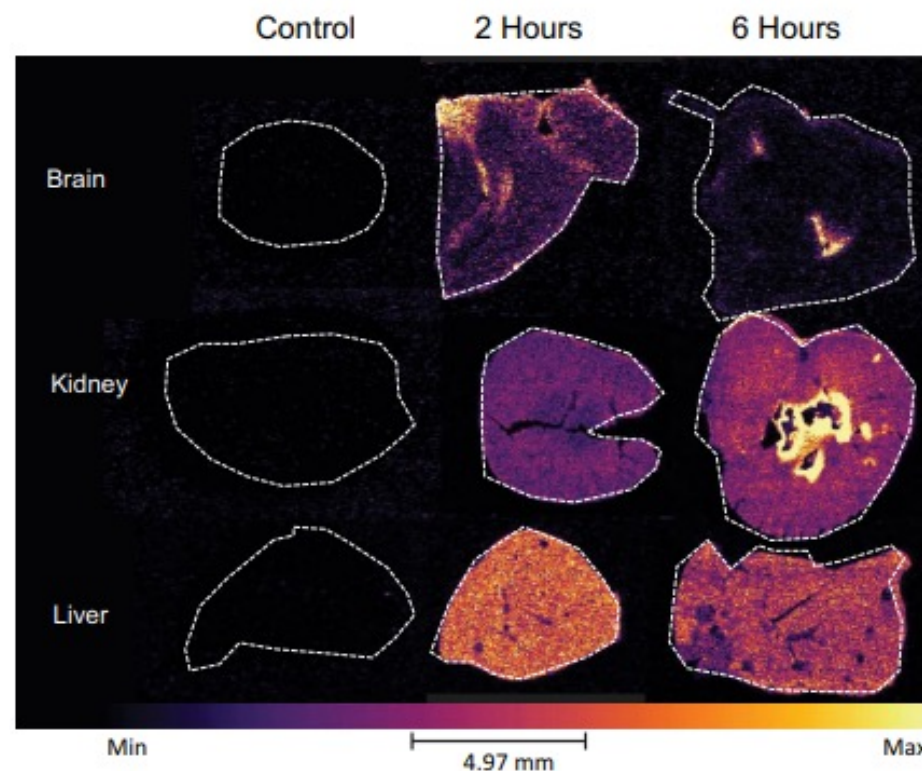


Figure 20. Visualization of the drug Moxifloxacin in control, and tissues dosed for 2 and 6 hours in brain, kidney and liver tissue sections



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Increasing Speed and Sensitivity; With A Unique DESI Targeted MS Imaging System

Mass spectrometry imaging (MSI) has been successfully used to localize pharmaceutical compounds and metabolites, directly from dosed tissue section. However, due to tissue complexity, presence of salt and low-level analytes, the detection can be challenging especially at therapeutic levels.

Desorption Electrospray Ionization (DESI) MSI is typically known for the mapping of small molecules directly from tissue sections and has been proven to be successful when used on Time-of-Flight (TOF) based mass spectrometers, in particular for untargeted analysis. Tandem quadrupole (TQ) mass spectrometers, on the other hand, are renowned for their sensitivity, speed and quantitative robustness for targeted applications using Multiple Reaction Monitoring (MRM) modes of acquisition, and are widely adopted for drug quantitation. With a combination of the two technologies a remarkable and unique technique emerges to perform sensitive, fast, and quantitative MSI directly on surfaces.

Here we present the limit of detection (LOD) of several pharmaceutical compounds when spotted on tissue using targeted multiple reaction monitoring (MRM) mode of acquisition on a DESI TQ-MS, as well as the linearity of the dilution series.

Benefits and Highlights

- DESI allows for the efficient ionization of a wide range of compounds without the need of further sample preparation and is ideally suited to imaging a wide range of drug and drug metabolites
- Workflow could be used for semiquantitative analysis

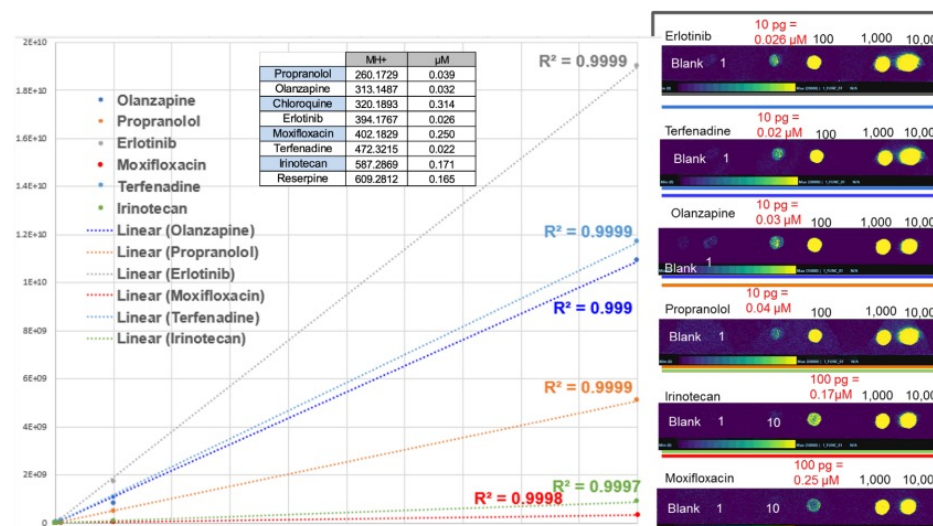


Figure 21. DESI MRM dilution series images for each drug as well as the ROIs summed intensities plotted in excel for propranolol, olanzapine, erlotinib, moxifloxacin, terfenadine and irinotecan.



Read the Application Note

Links to other Useful Materials

- BioTech Startup Advances Preclinical Studies for Topical Osteoarthritis Drug with Waters ACQUITY Premier System and Xevo TQ-XS
- CDMO Pushes Bioanalytical Boundaries with Innovative Topical and Transdermal Services
- New University Laboratory Bridges Development Path for Promising Drug Compounds
- Protein Digestion, Get's Intelligent
- Quantifying Oligonucleotides

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