Improving Chromatographic
Separations Of Biopharmaceuticals
With MaxPeak High Performance
Surfaces (HPS) Technology

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Analytical Solutions Using MaxPeak High Performance Surfaces (HPS) Technology for Biopharmaceutical Therapeutics

Biopharmaceutical therapeutics have consistently been the fastest growing segment within the pharmaceutical space and require advances in analytical testing for efficient discovery, development, and manufacturing to meet patients' needs. As these complex molecules usher in a new era of innovative medicine, scientists working with legacy instrumentation have increasingly faced challenges associated with these therapeutics. These challenges are commonly caused by non-specific adsorption of analytes containing electron-rich functional groups onto stainless-steel components typically found in legacy instruments. In practice, non-specific adsorption manifests as poor reproducibility, broad peak shapes, and reduced sensitivity and may call into question the accuracy of results. With analytical complexity continuing to grow in the biopharmaceutical space, a holistic solution is required to address these analytical challenges. To address these concerns, Waters has developed a first-of-its-kind surface chemistry technology, called MaxPeak™ High Performance Surfaces (HPS).

MaxPeak HPS Technology has been incorporated into Waters™ Instrument and Column Hardware to deliver the ultimate chromatographic performance for biopharmaceutical therapeutics. This novel advancement features a chemically resistant hybrid organic/inorganic barrier applied to metal surfaces along the flow path to reduce metal-based adsorption, a phenomenon that has hindered performance in liquid chromatography. MaxPeak HPS Technology provides improvement in resolution, sensitivity, accuracy, and robustness of chromatographic analyses to give scientists higher confidence in results, so they can make breakthrough discoveries and solve problems that matter.

At Waters, we are committed to providing scientists with stronger analytical capabilities to improve reproducibility and efficiency of biopharmaceutical analysis. In this biopharmaceutical eBook, our scientists' latest application notes have been compiled to showcase the implementation of MaxPeak HPS Technology for increased performance and efficiency in the analysis of intact proteins, peptides, nucleotides, glycans, and cell culture. By utilizing MaxPeak HPS Technology, a universal platform for liquid chromatography is achieved to meet the evolving needs of biopharmaceutical therapeutics.

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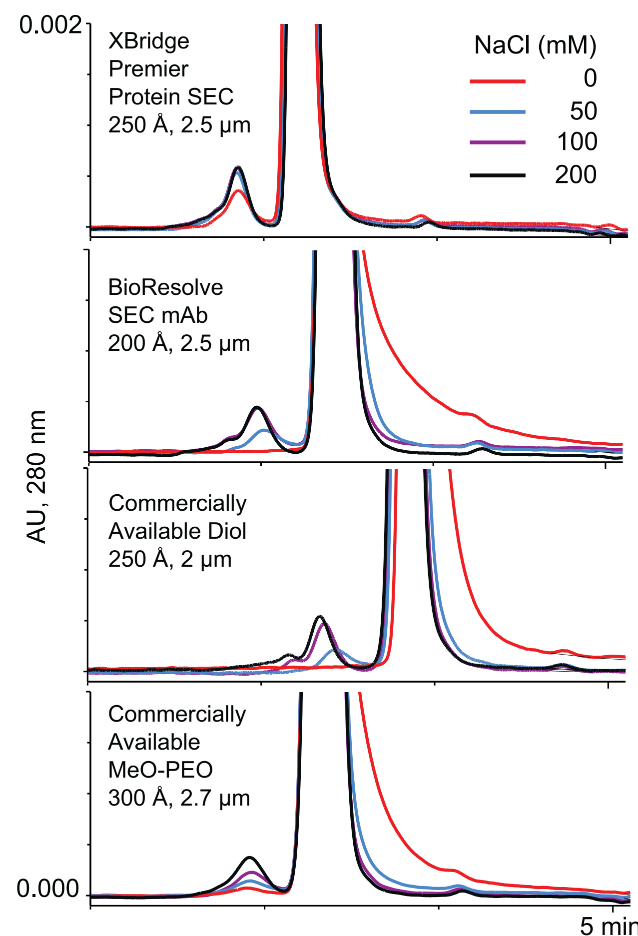
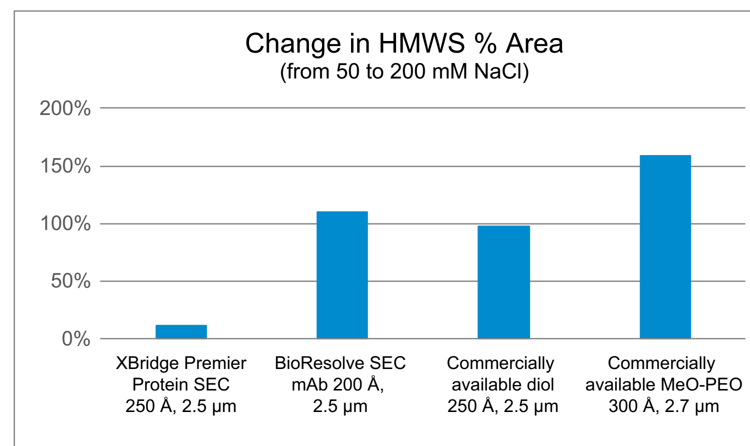
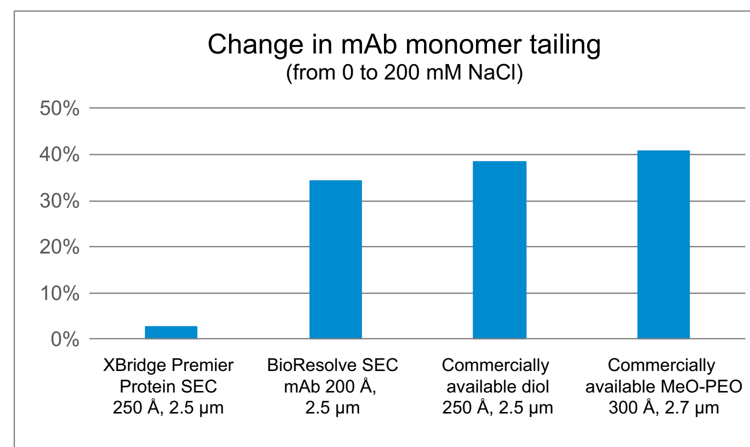
Waters ACQUITY and XBridge Premier Protein SEC 250 Å Columns: A New Benchmark in Inert SEC Column Design







Comparison of ionic secondary interactions performance using NISTmAB (RM 8671) and a 100 mM sodium phosphate pH 6.8 mobile phase containing varying amounts of added NaCl salt.

One of the major limitations in protein size-exclusion chromatography (SEC) is the presence of undesired secondary interactions stemming from a lack of packing material and column hardware inertness. Due to their highly active surfaces, proteins (including mAbs, ADCs, and other biotherapeutic molecules) have a propensity for interaction with metal-oxide surfaces present in column hardware, both hydrophobic and electrostatically active sites present on silica and hybrid silica particles. These undesired secondary interactions create significant challenges for resolving protein aggregates, monomers, and fragments from one another, as well as for the accurate quantitation of these species. While there are several commercially available SEC columns that attempt to address these problems, either through column materials or protein pre-conditioning, most still require an appreciable amount of method development to achieve optimal results. Use of high ionic strength mobile phases and the addition of organic solvents are known to help suppress secondary interactions but are in many ways limiting and can make a chromatographer's work more challenging. Improved column inertness would reduce the need for such measures and provide better method flexibility and robustness. To that end, Waters has developed a novel ethylene bridged-hybrid particle with a high-coverage hydroxy-terminated polyethylene oxide (BEH-PEO) surface and coupled it with a first of its kind hydrophilic high-performance surfaces (HPS) column hardware. A holistic solution to the problem of undesired secondary interactions in SEC is thereby achieved. The corresponding Waters XBridge™ and ACQUITY™ Premier Protein SEC 250 Å Columns bring a new level of inertness to protein SEC.

APPLICATION BENEFITS

- Reliable protein size variant analysis from MW ~10,000 to 650,000 Daltons
- Reduced undesired secondary interactions (ionic and hydrophobic) through the synergistic use of hydrophilic MaxPeak HPS Technology hardware with ACQUITY Protein SEC 250 Å, 1.7 µm and XBridge Protein SEC 250 Å, 2.5 µm particles
- Versatility for the use of physiological buffers to precisely quantitate protein aggregates, monomers, and fragments

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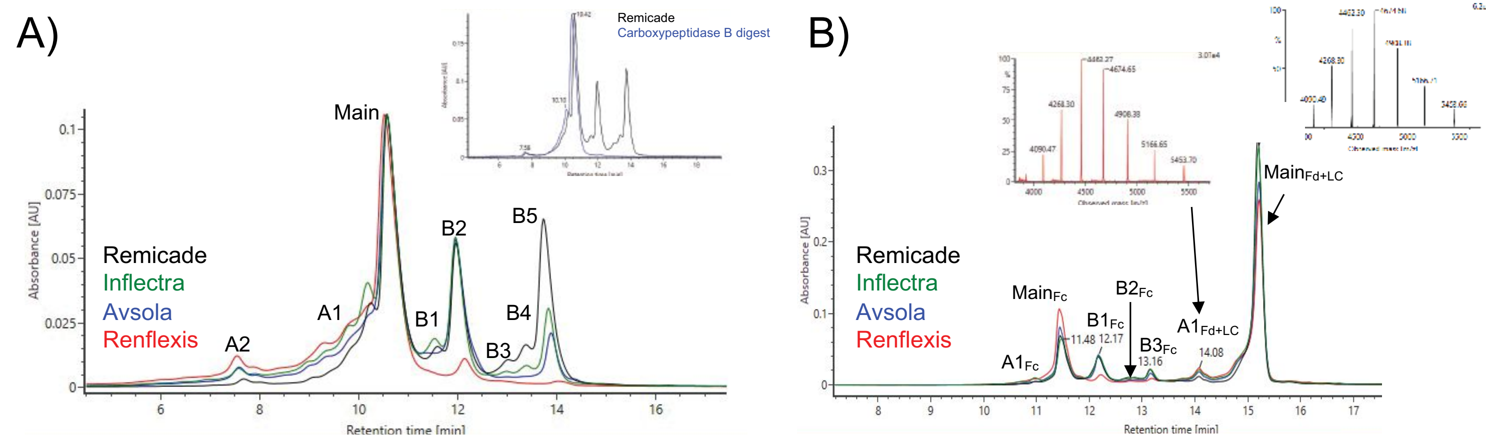
Comprehensive Biosimilar Comparability Assessment via Intact and Subunit RP-MS and IEX-UV-MS Using the Xevo G3 QTof System



UV overlay (280 nm) of (A) Intact IEX-MS analysis and (B) Subunit IEX-MS of infliximab biosimilars. Inset of (A) shows Remicade digested with Carboxypeptidase B for removal of C-terminal lysine species as orthogonal method to confirm these variants. Insets of (B) show combined raw spectra for A1Fd+LC and MainFd+LC peaks, MaxEnt1 deconvoluted "masses, which are near isobaric.

With the increasing prevalence of biosimilar monoclonal antibodies (mAbs) in the drug development landscape, the need for robust liquid chromatography-mass spectrometry (LC-MS) instruments and streamlined characterization workflows is critical. The Xevo™ G3 QTof Mass Spectrometer coupled with an ACQUITY Premier UPLC™ System offers the sensitivity, mass resolution, spectrum quality, and mass accuracy for large molecules to ensure accurate characterization of mAbs at intact and subunit levels. The hardware coupled with the waters_connect™ Informatics platform offers dedicated workflows for intact mAb analysis enabling streamlined reliable data analysis in a compliance-ready environment. This study demonstrates the use of Xevo G3 QTof platform to perform a comparability analysis for infliximab innovator Remicade® and three biosimilars (Inflectra®, Avsola®, and Renflexis®). The samples were analyzed at intact and IdeS subunit levels via reversed phase (RP) LC-MS and native ion exchange (IEX) LC-MS. The resulting data were processed with UNIFI™ and INTACT Mass applications within waters_connect. mAb product quality attributes such as N-glycosylation, C-terminal lysine variants, and charge variants were quantified and compared among the biosimilars.

APPLICATION BENEFITS

- Xevo G3 QTof offers updated ion optics, extended mass calibration range, and improved quantification capabilities for large molecule applications.
- Confident profiling of product quality attributes at intact and subunit level using ACQUITY Premier BEH™ C_4 and BioResolve™ SCX mAb Columns.
- Flexibility of informatics platform for use in both GxP and non-GxP environments
- Use of intelligent data capture (IDC) improves spectral quality and reduces file size

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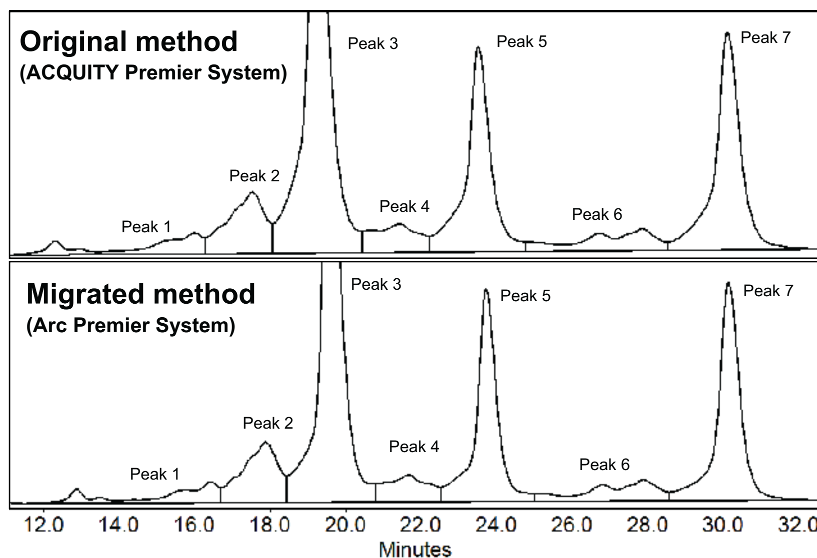
Obtaining Equivalent IEX Chromatographic Performance Through Automated Method Scaling Using Waters Column Calculator



Relative retention time

N=5	BioResolve CX pH concentrates			MES salt buffer		
	ACQUITY Premier	Arc Premier	Absolute difference	ACQUITY Premier	Arc Premier	Absolute difference
Peak 1	0.834	0.841	0.007	0.823	0.808	0.015
Peak 2	0.914	0.915	0.001	0.890	0.877	0.013
Peak 3	1.000	1.000	0.000	1.000	1.000	0.000
Peak 4	1.117	1.108	0.009	1.106	1.094	0.012
Peak 5	1.226	1.213	0.013	1.219	1.219	0.000
Peak 6	1.453	1.427	0.026	1.454	1.450	0.004
Peak 7	1.571	1.542	0.029	1.561	1.570	0.009

Relative % peak area

N=5	BioResolve CX pH concentrates			MES Salt Buffer		
	ACQUITY Premier	Arc Premier	Absolute difference	ACQUITY Premier	Arc Premier	Absolute difference
Peak 1	4.408	4.486	0.078	4.258	4.644	0.386
Peak 2	8.106	8.628	0.522	7.480	7.558	0.078
Peak 3	38.410	38.316	0.094	38.324	38.448	0.124
Peak 4	4.704	4.660	0.044	5.670	5.714	0.044
Peak 5	18.362	18.288	0.074	17.652	17.530	0.122
Peak 6	5.396	5.350	0.046	5.594	5.546	0.048
Peak 7	20.608	20.264	0.344	21.020	20.560	0.460

Relative retention time and relative percent peak area comparison between the original and migrated method using pH and salt-based gradients for Remicade

As upstream workflows are developed and refined, associated analytical methods are eventually migrated downstream to support process development and routinely monitor product and process attributes associated with the active pharmaceutical ingredient. For biotherapeutics, monitoring of charge variants associated with monoclonal antibodies (mAbs) is critical to establish product stability and process consistency. In this respect, ensuring assay results remain consistent as methods are migrated downstream can save time spent on qualification and validation activities. In this application note, an ion-exchange chromatography (IEX) method for mAbs was scaled using the Waters Column Calculator from an ACQUITY Premier System (UPLC) to an Arc™ Premier System (UHPLC) representative of upstream and downstream LC configurations. Results from this study demonstrate that with appropriate scaling, methods can be migrated to downstream workflows, while retaining the chromatographic performance in terms of selectivity and peak area percent.

APPLICATION BENEFITS

- ACQUITY Premier and Arc Premier systems deliver consistent results using pH or salt gradients.
- BioResolve SCX mAb Columns are offered in a variety of dimensions to support IEX workflows across UPLC and UHPLC platforms.
- BioResolve CX pH Buffer Concentrates enable reduced preparation and bench time to achieve robust and reproducible cation-exchange separation.
- The Waters Column Calculator offers straightforward method scaling with minimal user input.

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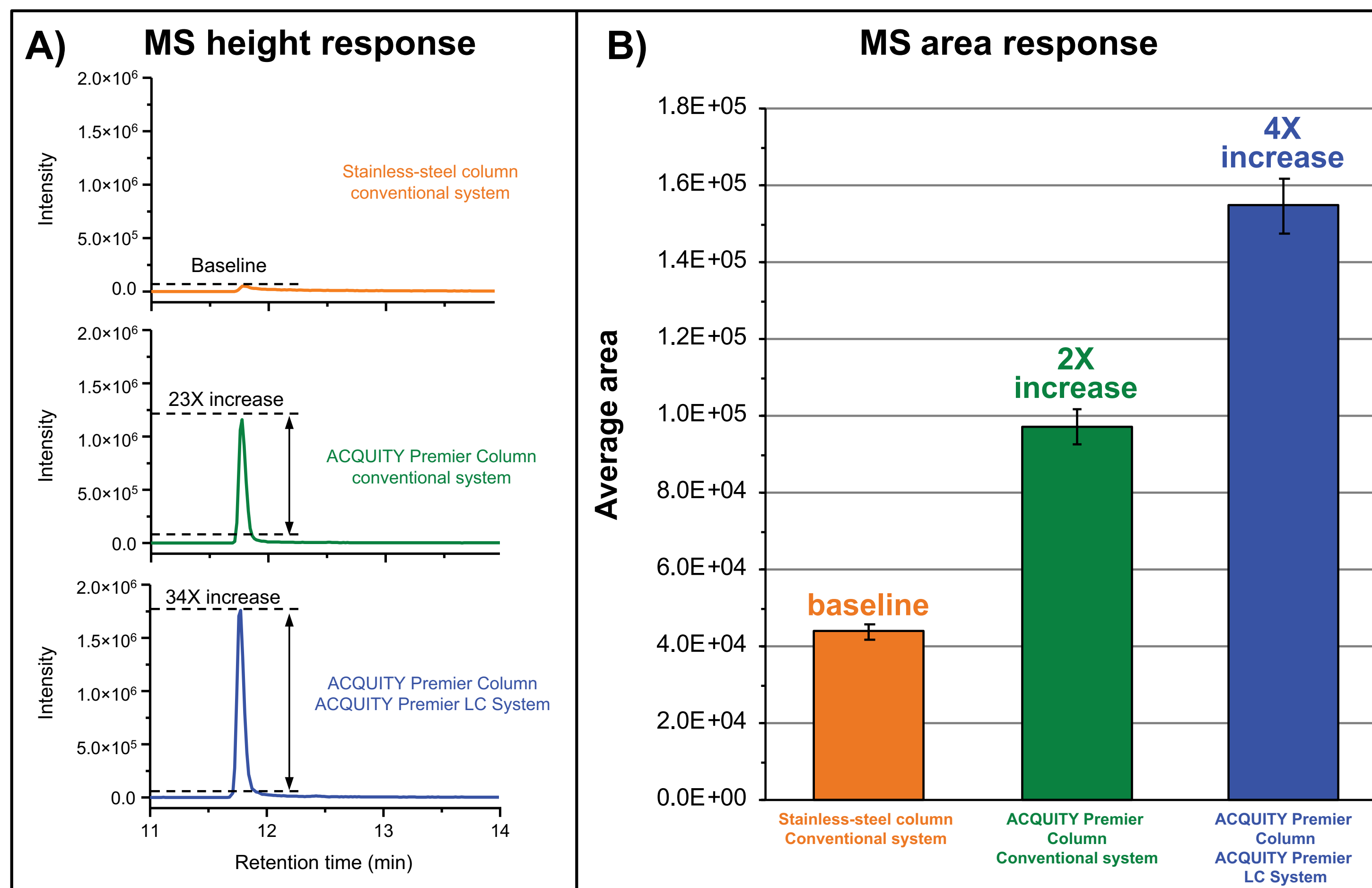
Increasing Recovery and Chromatographic Performance of Acidic Peptides Using Waters ACQUITY Premier Solution



An extracted ion chromatogram (XIC) of the T14 peptide was used to determine MS-response for a conventional LC system and column (top panel), a conventional LC system configured with an ACQUITY Premier Column (middle panel), and a ACQUITY Premier LC System configured with an ACQUITY Premier Column (bottom panel). Mean peak area and standard deviation of corresponding XICs for the T14 peak were calculated using a set of three replicate injections.

Analyte/surface adsorption in liquid chromatography (LC) is a contributing factor in poor peak shape, tailing, and diminished recovery of compounds in LC-based techniques. In this study, the performance of the ACQUITY Premier Solution featuring MaxPeak High Performance Surfaces (HPS) Technology was evaluated in its ability to mitigate metal-induced adsorption phenomena of an "acidic" peptide (sequence: VDNALQSGNSQESVTEQDSK, pI=3.9). Using an RPLC/MS-based technique, up to a 34-fold increase in MS detector response was observed for the acidic peptide. This was in part attributed to the significant reduction in tailing observed when using the ACQUITY Premier System with MaxPeak HPS Technology in comparison to conventional hardware. Furthermore, up to a fourfold increase in peak area was observed when using the ACQUITY Premier UPLC System and Column, demonstrating the MaxPeak HPS Technology also improved the recovery of the metal-sensitive peptide. The chromatographic performance gains observed with ACQUITY Premier Solution featuring MaxPeak HPS Technology resulted in a threefold increase in high-energy b/y fragment ions (11 vs. 36) when compared to the conventional LC system and column. In summary, this study demonstrates how the chromatographic performance gains observed with ACQUITY Premier Solution, featuring MaxPeak HPS Technology, can increase productivity in the lab and improve data quality for metal-sensitive analytes.

APPLICATION BENEFIT

- The ACQUITY Premier Solution significantly improves recovery, peak shape, and reproducibility of peptides containing acidic amino acid residues compared to stainless-steel hardware.

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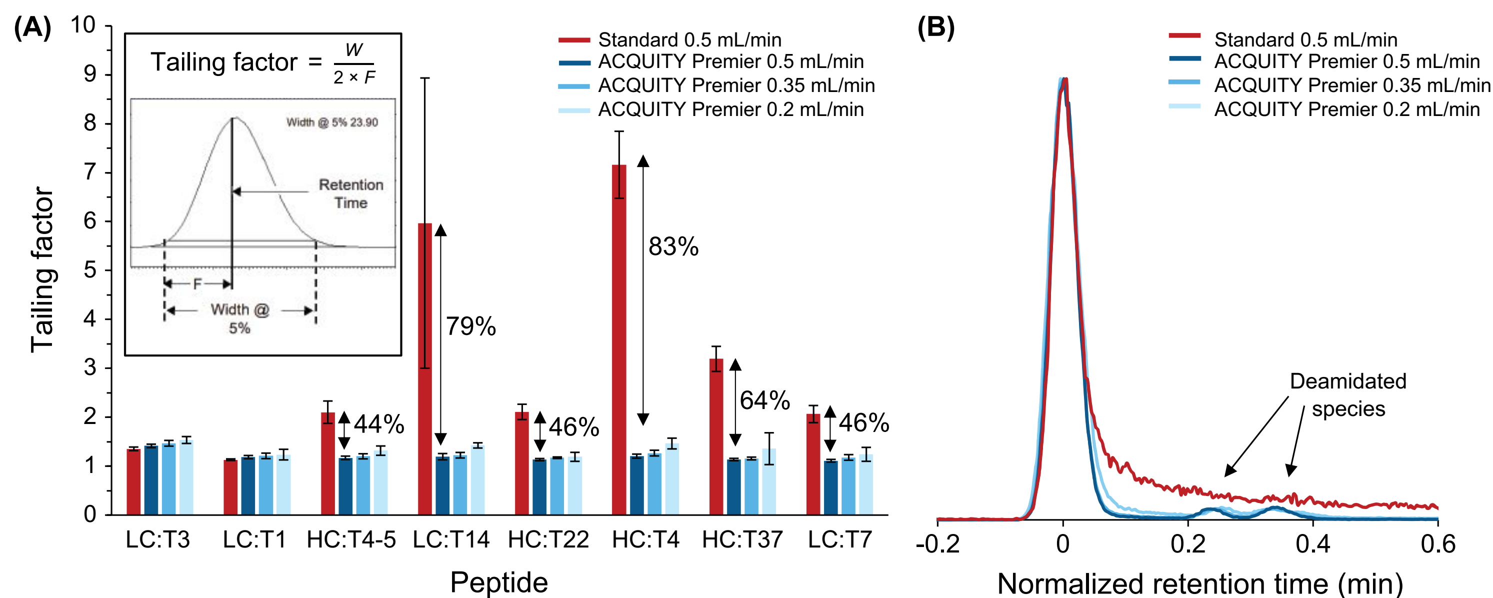
Improving Peptide Mapping Studies and Reducing Assay Failures Through Reproducible Performance Using the ACQUITY Premier UPLC System (BSM)



Evaluation of the performance of MaxPeak HPS Technology, including (A) calculated tailing factor for one basic peptide (LC:T3) and seven acidic peptides on a standard CSH C_{18} Column at 0.5 mL/min and an ACQUITY Premier CSH C_{18} Column at three flow rates, and (B) example of how the reduction of tailing with MaxPeak HPS Technology enables detection of low-abundance species, showing HC:T37 and its two low-abundance deamidated forms. Error bars represent standard deviation across three systems with three replicate injections each.

Reproducibility of liquid chromatography (LC) systems is critical in peptide mapping workflows to simplify sample comparability and ensure accurate peak assignment. In this study, the performance of the ACQUITY Premier System, the most recent evolution of the Waters ACQUITY UPLC bioseparations platform, was investigated. This comprehensive study evaluated the retention time reproducibility across three systems and nine LC separation conditions to evaluate flow rate and gradient impact on assay reproducibility. The results indicate the ACQUITY Premier System is able to deliver consistent retention times with standard deviations of less than one second for the majority of peptides investigated under each condition set. Furthermore, the ACQUITY Premier System with MaxPeak HPS Technology was shown to substantially improve chromatographic performance by reducing nonspecific peptide adsorption, enabling accurate detection and quantitation of lower-abundance impurities. Overall, these results demonstrate that the ACQUITY Premier System is able to consistently and reproducibly deliver results for peptide mapping workflows that support the needs of development and quality control organizations.

APPLICATION BENEFITS

- ACQUITY Premier System delivers high inter- and intra-system precision of retention time across a broad set of conditions.
- MaxPeak HPS Technology improves chromatographic performance by reducing peak tailing.
- Robust performance of the ACQUITY Premier System enables high-confidence attribute analyses and impurity assays.

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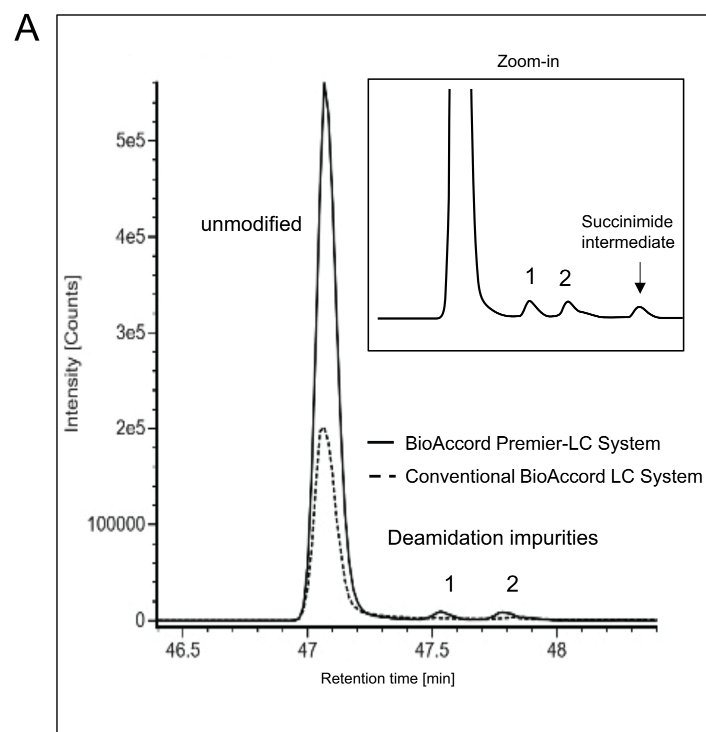
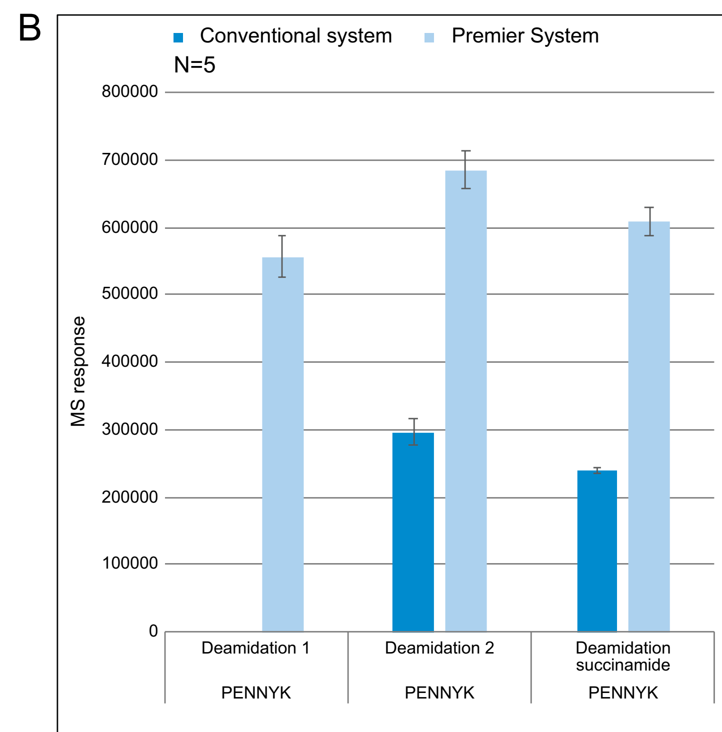
The BioAccord System With ACQUITY Premier for Improved Peptide CQA Monitoring





Recovery Comparison. A) Extracted ion chromatograms (XIC) for the HC:T37 peptide fragment and associated impurities from a tryptic digest of the NISTmAb reference material separated on a conventional BioAccord LC-MS System (dashed line) and a BioAccord LC-MS System with an ACQUITY Premier System featuring MaxPeak HPS Technology (solid line). B) Total normalized peak area for the HC:T37 impurities were calculated for a set of five injections on both systems.

Peptide MAM is an LC-MS-based assay for direct biotherapeutic product attribute analysis that is increasingly used in protein biotherapeutic quality assessment. As part of lifecycle management, assays need to remain accurate and consistent to ensure continued drug product quality and safety. In this respect, robust system performance plays a critical role in MAM data quality. Non-specific adsorption of acidic peptides to metal surfaces is a well-known phenomenon affecting LC-MS analyses, causing asymmetric peaks, loss of peptides, and increased variability in detector response for quantitative measurements. This work demonstrates the performance gains of the BioAccord™ LC-MS System configured with the inert ACQUITY Premier System resulting in increased peptide recovery and robust MS response, with more reproducible results for attributes spanning three orders of magnitude in dynamic range. The improved performance from MaxPeak High Performance Surfaces (HPS) Technology demonstrates that the BioAccord LC-MS System with an ACQUITY Premier System controlled by the compliant-ready waters_connect informatics platform is optimally suited as a LC-MS platform for MAM-based assays.

APPLICATION BENEFITS

- Improved acidic peptide recovery from MaxPeak HPS Technology in systems and columns
- Peptide quality attribute monitoring at low loading levels
- Stable MS signal at high and low measurement levels
- Lowered %RSD levels for monitored attributes

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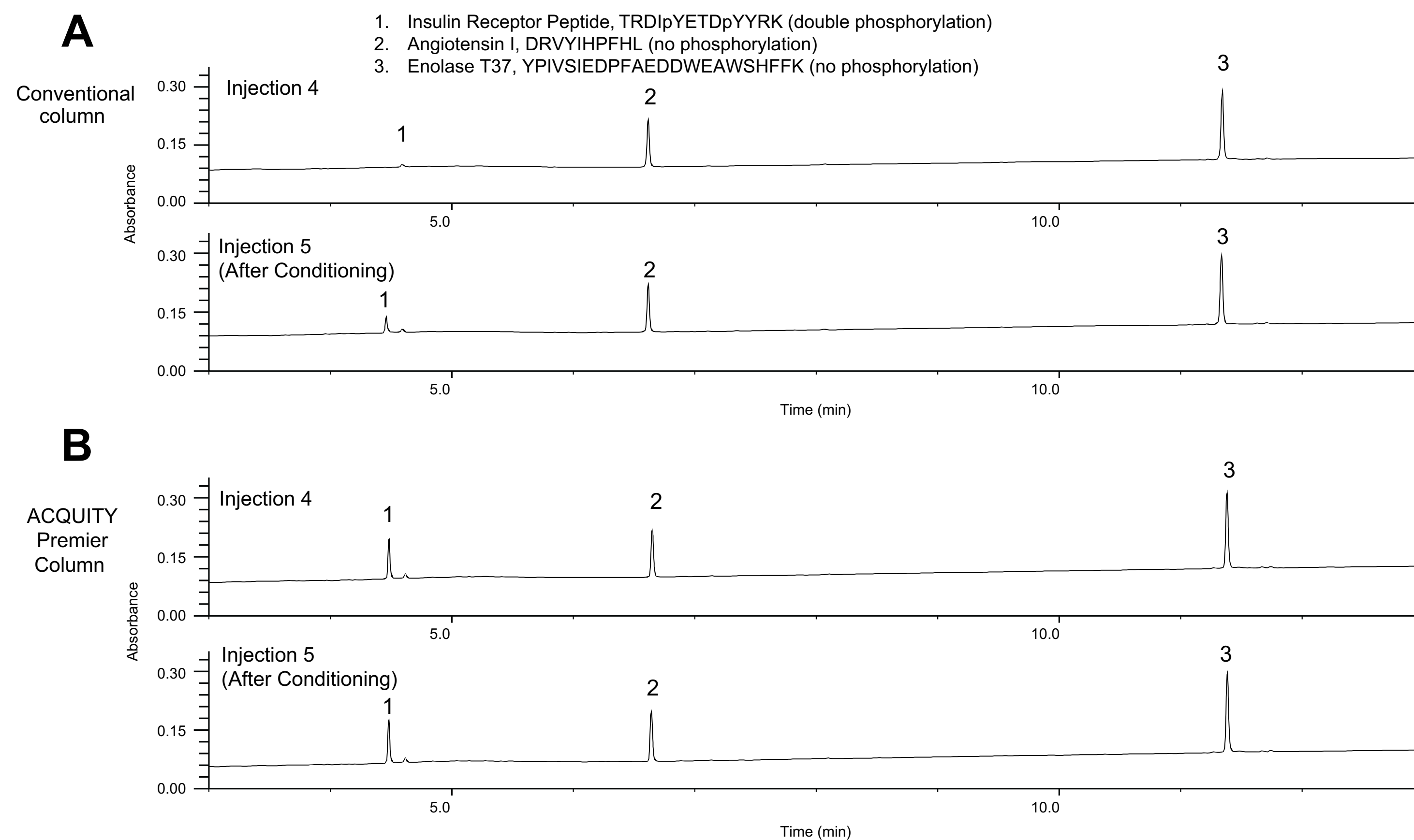
Enhancing Phosphopeptide Quantitation Using ACQUITY Premier Peptide CSH C_{18} Columns



UV chromatograms of the fourth injection (before column conditioning) and fifth injection (after column conditioning) of an equimolar mixture of angiotensin I, enolase T37, and doubly phosphorylated insulin receptor obtained using (A) an ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column or (B) an ACQUITY Premier CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column.

During LC separations, many analytes, especially those bearing a negative charge, can adsorb to electron-deficient metal-oxide surfaces and thereby be challenging to robustly analyze. In the case of peptides, these compounds may intrinsically contain acidic, negatively charged residues like aspartic acid and glutamic acid. Further still, post-translational modifications, such as phosphorylation, can add acidic character to peptides and exacerbate adsorptive losses. Some common methods to circumvent this challenge are the use of mobile phase additives or chelators. Strong acids have been observed to improve the recovery of phosphorylated compounds, yet this method can be detrimental to those analyses requiring MS detection. Use of chelating agents, which can also help prevent metal-analyte adsorption, has drawbacks in the form of reduced MS intensity or solubility issues. Recent studies have shown that ACQUITY Premier Columns and their MaxPeak High Performance Surfaces (HPS) Technology can be used to enhance the recovery and reproducibility of analytes such as peptides. This technology has been demonstrated to mitigate metal-analyte interactions that could aggravate sample loss and has been proven to be a more effective hardware technology for this purpose when compared to titanium. In this application note, we further demonstrate the potential of ACQUITY Premier Columns for use in the analysis of phosphorylated peptides, which might be of interest for proteomics analyses or studies involving kinase inhibitors.

APPLICATION BENEFITS

- Improved recovery versus conventional column technologies
- Minimal to no column conditioning required
- Better reproducibility and method robustness
- Higher quality MS spectra

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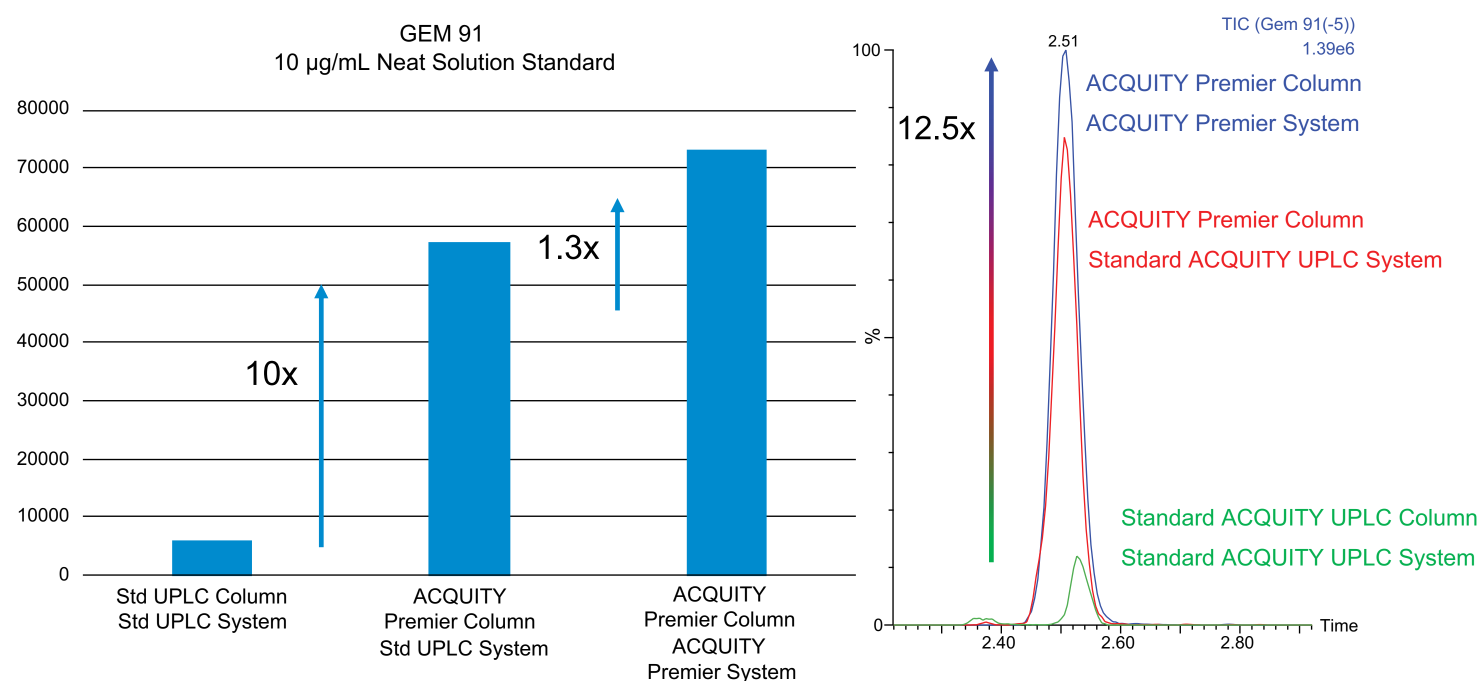
Utilization of the ACQUITY Premier System and Column for Improved Oligonucleotide Bioanalytical Chromatographic Performance



Demonstration of improved chromatographic performance for GEM91 using the ACQUITY Premier System and ACQUITY Premier Column as compared to the standard ACQUITY UPLC I-Class System and standard UPLC Column.

Among the many LC-MS analytical method development challenges for oligonucleotides, their well-known propensity to adsorb to metals, due to their polyanionic nature, is often the most problematic. This metal interaction negatively impacts chromatographic performance, often resulting in poor peak shape and issues with analyte recovery and reproducibility, ultimately limiting overall performance of the analytical method. Conditioning or passivation of LC systems and columns, using high concentrations of the oligonucleotide to block sites of adsorption, is often used. While effective, this passivation is not permanent. As an alternative, use of chelating reagents in mobile phases, such as EDTA, is often used. While also effective, use of chelating additives often negatively impacts LC-MS assays, suppressing MS signal and limiting sensitivity. The work presented here demonstrates the improved oligonucleotide bioanalytical quantitative performance using the ACQUITY Premier UPLC System and ACQUITY Premier Oligonucleotide C_{18} Column. The ACQUITY Premier System with MaxPeak HPS Technology was specifically designed to prevent non-specific adsorption due to ionic interactions, significantly improving oligonucleotide peak shape, recovery, and overall reproducibility of the analytical method without the need for system or column passivation.

APPLICATION BENEFITS

- Use of the ACQUITY Premier System and ACQUITY Premier Oligonucleotide C_{18} Column yielded improved oligonucleotide recovery and peak shape, ultimately improving method detection limits and reproducibility.
- Incorporation of the MaxPeak HPS Technology eliminated the need for lengthy system and column passivation, saving costly ion-pairing mobile phase reagents, and maximized system uptime.

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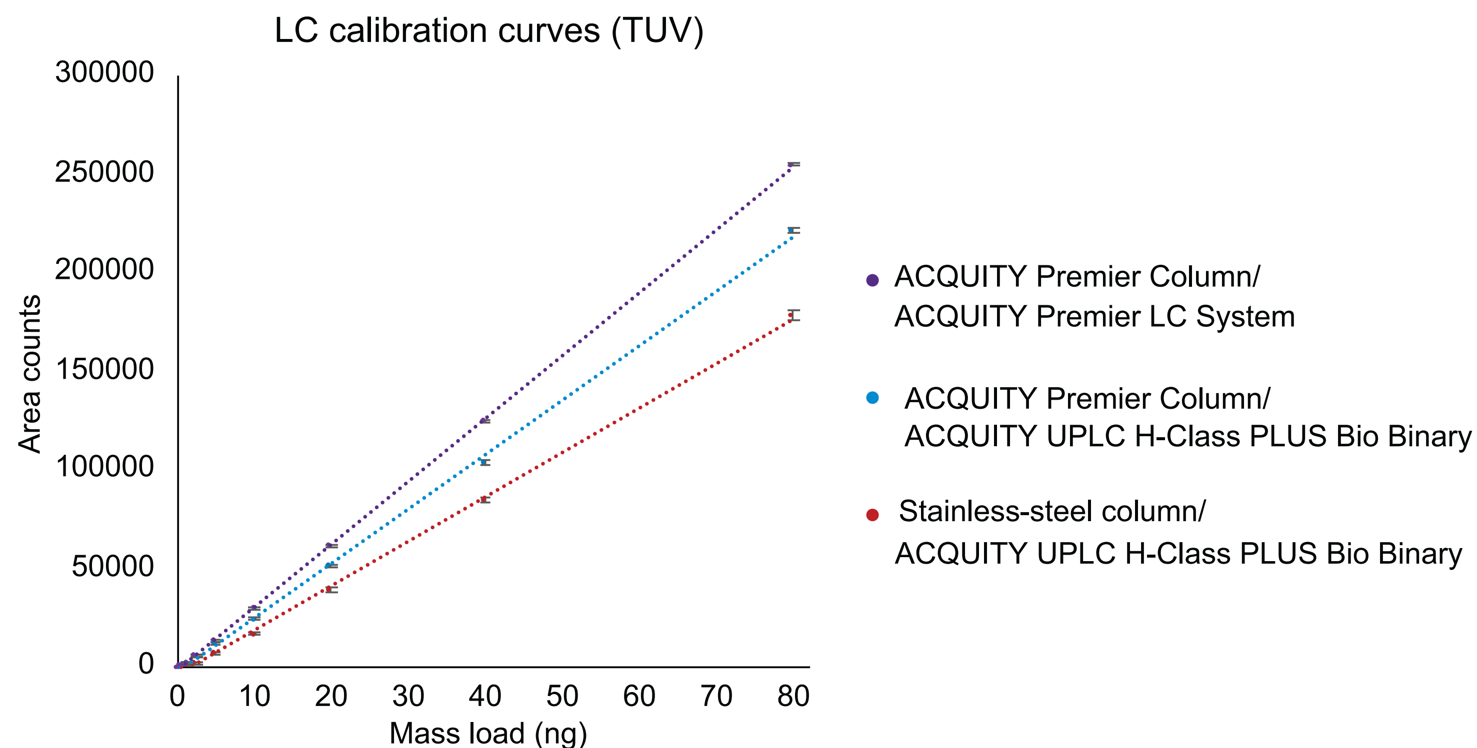
Improving Recovery and Quantitation of Oligonucleotide Impurities Using MaxPeak HPS Technology



Calibration curves of GEM91 using a stainless-steel column and conventional system (red), an ACQUITY Premier Column and conventional system (blue), and an ACQUITY Premier Column and System (purple). The different slopes indicate distinct differences in recovery using the columns and systems tested.

Ion-pair reversed-phase chromatography is a commonly used technique for the separation of synthetic oligonucleotide impurities. Analysis of oligonucleotides is notoriously difficult due to their high affinity for metallic surfaces when using traditional ion-pairing method conditions. ACQUITY Premier Columns and LC Systems were designed with MaxPeak High Performance Surfaces (HPS) Technology to address the variability in the analysis of metal-sensitive analytes such as oligonucleotides. The ACQUITY Premier Solution offers out-of-the-box performance without the need for conditioning or passivation, as would be required by stainless-steel columns and LC systems having a metal-containing flow path. Furthermore, the ACQUITY Premier Solution demonstrated improved recovery for greater sensitivity and limits of detection and offered more reliable quantitation of trace-level impurities in optical workflows.

APPLICATION BENEFITS

- Out-of-the-box performance without the need for conditioning or passivation
- Extended dynamic range with increased linearity
- Enhanced repeatability and quantitation of trace-level impurities
- Increased recovery across all oligonucleotide types evaluated

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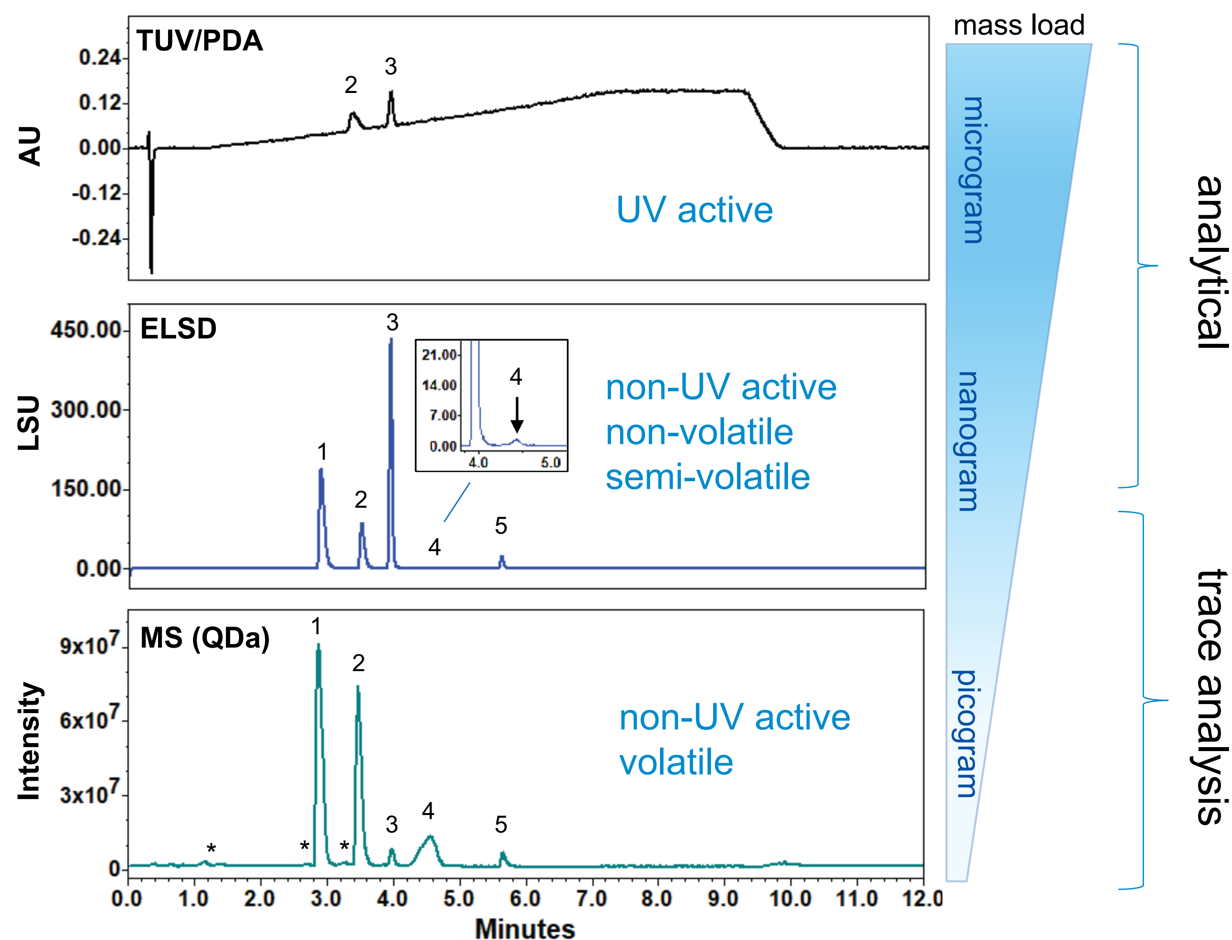
Lipid Nanoparticle Analysis: Leveraging MS to Reduce Risk



Detector response is shown across 3 detector types for a panel of lipids representative of components used in the production of lipid nanoparticles. Peaks 1-5 are SM102, DOTMA, cholesterol, DMG-PEG2000, and DSPC. SM102, cholesterol, DMG-PEG200, and DSPC were prepared in a molar ratio of 50:38.5:1.5:10 representative of a formulated LNP sample.

Lipid nanoparticles (LNPs) represent an elegant solution in the delivery of gene-based therapeutics that can be challenging to execute. As a multi-component biologic composed of lipid species with varying properties that impact chromatography and detector response, new approaches are needed in their characterization and monitoring. This application note proposes a liquid chromatography (LC)-based optical/mass-spectrometry (MS) dual-detector platform with improved sensitivity and diagnostic power for lipid nanoparticle workflows. In this workflow, the ACQUITY QDa™ Mass Detector provides complementary mass data for raw material impurity screening, MS spectral library-based lipid component confirmation, and stability monitoring. As an Empower™ Software-based method, the proposed workflow is easy to deploy in both non-regulated and regulated environments alike for efficient development and migration of lipid nanoparticle workflows.

APPLICATION BENEFITS

- MS offers lower limits of detection and increased diagnostic power for impurity analyses.
- Complementary mass data offers orthogonal detection and confirmation of lipid species.
- Empower Software-based workflows ensure compliance and straightforward method migration.

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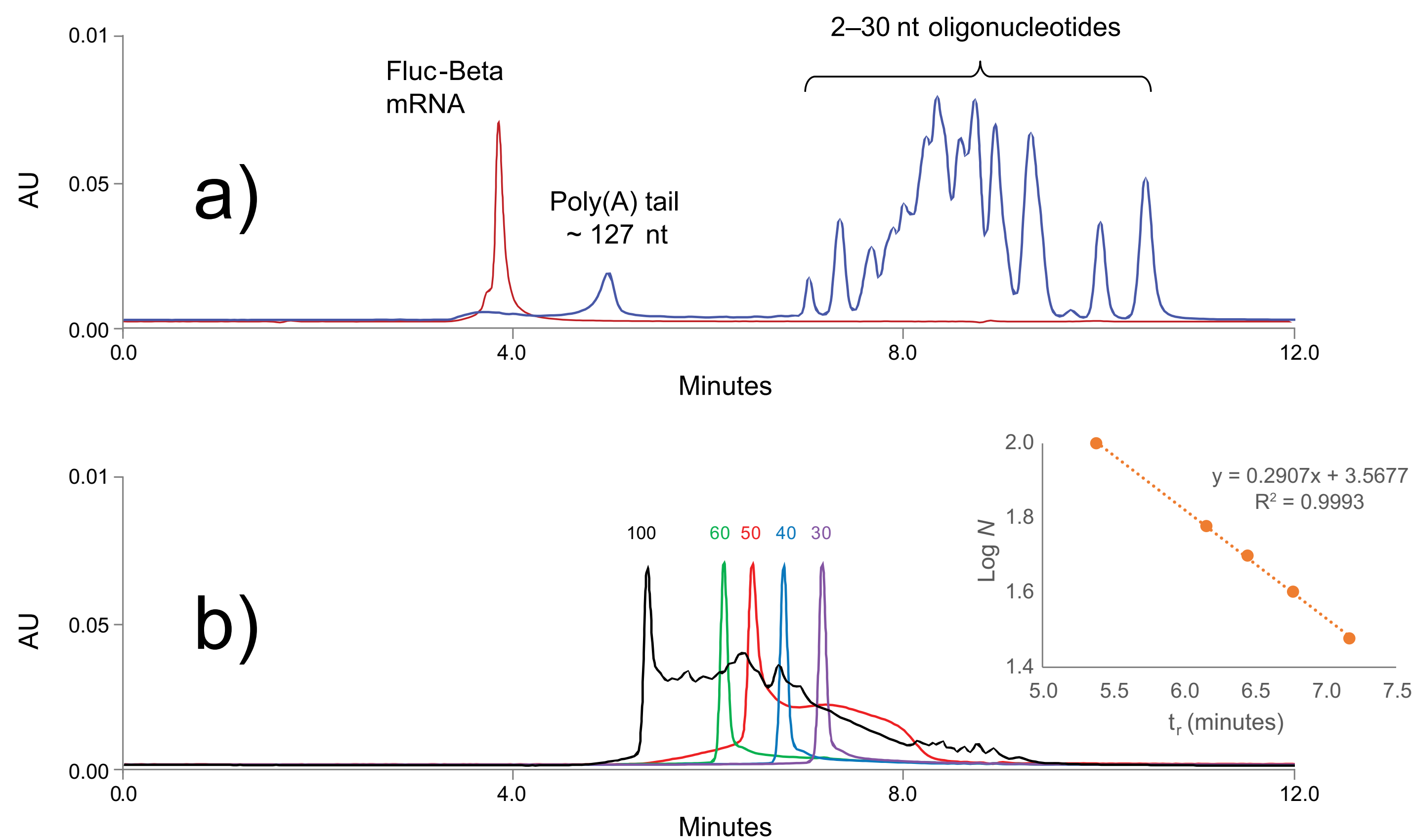
Size-Exclusion Chromatography Method for Poly(A) Tail Analysis of mRNA



a) 1970 nt Fluc-beta mRNA (red chromatogram) was digested with RNase T1. The digestion products, poly(A) tail and 2–30 nt short oligonucleotides (blue chromatogram) were separated with an ACQUITY Premier Protein SEC 250 Å, 4.6 x 150 mm, 1.7 µm Column. b) SEC calibration with synthetic 30, 40 , 50, 60, and 100 nt oligoribonucleotide(A) standards. Linear calibration is shown in the inset. An extrapolation of the calibration curve is used to approximate the length of the even larger poly (A) tail.

The first-in-class therapeutic messenger RNA (mRNA) vaccines were successfully developed against SARS-CoV-2 virus. Therapeutic mRNA molecules can also be used for protein replacement therapy or vaccination approaches in cancer treatment. Rapid advances of mRNA technology require development of analytical methods. Current mRNA methods include analysis of 5′ capping status, primary mRNA sequence, process impurities, and the 3′ poly(A) tail. In this application note, we describe a robust and simple method for poly(A) tail length measurements. The method utilizes the digestion of an mRNA molecule with RNase T1 to liberate the poly(A) tail. The digestion products and poly(A) tail are then separated by size-exclusion chromatography (SEC). The poly(A) tail length is estimated using an SEC calibration constructed from nucleic acid standards.

APPLICATION BENEFITS

- Fast and robust method for analysis of poly(A) tail length
- Simple SEC-UV method suitable for QC testing

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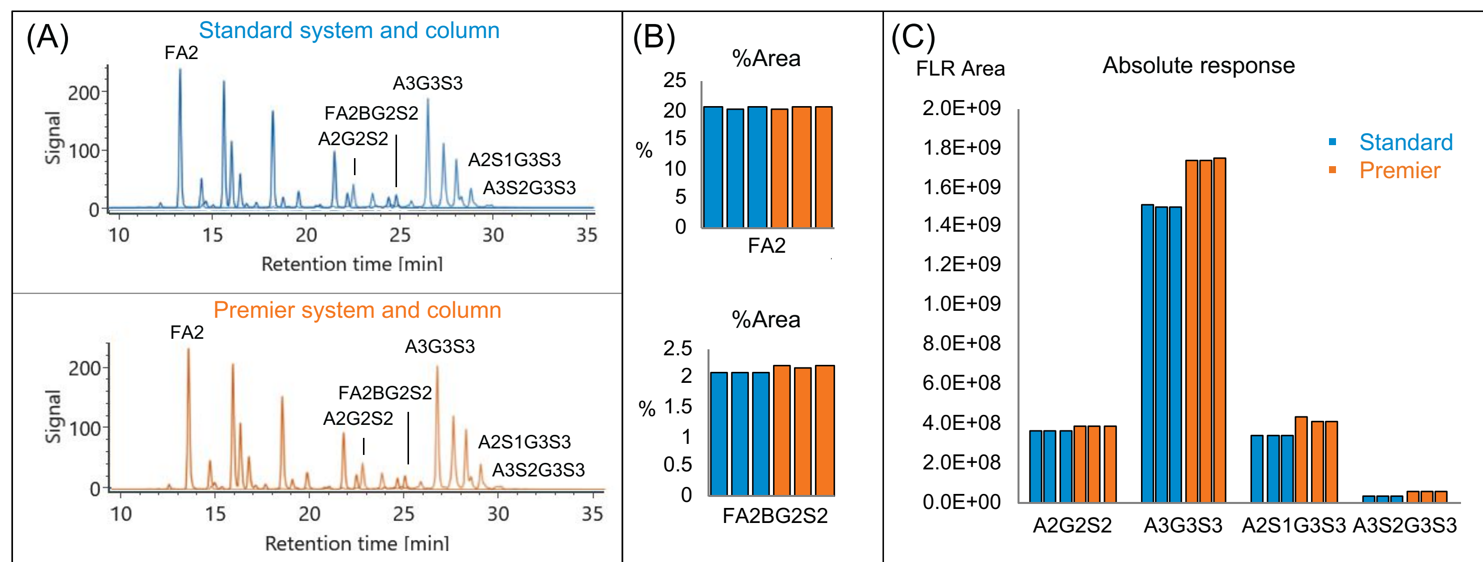
Improving Released N-glycan Analysis in Biotherapeutic Development Using the ACQUITY Premier Solution with MaxPeak High Performance Surfaces (HPS) Technology



The recovery of sialylated glycans is slightly better using the BioAccord System with ACQUITY Premier LC and Column. (A) overlaid chromatograms of the separation of neutral and sialylated RFMS labeled glycan performance test standards (GPTS) on Standard vs. ACQUITY Premier System and Column. (B) Comparison of relative abundances of a neutral glycan, FA2, vs. a sialylated glycan, FA2BG2S2 in the neutral GPTS. (C) Comparison of the absolute response of the representative glycans in the sialylated GPTS

Characterization and monitoring of glycosylation are necessary during the development and manufacturing of biotherapeutics to ensure that drug products are consistent, safe, and efficacious. As a critical quality attribute, glycosylation is commonly assessed via fluorescent labeling of released N-linked glycans, followed by hydrophilic interaction chromatography (HILIC) and fluorescence (FLR) detection. With the development of FLR- and MS-friendly glycan labeling reagents, such as the *RapiFluor*-MS™ (RFMS) technology, combined FLR/MS analysis has become increasingly common – even for routine analysis. Biopharmaceutical laboratories have developed robust standard analytical procedures for the analysis of glycosylation. However, acidic glycans, especially complex phosphorylated glycans, can demonstrate low recovery during HILIC-FLR/MS analysis due to ionic interactions with metal surfaces. To address this issue, we optimized the existing HILIC chromatographic separation method for RFMS labeled N-glycans using the new BioAccord LC-MS System and ACQUITY Premier Glycan BEH Amide Column that can protect against these unwanted analyte-surface interactions.

APPLICATION BENEFITS

- Enhanced recovery of acidic glycans that have stronger interactions with metal surfaces
- Improved recovery and peak shape of phosphorylated glycans that are affected by these interactions
- Automated glycan peak assignment capability assisted by calibrated glycan retention time using the existing UNIFI Glycan Scientific Library

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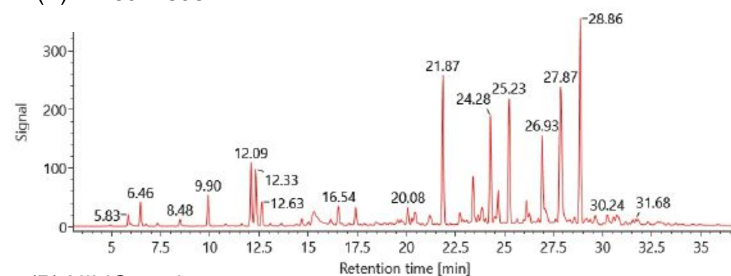
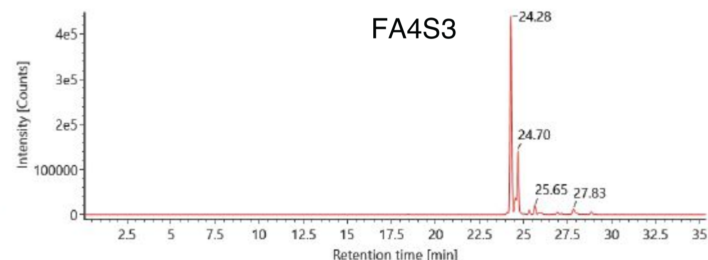
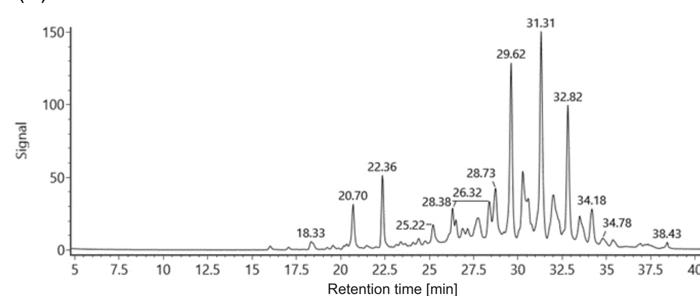
Released Glycan Analysis of Erythropoietin Using the ACQUITY Premier Glycan BEH C_{18} AX Column and BioAccord System with ACQUITY Premier

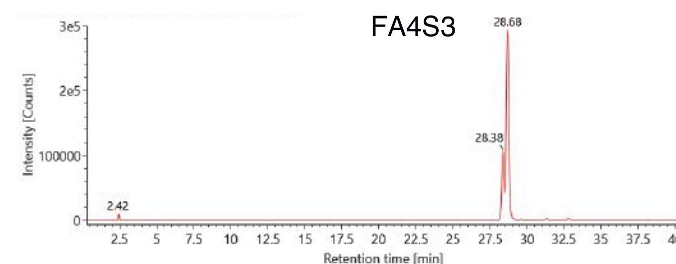
(A) Mixed mode





(B) HILIC mode





Comparison of EPO released glycan analyses using (A) HILIC mode and (B) mixed mode separation. The charge based mixed mode separation provides higher resolution for isomeric sialylated glycans. One example is the separation of fucosylated tetra-antennary glycans bearing three sialic acids, FA4S3.

The abundance and structure of highly-sialylated glycans can factor into the clearance rate and in-vivo activity of many glycosylated biotherapeutics. Given this, accurate analytical characterization and monitoring methods are required to ensure drug product quality. For released glycan characterization, hydrophilic interaction chromatography (HILIC) with fluorescence and mass spectrometry detection is widely used. However, the abundance, heterogeneity, and various isomeric forms of highly sialylated glycans limit the chromatographic resolving power when using traditional HILIC-based separations. In this work, we demonstrate how analyte charge can be leveraged to improve the chromatographic selectivity and detection of fluorescently tagged highly-sialylated glycans by using mixed mode chromatography within the integrated LC-FLR-MS BioAccord LC-MS System with an ACQUITY Premier System.

APPLICATION BENEFITS

- Improved chromatographic resolution for highly-sialylated, highly complex glycans
- Charge-based glycan profile for highly-sialylated glycans improved compared to traditional HILIC methods
- Improved sensitivity for highly-sialylated glycans using FLR and MS detection
- Fragmentation capability of the BioAccord LC-MS System provides additional glycan structural information for assignments

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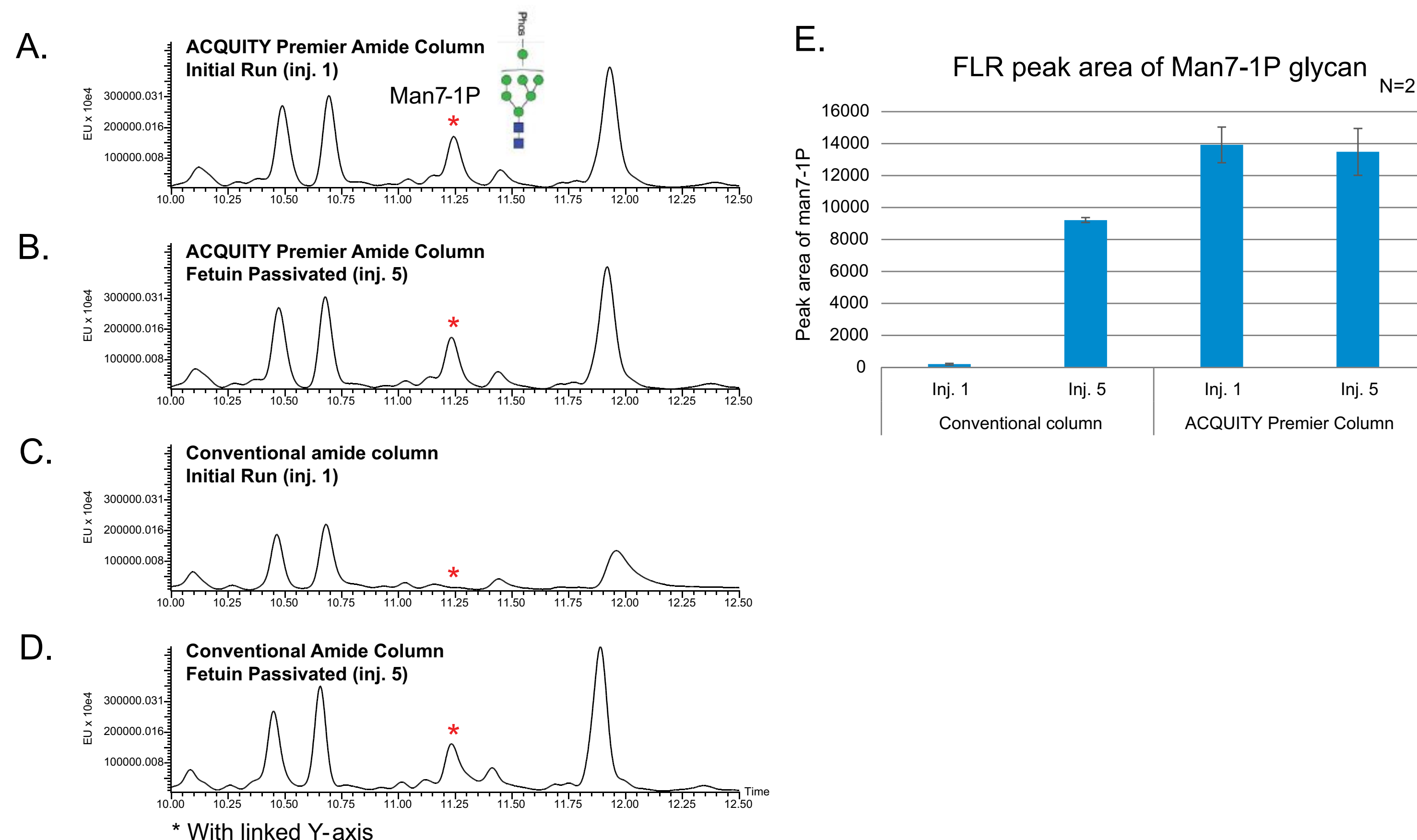
MaxPeak High Performance Surfaces Technology Improves HILIC Profiling of Released N-glycans



*Recovery comparison of phosphorylated glycans using an ACQUITY Premier Glycan Amide Column and a conventional Amide Column. A, B) FLR detection of a singly phosphorylated glycan (Man7-1P, labeled with *) using an ACQUITY Premier Glycan BEH Amide Column before and after passivation; C, D) FLR detection of singly phosphorylated glycan using a conventional steel column before and after passivation; E) Recovery of Man7-1P glycan based on FLR peak area.*

The chromatographic analysis of metal-sensitive compounds can be negatively impacted by the stainless-steel column hardware. Because of adsorption and secondary interactions, such compounds can exhibit asymmetric peaks and poor recovery, especially under demanding, low ionic strength separations. To address this issue, MaxPeak High Performance Surfaces (HPS) Technology was developed. This technology provides a highly effective surface barrier that prevents analytes from undergoing undesired interactions with the metal surfaces of a chromatographic column. In terms of released N-glycans, these sorts of problems can arise with acidic N-linked glycans, like those containing multiple sialic acids or phosphorylated mannose residues. Acidic N-linked glycans are often assigned as critical quality attributes in certain biotherapeutics because they can affect stability, efficacy, and immunogenicity. To carefully control and monitor these moieties, N-glycans are frequently released and analyzed by hydrophilic interaction chromatography (HILIC) and mass spectrometry. In some situations, with this technique, it has been a challenge to achieve good peak shape and recovery of acidic N-linked glycans while maintaining MS compatibility. With ACQUITY Premier Glycan BEH Amide Columns that are equipped with MaxPeak HPS Technology, it is now possible to recover highly-sialylated and phosphorylated glycans from ultrahigh pressure HILIC chromatography without any dependence or requirement for column conditioning.

APPLICATION BENEFITS

- Novel ACQUITY Premier Glycan BEH Amide Column with MaxPeak HPS Technology to provide desired performance and improved recovery of glycans starting with the first injection on a new column
- Accurate profiling of highly-sialylated and phosphorylated glycans without time-consuming column condition and passivation
- Better reproducibility of retention times for sialylated glycans versus a conventional stainless-steel hardware column
- MS-grade ammonium formate concentrate and the *RapiFluor*-MS Glycan Performance Test Standards to help establish an easy-to-implement released N-glycan approach

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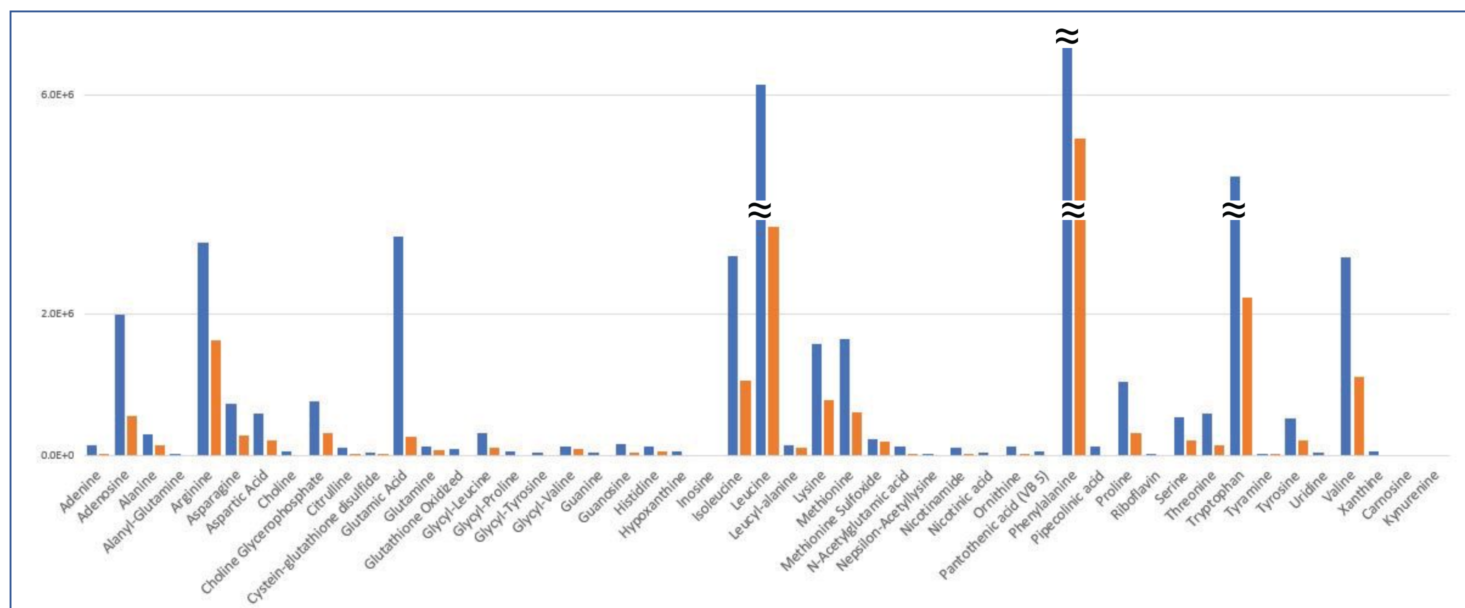
Monitoring Nutrients and Metabolites in Microbial Culture Media Using the BioAccord LC-MS System with ACQUITY Premier



Response comparison of 50 compounds detected in both LB broth (orange bars) and Terrific broth (blue bars).

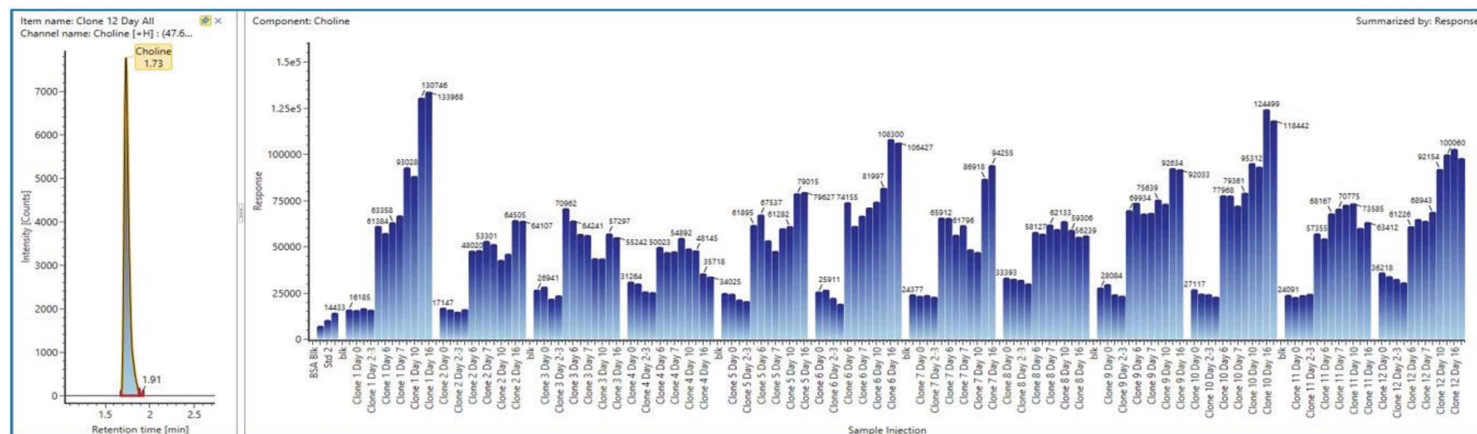
Along with mammalian cell culture systems, microbial based fermentation requires media to support the growth and maintenance of microorganism and high-quality protein production. In this application brief, we describe the application of the liquid chromatography-mass spectrometry (LC-MS) methodology and workflow developed for cell culture media using the BioAccord LC-MS System for nutrient and metabolite monitoring in growth media for microbial-based bioprocessing. The method package includes a comprehensive reversed-phase LC-MS method; a 200-plus compound library; a simple, stepwise workflow for data review including trend plots; a suite of tools for unknown screening; multivariate data analysis tools; and reporting template. This media monitoring is a supplement to product quality analysis that BioAccord LC-MS System provides, which includes intact protein analysis, peptide mapping and monitoring (including Multiple Attribute Method), released glycan analysis, and oligonucleotide mass confirmation.

APPLICATION BENEFITS

- Accelerated media development and monitoring is enabled by the direct measurement of microbial growth media attributes with dedicated workflows using the BioAccord LC-MS System, MaxPeak High Performance Surfaces (HPS) Technology, and the waters_connect informatics platform.
- Enable decision-making with the acquisition of process inputs and product quality output on a single LC-MS platform supporting media as well as product quality attributes monitoring, including intact protein analysis, peptide MAM, released glycan, and oligonucleotide mass confirmation.

Read The Full Application Note

Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System With ACQUITY Premier



An example of abundance changes of choline in spent cell culture media is shown by the trending plot bar chart for 12 different bioreactors across multiple days.

In biotherapeutics process development, cell culture media solution provides essential building blocks and nutrients for cell health and biotherapeutics production. Monitoring the feed components and metabolites can provide information on cell growth, biotherapeutic titer, and product quality. The BioAccord LC-MS System has been used to support product quality analysis such as intact protein analysis, peptide MAM, released glycan, and oligonucleotide mass confirmation. In this application the BioAccord LC-MS System is used to monitor the nutrients and metabolites in cell culture media. The method package includes a comprehensive reversed-phase LC-MS method, a 200-plus compound library; a simple, stepwise workflow for data review, including trend plots; a suite of tools for unknown screening; multivariate data analysis tools; and reporting template. The method has been applied in spent media analysis for clone selection and process optimization.

APPLICATION BENEFITS

- Excellent chromatographic performance delivered by MaxPeak HPS Technology implemented for both the column and UPLC system hardware
- Rich full-scan HRMS data using the simple-to-use BioAccord LC-MS System
- Inclusion of a200-plus compound library, comprising amino acids, vitamins, nucleic acids/bases, nucleotides, metabolites, and other compounds of interest in bioprocessing development
- Streamlined function and class-based workflow to facilitate stepwise data review
- A single compliant-ready informatics package supporting data acquisition, data review, elucidation of unknowns, report template, and multivariate data analysis

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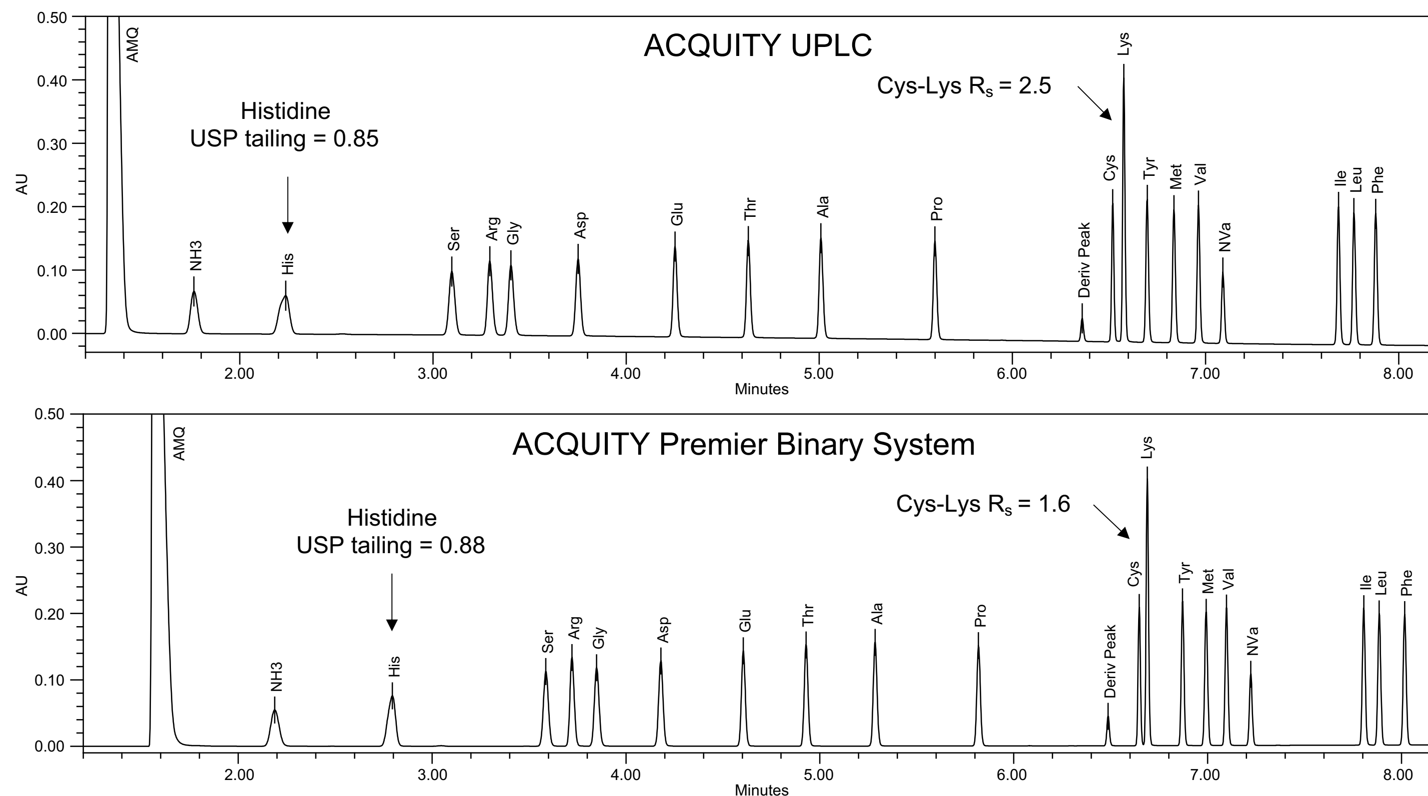
Instrument Considerations for Successful Adaptation of Amino Acid Analysis Methods that Utilize Pre-Column Derivatization from an ACQUITY UPLC to an ACQUITY Premier Binary System



Example chromatograms for the amino acid standard (500 µM) obtained on the ACQUITY UPLC using the original method and on the ACQUITY Premier Binary System using the adapted method conditions.

Amino acid analysis (AAA) using high-performance liquid chromatography has its own unique challenges due to the chemical and physical properties of the analytes. Since most amino acids have no chromophore, a derivatization technique, such as AccQ•Tag™, is often used for analysis by UV. In addition, the varied chemical properties make separation of a wide range of amino acids challenging. The AccQ•Tag Solution was released in 2007 on the Waters ACQUITY UPLC System to provide a complete solution for amino acids in a variety of matrices. However, with improvements in instrumentation, the migration of the methods used for analysis of amino acids to state-of-the-art systems was needed. Specifically, laboratories may desire the benefits of using MaxPeak High Performance Surfaces Technology but also need the ability to migrate migrate legacy methods, such as those used for amino acid analysis.

APPLICATION BENEFITS

- AccQ•Tag Ultra Chemistry Kit includes column, standards and reagents, and eluents for fast, reliable, and reproducible amino acid derivatization, separation, and quantification.
- ACQUITY Premier UPLC System provides exceptional precision for challenging gradients and increased speed for high-throughput analysis.
- MaxPeak HPS Technology increases analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.
- All critical performance characteristics are maintained, including peak shape, resolution, linearity, limit of quantification, and intrainterday precision after method adaptation.

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Learn more about the products, solutions, and services featured in this eBook.

SYSTEMS

- ACQUITY Premier System
- ACQUITY QDa Mass Detector
- Arc Premier System
- BioAccord LC-MS System
- Xevo G3 QTof

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