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Unbiased Biotherapeutic Analysis: Achieved with Greater Efficiency and Higher Confidence in Results

Scott J. Berger and William J. Warren

WCBP 2022 Waters Corporation Technical Seminar

Overcoming the Risks of Bias in Biopharmaceutical Analysis

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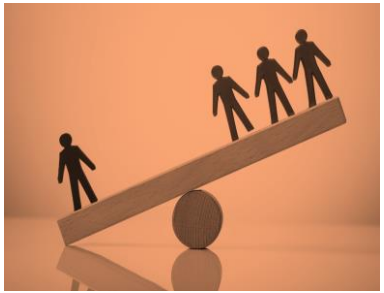
- Bias spans from sample prep to analysis to data processing to reporting.
  - Systematic: Instruments/Consumables, Method Development, SOPs
  - Non-systematic: Human-factors, Method Robustness
- Costs of Bias
  - Accuracy of Results (Actionable knowledge)
  - Assay Failure / Validation Issues
  - Challenges in Method Transfer
- Overcoming / Limiting Bias
  - Systematic: Better Instruments, AQbD, Quality Culture, Standards
  - Non-Systematic: Automation, AQbD, Validation / Verification / System Suitability



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Overcoming Bias in Sample Preparation with Automation and Smart-Connected Labs

Manual Processes are a Challenge to Reproducible Sample Prep and Lab Workflows

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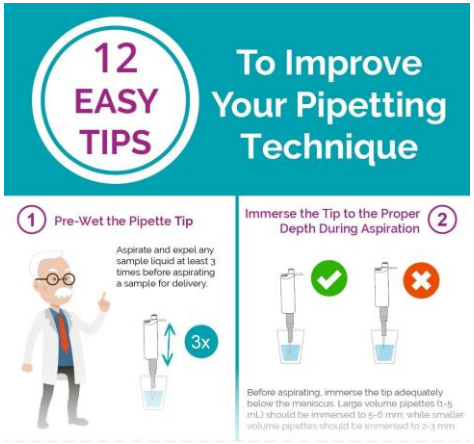

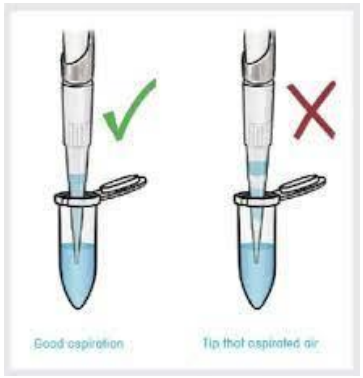

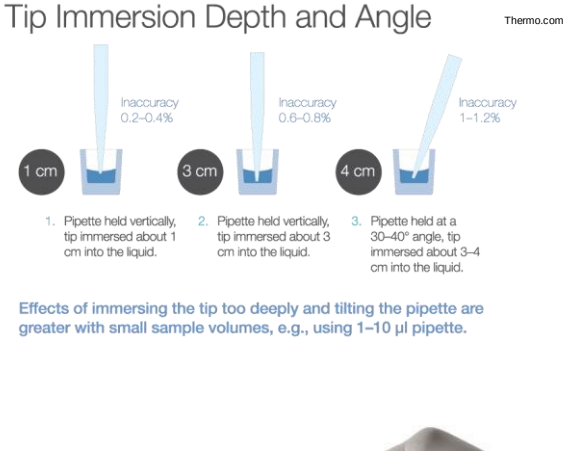

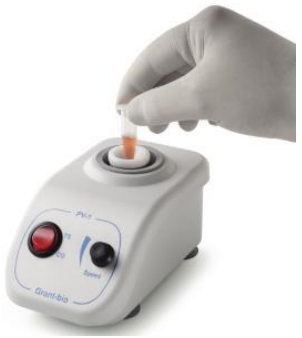

Andrew Alliance Connected Lab Solutions

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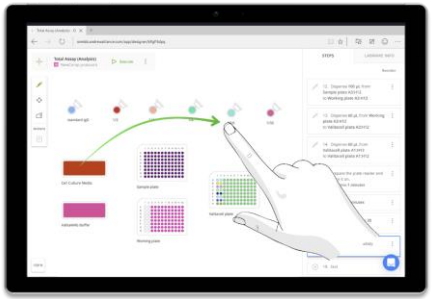
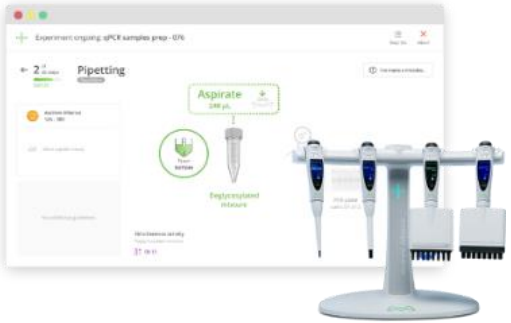
OneLab
design & execute

- Connected Lab Ecosystem
  - Enables total lab connectivity
  - Ease of use
  - Compliant ready



Pipette
easy pipetting

- Smart electronic pipettes
  - Browser-controlled
  - Improving productivity
  - Improving reproducibility



Andrew
the pipetting robot

- Connected liquid handling robot for complete laboratory workflows
  - Automation
  - Reproducibility
  - Fully traceable experiments

OneLab
design & execute

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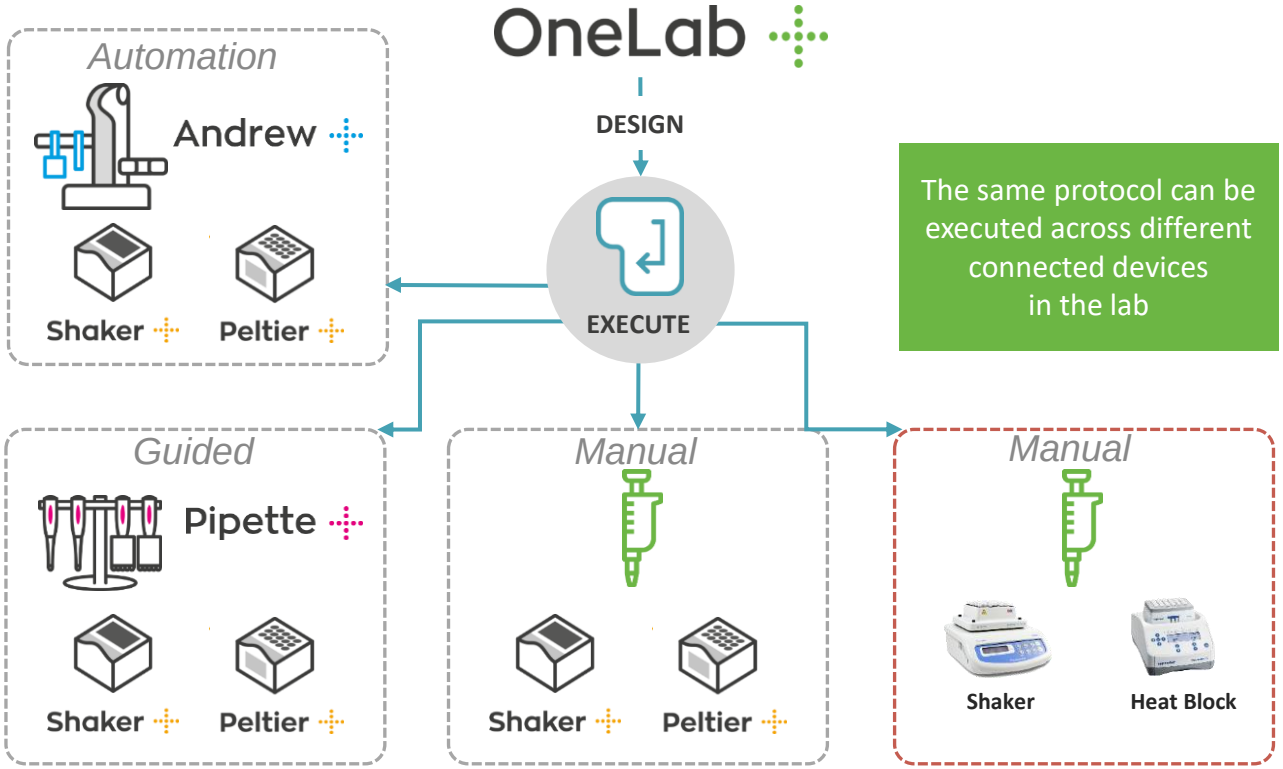
Create Device Independent Protocols



Automation Solutions: Adding Consistency – Reducing Effort

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Your Lab's Most Valuable Partner

Andrew+ the Pipetting Robot ensures reproducible and fully traceable experiments by automating tasks – from repetitive, time-intensive pipetting to complete laboratory workflows – using conventional pipettes and labware.

Andrew
the pipetting robot

Standard Curve
Create a series of standards of increasing concentration in order to produce a calibration curve.

Serial Dilution
OneLab™ calculates required volumes and concentrations, taking full account of sample viscosity and dilution of samples.

Concentration Normalization
OneLab's Normalization Wizard automates the production of concentration normalization volumes, typically saving >80% time required for what is often a highly laborious process.

Microplate Reformatting
The Andrew+™ Pipetting Robot is equipped to handle a wide range of aliquoting operations between different types of microplate, microtube, vials, and racked HPLC tubes.

Andrew
the pipetting robot

Biopharma Application Examples

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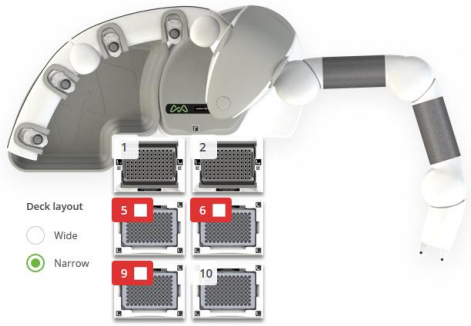
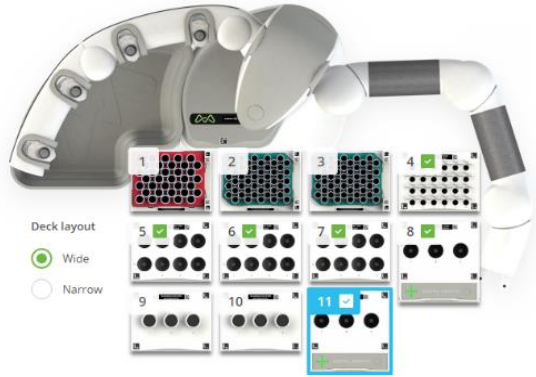
AccQ-Tag Ultra LC Amino Acid Analysis

GlycoWorks LC-MS N-Glycan Analysis

ValitaTITRE mAb Quantification



96-Well PCR Master Mix Setup



NucleoBond® Xtra Midi DNA Purification

- **Pipetting**
- **Dilution**
- **Formatting**
- **Normalization**
- **Endotoxin**
- **Heating, Cooling, Shaking, Vacuum processing ….**

Waters is helping to bring all the pieces together

Improving the Productivity of Glycan Analysis

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Sample Prep

Automated GlycoWorks ***Rapi*Fluor-MS** Preparation

- Automated sample prep
- Traceability in OneLab+
- Process 8-48 samples/ run
- Novel chemistry labeling
- Efficient fluorescence
- Enhanced MS ionization

Separation and Detection

BioAccord™ SYSTEM

ACQUITY glycan separation technology

- Easy to deploy and operate
- Robust performance
- One-click start-up
- Minimal training & low maintenance
- Qualification supported

Informatics

UNIFI glycan workflow

- Ready to use workflow
- Automated
- Compliant ready
- Full audit trail

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Reducing Bias in Biopharmaceutical LC and LC-MS Analysis

Reducing Unwanted Analyte Adsorption and Secordary Interactions: Ethylene Bridged Hybrid (BEH) Particle Technology

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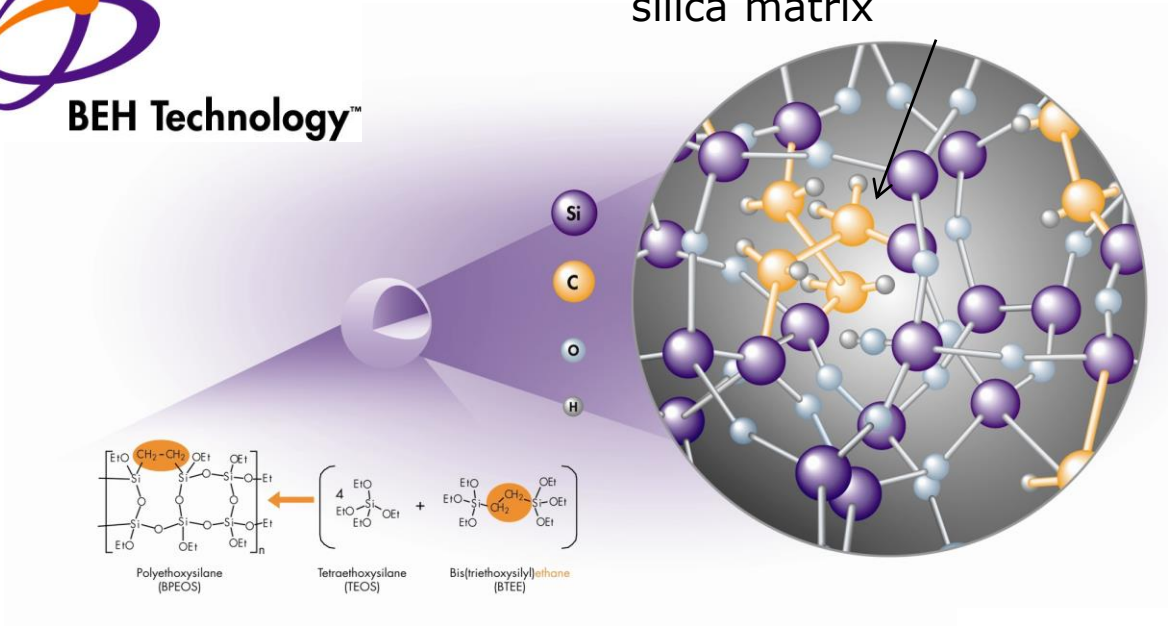
BEH Technology™



- Chronographic media contains a high percentage of total LC surface area.
- Negatively charged silanols on traditional silica particles interact with basic groups leading to poor recovery and poor peak shape.
- Organic-hybrid particles enabled stronger particles, wider pH stability and vastly reduced silanol activity.

Anal. Chem. **2003**, *75*, 6781-6788 U.S. Patent No. 6,686,035

BEH Catalyzed the Development of a Portfolio of Innovative Chemistries for Biopharmaceutical Characterization

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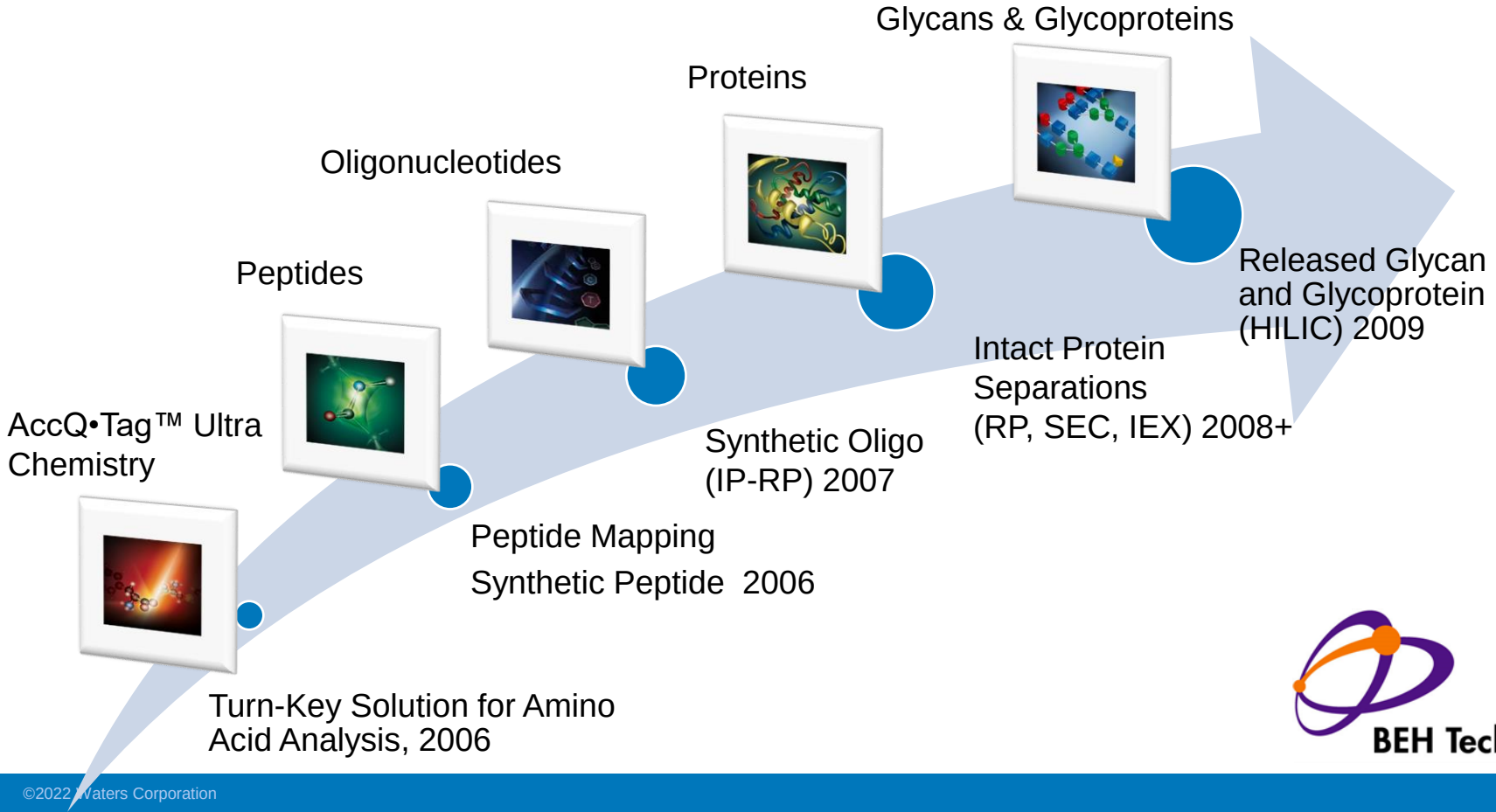

BEH Technology™

Building a Better SEC Column with BEH Technology

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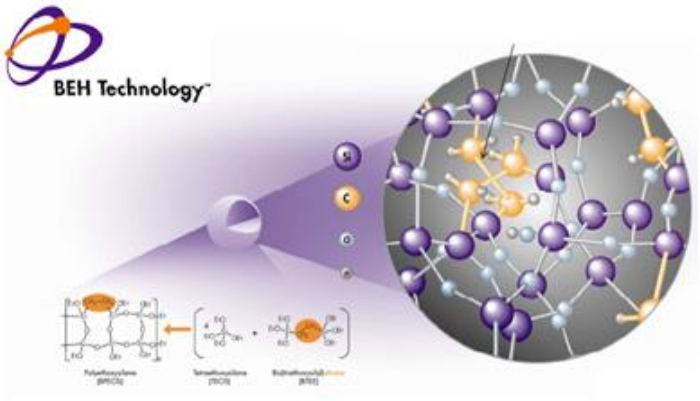
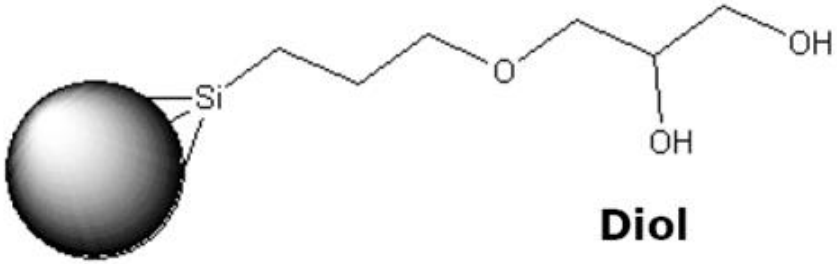
Bridged Ethyl Hybrid base particles combined with an effective diol bonding provide a stable chemistry with minimal secondary interactions.





Diol

Recovery of Lysozyme, a highly basic protein
Silica vs BEH SEC Particles

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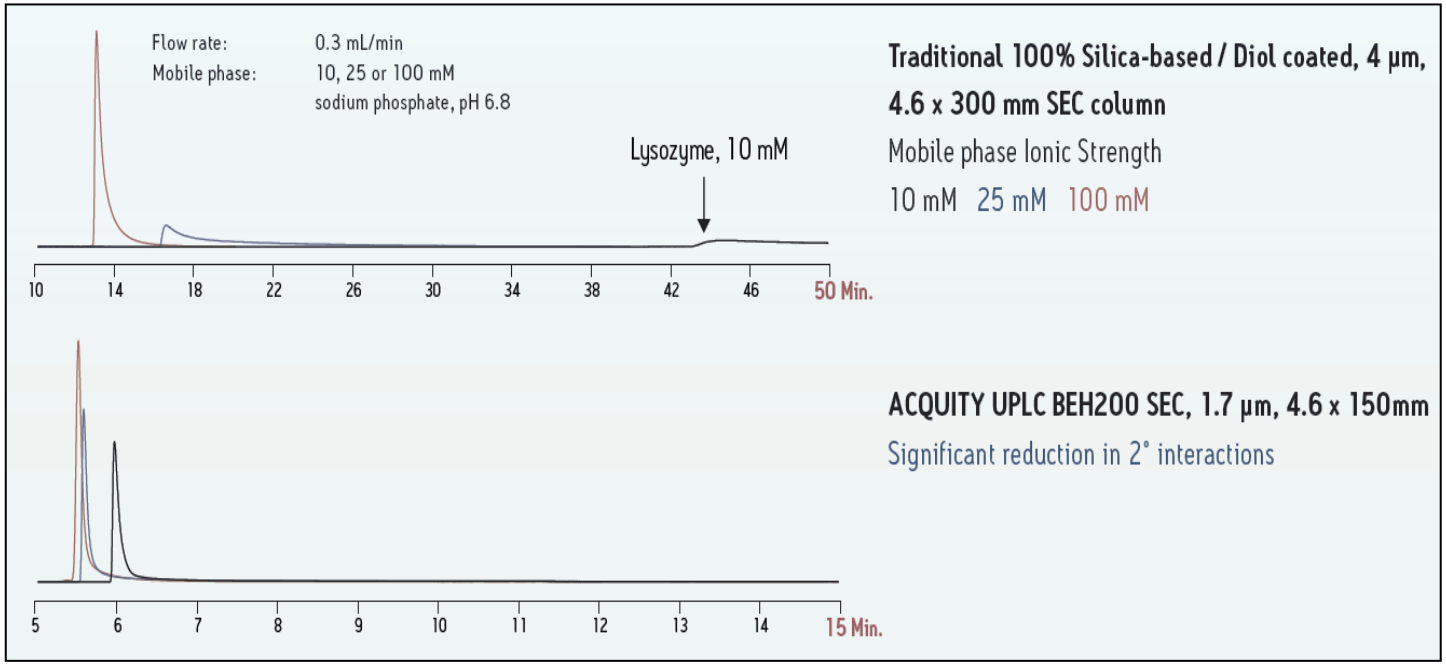

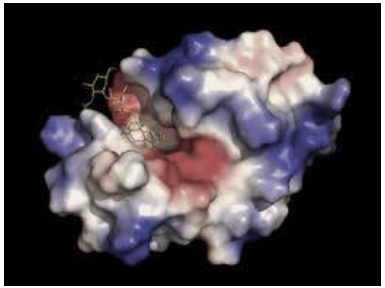

Lysozyme pI ~ 10.5

Flow rate: 0.5 mL/min; Mobile phase: 10, 25 or 100 mM sodium phosphate, pH 6.8

MAXPEAK™
HIGH PERFORMANCE SURFACES

MaxPeak™ Premier High-Performance Surfaces increase analyte recovery, sensitivity, and reproducibility by minimizing analyte / surface interactions on systems and columns that can lead to sample losses and poor separations.

Waters MaxPeak™ High Performance Surfaces better addresses the non-specific adsorption problem without creating additional complications

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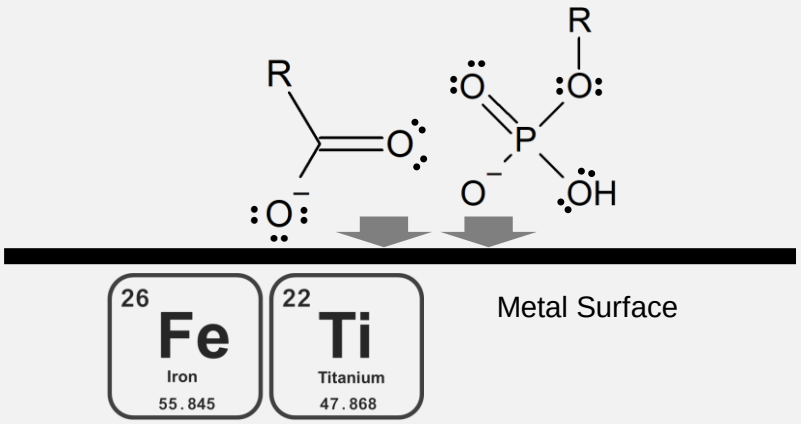
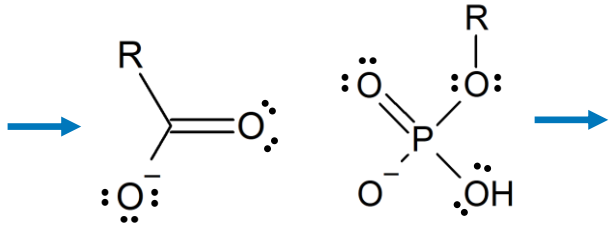


On conventional LC systems, metal sensitive analytes are adsorbed on to metal surfaces



MAXPEAK™
HIGH PERFORMANCE SURFACES

Waters MaxPeak High Performance Surface is designed to minimize metal-analyte interactions

A Better Solution from Waters R&D

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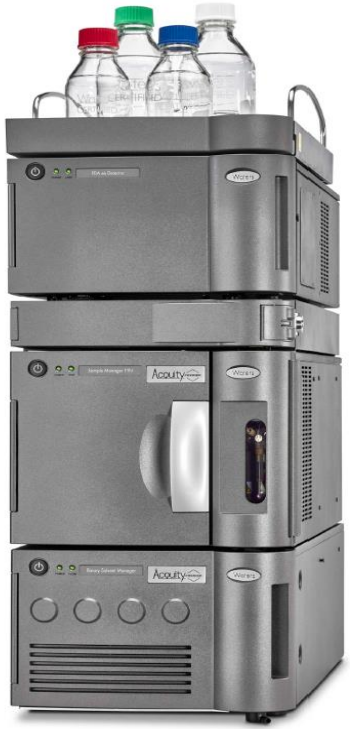
MAXPEAK™
HIGH PERFORMANCE SURFACES

Vials & Plates

QuanRecovery™
WITH MAXPEAK HPS™

Acquity™ PREMIER
COLUMNS AND SYSTEMS
with MAXPEAK™
HIGH PERFORMANCE SURFACES



HPS Technology: Advancing UHPLC and UPLC forward.

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Routine Anaysis → **Sample Complexity**

Arc™ HPLC

HPLC Dependability

Optimized for 3.5 – 5 µm particles

Arc™ PREMIER

UHPLC Flexibility

Optimized for 2.5 – 3.5 µm particles

Acquity PREMIER

UPLC Performance

Optimized for sub-2 µm particles

Arc Premier System

- Based on the biocompatible ACQUITY Arc Bio System
- Quaternary or Binary Solvent Management
- Deploy 2.5 µm particle columns to boost the chromatographic performance
- Modernize legacy HPLC methods within regulatory guidelines
- Ideal inlet for SQ, QQQ Mass Detection
- Empower and MassLynx Control
- MaxPeak HPS Technology for optimal separations of metal sensitive molecules
- Reduce or eliminate system passivation for oligonucleotides and other metal sensitive / acidic molecules

Arc™ PREMIER

Backed by Waters world-class technical and application support, with unrivaled chromatographic fidelity and informatics software

ACQUITY Premier System

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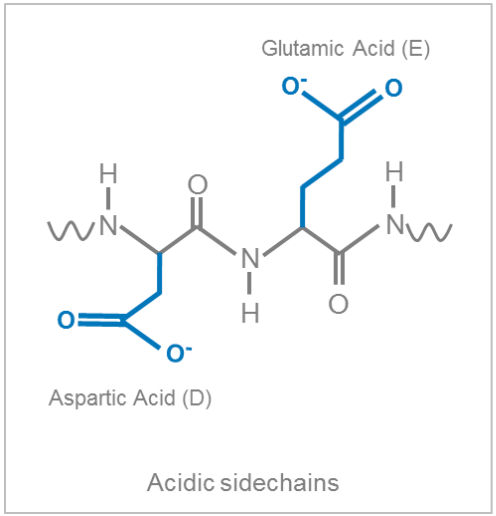
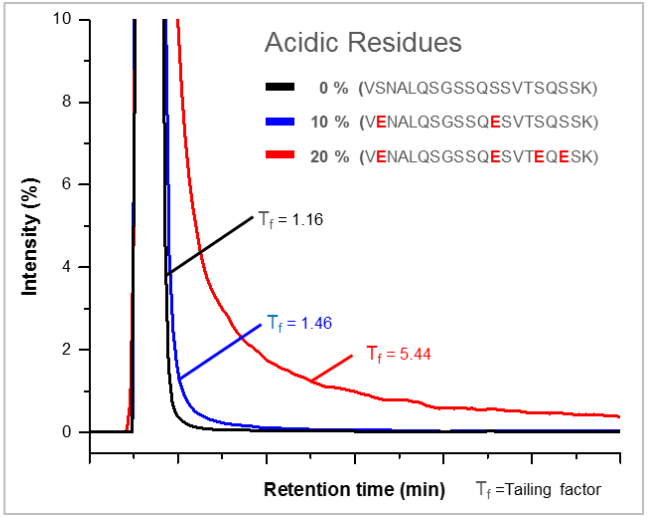
- Based on the biocompatible ACQUITY H-Class PLUS Bio and H-Class Binary PLUS Bio
- Quaternary or Binary Solvent Management
- Deploy sub 2.0 µm particle columns to maximize chromatographic performance and minimize analysis time.
- Ideal inlet for SQ, QQQ, and HRMS Detection
- Empower, MassLynx, waters_connect Control
- MaxPeak HPS Technology for optimal separations of metal sensitive molecules
- Reduce or eliminate system passivation for oligonucleotides and other metal sensitive / acidic molecules

Acquity™ PREMIER

Backed by Waters world-class technical and application support, with unrivaled chromatographic fidelity and informatics software

Increasing peptide acidic characteristics leads to increased tailing on conventional chromatography systems





Acidic species in protein separation

- Commonly seen due to deamidation and other modifications
- Accurate analysis required in product characterization and PQA monitoring
- Waters ACQUITY Premier Solution designed to improve separation and recovery of acidic species

ACQUITY Premier Column improves the separation of acidic peptides such as the PENNYK peptide

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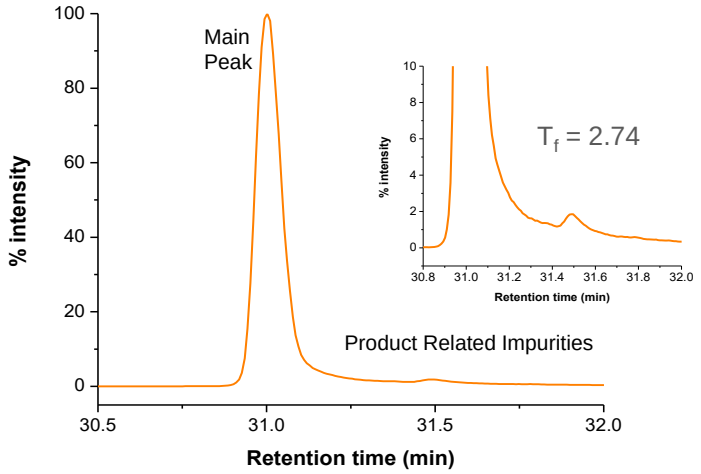
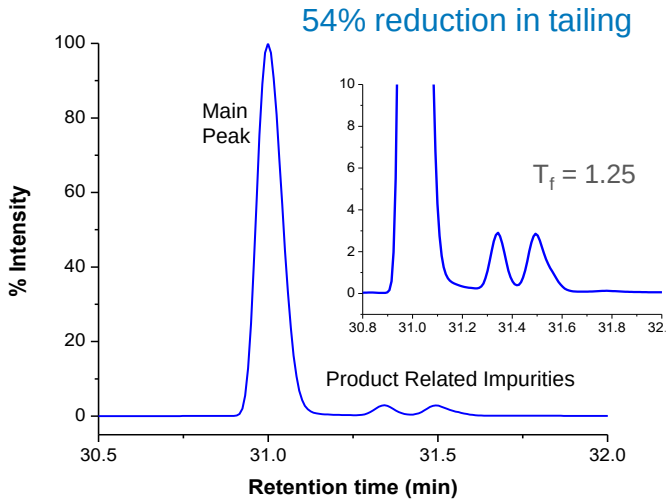
Conventional Column / System



ACQUITY Premier Column / System



mAb T37 peptide: GFYPSDIAVEWESNGQPENNYK Acidic residues

Acquity PREMIER

ACQUITY PREMIER:
Reproducible Performance from Injection #1

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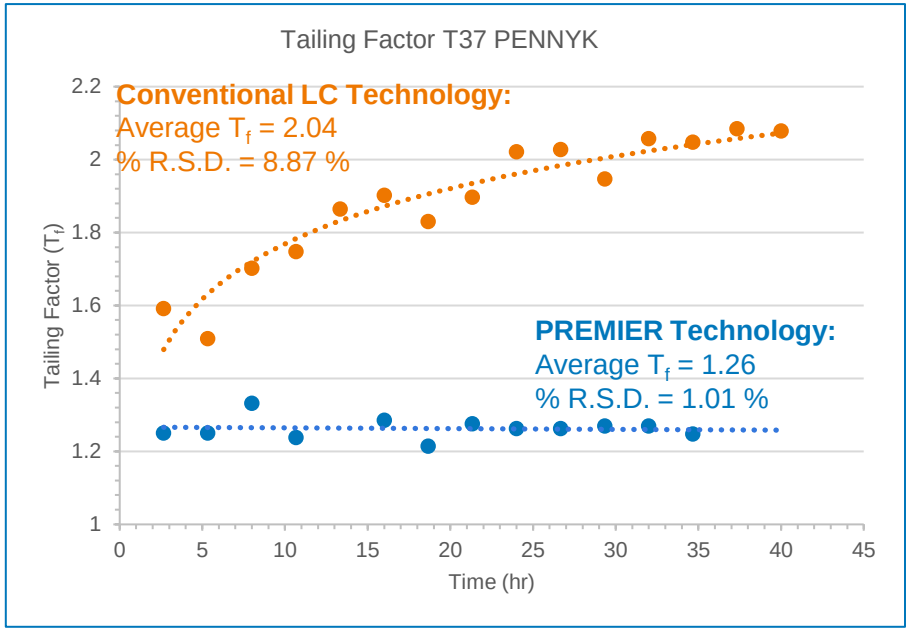
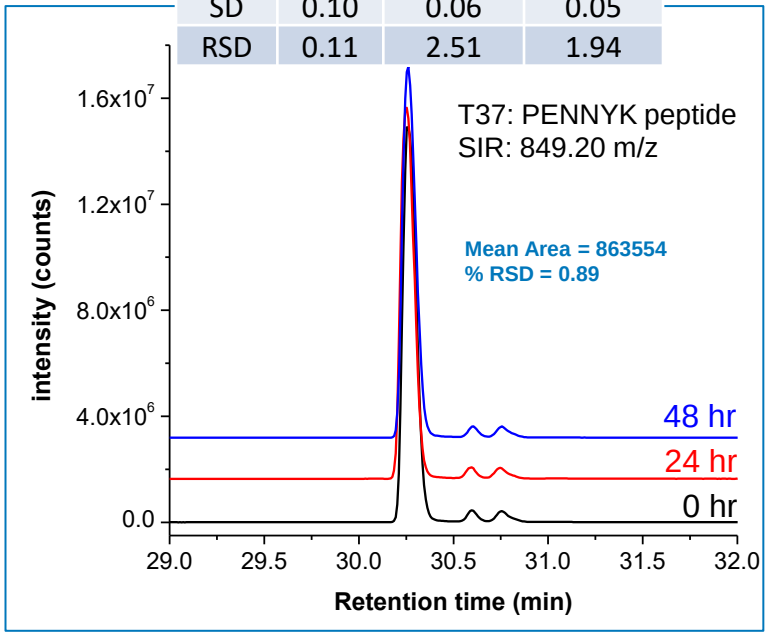
Acid passivation does not last and needs to be repeated

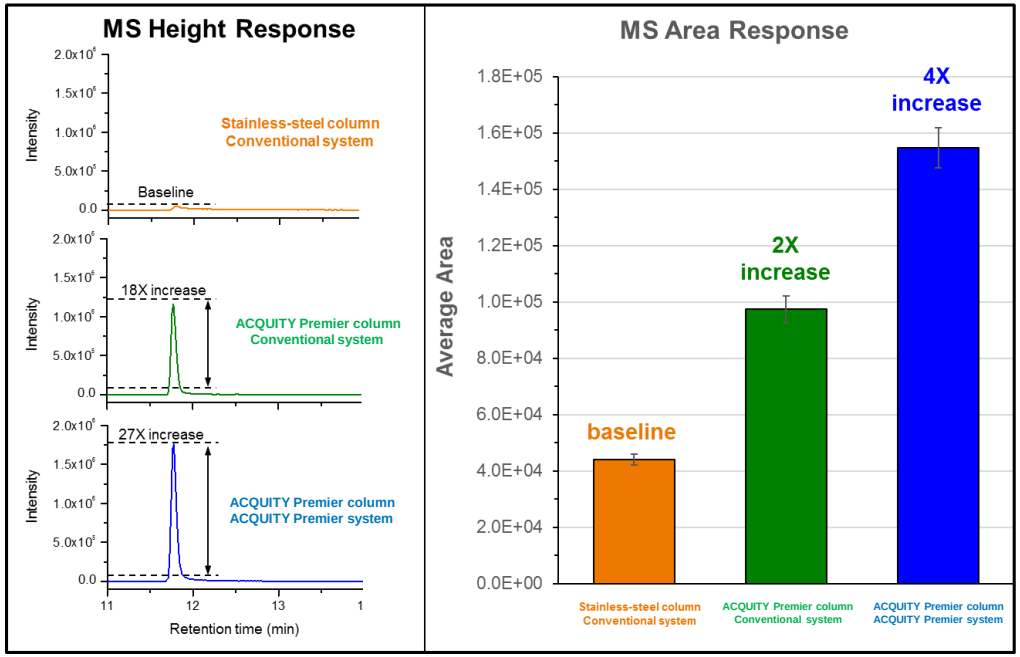


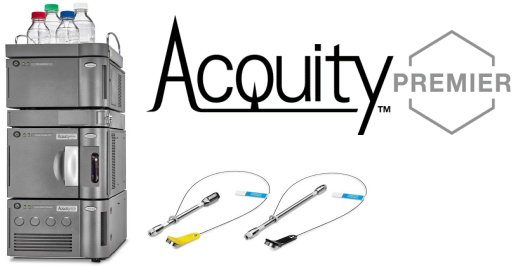
Area N=15	Native (%)	Deamd. 1 (%)	Deamd. 2 (%)
Avg.	94.90	2.31	2.79
SD	0.10	0.06	0.05
RSD	0.11	2.51	1.94



Acid passivated metals
ACQUITY PREMIER

ACQUITY Premier Column and ACQUITY Premier System work synergistically to improve acidic peptide recovery





- Consistent relative quantification of low level PTM such as deamidation can be challenging
- ACQUITY Premier solution improves consistency and accuracy of monitoring low abundance PTMs

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Making LC-MS Attribute Based Analysis Accessible to All

The BioAccord LC-MS System with ACQUITY Premier

The First SmartMS Enabled Biopharmaceutical LC-MS System

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Redefining performance
Purposefully designed system to address challenges

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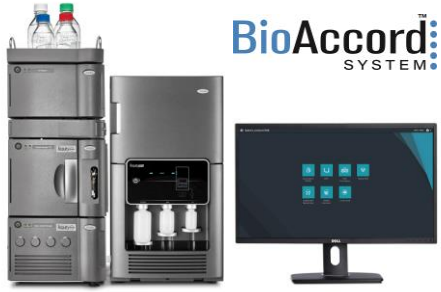
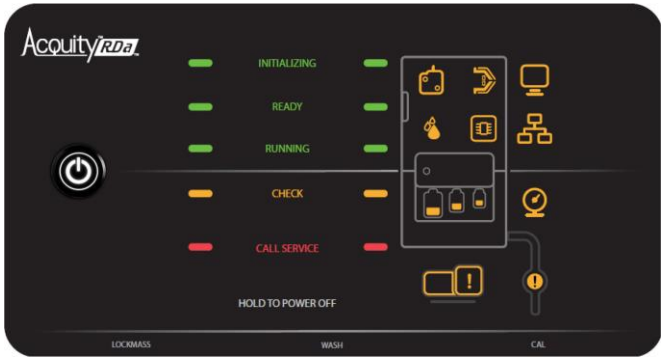
Easy to Deploy and Operate

- Accessible to non-MS experts, reduced training time
- Intuitive user interface, with on-system status display
- Minimal footprint



The first SmartMS-enabled Biopharmaceutical System



Redefining performance
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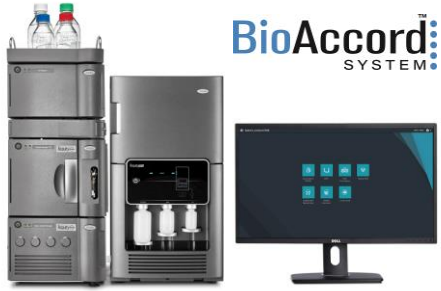

Maximized Up-time

- Automated and consistent system set up
- User intelligently guided to address issues
- Reduced time spent on troubleshooting

Redefining performance
Purposefully designed system to address challenges

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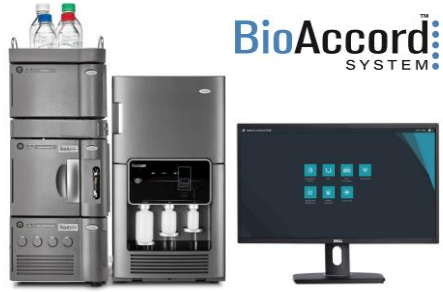
- Minimal footprint

Maximized Up-time

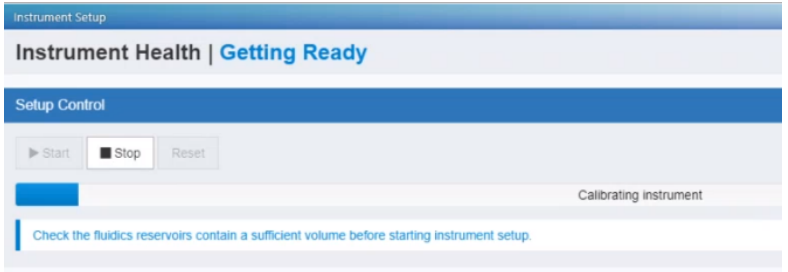
- Automated and consistent system set up
- User intelligently guided to address issues
- Reduced time spent on troubleshooting

Confidence in Data Quality

- System ensures it always runs at required performance
- Generate good data for every injection
- Prevent wasting samples



The first SmartMS-enabled Biopharmaceutical System



waters_connect: New Applications for Peptide MAM and Intact Mass Analysis

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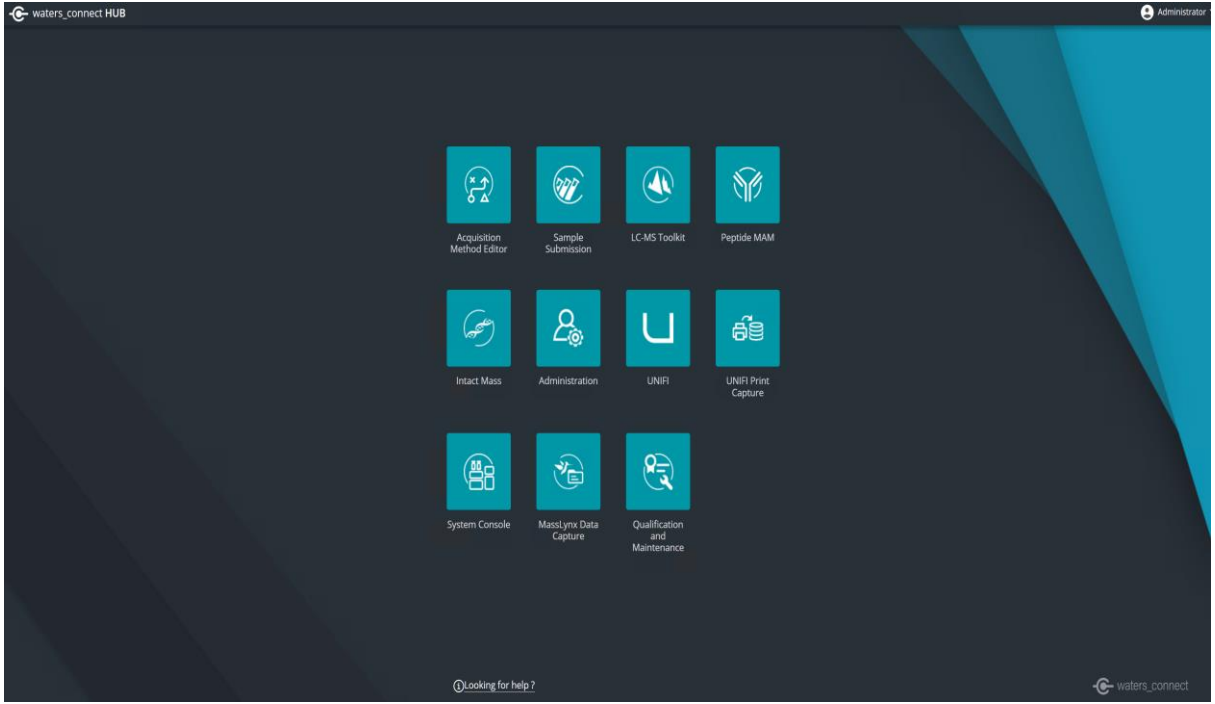

Supplementing the core UNIFI application workflows

- Intact Mass
- Peptide Mapping
- Released Glycan
- Accurate Mass Screening

Peptide MAM

Released Q4 2020

INTACT Mass

New for 2022

Harmonizing Workflows across Development, Manufacturing & QC

ENSURING DATA INTEGRITY, OPERATIONAL EFFICIENCY AND SCALABILITY

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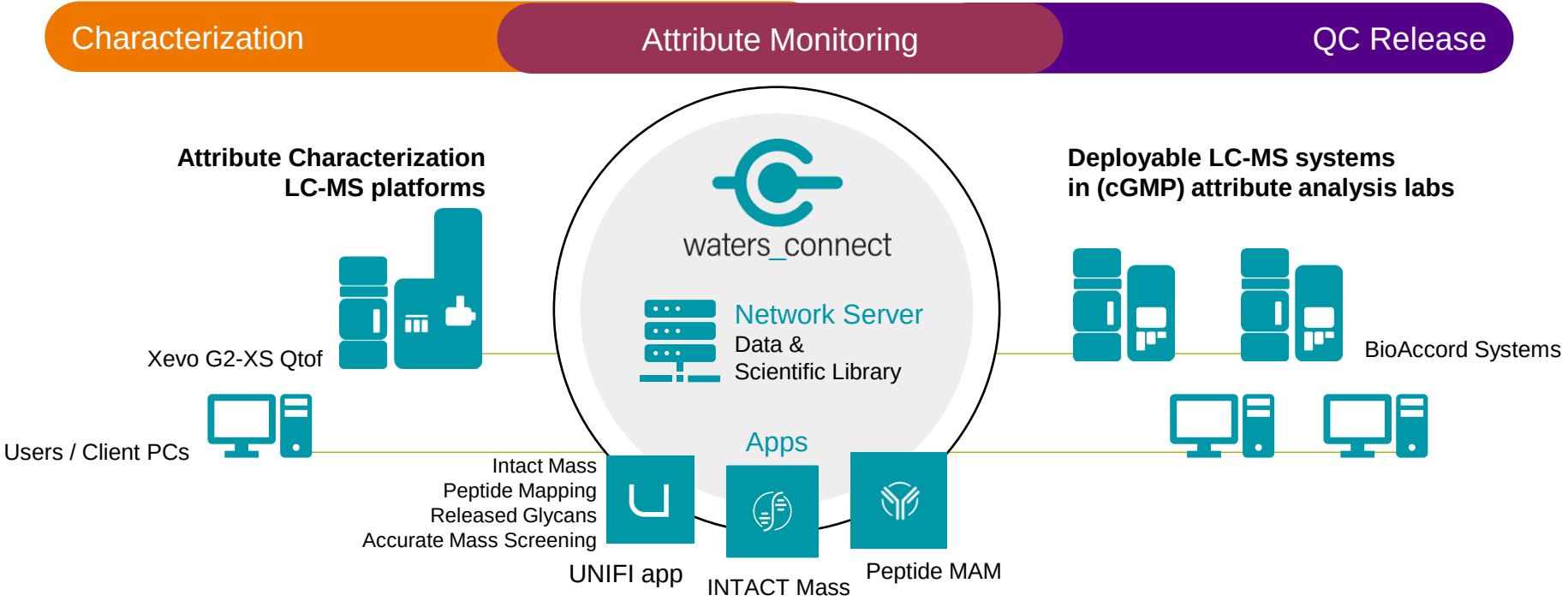

- ✓ Integrated compliance-ready data acquisition, processing and reporting
- ✓ A shared ecosystem that enables data traceability and transferability

Characterization: Identifying product quality attributes
Case study: NIST mAb peptide mapping

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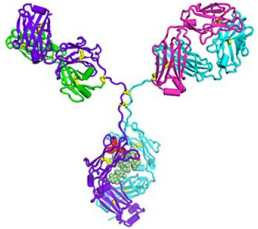
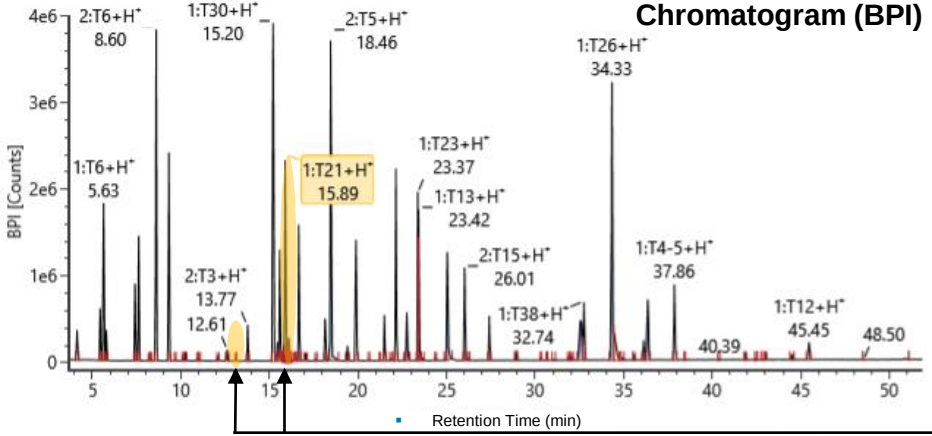
NISTmAb



- RM8671
- Sample digested with trypsin following reduction & alkylation

Chromatogram (BPI)



Identify and map product quality attributes e.g. 1:T21 DTLMISR

Chain 1	DIQMTQSPST	LSASVGDRVT	ITCSASSRVG	YMHWYQQKPG	KAPKLLIYDT	SKLASGVPSR	FSGSGSGTEF
	TLTISSLQPD	DFATYYCFQG	SGYPFTFGGG	TKVEIKRTVA	APSVFIFPPS	DEQLKSGTAS	VVCLLNNFYP
	REAKVQWKVD	NALQSGNSQE	SVTEQDSKDS	TYSLSSTLTL	SKADYEKHKV	YACEVTHQGL	SSPVTKSFNR
	GEC						
Chain 2	QVTLRESGPA	LVKPTQTLTL	TCTFSGFSLS	TAGMSVGWIR	QPPGKALEWL	ADIWWDDKKH	YNPSLKDRLT
	ISKDTSKNQV	VLKVTNMDPA	DTATYYCARD	MIFNFYFDVW	GQGTTVTVSS	ASTKGPSVFP	LAPSSKSTSG
	GTAALGCLVK	DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	GLYSLSSVVT	VPSSSLGTQT	YICNVNHKPS
	NTKVDKRVEP	KSCDKTHTCP	PCPAPELLGG	PSVFLFPPKP	K**DTLMISR**TP	EVTCVVVDVS	HEDPEVKFNW
	YVDGVEVHNA	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ
	VYTLPPSREE	MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW
	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK				

High quality sequence coverage 94% with, Mass error ±10 ppm, Containing ≥3 b/y fragment ions

Transfer selected product quality attributes from characterization to the scientific library

Component Summary

	Protein name	Fragment label	Peptide	Modifiers	Observed RT (min)	Observed m/z	Observed mass (Da)	Mass error (ppm)	Response	Matched 1st Gen Primary...
14	NISTmAb	1:T21	DTLMISR		15.90	418.2206	835.4340	-0.3	26678350	8
15	NISTmAb	1:T21&	DTLMISR	Oxidation M [4]	13.10	426.2169	851.4266	-3.0	1134178	4

Characterization / Peptide mapping results

waters_connect Scientific Library is populated with critical quality attributes information

- Unmodified and modified peptides are sent to the scientific library for NISTmAb
- These peptide lists can be updated and shared between multiple systems as product knowledge increases

Simplified design: Peptide MAM guided workflow

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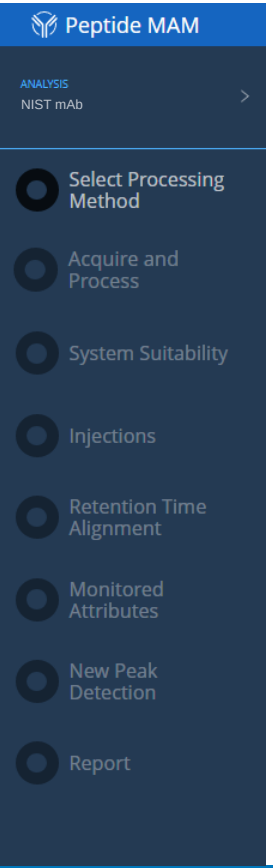

Acquisition and processing

- System Suitability Test criteria (SST)
- Peptide attribute list
- Processing criteria
- New peak detection criteria

Data reviewing and results reporting

- System Suitability Testing results (pass/fail)
- Peak review (chromatograms)
- Peptide attributes results (limit checks)
- %mod. summary table
- New peak detection verification
- Reporting

Peptide MAM Guided Workflow

Seamless jump from Peptide MAM to Sample Submission app

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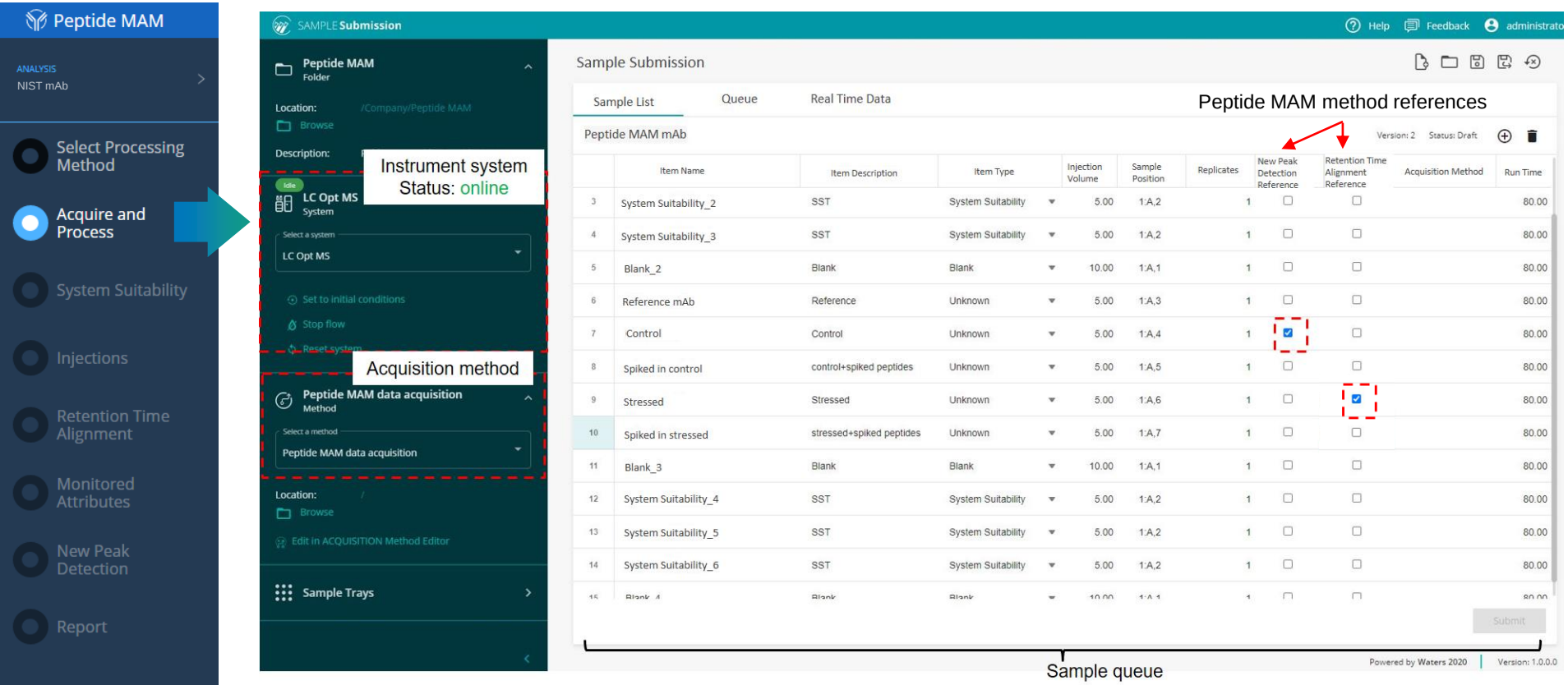

Rapid evaluation of system performance based on custom SOP criteria

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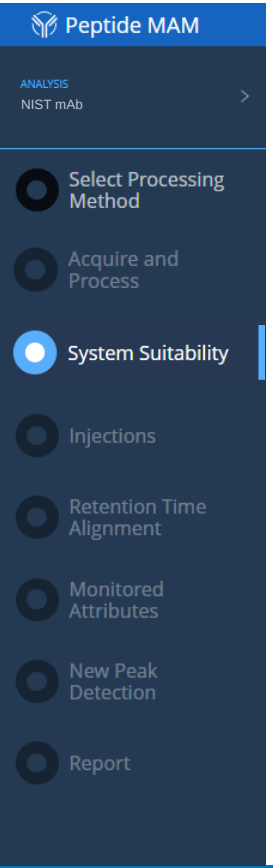

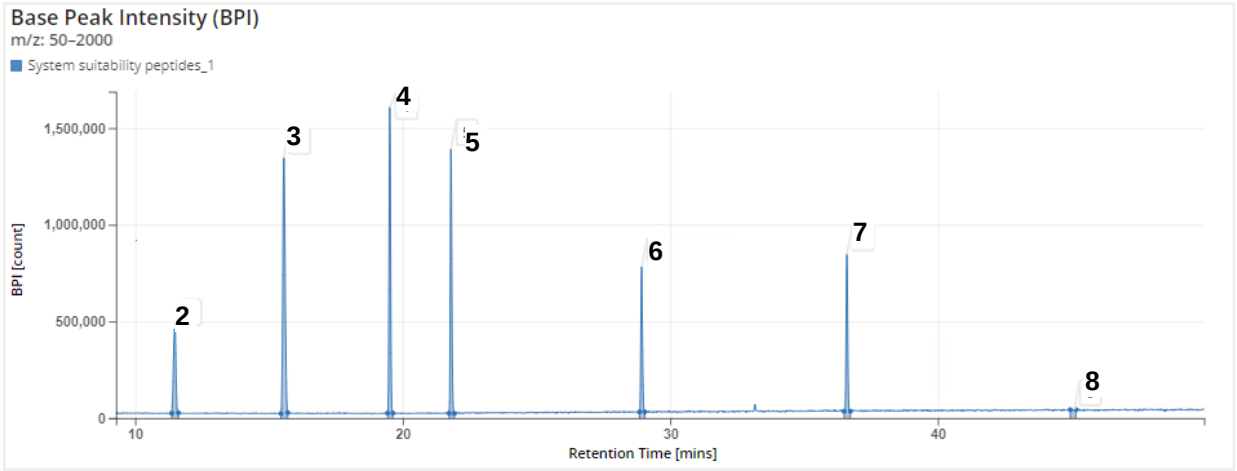

User defined pass/fail criteria

- mass error
- RT
- intensity
- peak width

Pass/Fail	Name	Observed Mass (Da)	Expected Mass (Da)	Mass Error (ppm)	Retention Time (min)	Expected Retention Time (min)	Retention Time Error (min)	Response	Peak Width FWHM (min)
Passed	2	898.4688	898.4668	2.2407	11.48	11.48	0.00	17,243,032	0.11
Passed	3	1,059.5648	1,059.5624	2.2614	15.56	15.57	-0.01	38,913,908	0.11
Passed	4	1,045.5397	1,045.5367	2.8838	19.52	19.52	0.00	35,682,024	0.07
Passed	5	1,295.6830	1,295.6794	2.7995	21.81	21.82	-0.01	29,586,450	0.07
Passed	6	1,757.9379	1,757.9294	4.8465	28.94	28.96	-0.02	35,185,048	0.08
Passed	7	1,871.9666	1,871.9641	1.3305	36.61	36.64	-0.03	24,767,486	0.09

Robust and Reproducible Quantification via Automated Retention Time Alignment

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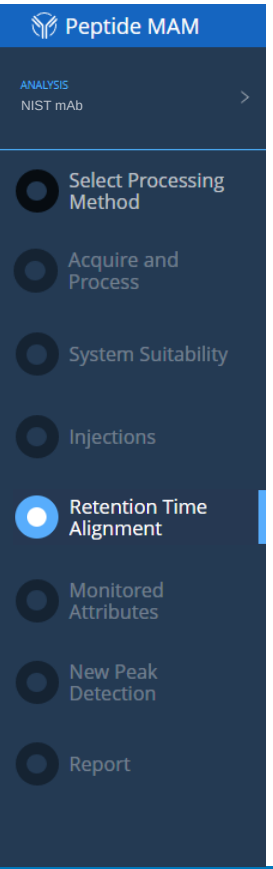

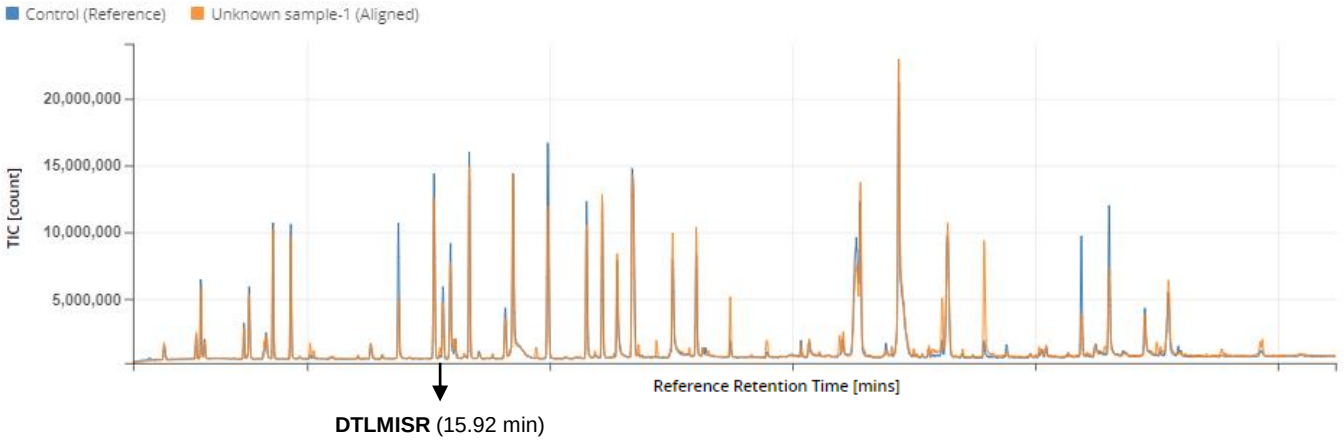

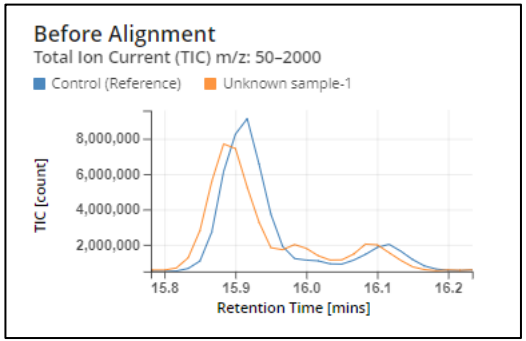

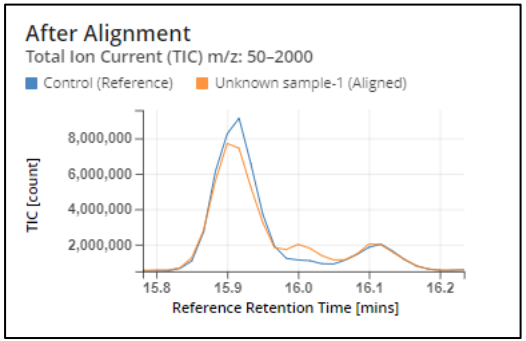

Maximized inter-system reproducibility and inter-day repeatability.

- Alignment prior to peak assignment.
- Automatic correction of possible offsets.

Increased productivity with simplified results review

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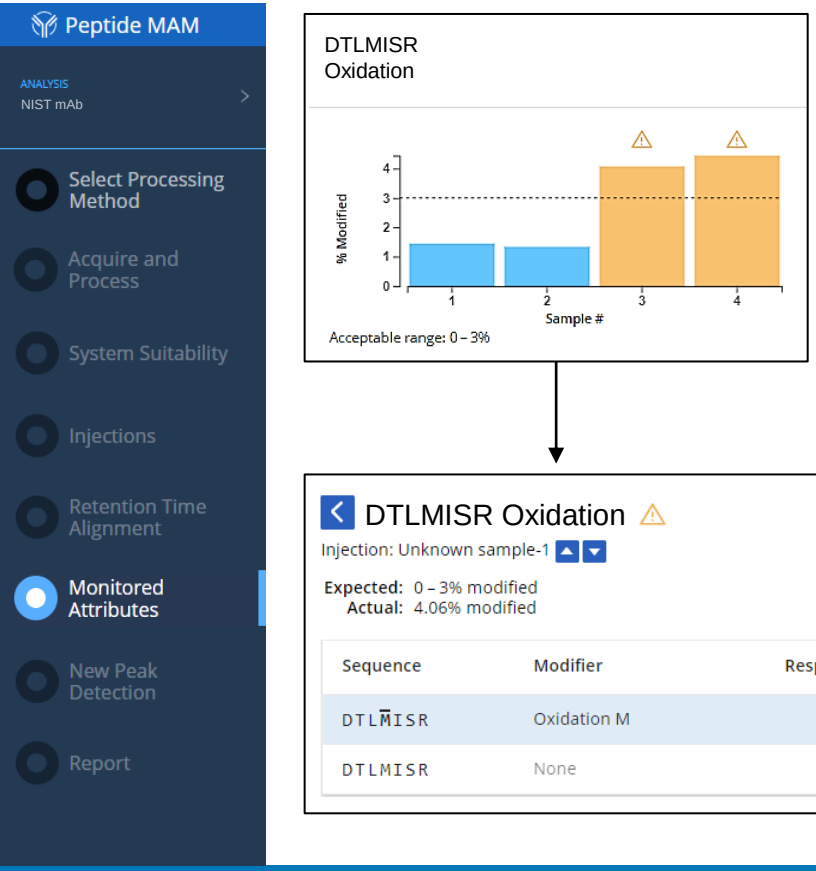
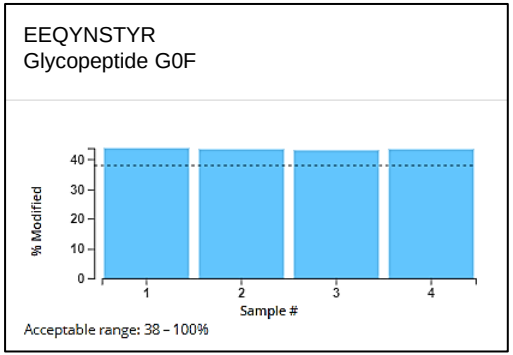
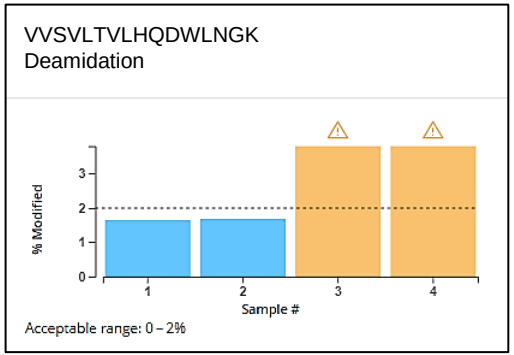
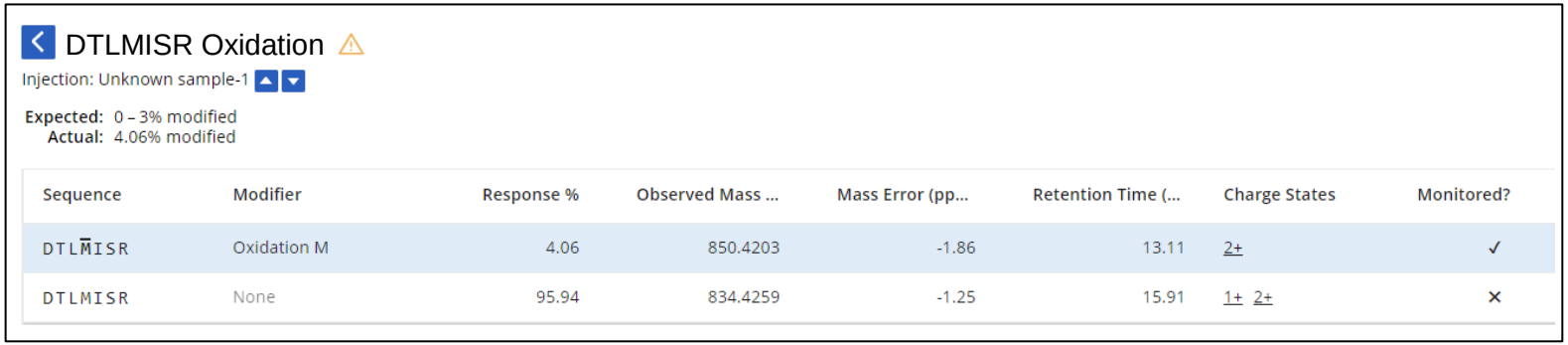






Samples and attributes out-of-specifications are clearly tagged.



Effective New Peak Detection
New peaks discovered in each sample vs. reference control

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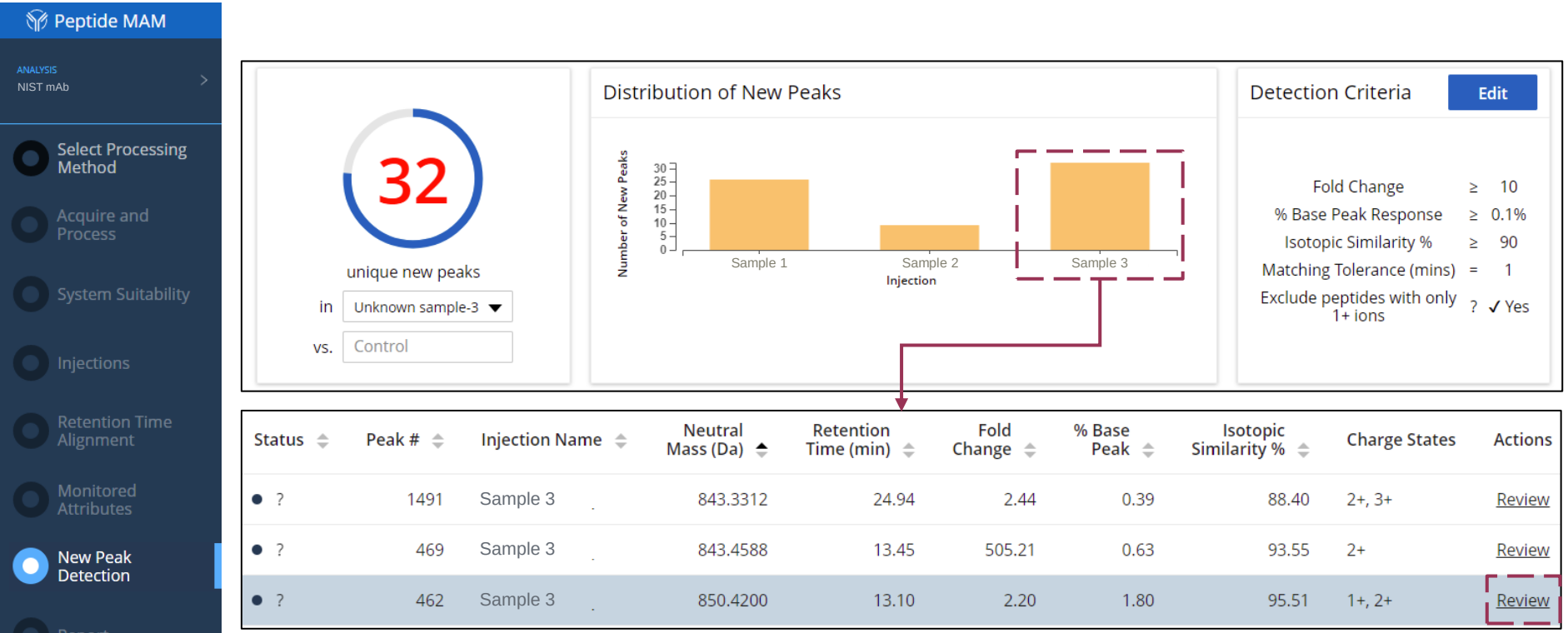

Status	Peak #	Injection Name	Neutral Mass (Da)	Retention Time (min)	Fold Change	% Base Peak	Isotopic Similarity %	Charge States	Actions
?	1491	Sample 3	843.3312	24.94	2.44	0.39	88.40	2+, 3+	Review
?	469	Sample 3	843.4588	13.45	505.21	0.63	93.55	2+	Review
?	462	Sample 3	850.4200	13.10	2.20	1.80	95.51	1+, 2+	Review

Clear, easy to share information with attribute-centric reporting

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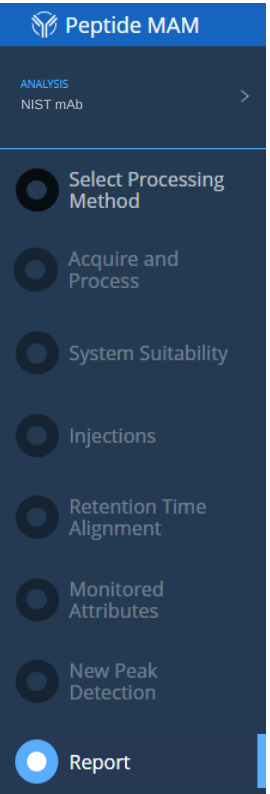

Peptide MAM Report

Generated By: waters_connect Administrator
Generated Date: 24 Jun 2021 12:20
Processing Date: 24 Jun 2021 11:46

Analysis

Name: Peptide MAM Data 2021
Version: 1
Location: Company/MAM/Demo data
Description: Reprocess data from 2020

Processing Method

Name: MAM Demo Data processing
Version: 5
Location: Company/MAM/Demo data
Description: 2021-03-31

Injection List

#	Injection Name	Type	Acquisition Status	Injection Volume (µl)
1	System suitability peptides_1	SST	Complete	1.00
2	System suitability peptides_2	SST	Complete	1.00
3	System suitability peptides_3	SST	Complete	1.00
4	Blank_2	Blank	Complete	10.00
5	Control	Unknown	Complete	10.00
6	Unknown sample-1	Unknown	Complete	10.00
7	Unknown sample-2	Unknown	Complete	10.00
8	Unknown sample-3	Unknown	Complete	10.00
9	Blank_3	Blank	Complete	10.00

System Suitability

System suitability peptides_1

Pass/Fail	Name	Observed Mass (Da)	Mass Error (ppm)	Retention Time (min)	Retention Time Error (min)	Response	Peak Width FWHM (min)
Pass	2	898.4688	2.2407	11.48	0.00	17,243,032	0.11
Pass	3	1,059.5648	2.2614	15.56	-0.01	38,913,908	0.11
Pass	4	1,045.5397	2.8838	19.52	0.00	35,682,024	0.07
Pass	5	1,295.6830	2.7995	21.81	-0.01	29,586,450	0.07
Pass	6	1,757.9379	4.8465	28.94	-0.02	35,185,048	0.08
Pass	7	1,871.9666	1.3305	36.61	-0.03	24,767,486	0.09

Take informed decisions based on critical results:

system suitability,
attribute monitoring with faulting samples,
new peaks detected,
audit trail and contextual information,
…

Monitored Attributes

Attribute Definitions

Attribute Name: DIQMTQSPSTLSASVGDR oxidation
Acceptable Range: Less than 2%

Found?	Monitored?	Sequence	Modifications	Retention Time (min)	Charge States
✓	No	DIQMTQSPSTLSASVGDR		22.76	2+, 3+, 4+
✓	Yes	DIQ**M**TQSPSTLSASVGDR	Oxidation M[4]	22.77	2+, 3+

Results - % Modified

⚠ = Outside acceptable limits

Injection Name	Glycopeptide G0F-GlcNAc (6/6 forms)	Glycopeptide G1F-GlcNAc (6/6 forms)	Glycopeptide Man5 (6/6 forms)	MISR ox (2/2 forms)	VVSVL misC deami (4/4 forms)
Control	2.36	2.56	0.86	1.47	0.82
Unknown sample-1	2.16	2.46	0.83	⚠ **4.12**	2.23
Unknown sample-2	2.35	2.49	0.92	1.36	0.89
Unknown sample-3	2.27	2.47	0.74	⚠ **4.54**	2.30

waters_connect: New Applications for Peptide MAM and Intact Mass Analysis

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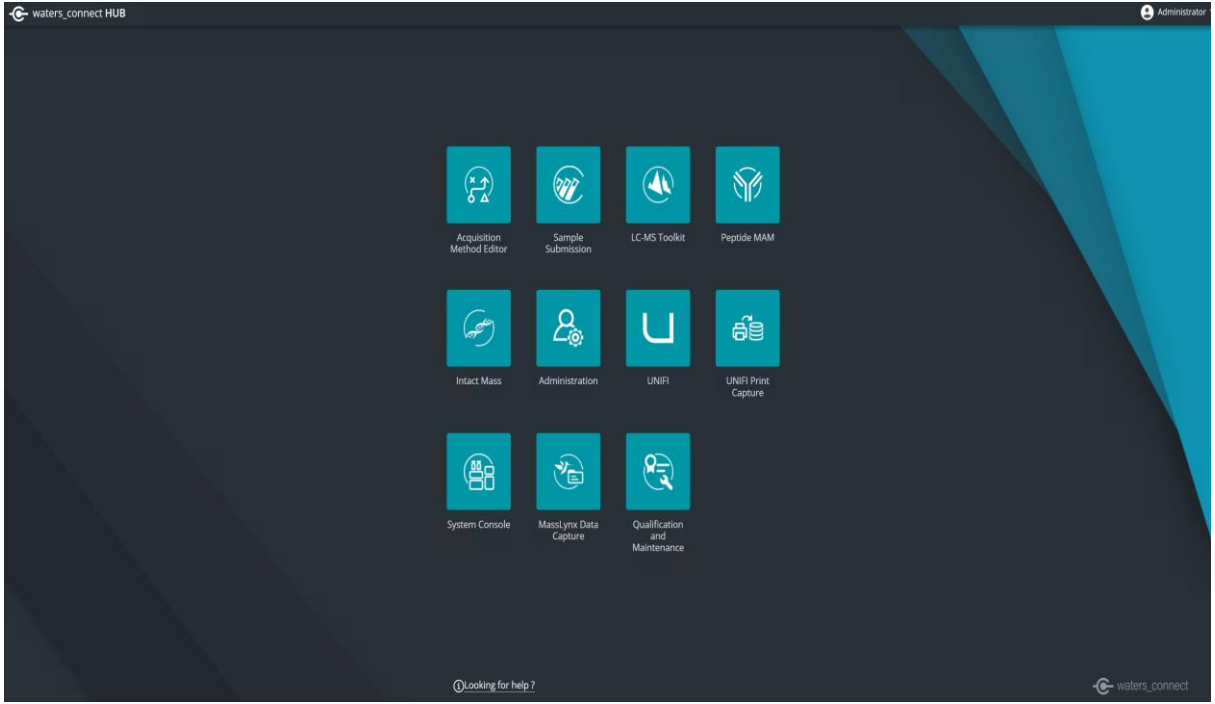

Supplementing the core UNIFI application workflows

- Intact Mass
- Peptide Mapping
- Released Glycan
- Accurate Mass Screening

Peptide MAM

Released Q4 2020

INTACT Mass

New for 2022

Automating Intact Mass LC-MS

Simple, Flexible, Efficient

INTACT **Mass**

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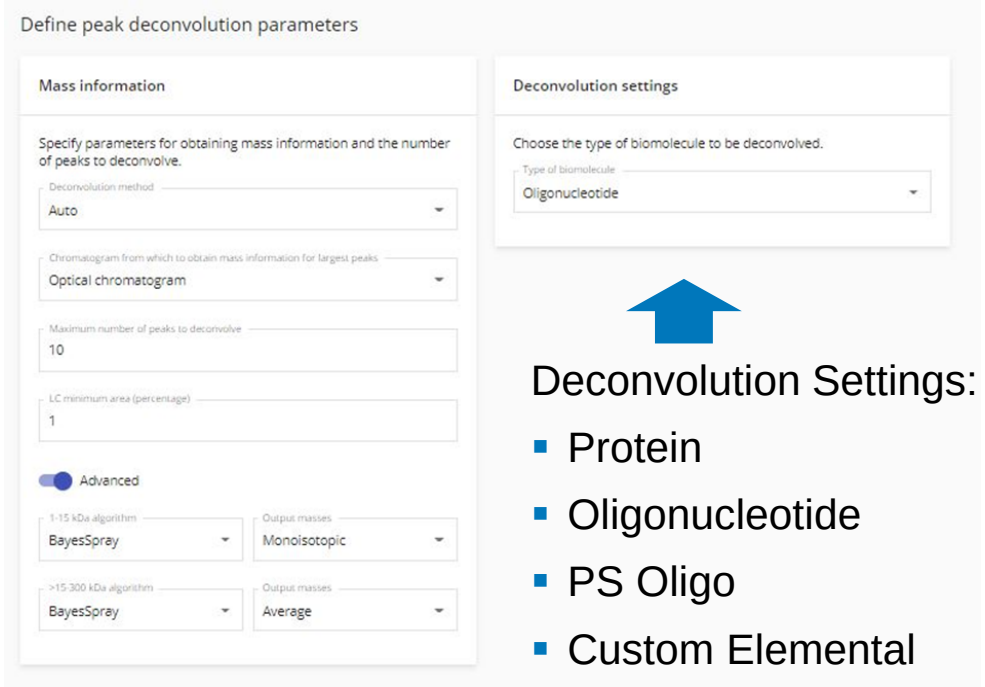
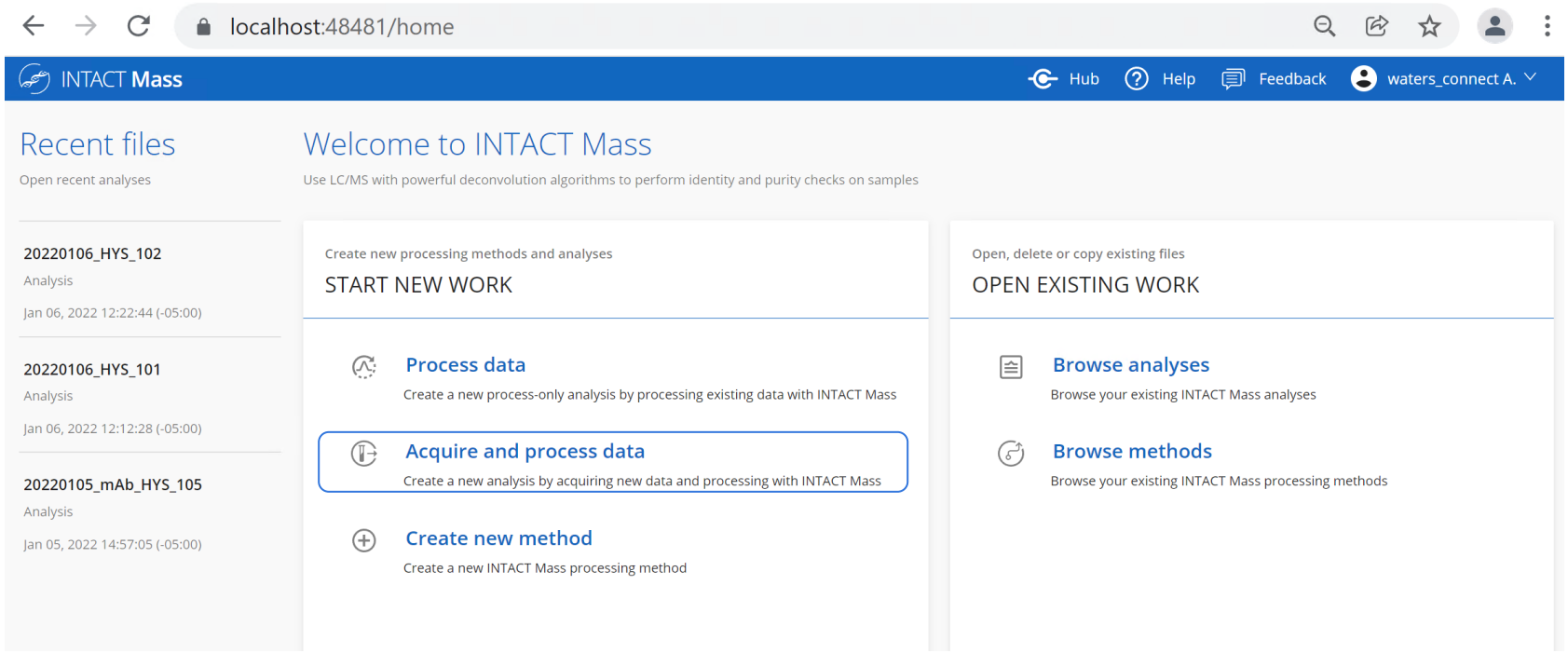
- Goal: Mass Confirmation and Purity for each Injection
  - Simple: Single Sample to Plate-based Analysis
  - Flexible: Purity using optical chromatogram, TIC, or within a mass spectrum
- Automating LC-MS Deconvolution
  - Simple: Automate Peak Picking (UV/TIC)
  - Simple: Automatically determines input and output mass ranges, optimal deconvolution conditions
  - Simple: Reduces human error , and non-specialists can obtain deconvolved mass information
  - Efficient: Create generic methods to analyse a wide range of molecules efficiently
- Assigning ID for products and impurities using variable modifications
  - Flexible: Product related substances/impurities identified
  - Efficient: Fine control of potential combinations of modifications
- Automated Acquire and Process Workflow
  - Speed: Multiplex Processing (Multiple Spectra) occurs in parallel with acquisition
  - Flexibility: Expected masses and molecule IDs provided in sample list

Automating LC-MS Deconvolution

Setting	Deconvoluted Mass Range	Typical Use
BayesSpray Monoisotpic	1 – 15 kD	Peptides Small Oligos
BayesSpray Average Mass	15 – 300 kD	Proteins Large Oligos
MaxEnt 1 Average Mass	15 – 300 kD	Proteins Large Oligos
Unprocessed	>1 kDa	Small Molecules



Automated Acquisition and Data Processing



Imported Sample List from .csv File

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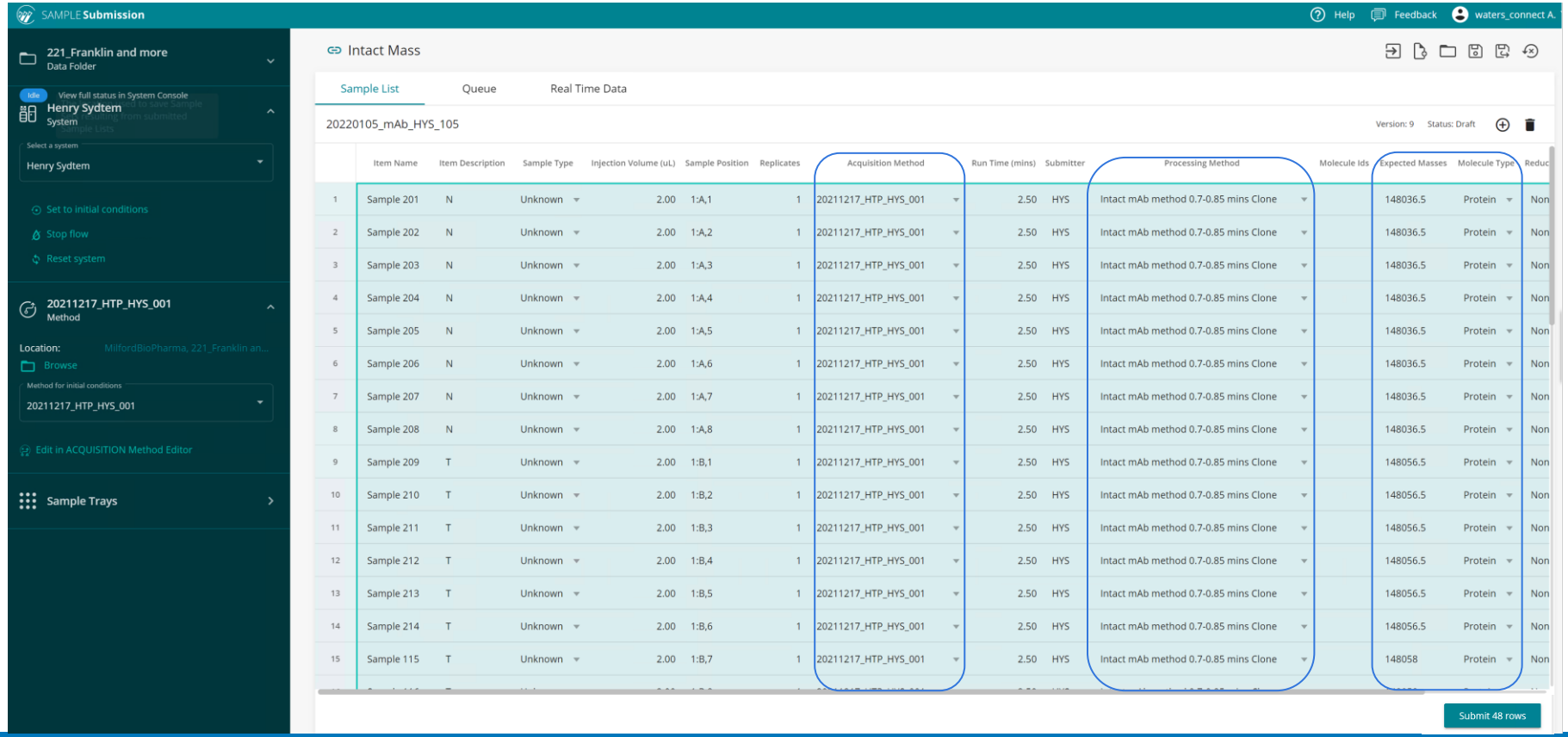

Higher Throughput: Fast Desalting Method Coupled with Scheduled Lock Mass Acquisition

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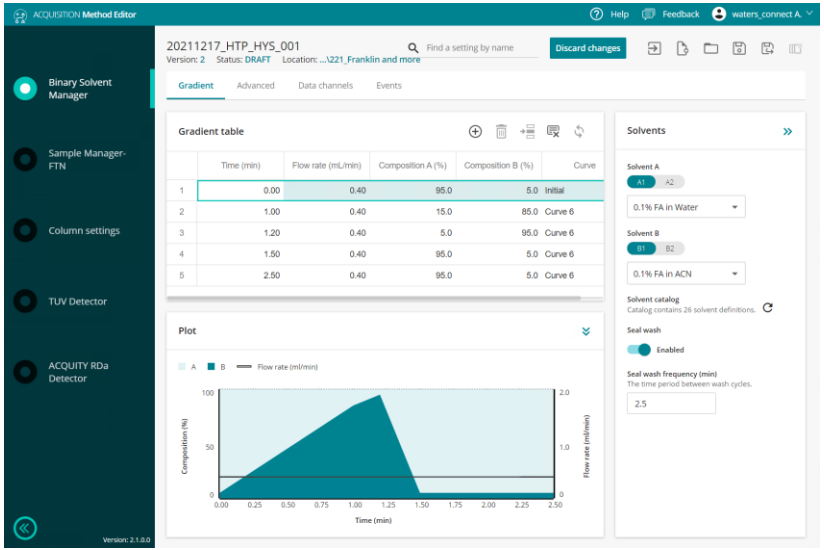
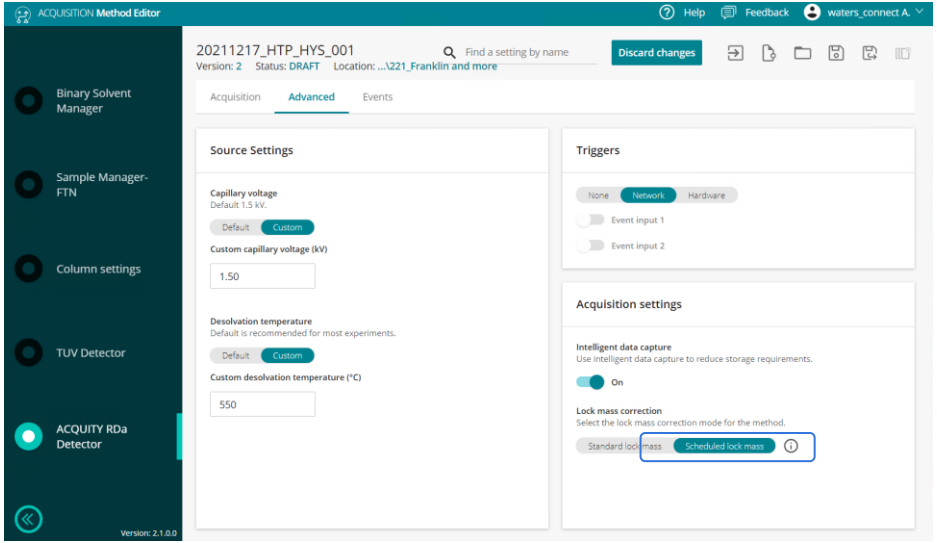
2.5 mins fast LC gradient



Scheduled Lock Mass minimizes inter-run system-checks



"Scheduled lock mass" ~17.5 seconds/injection vs. "standard lock mass" ~2 mins/injection.

Data for a single Injection of NISTmAb

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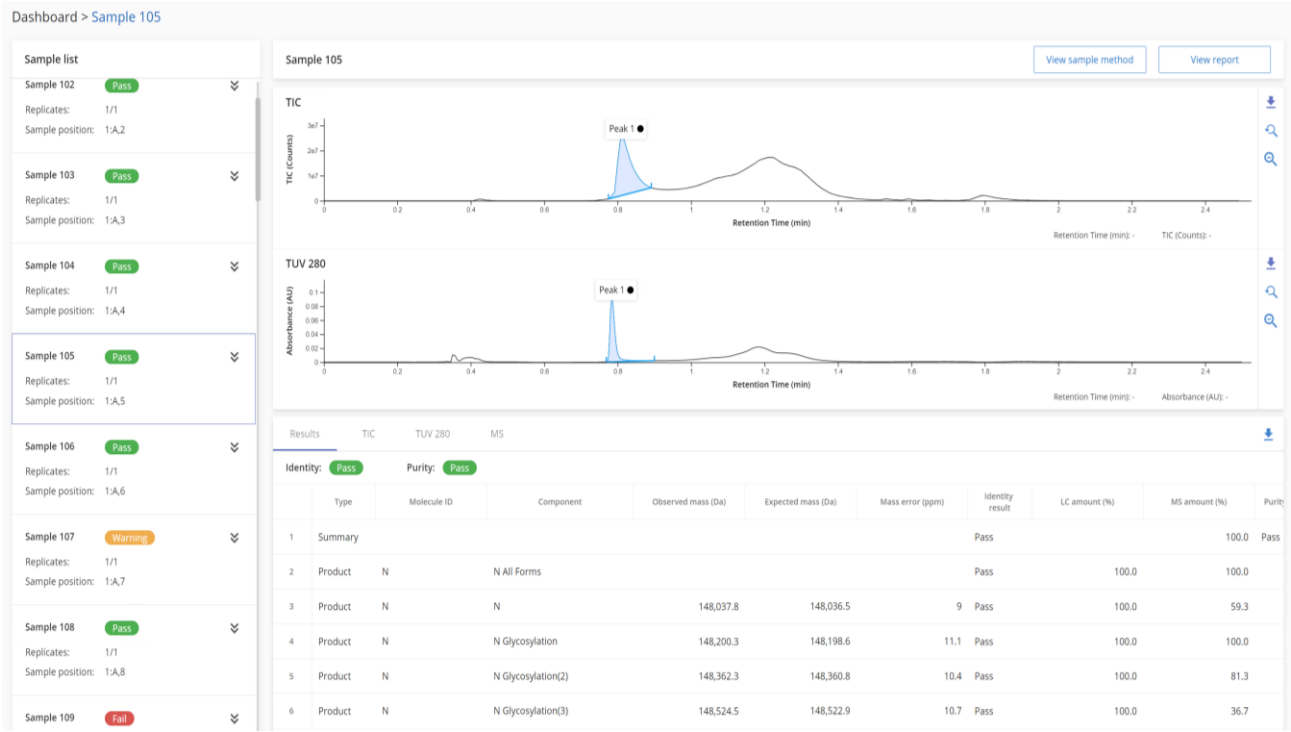

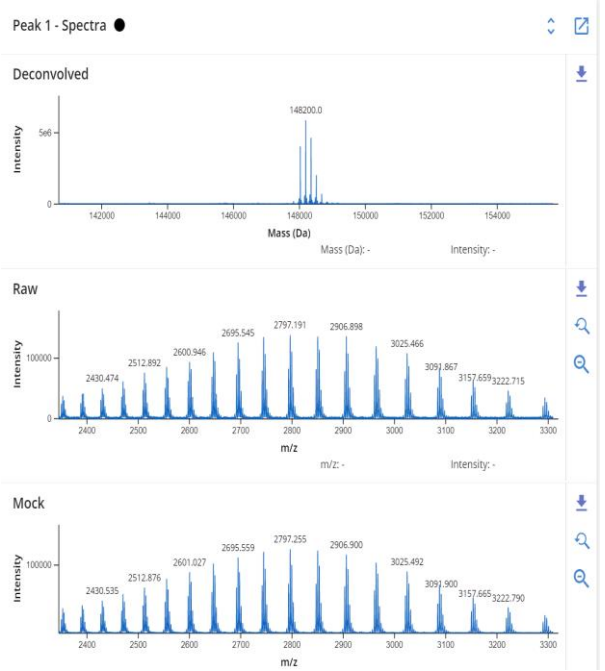

Review of a 48-Well Plate Intact Mass Sample Set

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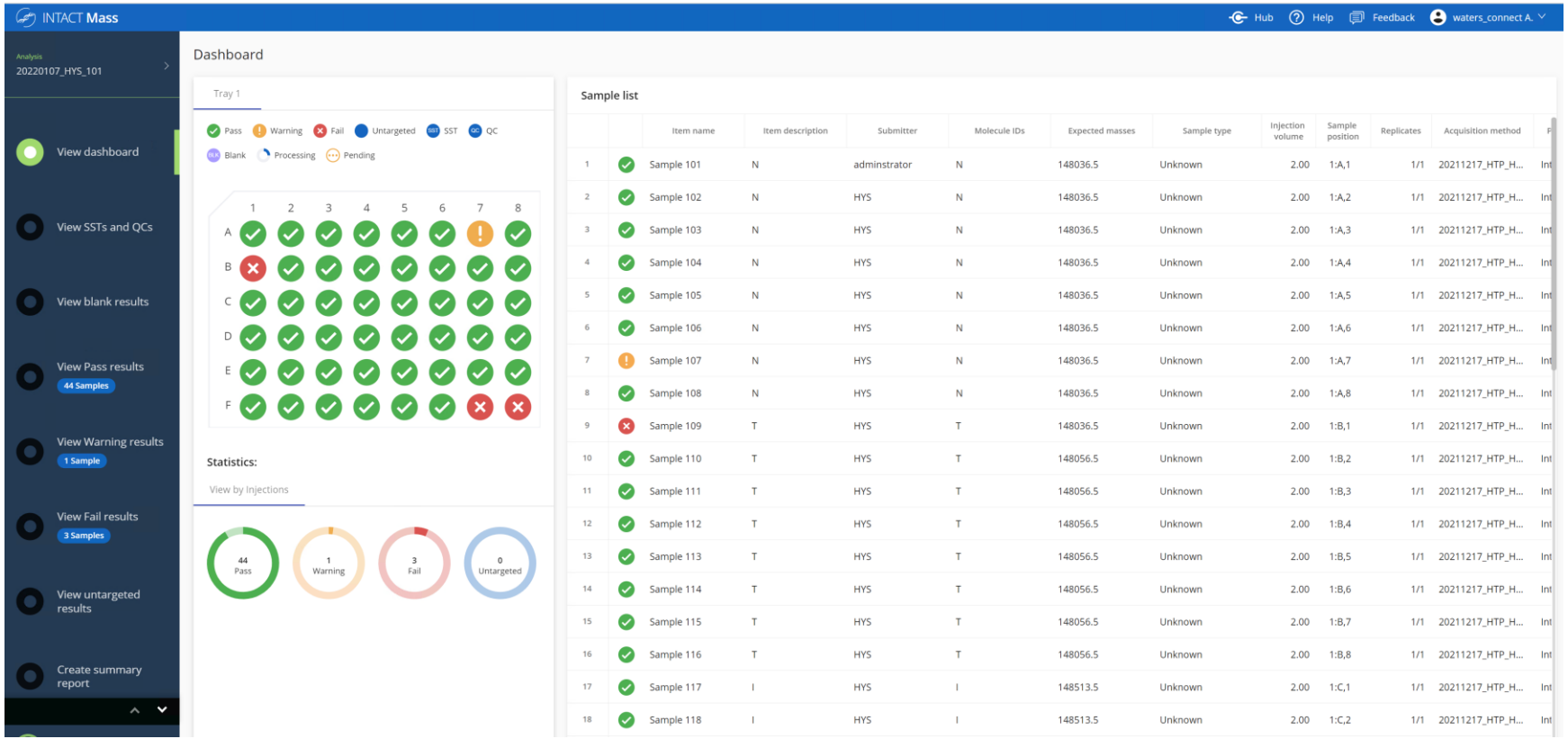

Sample-Centric Report

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< Sample 105 Sample report

INTACT Mass

Version: 1.0.0.0

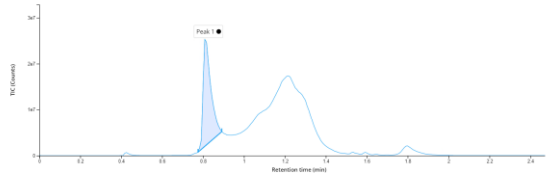
Analyst name: administrator
Analysis name: 20220107_HYS_101
Sample set name: 20220105_mAb_HYS_105 - v2

Sample 105
Pass

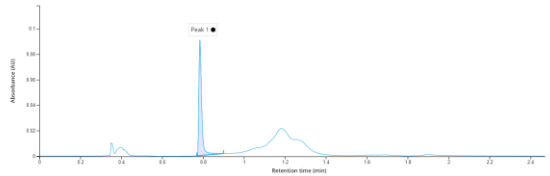
Created by: administrator Time acquired: Jan 06, 2022 02:23:12 (+00:00) Time processed: Jan 07, 2022 16:52:34 (-05:00) Time report generated: Jan 07, 2022 17:25:17 (-05:00)

Item description:	N	Molecule IDs:	N
Sample position:	1:A,5	Expected masses:	148036.5
Sample type:	Unknown	Submitter:	HYS
Injection volume:	2	Molecule type:	Protein
Replicates:	1	Reduction state:	None
Acquisition method:	20211217_HTP_HYS_001	Enzymatic treatment:	None
Processing method:	Intact mAb method 0.7-0.85 mins Clone - v3	Acquisition status:	Complete

TIC

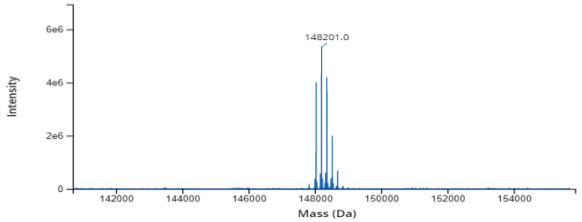


TUV 280

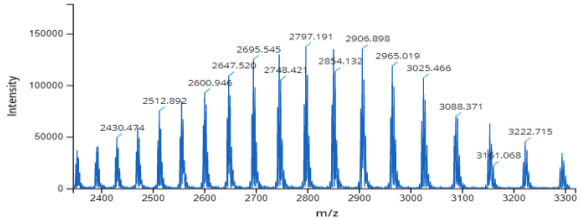


Peak 1 - Spectra

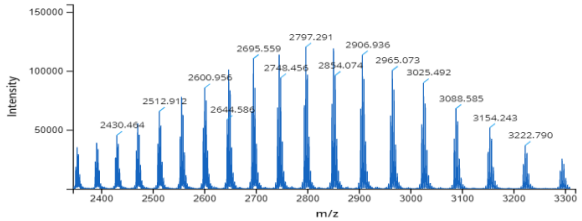
Deconvolved



Raw



Mock



Reducing Bias and Increasing Efficiency and Robustness of Biopharmaceutical Analysis

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1

Automating Lab Processes to Reduce Errors and Drive Efficiencies

From Sample Prep to Data Processing to Data Review and Reporting

2

Improving Technologies to Deliver Unbiased Results

Building the Smart-connected lab, with automated self-calibrating and self-diagnosing systems, and utilizing innovative material science to deliver all analytes effectively to detection.

3

Simplifying Operations to Increase Accessibility and Robustness

There is still a need for highly flexible research technology that pushes boundaries and requires expert interaction, but routine analysis should be engineered to enable non-experts to generate quality results.

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MaxPeak™ Premier High-Performance Surface Technologies for LC-Based Bioseparations

Bill Warren
Principal Product Manager

MAXPEAK™
HIGH PERFORMANCE SURFACES

Agenda

- Confidence in Results and Analyte Losses
- Introducing MaxPeak Premier HPS Technology for Bioseparations
- Challenges and Premier Solutions for:
  - **Protein Size Variant Analyses (SEC)**
  - Peptide Separations (Reversed Phase)
  - Nucleic Acid Analyses (Reversed Phase)
  - Glycoprotein / Release Glycan Analyses (HILIC)
- More Information and Additional Educational Assets

What Gives You CONFIDENCE in LC-Based Biomolecule Analyses?

- Highly reproducible?
  - Low RSD's
  - Analyte detection is complete and predictable
- Data from LC-based biomolecule analyses is reliable to address internal critical quality attribute (CQA) and regulatory needs
  - Free from unexpected surprises or repeat testing

There Are the Wonderful Days …

- Develop and Validate a New Method
  - Necessary Component Resolution
  - Method Linearity
  - Adequate LOD and LOQs
  - Required Batch to Batch and Column to Column Consistence
  - **Results NOT biased by LC Separation !**

There Are the Not So Wonderful Days …

- We need to analyze some samples urgently.
  - Inject the sample onto the column and … see NOTHING
  - Inject it again … NOTHING
- What is going on?
  - Try to passivate the column, and the system
  - Inject higher concentrations of sample
- We lose … confidence

What Happens When You DON'T Have Confidence in Your Results?

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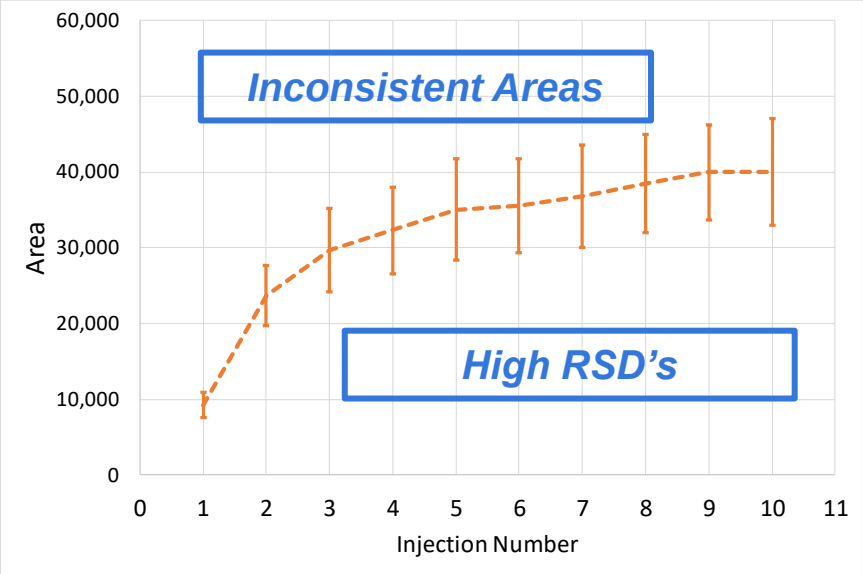
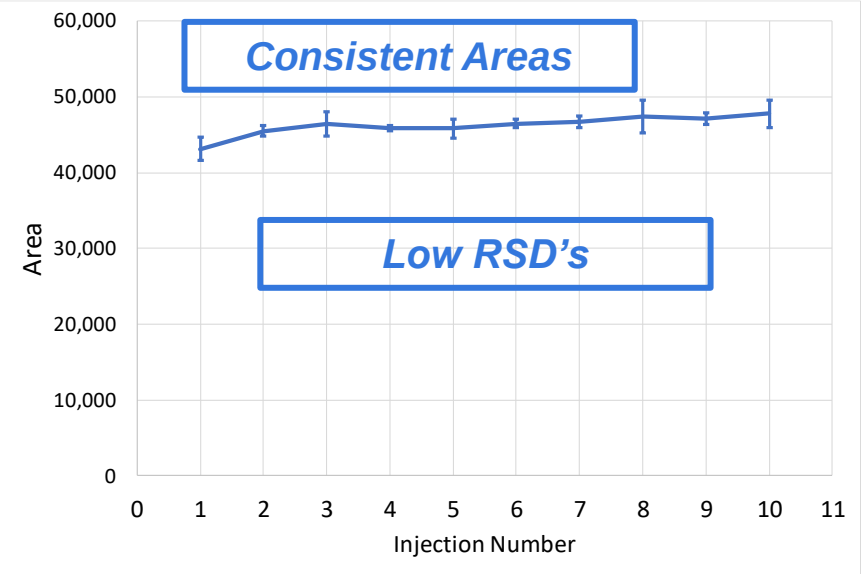
What happens when you are getting this…



But you need this….



What is the cause of this? What can we do to reduce this problem?

Biomolecules Can Bind to Surfaces

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- Non-Specific Binding (NSB) and Non-Specific Adsorption (NSA) can be a problem
  - *Any binding or adsorption that was NOT intended*
  - Molecules tend to adhere to ***any*** exposed surfaces.
  - ***Any*** chemical interaction can be the source of binding, *but most dominantly…*
    - Polarity-based interactions, *"Like" attracts "like"*
      - *e.g., hydrophobic attraction*
    - Ionic interactions, *Positive and negative charge attraction*
      - *e.g., coulombic attraction*

How Do Scientists Solve This Problem Today?

Solution	How does it work?	What is the consequence?
Passivation of surfaces with acid	Removes free iron from steel surface	• Time consuming • Strong acids • Not stable, needs to be repeated
Passivation of surfaces with sample or matrix	Analyte or matrix coats reactive surfaces	• Time consuming • Not stable, needs to be repeated

PEEK or PEEK lined steel columns	Replaces metal with non-reactive material	• PEEK alone is not high pressure tolerant
		• PEEK materials have:
		  • Higher dimensional variability
		  • Lower frit permeability
		  • Incompatible with some solvents

Titanium in columns or parts	It doesn't. Titanium is a metal!	• Analyte loss
Industrial coatings	Covers the metals with material, e.g silicates/other materials	• MS bleed, and other unexpected problems • These were never designed for LC and LC-MS applications!
Additives in mobile phases	Chelates with metals to prevent analyte adsorption	• Ion suppression and other unknown effects • Continued use necessary • Possible solubility issues

A Technology Brand was Created…

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MAXPEAK™
HIGH PERFORMANCE SURFACES

MaxPeak™ Premier High-Performance Surfaces are new and innovative technologies designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte / surface interactions that can lead to sample losses.

Analytical Tools and Solutions for Bioseparations

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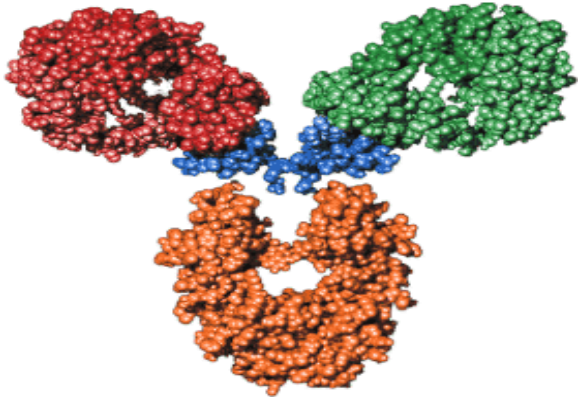
Understanding Primary Structure



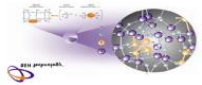
Impurities
Host Cell Protein
Excipients Analysis (e.g., Polysorbates

Purity, Content, Glycosylation

Higher Order Structure
HDX-MS
SEC-MALS







Reversed Phase	Peptide Mapping	Amino Acid Analysis	Hydrophobic Interaction	Size Exclusion	Glycan HILIC	Ion Exchange
• Separate oxidized, fragmented species • Intact Mass • Reduced mAb characterization	• mAb Identity • PTM characterization • Disulphides • MAM	• Understanding molar absorptivity • Protein Concentration • *(Quantitative AAA)*	• Separate based on hydrophobicity under non denaturing conditions	• Separation based on size of molecule • Dimers/Aggregation • High Molecular Weight • Low Molecular Weight • Clips	• Glycosylation Pattern • Released N-Glycan • Orthogonal methods for Intact, Subunit, Glycopeptide Mapping	• Separation Based on charge • Monitor deamidation, sialyaltion, pyroglutamate

MAXPEAK™ PREMIER

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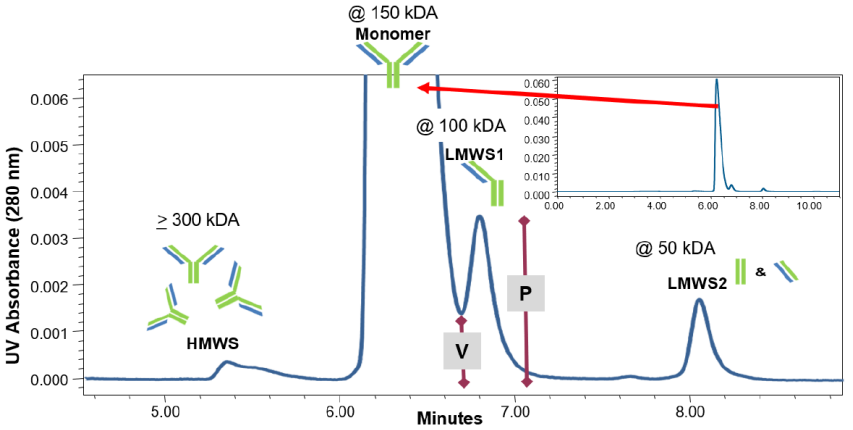
SEC of Proteins



Protein Size Variant Analyses Using SEC

The Challenge of Non-Desired, Secondary Interactions

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- **Size Exclusion Chromatography**
  - Separations based on biomolecules size in solution using columns containing appropriate pore size SEC particles
  - Isocratic separation, comparatively low resolving vs techniques such as reversed phase
  - Challenging to separate compounds less than 2X different in size
  - SEC method development frequently required to develop a robust protein size variant separtion

- **Most commercialized LC based SEC columns contain of silica-based, diol-bonded particles of defined pore and particle size packed in stainless steal hardware**

- **Non-desired, secondary ionic and hydrophobic interactions between proteins and SEC particles and stainless column hardware are challenges to obtaining platform based, robust analyses**

Introducing MaxPeak™ Premier Protein SEC 250Å Columns and Guard for Protein Size Variant Analyses

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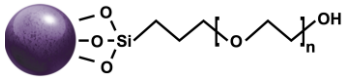
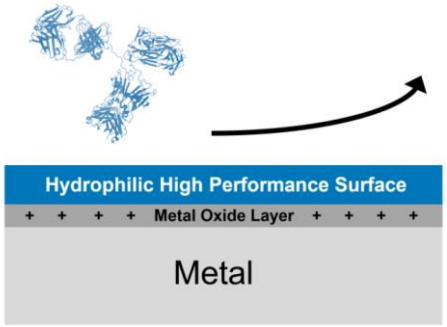
A: BEH-PEO SEC Technology



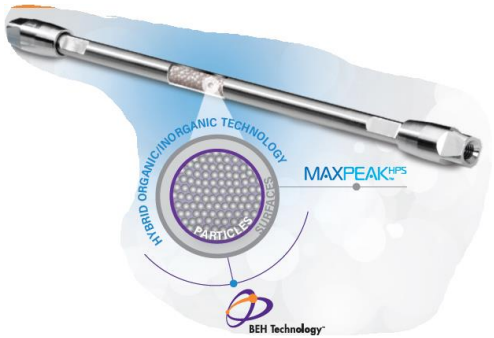
plus

B: MaxPeak Premier SEC Technology



equals

MaxPeak Premier Protein
SEC 250A Columns and Guard



Use of MaxPeak Premier Protein SEC 250Å Column Technology to Reduce Undesired Secondary Interaction (A) Hydroxy terminated PEO bonded BEH particles with low ionic and low hydrophobic secondary interactions. (B) MaxPeak High Performance Surface (HPS) with hydrophilic properties to minimize secondary interactions between biomolecules and metallic column hardware and REDUCE Costly Method Development Investment.

SEC Challenges for Reliable Biotherapeutic Protein Size Variant Analyses

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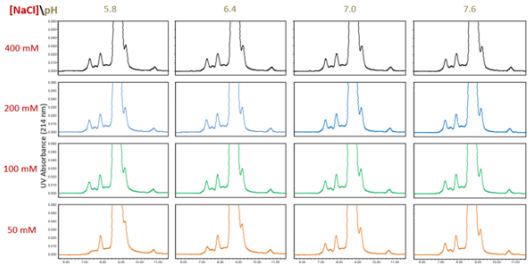
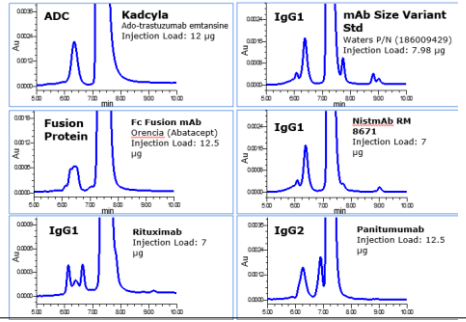
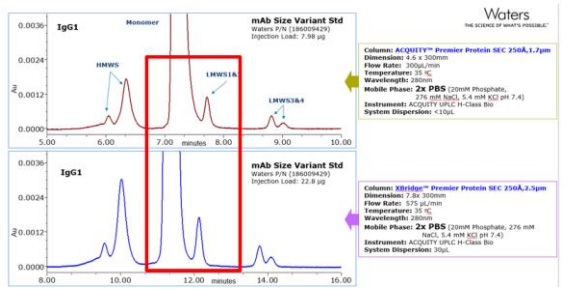
Long SEC Method Development Time (Desire to have Platform Method)



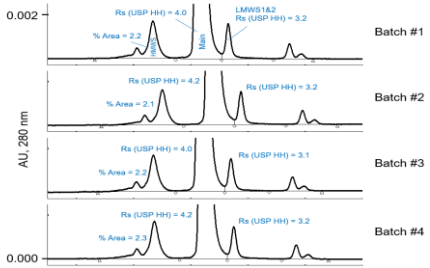
Out of the Box Performance for Protein Aggregate, Monomer, and Fragment SEC Analysis



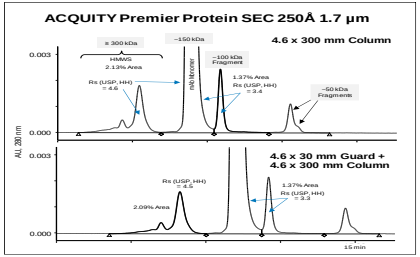
The option to have speed (1.7 um) and scalability (2.5 um)



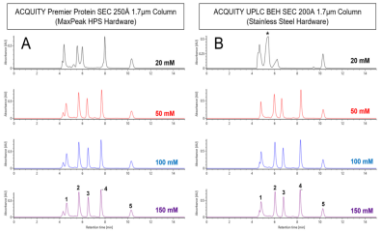
Batch to Batch and Consistency



Decrease Cost per Analyses with Guard

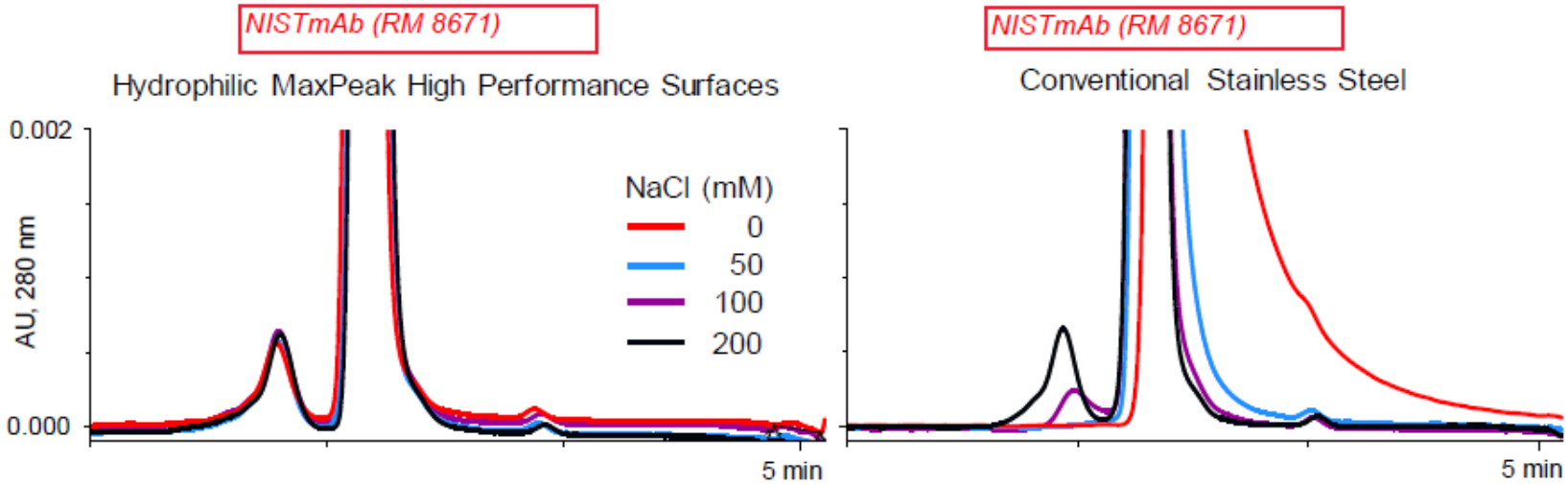


Improved SEC / MS Analysis of Native mAbs



QR Code Enabled for Column Specific and Authenticity Information

Influence of SEC Column Hardware on mAb Analysis



XBridge™ Protein SEC BEH-DIOL 250Å 2.5 µm stationary phase was packed into both hydrophilic MaxPeak High Performance Surfaces hardware (left) and conventional stainless-steel hardware (right) to clearly demonstrate the benefit afforded by the HPS hardware.

Influence of SEC Particle on ADC Analysis

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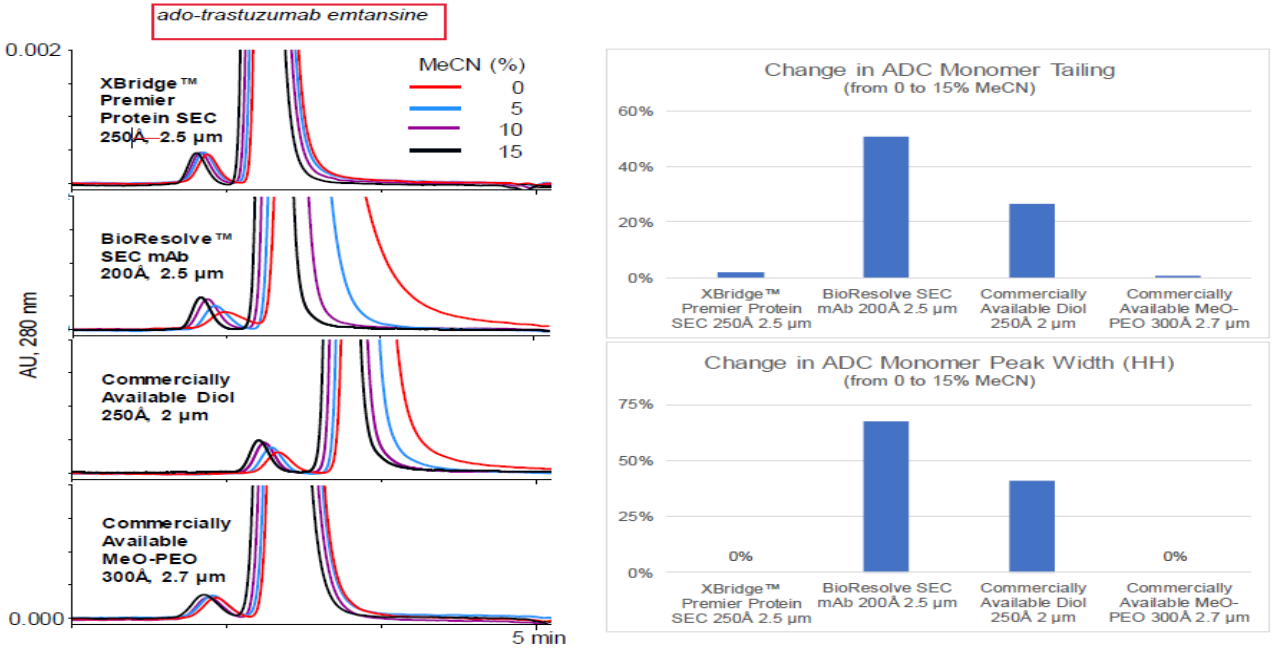

Comparison of hydrophobic secondary interactions performance using the ADC ado-trastuzumab emtansine. Upon increase of organic concentration, the degree of change observed for the XBridge™ Premier Protein SEC 250Å 2.5 µm column is again negligible, with outstanding peak shape from 0 to 15% MeCN

Shorten SEC Method Development:
Is a SEC Platform Method Possible?

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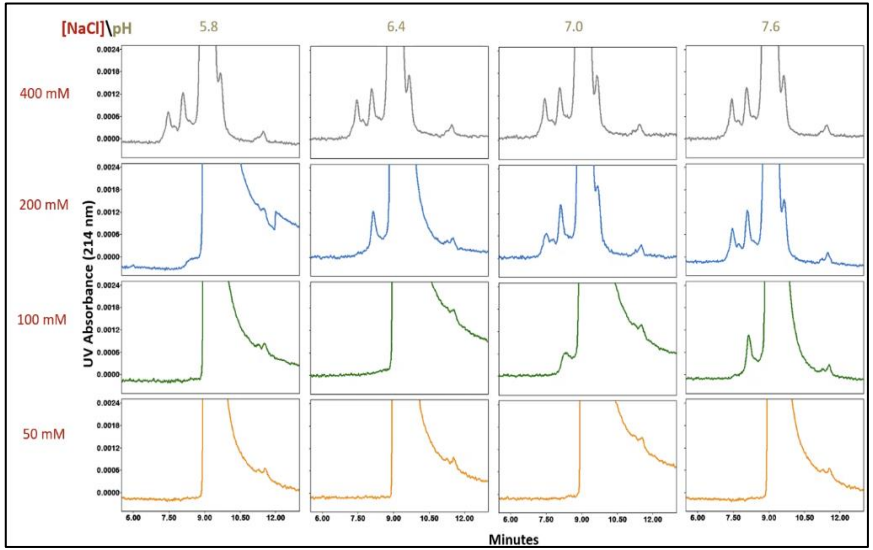
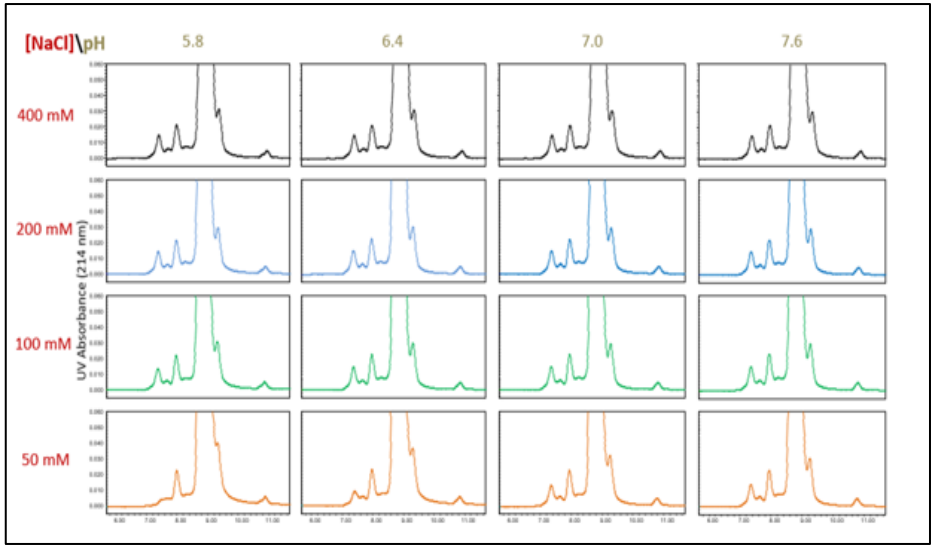
Traditional, Diol-Bonded, SEC 200Å,
2.5 µm Column in Stainless Steel
(SEC Method Development needed)



XBridge Premier Protein SEC 250Å,
(BEH-PEO) 2.5 µm Column
(Less Independent on pH and Salt
so less method development)



Out of Box Performance of Size Based Protein Variant Separations
(Approx. 10,000 to 650,000 Daltons)

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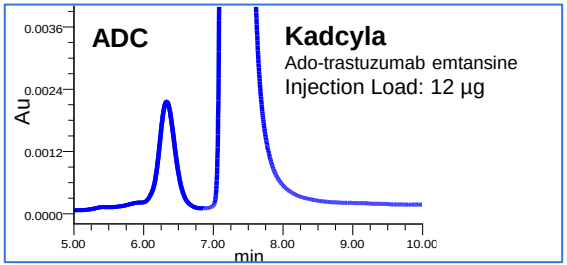
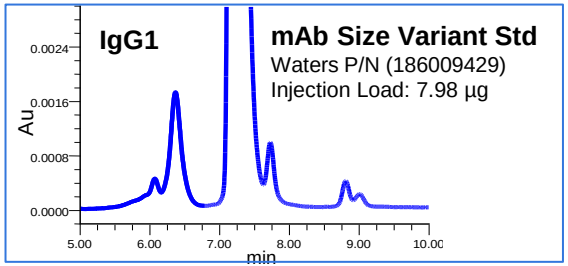
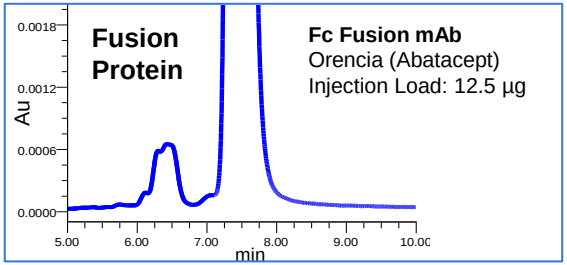
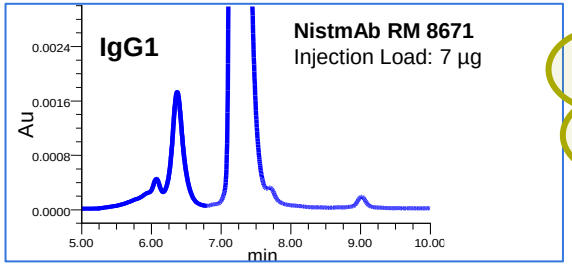
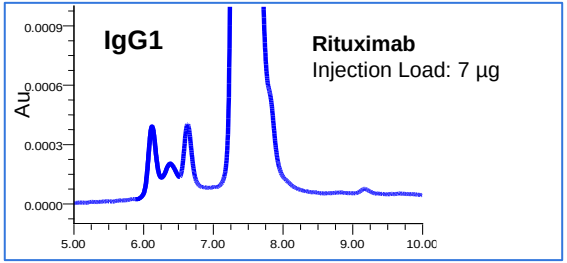
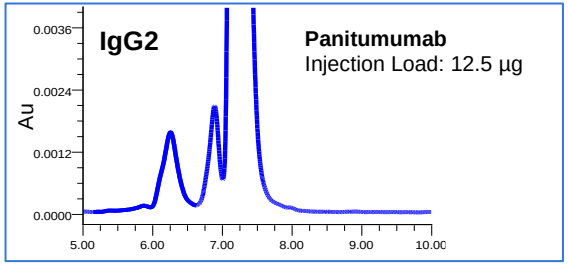






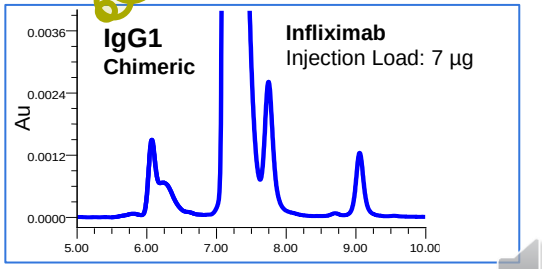






Column: ACQUITY™ Premier Protein SEC 250Å,1.7µm Dimension: 4.6 x 300mm
Flow Rate: 0.3 mL/min
Temperature: 35 °C
Wavelength: 280nm
Mobile Phase: 2xPBS (20mM Phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument: ACQUITY UPLC H-Class Bio
System Dispersion: <10µL

2X Phosphate Buffered Saline (PBS) shown here to give excellent resolutions and estimations of aggregates and fragments for several classes of proteins.



SEC Challenges for Reliable Biotherapeutic Protein Size Variant Analyses

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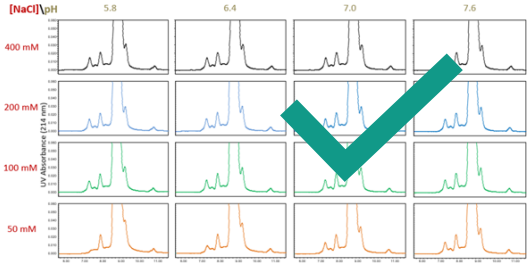
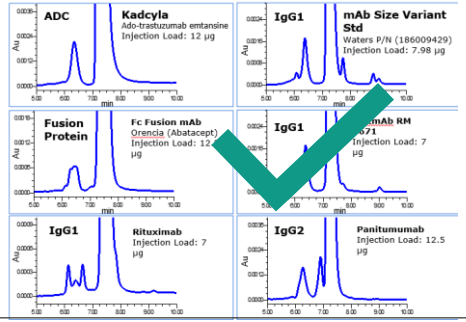
Long SEC Method Development Time (Desire to have Platform Method using PBS)



Out of the Box Performance for Protein Aggregate, Monomer, and Fragment SEC Analysis



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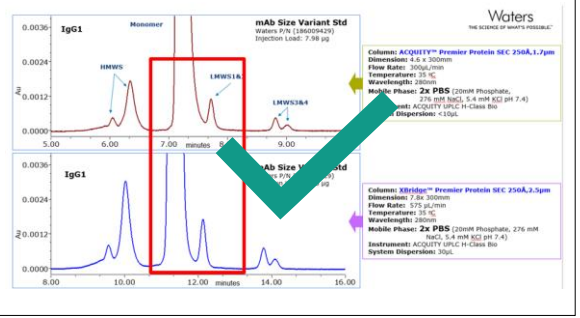

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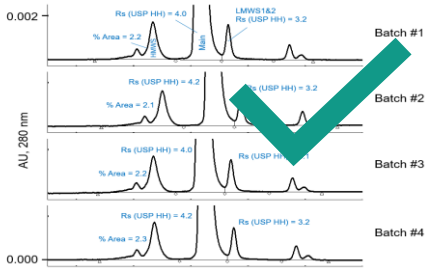
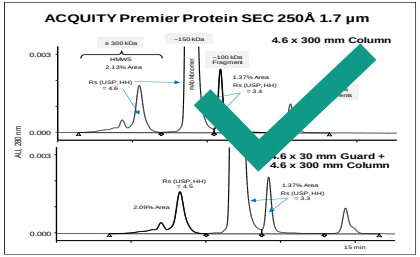
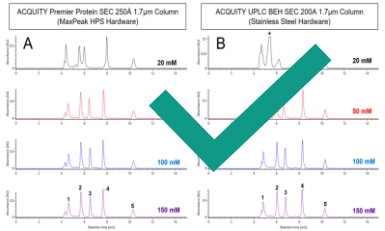


Decrease Cost per Analyses with Guard



Improved SEC / MS Analysis of Native mAbs



QR Code Enabled for Column Specific and Authenticity Information

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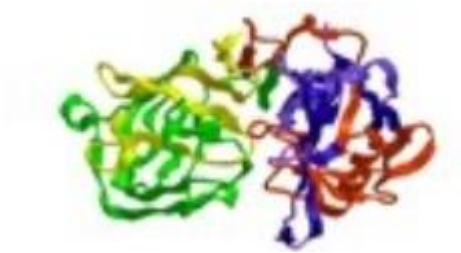
MAXPEAK™ PREMIER

Peptides



MaxPeak Premier for Peptide Mapping

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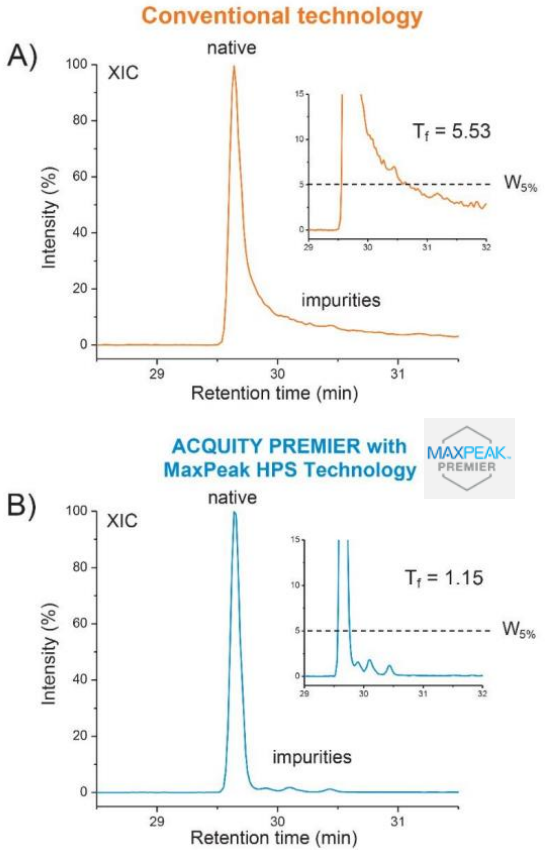

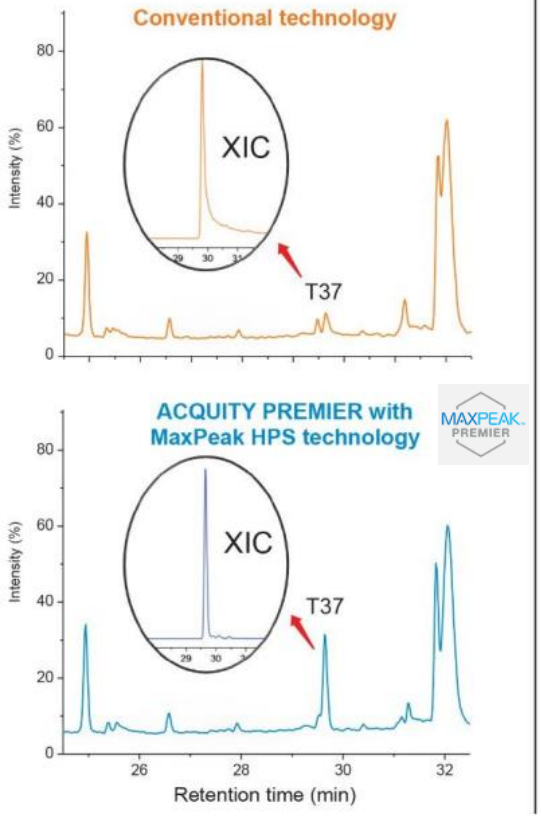

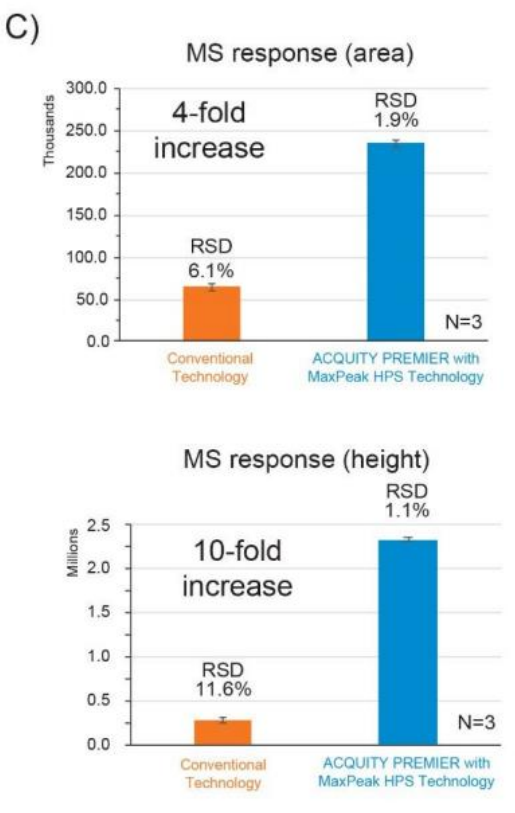

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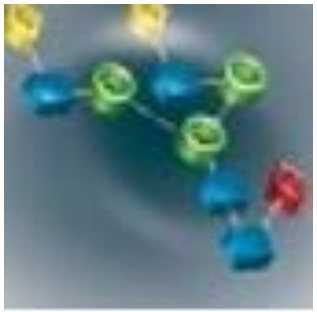
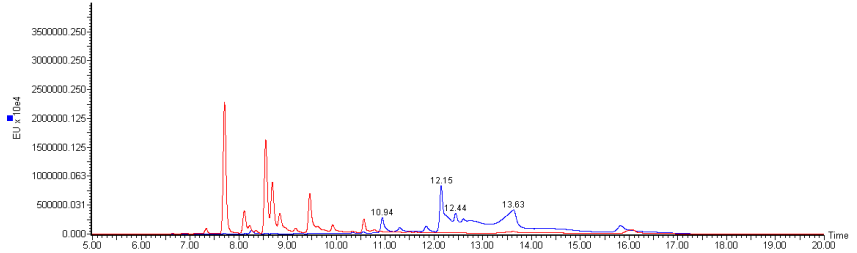
MAXPEAK™ PREMIER

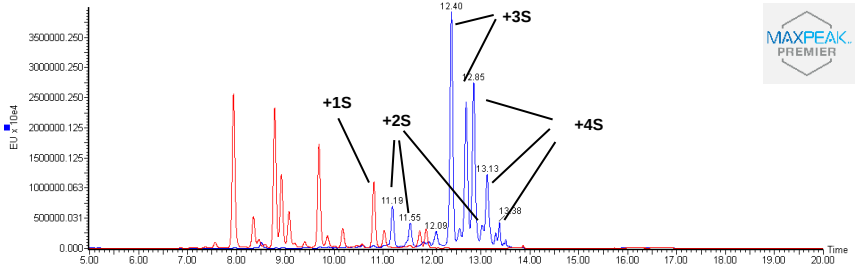
Glycans

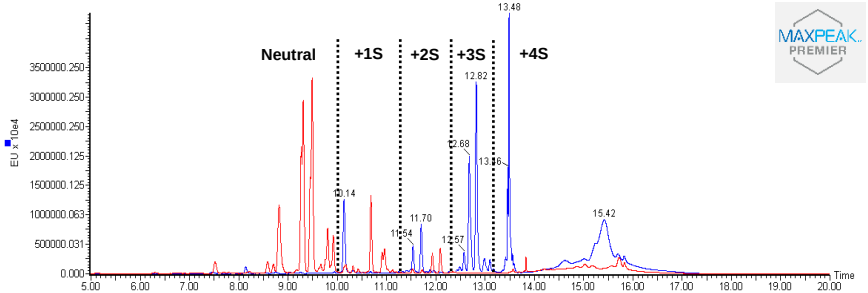


Representative Chromatogram from #1 Injection

Premier Glycan Amide and Glycan C18AX







ACQUITY Glycan Amide

- Column conditioning is critical

ACQUITY PREMIER Glycan Amide

- HILIC separation
- Ideal for mAb glycosylation profile
- 130Å and 300Å

ACQUITY PREMIER Glycan C18 AX

- Mixed-mode separation
- Ideal for sialylated and phosphorylated glycans
- Mobile phase and standard available

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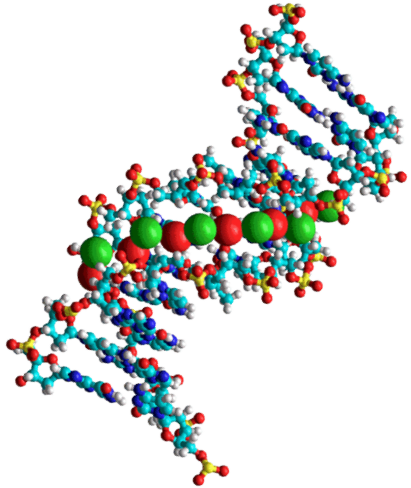
MAXPEAK™ PREMIER

Oligonucleotides



ACQUITY PREMIER:
Performance from Injection #1

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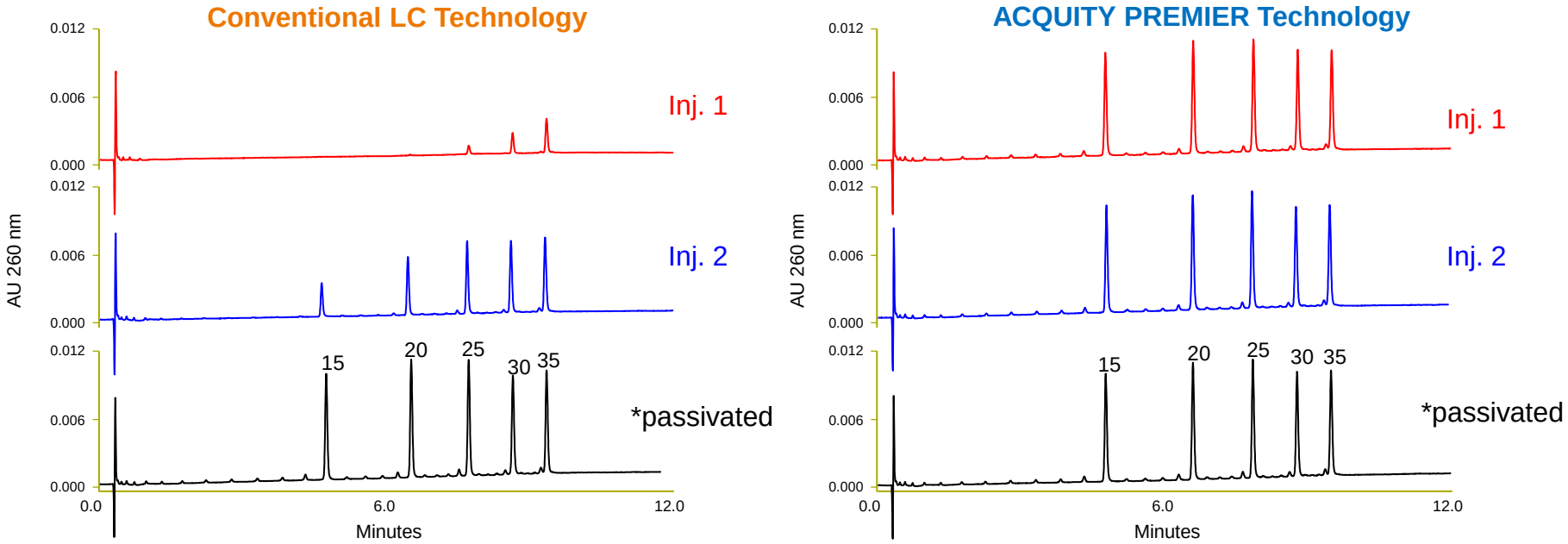
15-35mer Oligonucleotide Standard (oligodeoxythymidines)



QuanRecovery Vials and Plates with MaxPeak™ High Performance Surfaces (HPS)

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QuanRecovery™ WITH MAXPEAK™ HPS

- First product launched with MaxPeak HPS
- Reduces analyte losses from **non-specific binding due to hydrophobic interactions**
- Hydrophilic surface modification: no coating or extra chemicals on the surface
- Increased recovery, sensitivity, and repeatability in biomolecule analysis

300 µL injection vial

700 µL 96-well plate

Summary

- The risks caused by non-specific binding and non-specific adsorption, such as poor sensitivity, reproducibility, peak shapes and linearity, can be mitigated with MaxPeak ™ Premier High-Performance Surface (HPS)Technology

- MaxPeak™ HPS are new and innovative technologies designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses
  - XBridge and ACQUITY PREMIER columns enhance data quality by reducing biomolecule loses on column frits and tubing
  - QuanRecovery Vials and Plates reduce losses due to hydrophobic interactions

- Synergistic coupling of MaxPeak Premier HPS hardware and BEH, CSH, and HSS T3 particle technologies combine to deliver high quality, more robust bioseparations.

More Information and Additional Educational Assets

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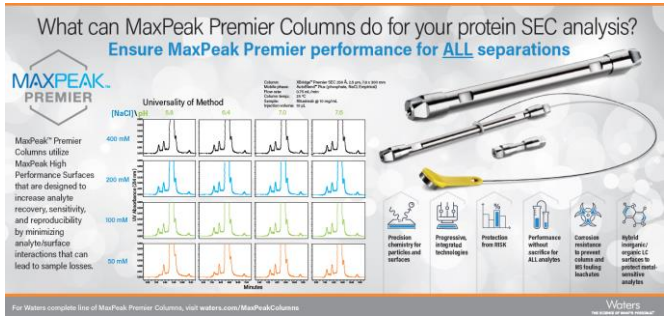
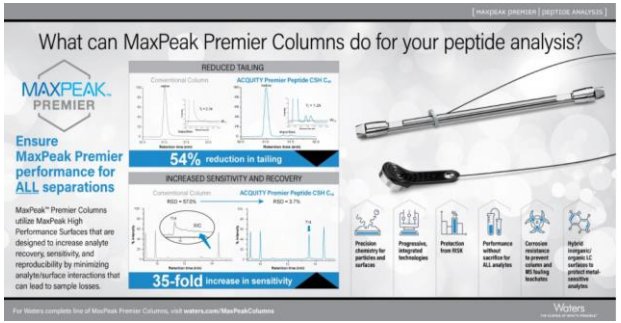
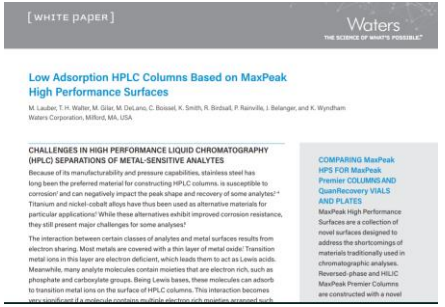
SEC Premier



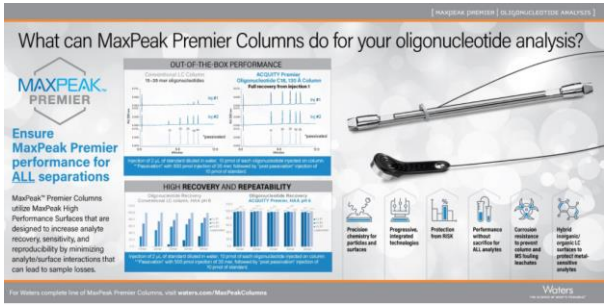
Peptide Premier



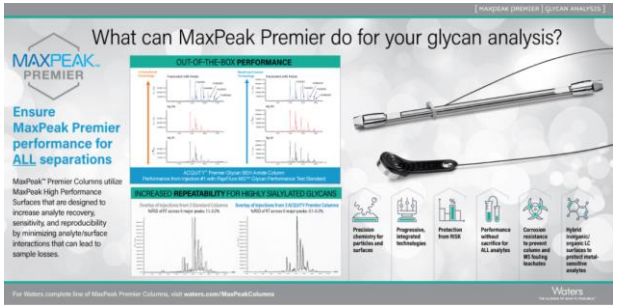
MAXPEAK Premier White Paper



Oligo Premier



Glycan Premier



MAXPEAK Premier HPS Ordering Guide

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

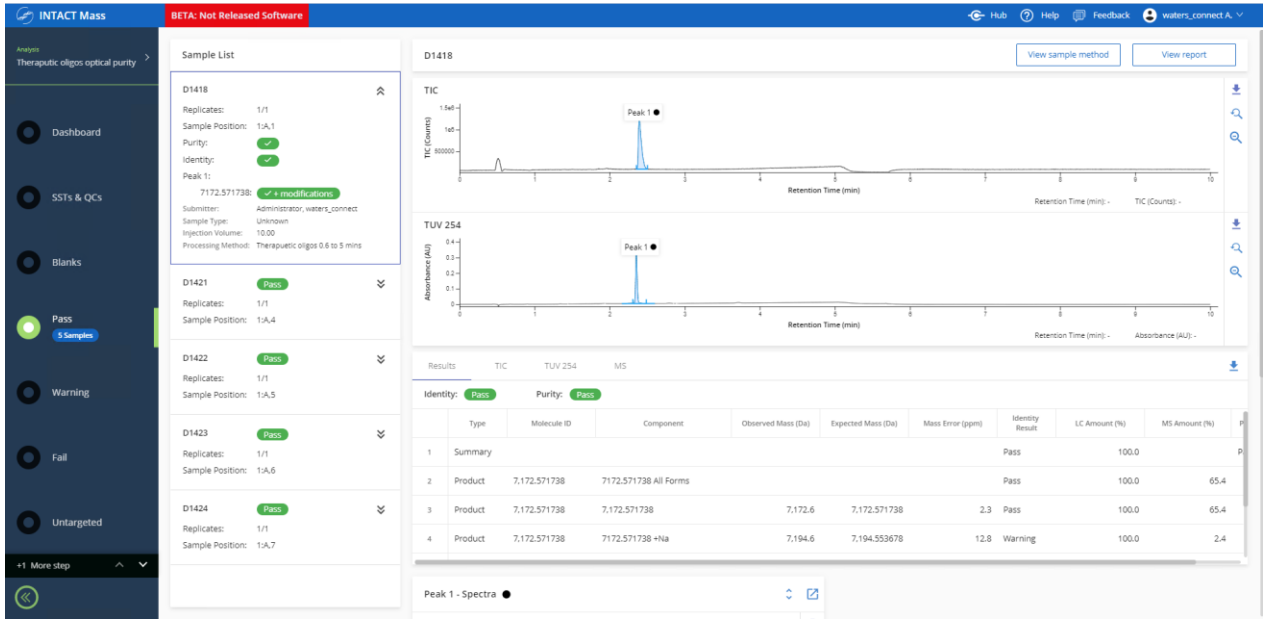
INTACT Mass

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Confidence
Mass Conformation & purity analysis of proteins, peptides & oligonucleotides.

Productivity
High throughput acquisition, deconvolution & reporting.

Accessibility
Compliance-ready, automated workflow.



- Automated MS deconvolution for product and impurity Mass Confirmation
- Quantitation of impurities via optical TIC, or MS spectral response

Automated…

- ✓ Parallel acquisition and processing
- ✓ Chromatographic peak identification
- ✓ Determination of deconvolution parameters
- ✓ Assignment of product modifications and impurities
- ✓ Report Generation

Harmonizing peptide attribute workflows from biotherapeutic attribute characterization to lot release

ENSURING DATA AND OPERATIONS CONTINUITY