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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

- 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



An abstract graphic featuring a complex network of interconnected nodes and lines, resembling a molecular structure or a data network. The nodes are represented by small circles in various shades of blue and grey, connected by thin, light blue lines. The background is a gradient of blue, with the network graphic being more prominent on the left side and fading towards the right.

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

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

12
EASY
TIPS

To Improve
Your Pipetting
Technique

1

Pre-Wet the Pipette Tip

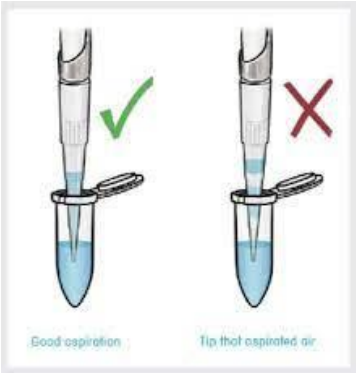
Aspirate and expel any sample liquid at least 3 times before aspirating a sample for delivery.

3x

2

Immerse the Tip to the Proper Depth During Aspiration

Before aspirating, immerse the tip adequately below the meniscus. Large volume pipettes (1-5 mL) should be immersed to 5-6 mm, while smaller volume pipettes should be immersed to 2-3 mm.



Tip Immersion Depth and Angle

Thermo.com

1 cm

Inaccuracy 0.2-0.4%

3 cm

Inaccuracy 0.6-0.8%

4 cm

Inaccuracy 1-1.2%

1. Pipette held vertically, tip immersed about 1 cm into the liquid.

2. Pipette held vertically, tip immersed about 3 cm into the liquid.

3. Pipette held at a 30-40° angle, tip immersed about 3-4 cm into the liquid.

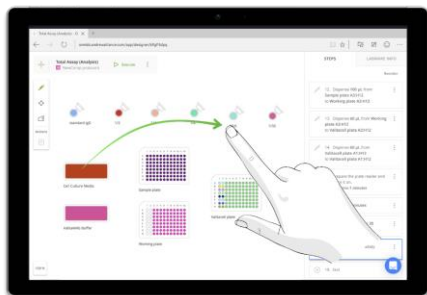
Effects of immersing the tip too deeply and tilting the pipette are greater with small sample volumes, e.g., using 1-10 µl pipette.



OneLab

design & execute

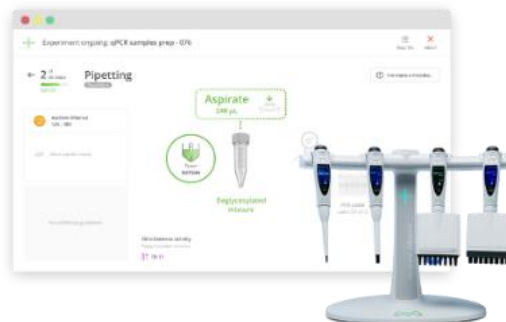
- Connected Lab Ecosystem
 - Enables total lab connectivity
 - Ease of use
 - Compliant ready



Pipette

easy pipetting

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

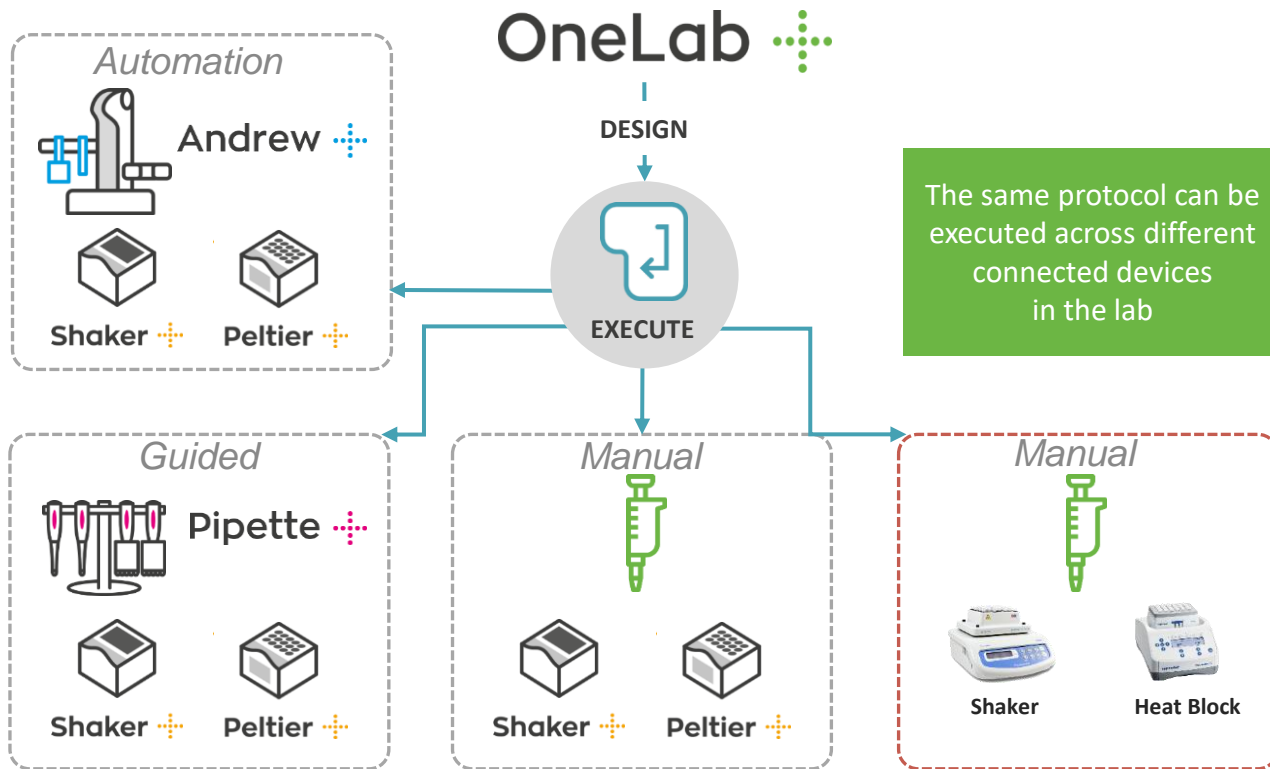


Andrew

the pipetting robot

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





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

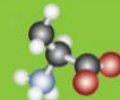
OneLab's Normalization Wizard automates the production of concentration normalization volumes, typically saving >80% time required for what is often a highly laborious process.

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.



ANTIBODY
PURIFICATION



AMINO ACID
ANALYSIS



RELEASED
N-GLYCAN ANALYSIS



mAb SUBUNIT
ANALYSIS





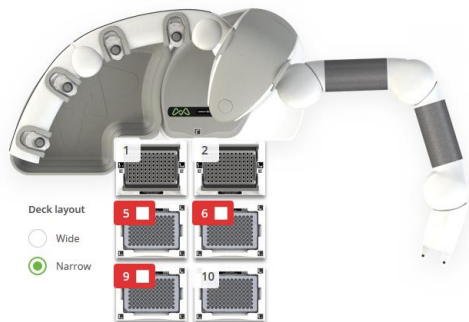
AccQ-Tag Ultra LC Amino Acid Analysis



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 **RapiFluor-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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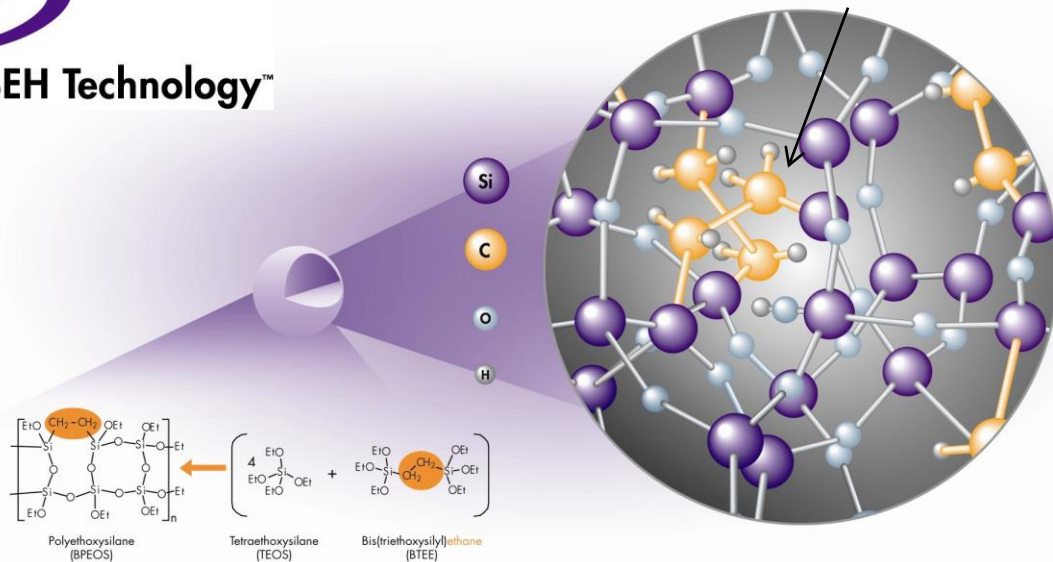
The background of the slide features a complex, abstract network diagram. It consists of numerous small, light blue circular nodes connected by thin, light blue lines, creating a web-like structure that spans the entire width of the slide. The nodes are of varying sizes, and the lines are of varying thicknesses, giving it a dynamic and interconnected appearance. A solid dark blue horizontal band is positioned across the middle of the slide, serving as a backdrop for the title text.

Reducing Bias in Biopharmaceutical LC and LC-MS Analysis

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



Bridged Ethanes within a silica matrix

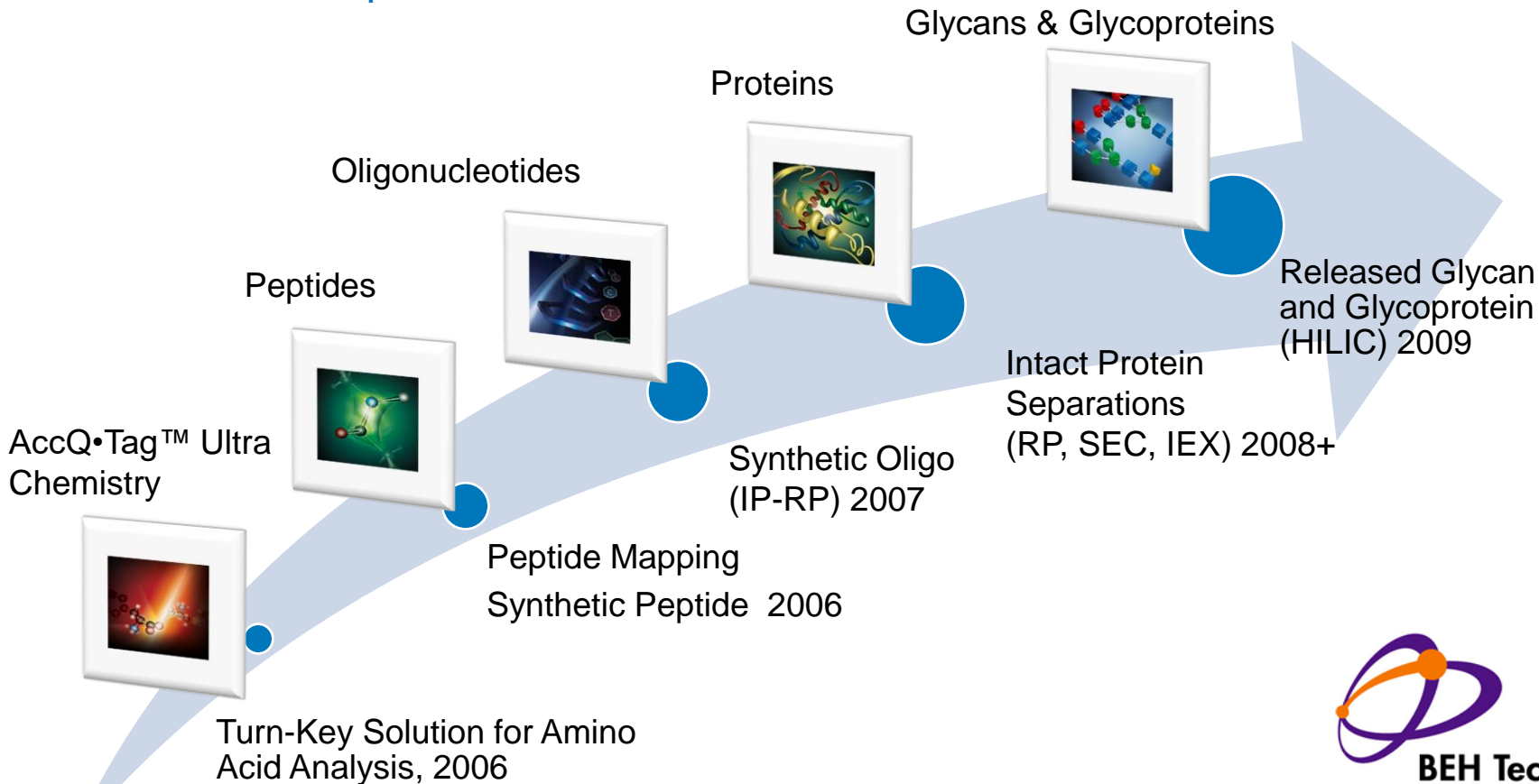


- Chronographic media contains a high percentage of total LC surface area.
- Negatively charged silanols on traditional silica particles interact with basic groups leading to poor recovery and poor peak shape.
- Organic-hybrid particles enabled stronger particles, wider pH stability and vastly reduced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

Anal. Chem. **2003**, 75, 6781-6788

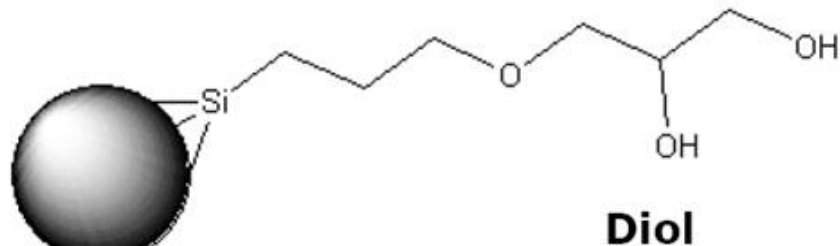
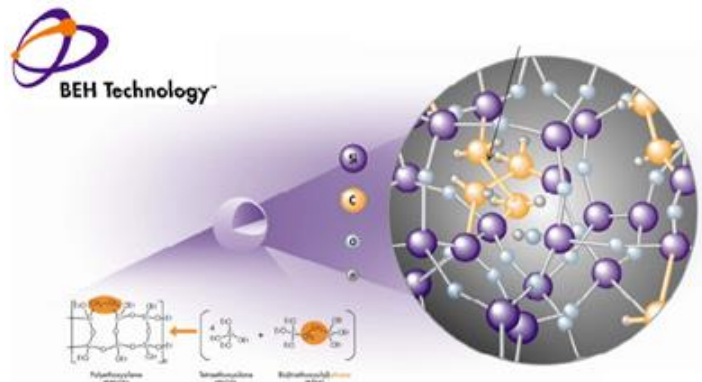
U.S. Patent No. 6,686,035

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



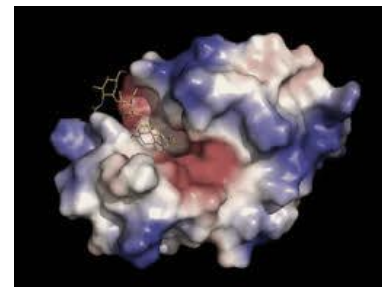
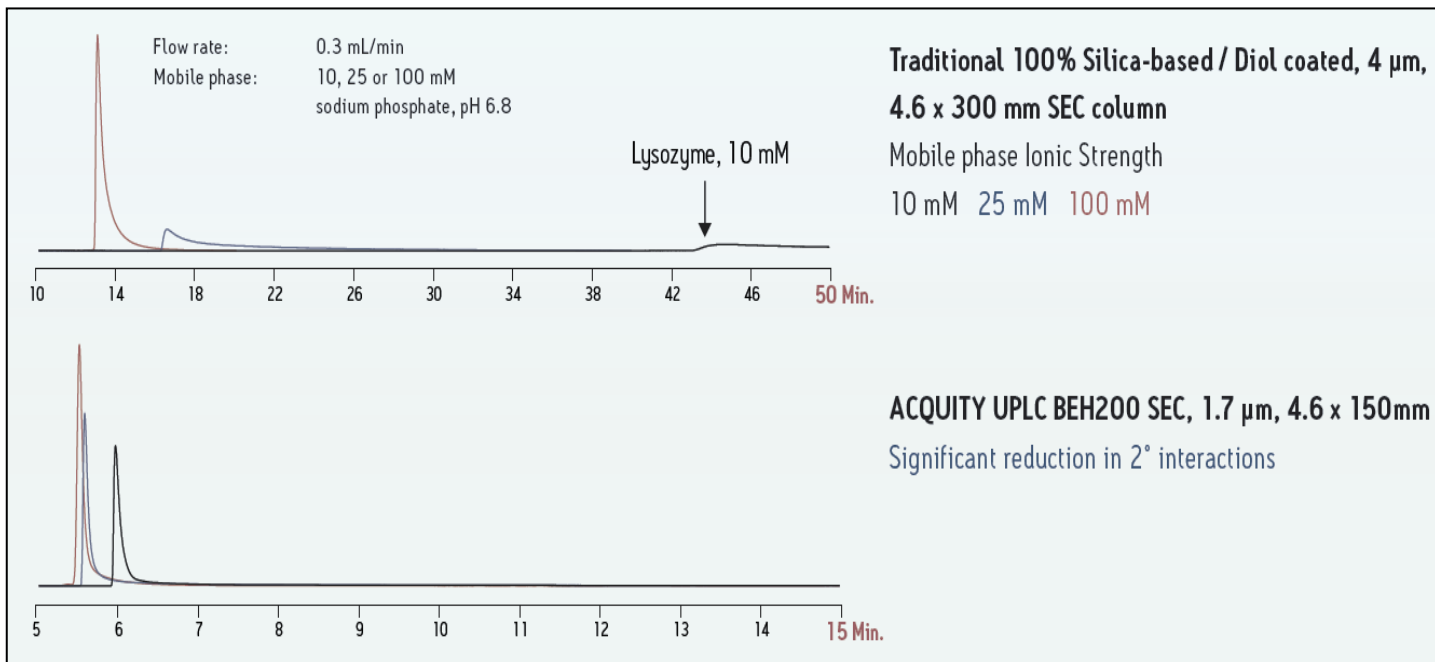
Building a Better SEC Column with BEH Technology

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



Recovery of Lysozyme, a highly basic protein

Silica vs BEH SEC Particles



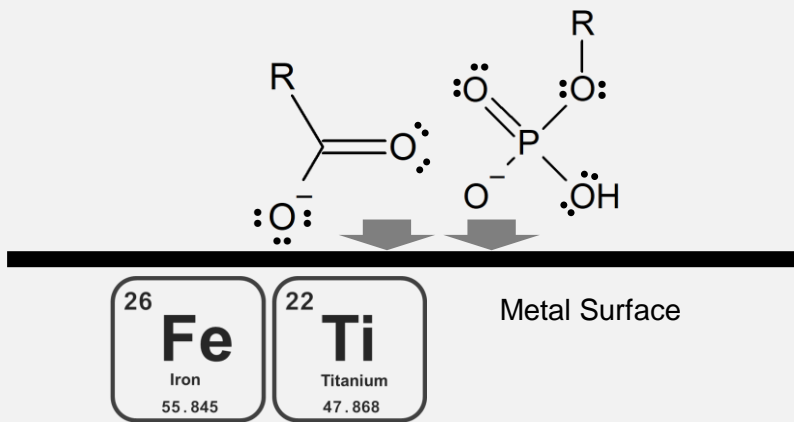
Lysozyme pI ~ 10.5

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

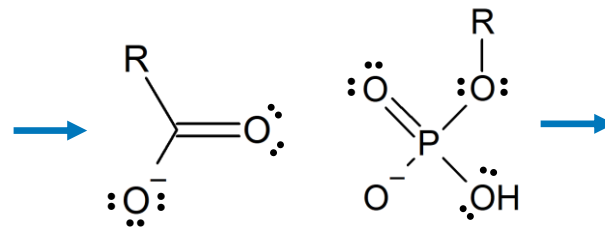


MaxPeak™ Premier High-Performance Surfaces increase analyte recovery, sensitivity, and reproducibility by **minimizing analyte / surface interactions on systems and columns** that can lead to sample losses and poor separations.

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



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



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 Analysis

Arc™ HPLC

HPLC Dependability

Optimized for 3.5 – 5 μm particles



Arc™ PREMIER

UHPLC Flexibility

Optimized for 2.5 – 3.5 μm particles



Sample Complexity

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 μ m 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



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

ACQUITY Premier System

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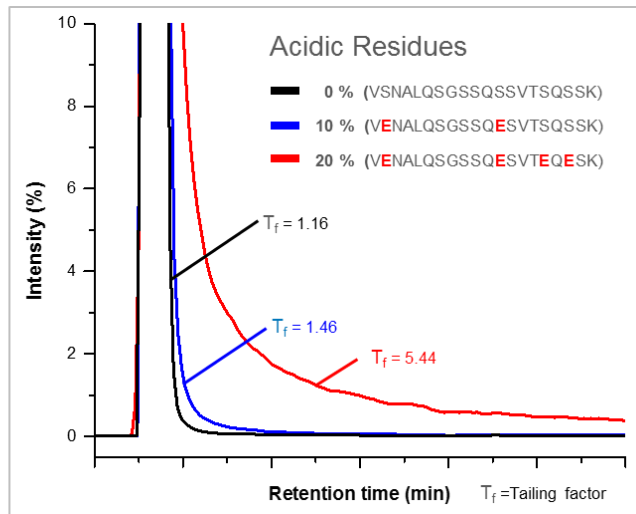
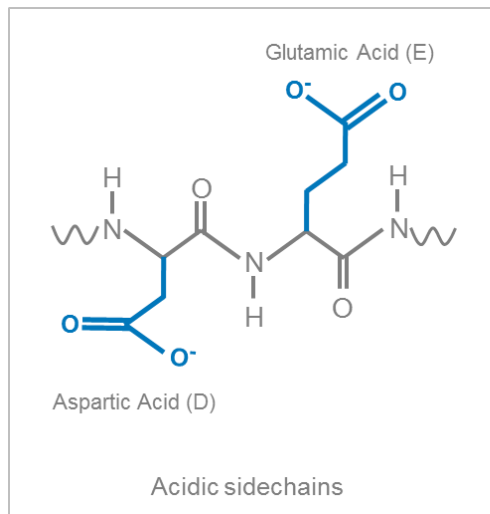


- Based on the biocompatible ACQUITY H-Class PLUS Bio and H-Class Binary PLUS Bio
- Quaternary or Binary Solvent Management
- Deploy sub 2.0 μm particle columns to maximize chromatographic performance and minimize analysis time.
- Ideal inlet for SQ, QQQ, and HRMS Detection
- Empower, MassLynx, waters_connect 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

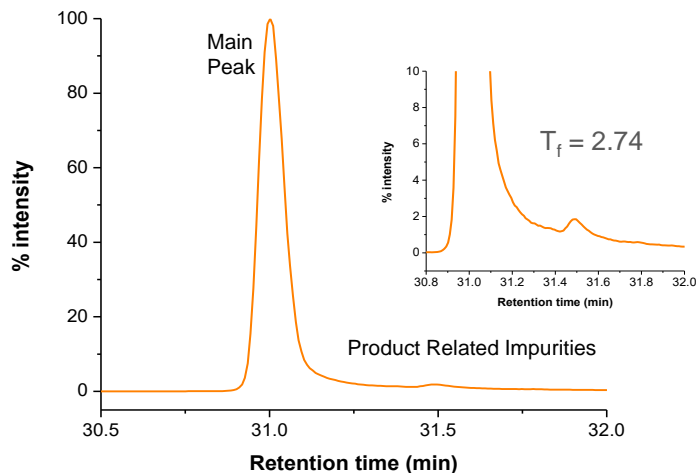


Acidic species in protein separation

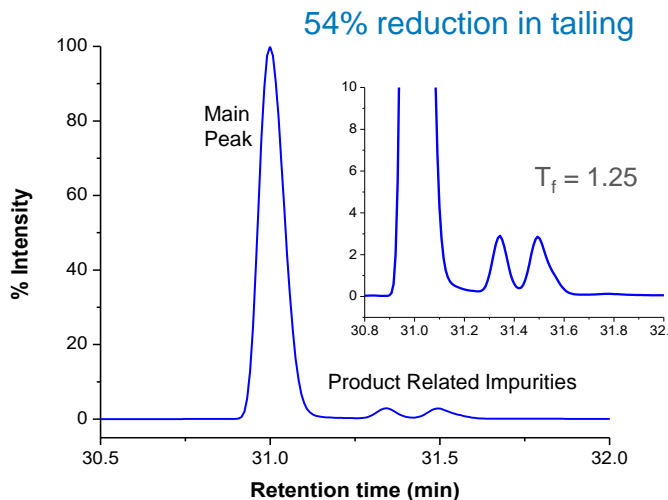
- Commonly seen due to deamidation and other modifications
- Accurate analysis required in product characterization and PQA monitoring
- Waters ACQUITY Premier Solution designed to improve separation and recovery of acidic species

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

Conventional Column / System



ACQUITY Premier Column / System



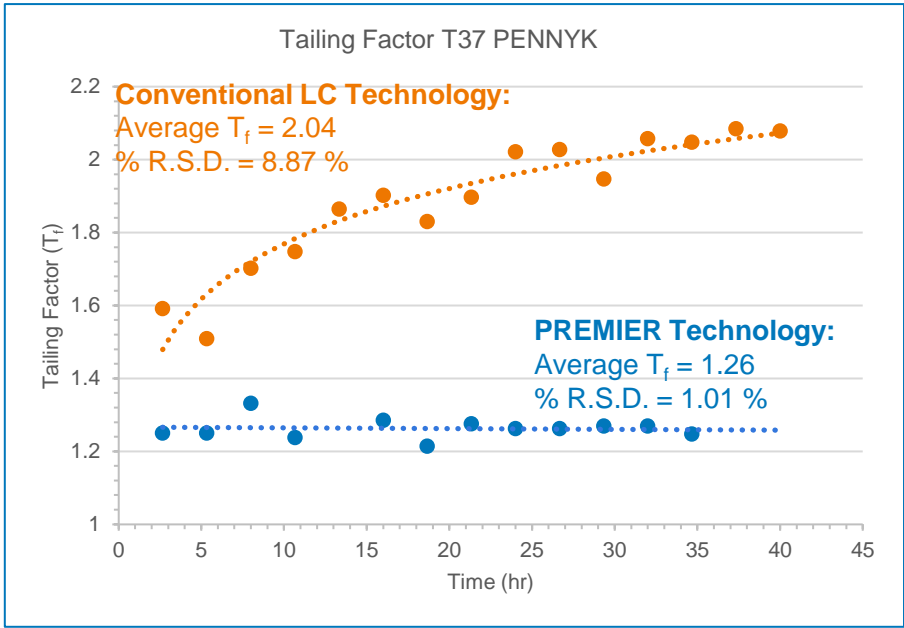
mAb T37 peptide: GFYPSDIAVEWESNGQPENNYK ■ Acidic residues



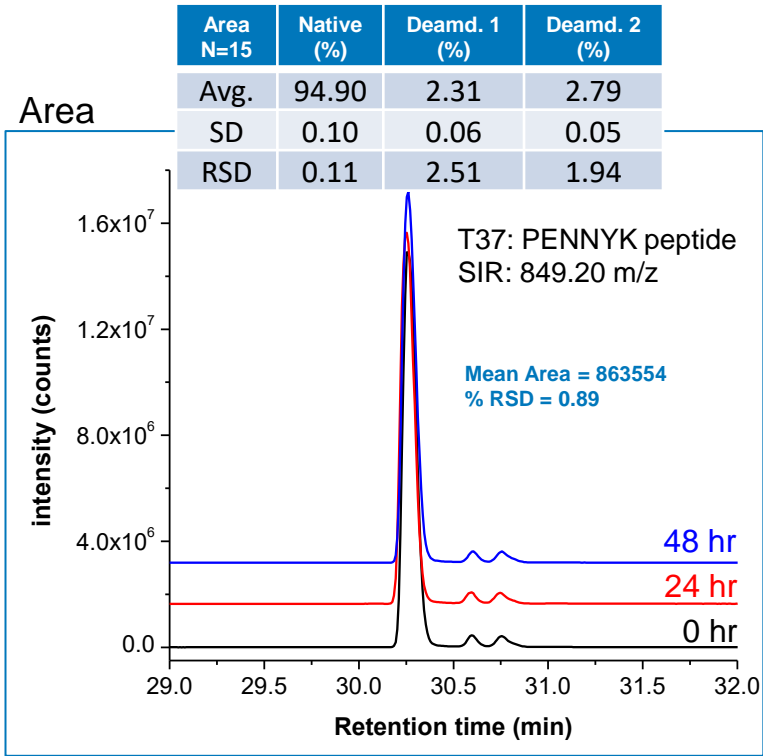
ACQUITY PREMIER:

Reproducible Performance from Injection #1

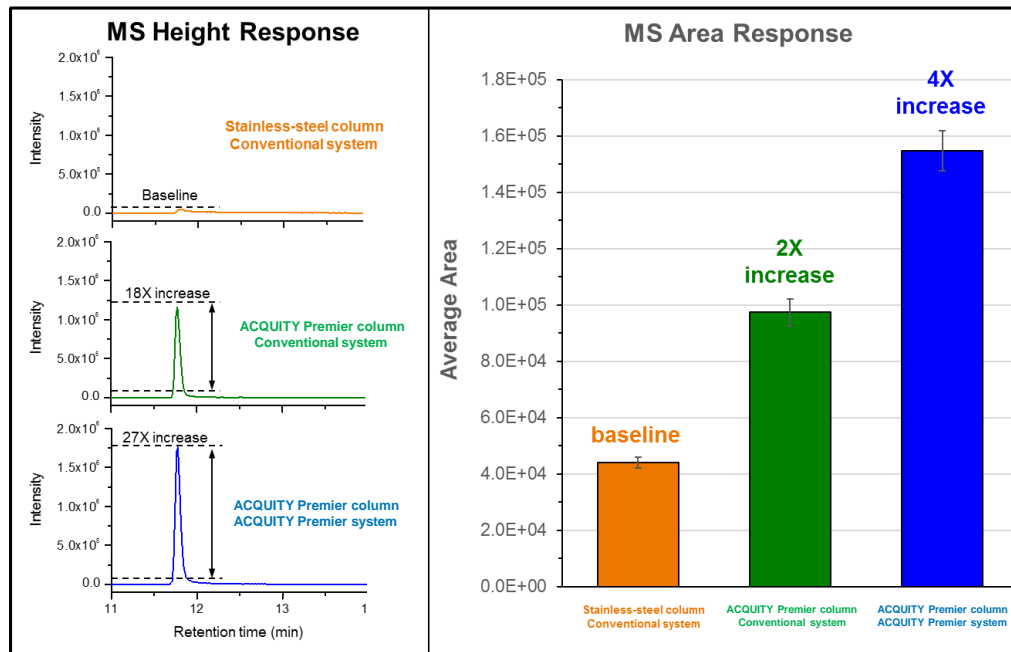
Acid passivation does not last and needs to be repeated



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

The background of the slide features a complex, abstract network diagram. It consists of numerous small, light blue circular nodes connected by thin, light blue lines, creating a web-like structure that spans the entire width of the image. The nodes are of varying sizes, and the lines form a dense, interconnected pattern. A solid dark blue horizontal band is superimposed over the center of the image, providing a high-contrast background for the white text.

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

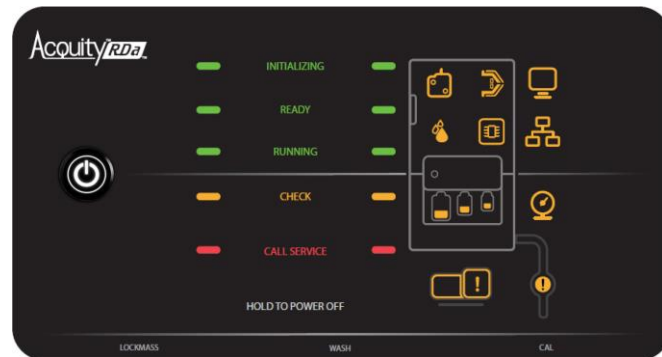
Easy to Deploy and Operate

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

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The first SmartMS-enabled
Biopharmaceutical System



Redefining performance

Purposefully designed system to address challenges

Easy to Deploy and Operate

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

Maximized Up-time

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

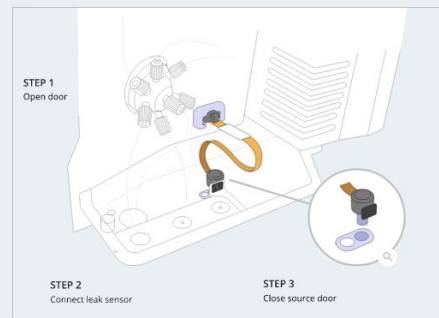
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Leak Detector Sensor Not Fitted

Follow instructions below. Further instructions and safety advisories can be found in the Overview and Maintenance Guide.

Fit sensor



Please click here if you have attempted the resolution

Redefining performance

Purposefully designed system to address challenges

Easy to Deploy and Operate

- Accessible to non-MS experts, reduced training time
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Maximized Up-time

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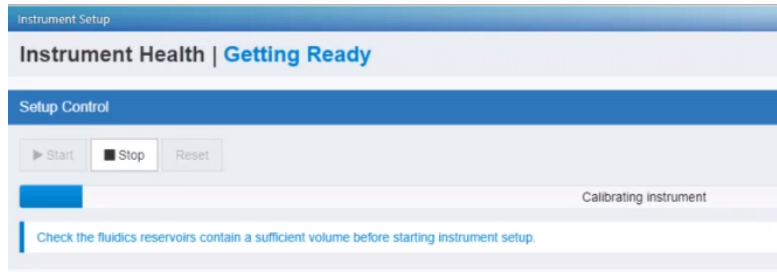
Confidence in Data Quality

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

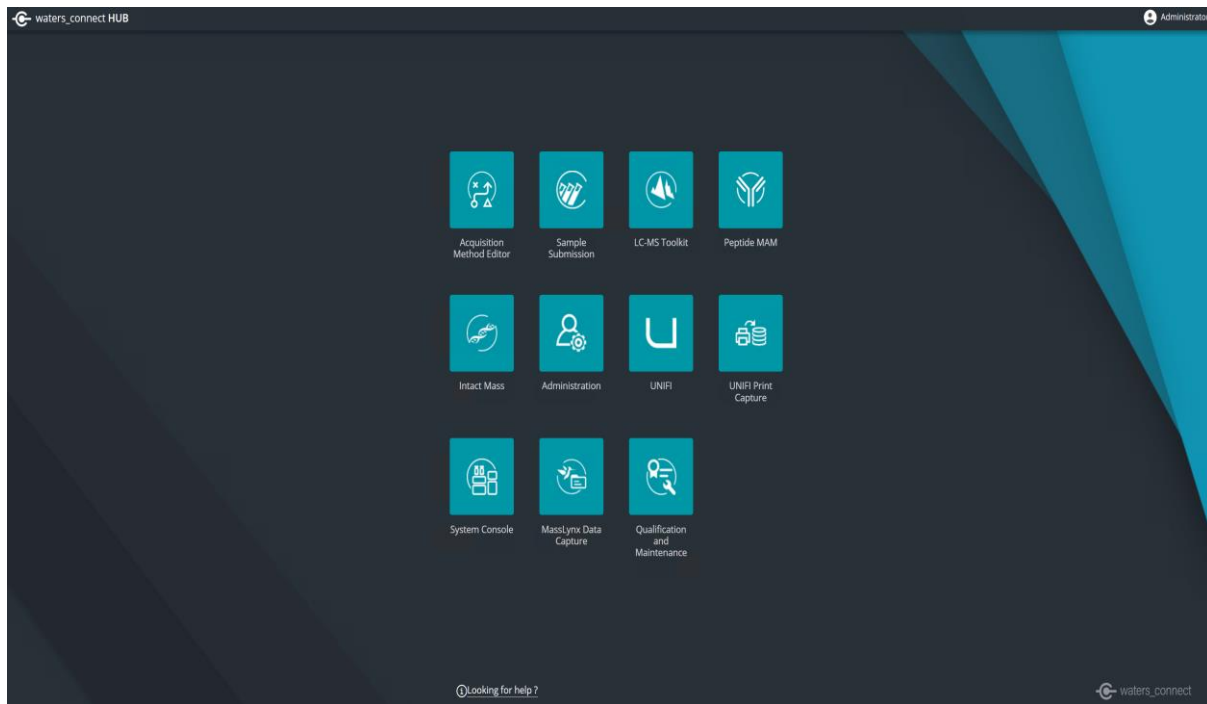
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



The first SmartMS-enabled
Biopharmaceutical System



waters_connect: New Applications for Peptide MAM and Intact Mass Analysis



Supplementing the core UNIFI application workflows

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



Released Q4 2020



New for 2022

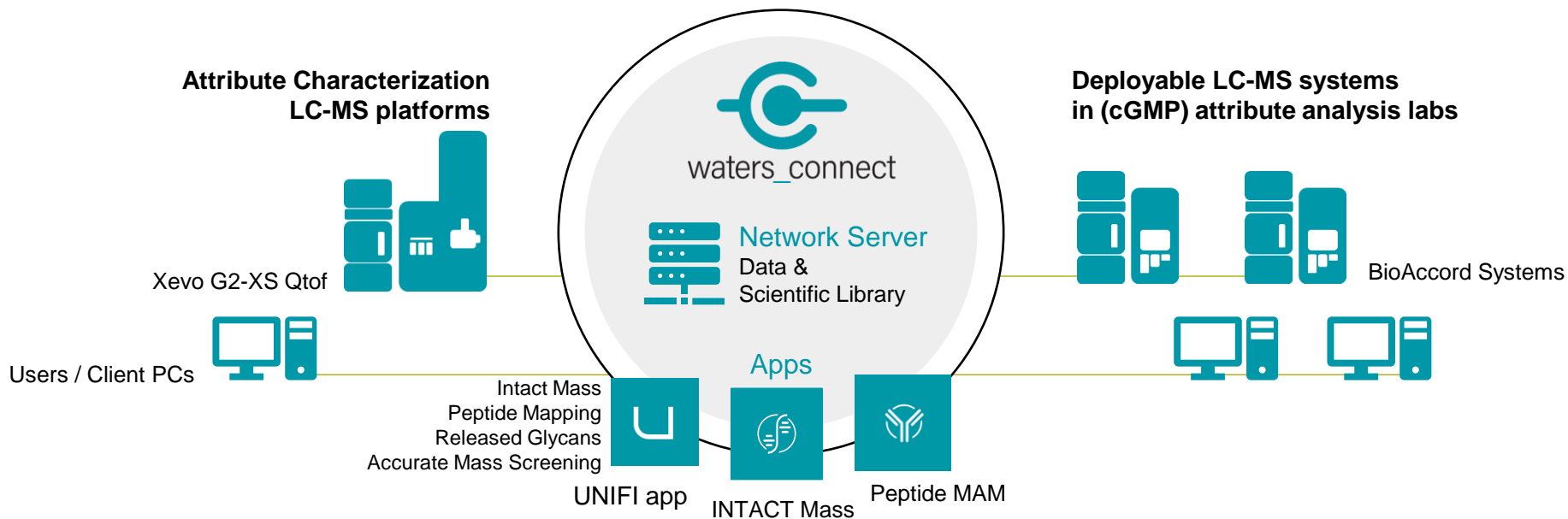
Harmonizing Workflows across Development, Manufacturing & QC

ENSURING DATA INTEGRITY, OPERATIONAL EFFICIENCY AND SCALABILITY

Characterization

Attribute Monitoring

QC Release

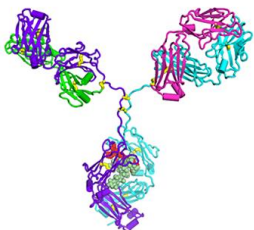


- ✓ Integrated compliance-ready data acquisition, processing and reporting
- ✓ A shared ecosystem that enables data traceability and transferability

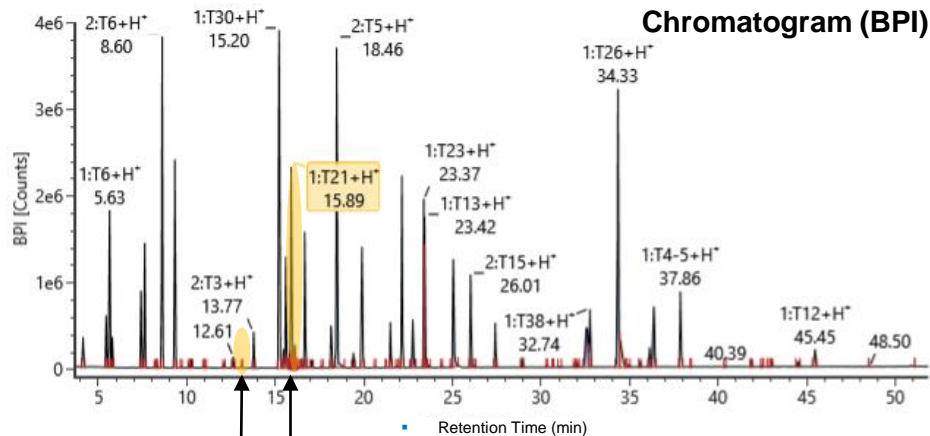
Characterization: Identifying product quality attributes

Case study: NIST mAb peptide mapping

NISTmAb



- RM8671
- Sample digested with trypsin following reduction & alkylation



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

Chain 1

DIQMTQSPST	LSASVGDRV	ITCSASSRVG	YMHYQQKPG	KAPKLLIYDT	SKLASGVPSR	FSGSGSGTEF
TLTISSLQPD	DFATYYCFQG	SGYPFTFGGG	TKVEIKRTVA	APSVFIFPPS	DEQLKSGTAS	VVCLLNFPYP
REAKVQWKVD	NALQSGNSQE	SVTEQDSKDS	TYSLSSTLT	SKADYEKHKV	YACEVTHQGL	SSPVTKSFNR

Chain 2

QVTLRSGPA	LVKPTQTLTL	TCTFSGFSL	TAGMSVGWIR	QPPGKALEWL	ADIWDDDKKH	YNPSLKDRLT
ISKDTSKNQV	VLKVTNMDPA	DTATYYCARD	MIFNFYFDVW	GQGTTVTVSS	ASTKGPSVFP	LAPSSKSTSG
GTAALGCLVK	DYFPEPVTVS	WNSGALTS	HTFPAVLQSS	GLYSLSSVVT	VPSSSLGTQT	YICNVNHKPS
NTKVDKRV	KSCDKTHTCP	PCPAPELLGG	PSVFLFPPKP	KDTLMISRT	EVTCVVVDVS	HEDPEVKFNW
YVDGVEVHNA	KTKPREEQYN	STYRVSVLT	VLHQDWLNGK	EYCKVSNKA	LPAPIEKTI	KAKGQPREPQ
VYTLPPSREE	MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTPPV	LDSGGSFFLY	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



Peptide MAM

ANALYSIS
NIST mAb

Acquisition and processing

Data reviewing and results reporting

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

Peptide MAM

ANALYSIS
NIST mAb

Select Processing Method

Acquire and Process

System Suitability

Injections

Retention Time Alignment

Monitored Attributes

New Peak Detection

Report

Peptide MAM

Folder

Location: /Company/Peptide MAM

Browse

Description:

Instrument system
Status: online

Set to initial conditions

Stop flow

Reset system

Acquisition method

Peptide MAM data acquisition Method

Select a method

Peptide MAM data acquisition

Location: /

Browse

Edit in ACQUISITION Method Editor

Sample Trays

Sample Submission

Sample List Queue Real Time Data

Peptide MAM method references

Version: 2 Status: Draft

	Item Name	Item Description	Item Type	Injection Volume	Sample Position	Replicates	New Peak Detection Reference	Retention Time Alignment Reference	Acquisition Method	Run Time
3	System Suitability_2	SST	System Suitability	5.00	1:A,2	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
4	System Suitability_3	SST	System Suitability	5.00	1:A,2	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
5	Blank_2	Blank	Blank	10.00	1:A,1	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
6	Reference mAb	Reference	Unknown	5.00	1:A,3	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
7	Control	Control	Unknown	5.00	1:A,4	1	<input checked="" type="checkbox"/>	<input type="checkbox"/>		80.00
8	Spiked in control	control+spiked peptides	Unknown	5.00	1:A,5	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
9	Stressed	Stressed	Unknown	5.00	1:A,6	1	<input type="checkbox"/>	<input checked="" type="checkbox"/>		80.00
10	Spiked in stressed	stressed+spiked peptides	Unknown	5.00	1:A,7	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
11	Blank_3	Blank	Blank	10.00	1:A,1	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
12	System Suitability_4	SST	System Suitability	5.00	1:A,2	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
13	System Suitability_5	SST	System Suitability	5.00	1:A,2	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
14	System Suitability_6	SST	System Suitability	5.00	1:A,2	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00
15	Blank_4	Blank	Blank	10.00	1:A,1	1	<input type="checkbox"/>	<input type="checkbox"/>		80.00

Submit

Sample queue

Powered by Waters 2020 | Version: 1.0.0.0

Rapid evaluation of system performance based on custom SOP criteria

Peptide MAM

ANALYSIS
NIST mAb

Select Processing Method

Acquire and Process

System Suitability

Injections

Retention Time Alignment

Monitored Attributes

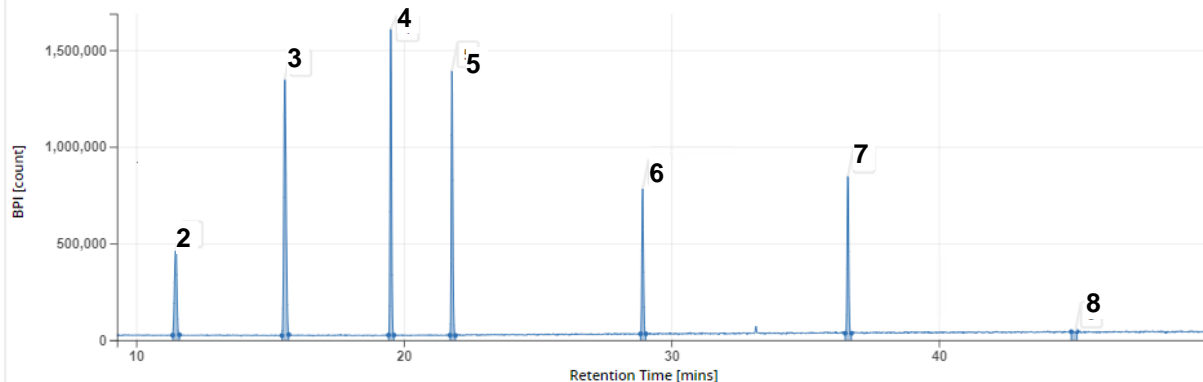
New Peak Detection

Report

Base Peak Intensity (BPI)

m/z: 50-2000

System suitability peptides_1



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

Peptide MAM

ANALYSIS
NIST mAb

Select Processing Method

Acquire and Process

System Suitability

Injections

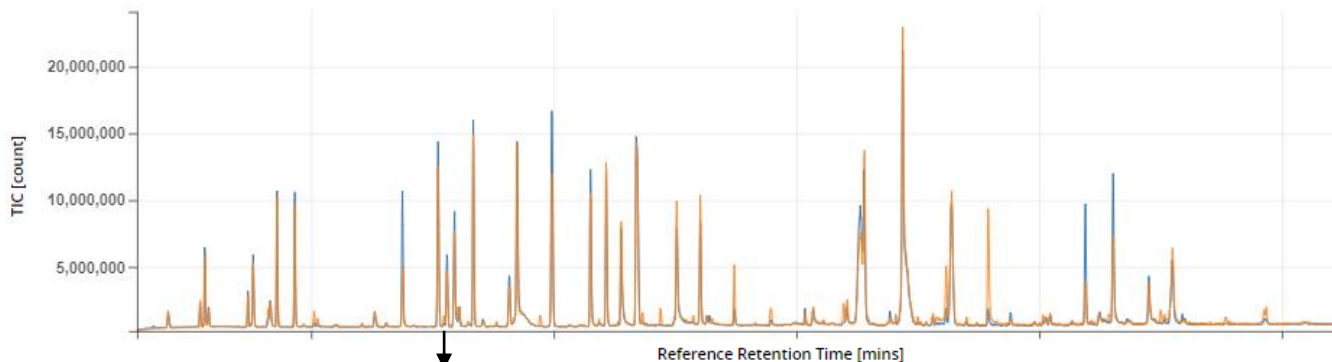
Retention Time Alignment

Monitored Attributes

New Peak Detection

Report

Control (Reference) Unknown sample-1 (Aligned)

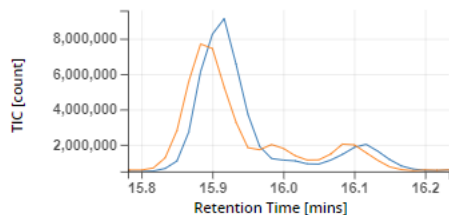


DTLMISR (15.92 min)

Before Alignment

Total Ion Current (TIC) m/z: 50-2000

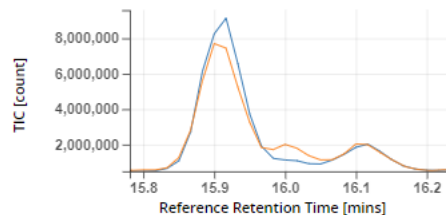
Control (Reference) Unknown sample-1



After Alignment

Total Ion Current (TIC) m/z: 50-2000

Control (Reference) Unknown sample-1 (Aligned)



Maximized
inter-system reproducibility
and inter-day repeatability.

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

Increased productivity with simplified results review

Peptide MAM

ANALYSIS
NIST mAb

Select Processing
Method

Acquire and
Process

System Suitability

Injections

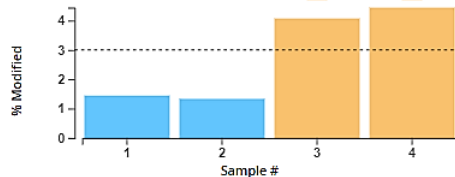
Retention Time
Alignment

Monitored
Attributes

New Peak
Detection

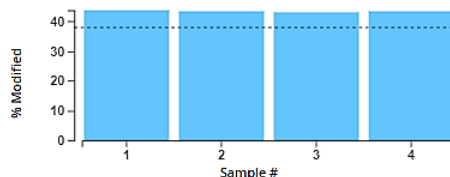
Report

DTLMISR
Oxidation



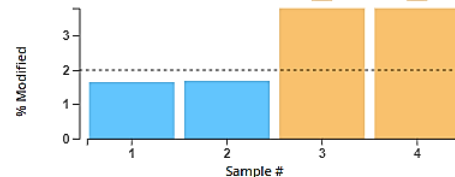
Acceptable range: 0 - 3%

EEQYNSTYR
Glycopeptide G0F



Acceptable range: 38 - 100%

VVSVLTVLHQDWLNGK
Deamidation



Acceptable range: 0 - 2%

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

< DTLISR Oxidation ⚠

Injection: Unknown sample-1 ▴ ▾

Expected: 0 - 3% modified
Actual: 4.06% modified

Sequence	Modifier	Response %	Observed Mass ...	Mass Error (pp...	Retention Time (...)	Charge States	Monitored?
DTLISR	Oxidation M	4.06	850.4203	-1.86	13.11	2+	✓
DTLISR	None	95.94	834.4259	-1.25	15.91	1+ 2+	✗

Effective New Peak Detection

New peaks discovered in each sample vs. reference control

Peptide MAM

ANALYSIS
NIST mAb

Select Processing
Method

Acquire and
Process

System Suitability

Injections

Retention Time
Alignment

Monitored
Attributes

New Peak
Detection

Report

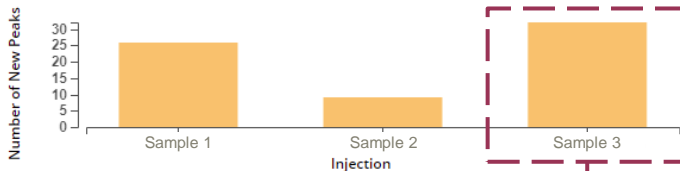


unique new peaks

in Unknown sample-3 ▼

vs. Control

Distribution of New Peaks



Detection Criteria

Edit

Fold Change ≥ 10
 % Base Peak Response $\geq 0.1\%$
 Isotopic Similarity % ≥ 90
 Matching Tolerance (mins) = 1
 Exclude peptides with only 1+ ions ? ☒ Yes

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

Peptide MAM

ANALYSIS
NIST mAb

Select Processing
Method

Acquire and
Process

System Suitability

Injections

Retention Time
Alignment

Monitored
Attributes

New Peak
Detection

Report

Take informed decisions based on critical results:
system suitability,
attribute monitoring with faulting samples,
new peaks detected,
audit trail and contextual information,

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

Monitored Attributes

Attribute Definitions

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

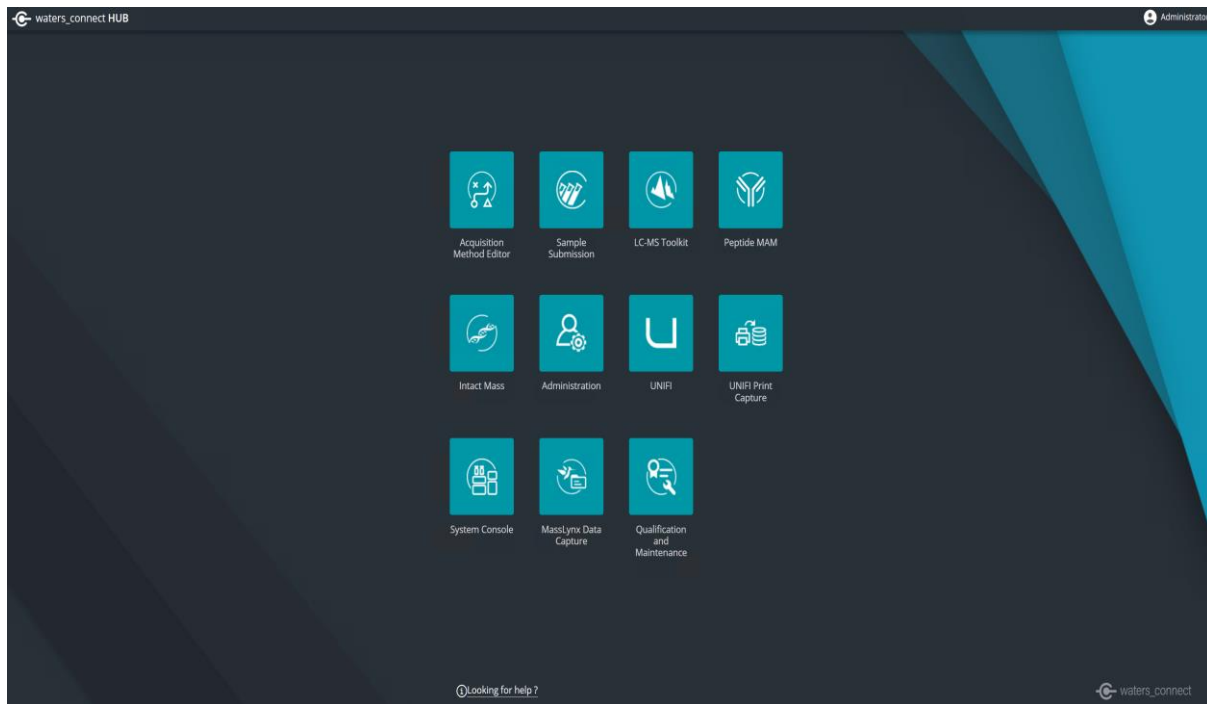
Found?	Monitored?	Sequence	Modifications	Retention Time (min)	Charge States
✓	No	DIQMTQSPSTLSASVGDR		22.76	2+, 3+, 4+
✓	Yes	DIQMTQSPSTLSASVGDR	Oxidation M[4]	22.77	2+, 3+

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)	VSVL 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



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**

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- **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



Define peak deconvolution parameters

Mass information

Specify parameters for obtaining mass information and the number of peaks to deconvolve.

Deconvolution method: Auto

Chromatogram from which to obtain mass information for largest peaks: Optical chromatogram

Maximum number of peaks to deconvolve: 10

LC minimum area (percentage): 1

☒ Advanced

1-15 kDa algorithm: BayesSpray Output masses: Monoisotopic

>15-300 kDa algorithm: BayesSpray Output masses: Average

Deconvolution settings

Choose the type of biomolecule to be deconvolved.

Type of biomolecule: Oligonucleotide

Deconvolution Settings:

- Protein
- Oligonucleotide
- PS Oligo
- Custom Elemental

Automated Acquisition and Data Processing

←

→

↺

localhost:48481/home

🔍

📄

☆

👤

⋮

🌀

INTACT Mass

🔄

Hub

?

Help

💬

Feedback

👤

waters_connect A. ▾

Recent files

Open recent analyses

20220106_HYS_102

Analysis

Jan 06, 2022 12:22:44 (-05:00)

20220106_HYS_101

Analysis

Jan 06, 2022 12:12:28 (-05:00)

20220105_mAb_HYS_105

Analysis

Jan 05, 2022 14:57:05 (-05:00)

Welcome to INTACT Mass

Use LC/MS with powerful deconvolution algorithms to perform identity and purity checks on samples

Create new processing methods and analyses

START NEW WORK

📄

Process data

Create a new process-only analysis by processing existing data with INTACT Mass

🔗

Acquire and process data

Create a new analysis by acquiring new data and processing with INTACT Mass

+

Create new method

Create a new INTACT Mass processing method

Open, delete or copy existing files

OPEN EXISTING WORK

📄

Browse analyses

Browse your existing INTACT Mass analyses

🔗


Browse methods


Browse your existing INTACT Mass processing methods


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45


Imported Sample List from .csv File


 **SAMPLE Submission**


 221_Franklin and more
Data Folder


 **Henry System**
View full status in System Console
Method for initial conditions
20211217_HTP_HYS_001


 Set to initial conditions


 Stop flow


 Reset system


 **20211217_HTP_HYS_001 Method**
Location: MilfordBioPharma, 221_Franklin an...


 Browse


 Method for initial conditions
20211217_HTP_HYS_001


 Edit in ACQUISITION Method Editor

 **Sample Trays**

 **Intact Mass**

 **Sample List**

 Queue

 Real Time Data

20220105_mAb_HYS_105

Version: 9 Status: Draft

	Item Name	Item Description	Sample Type	Injection Volume (uL)	Sample Position	Replicates	Acquisition Method	Run Time (mins)	Submitter	Processing Method	Molecule Ids	Expected Masses	Molecule Type	Reduc
1	Sample 201	N	Unknown	2.00	1:A,1	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
2	Sample 202	N	Unknown	2.00	1:A,2	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
3	Sample 203	N	Unknown	2.00	1:A,3	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
4	Sample 204	N	Unknown	2.00	1:A,4	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
5	Sample 205	N	Unknown	2.00	1:A,5	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
6	Sample 206	N	Unknown	2.00	1:A,6	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
7	Sample 207	N	Unknown	2.00	1:A,7	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
8	Sample 208	N	Unknown	2.00	1:A,8	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148036.5	Protein	Non
9	Sample 209	T	Unknown	2.00	1:B,1	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
10	Sample 210	T	Unknown	2.00	1:B,2	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
11	Sample 211	T	Unknown	2.00	1:B,3	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
12	Sample 212	T	Unknown	2.00	1:B,4	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
13	Sample 213	T	Unknown	2.00	1:B,5	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
14	Sample 214	T	Unknown	2.00	1:B,6	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148056.5	Protein	Non
15	Sample 115	T	Unknown	2.00	1:B,7	1	20211217_HTP_HYS_001	2.50	HYS	Intact mAb method 0.7-0.85 mins Clone		148058	Protein	Non

Submit 48 rows

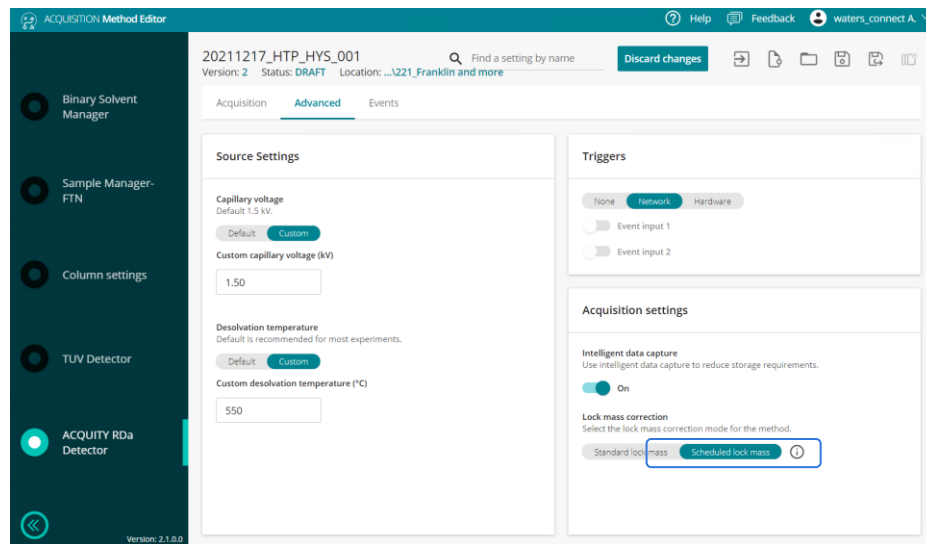
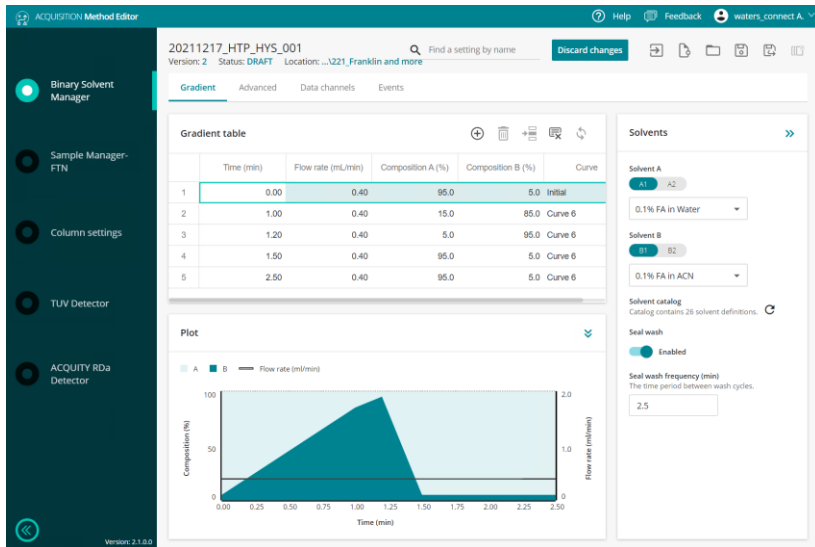
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46

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

2.5 mins fast LC gradient

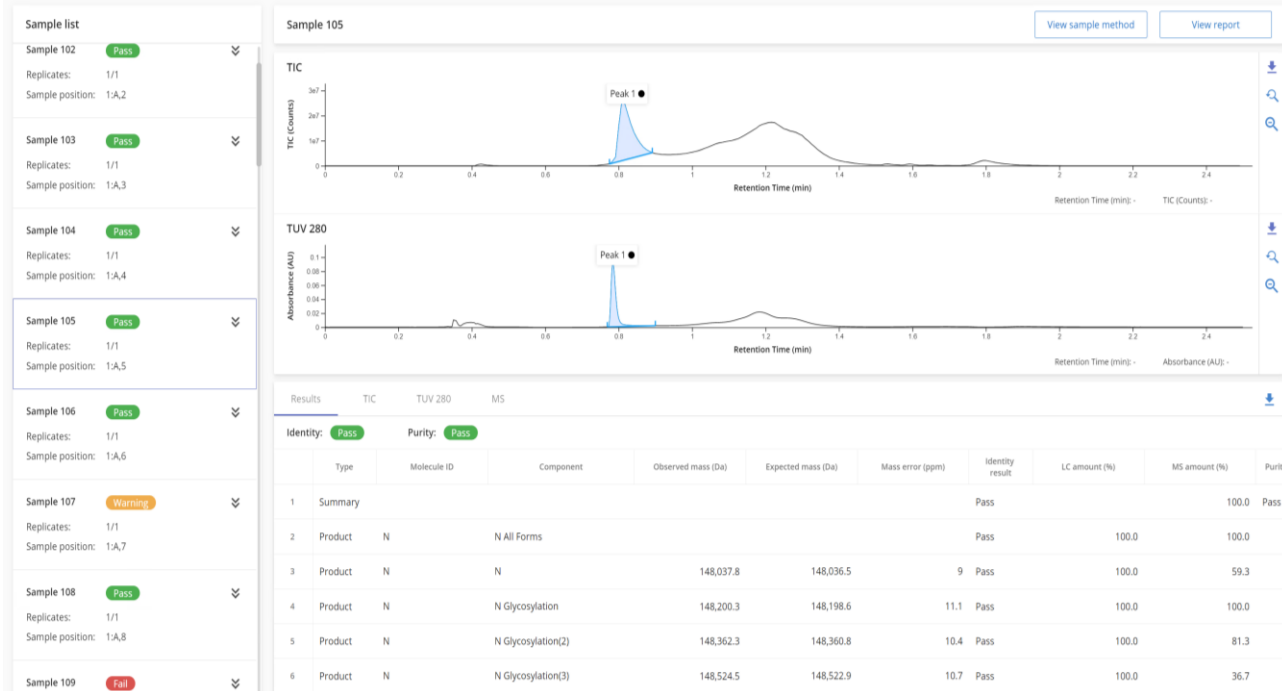
Scheduled Lock Mass minimizes inter-run system-checks



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

Data for a single Injection of NISTmAb

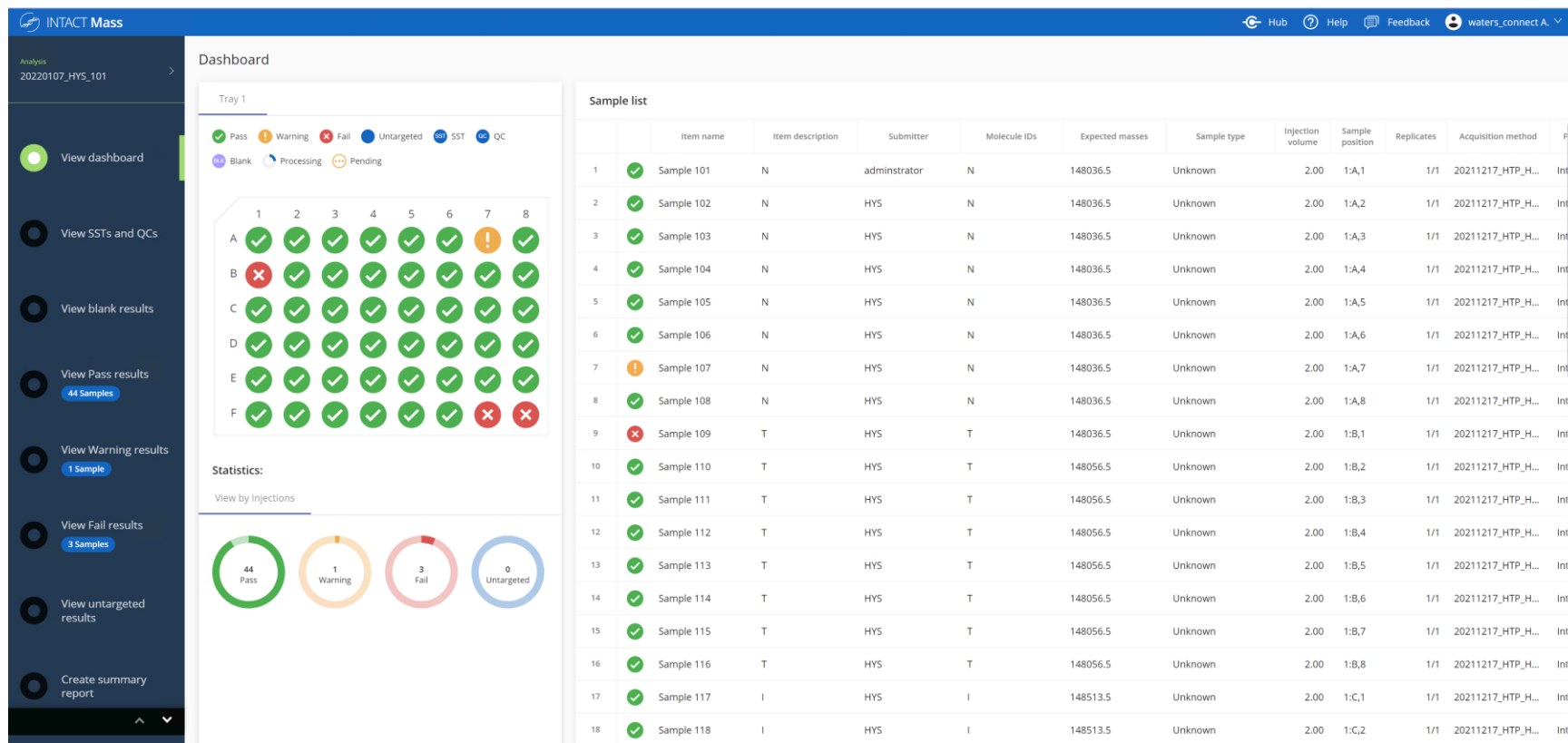
Dashboard > Sample 105



Peak 1 - Spectra

Deconvolved

Review of a 48-Well Plate Intact Mass Sample Set



Sample-Centric Report

< Sample 105 Sample report



Version: 1.0.0.0

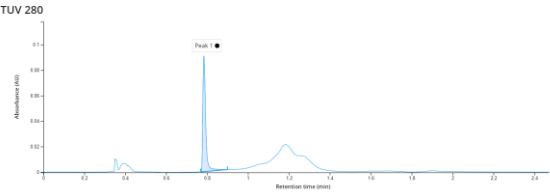
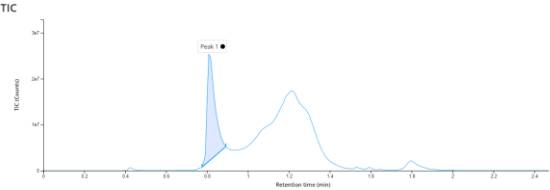
Analyst name: administrator
Analysis name: 20220107_HYS_101
Sample set name: 20220105_mAb_HYS_105 - v2

Sample 105

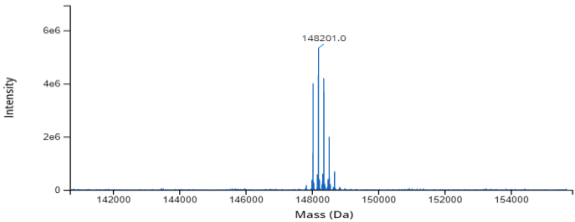
Pass

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)

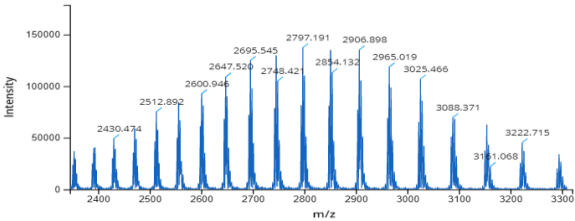
Item description:	N	Molecule IDs:	N
Sample position:	1A,5	Expected masses:	148036.5
Sample type:	Unknown	Submitter:	HYS
Injection volume:	2	Molecule type:	Protein
Replicates:	1	Reduction state:	None
Acquisition method:	20211217_HTP_HYS_001	Enzymatic treatment:	None
Processing method:	Intact mAb method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



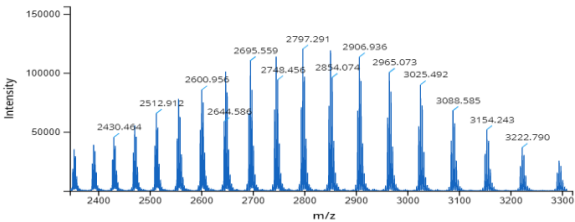
Peak 1 - Spectra ●
Deconvolved



Raw



Mock



Reducing Bias and Increasing Efficiency and Robustness of Biopharmaceutical Analysis

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.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

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

Bill Warren

Principal Product Manager

MAXPEAK™
HIGH PERFORMANCE SURFACES

- 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?



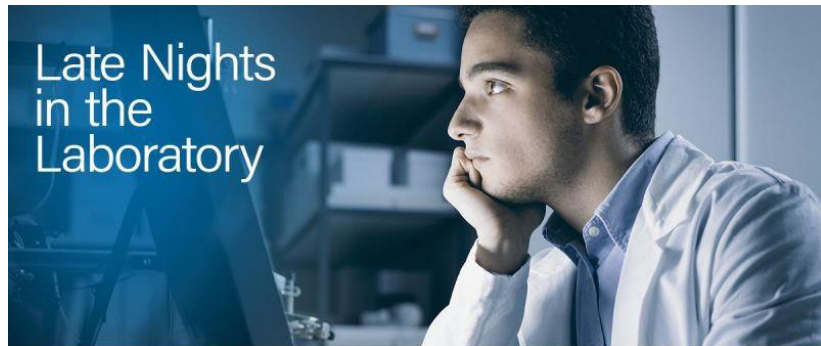
- Highly reproducible?
 - Low RSD's
 - Analyte detection is complete and predictable
- Data from LC-based biomolecule analyses is reliable to address internal critical quality attribute (CQA) and regulatory needs
 - Free from unexpected surprises or repeat testing

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 ...



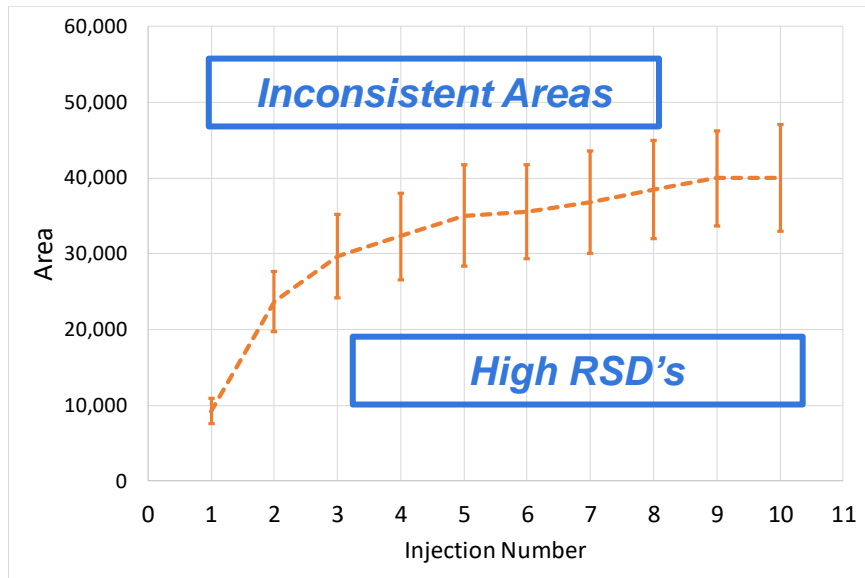
[READ THE BLOG NOW]

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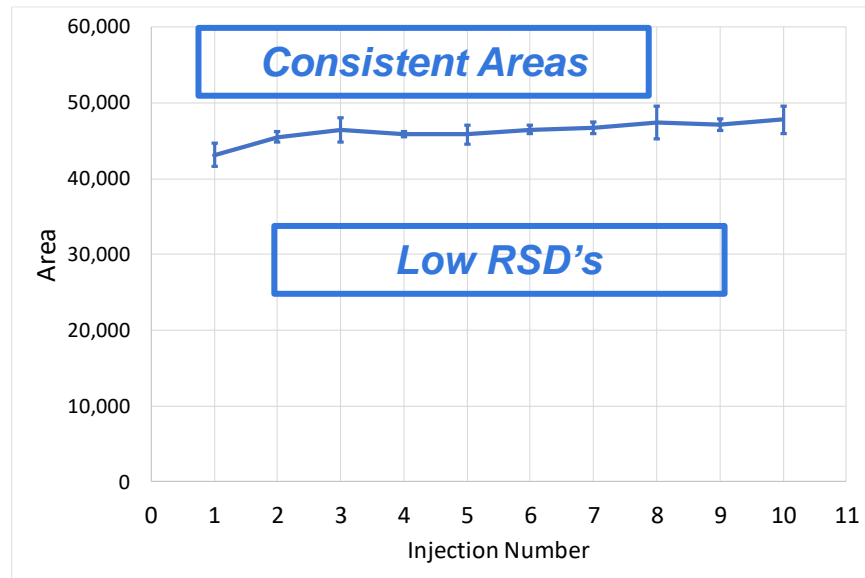
- We need to analyze some samples urgently.
 - Inject the sample onto the column and ... see NOTHING
 - Inject it again ... NOTHING
- What is going on?
 - Try to passivate the column, and the system
 - Inject higher concentrations of sample
- We lose ... confidence

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

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

- 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	<ul style="list-style-type: none"> • Time consuming • Strong acids • Not stable, needs to be repeated
Passivation of surfaces with sample or matrix	Analyte or matrix coats reactive surfaces	<ul style="list-style-type: none"> • Time consuming • Not stable, needs to be repeated
PEEK or PEEK lined steel columns	Replaces metal with non-reactive material	<ul style="list-style-type: none"> • PEEK alone is not high pressure tolerant • PEEK materials have: <ul style="list-style-type: none"> • Higher dimensional variability • Lower frit permeability • Incompatible with some solvents
Titanium in columns or parts	It doesn't. Titanium is a metal!	<ul style="list-style-type: none"> • Analyte loss
Industrial coatings	Covers the metals with material, e.g silicates/other materials	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Ion suppression and other unknown effects • Continued use necessary • Possible solubility issues

A Technology Brand was Created...



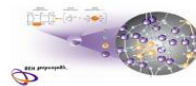
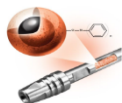
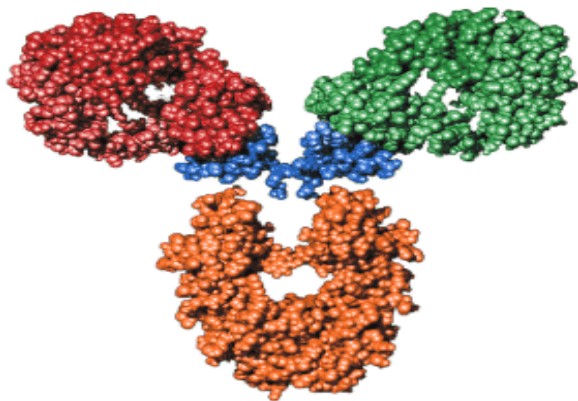
MaxPeak™ Premier High-Performance Surfaces are new and innovative technologies designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte / surface interactions that can lead to sample losses.

**Understanding
Primary Structure**

**Purity,
Content,
Glycosylation**

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

**Higher Order
Structure**
HDX-MS
SEC-MALS



Reversed Phase

- Separate oxidized, fragmented species
- Intact Mass
- Reduced mAb characterization

MAXPEAK
HIGH PERFORMANCE SURFACES

Peptide Mapping

- mAb Identity
- PTM characterization
- Disulphides
- MAM

MAXPEAK
HIGH PERFORMANCE SURFACES

Amino Acid Analysis

- Understanding molar absorptivity
- Protein Concentration
- (Quantitative AAA)

Hydrophobic Interaction

- Separate based on size of molecule
- Dimers/Aggregation
- High Molecular Weight
- Low Molecular Weight
- Clips

Size Exclusion

- Separate based on size of molecule
- Dimers/Aggregation
- High Molecular Weight
- Low Molecular Weight
- Clips

MAXPEAK
HIGH PERFORMANCE SURFACES

Glycan HILIC

- Glycosylation Pattern
- Released N-Glycan
- Orthogonal methods for Intact, Subunit, Glycopeptide Mapping

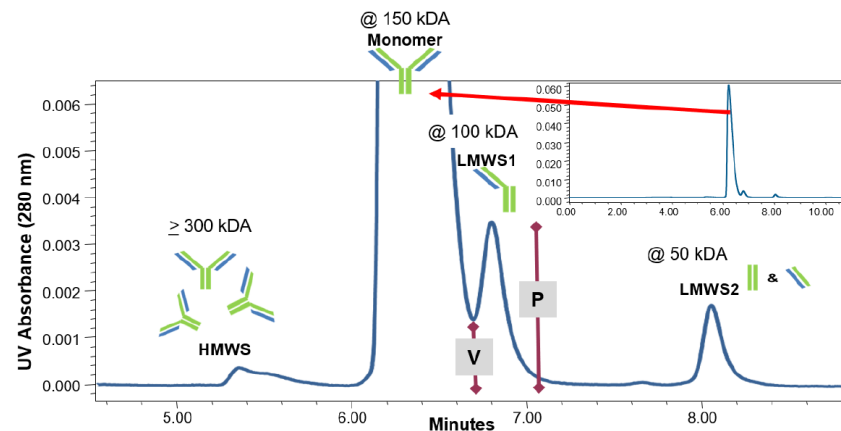
MAXPEAK
HIGH PERFORMANCE SURFACES

Ion Exchange

- Separation Based on charge
- Monitor deamidation, sialylation, pyroglutamate



SEC of Proteins



Protein Size Variant Analyses Using SEC

The Challenge of Non-Desired, Secondary Interactions

■ **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 separation

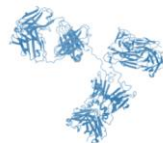
■ **Most commercialized LC based SEC columns contain of silica-based, diol-bonded particles of defined pore and particle size packed in stainless steel hardware**

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

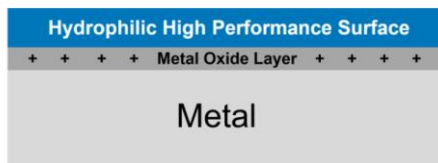
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MaxPeak Premier Protein SEC 250A Columns and Guard

plus



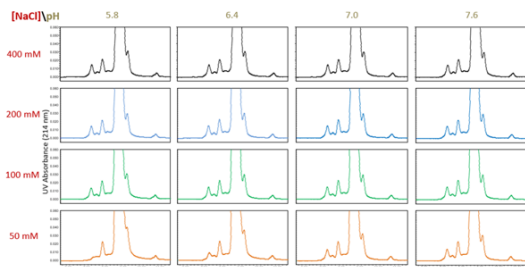
equals



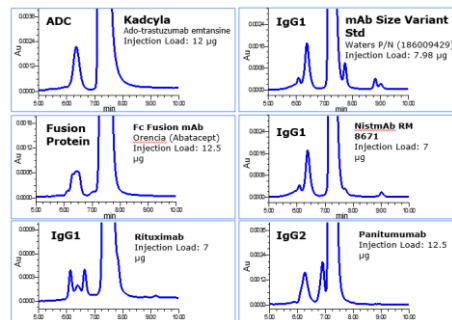
©2022 Waters Corporation

SEC Challenges for Reliable Biotherapeutic Protein Size Variant Analyses

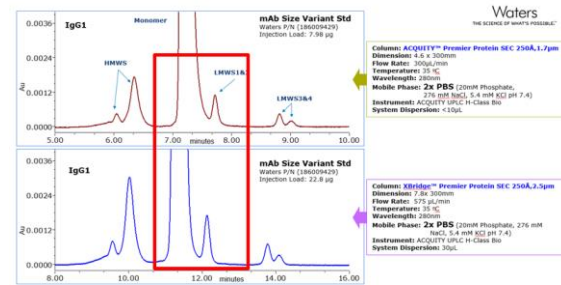
Long SEC Method Development Time (Desire to have Platform Method)



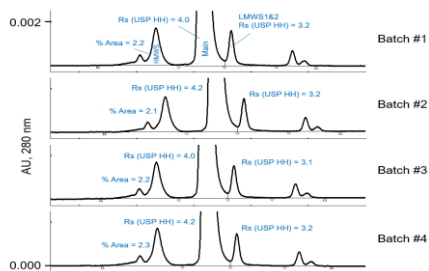
Out of the Box Performance for Protein Aggregate, Monomer, and Fragment SEC Analysis



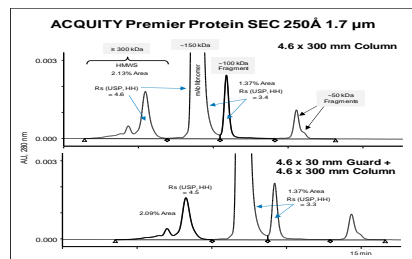
The option to have speed (1.7 μ m) and scalability (2.5 μ m)



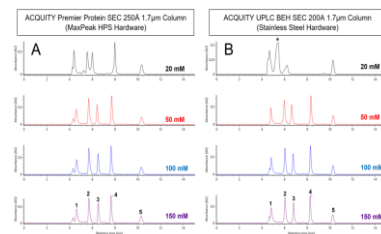
Batch to Batch and Consistency



Decrease Cost per Analyses with Guard



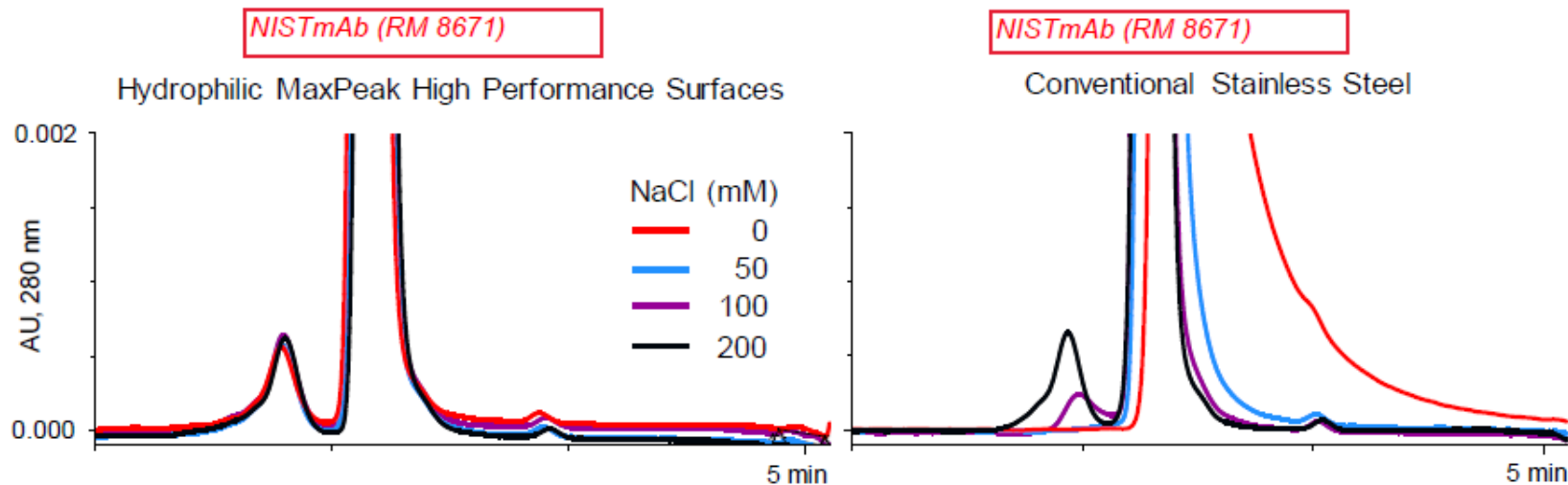
Improved SEC / MS Analysis of Native mAbs



QR Code Enabled for Column Specific and Authenticity Information

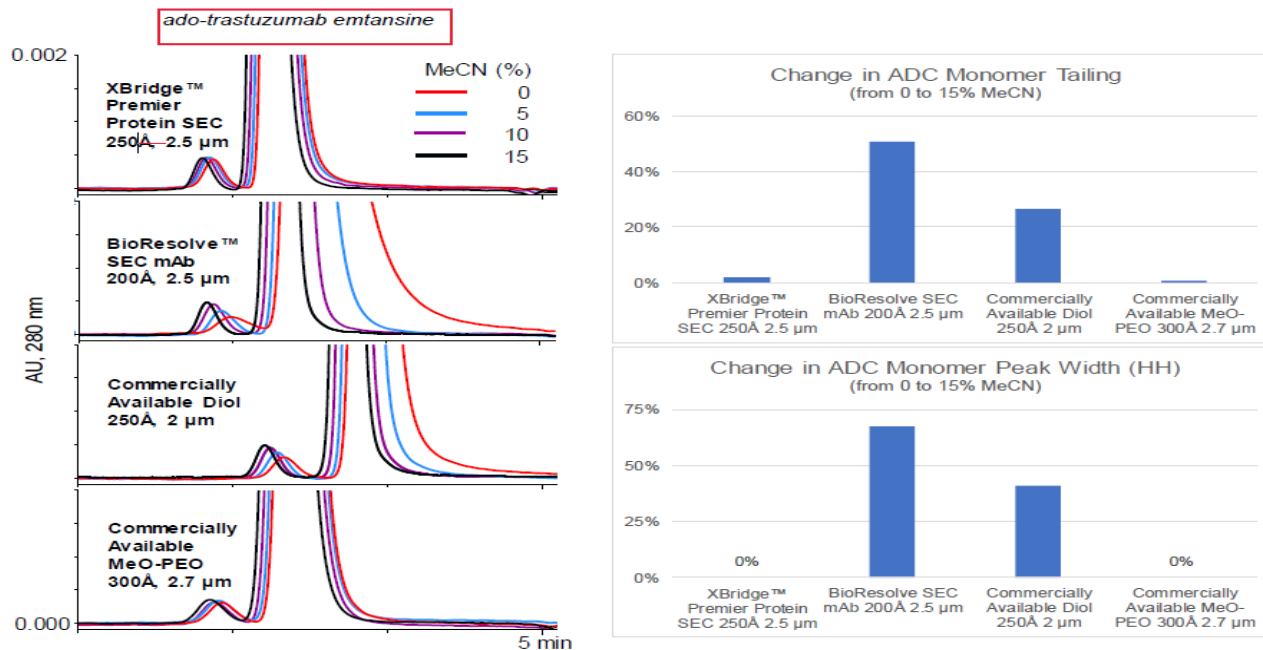


Influence of SEC Column Hardware on mAb Analysis



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.

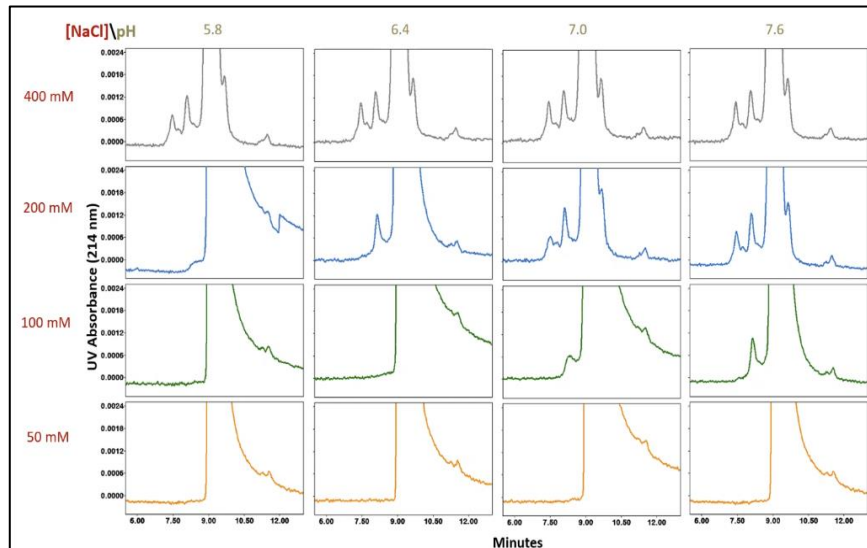
Influence of SEC Particle on ADC Analysis



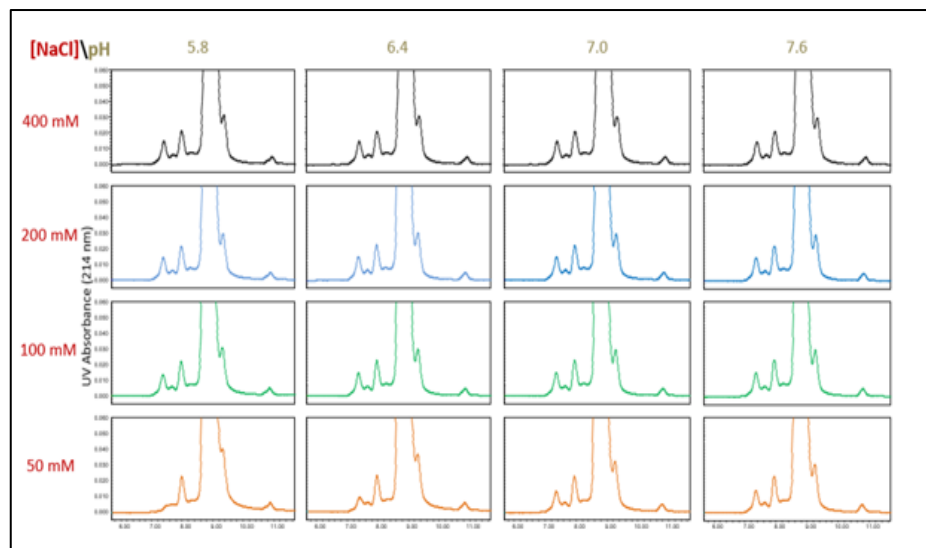
Comparison of hydrophobic secondary interactions performance using the ADC ado-trastuzumab emtansine. Upon increase of organic concentration, the degree of change observed for the XBridge™ Premier Protein SEC 250Å 2.5 µm column is again negligible, with outstanding peak shape from 0 to 15% MeCN

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

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

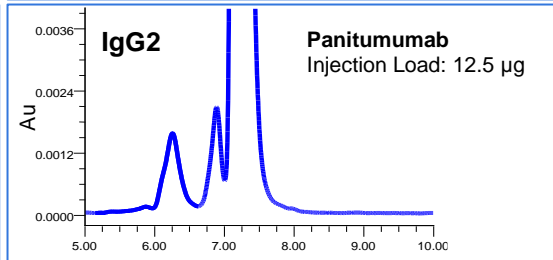
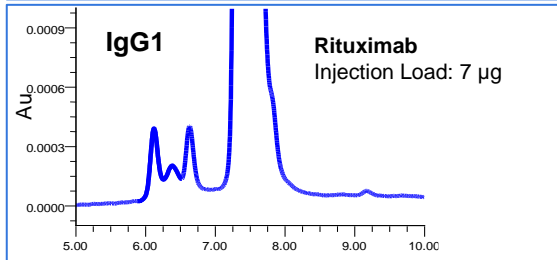
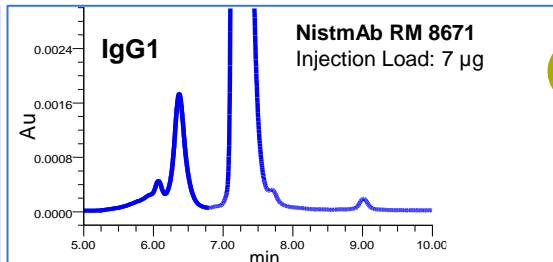
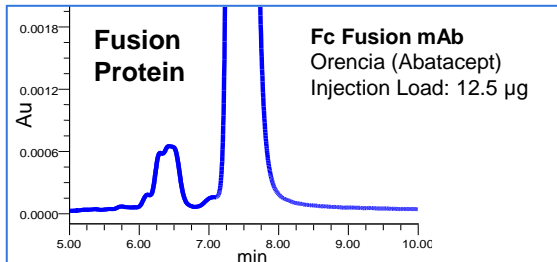
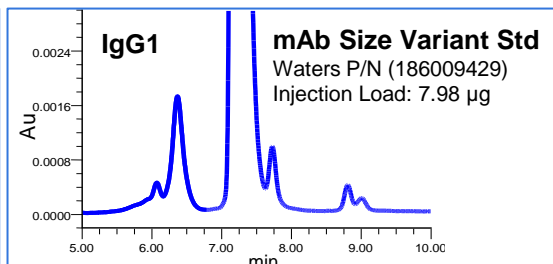
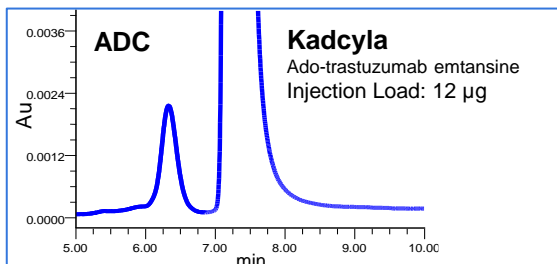
Temperature: 35 °C

Wavelength: 280nm

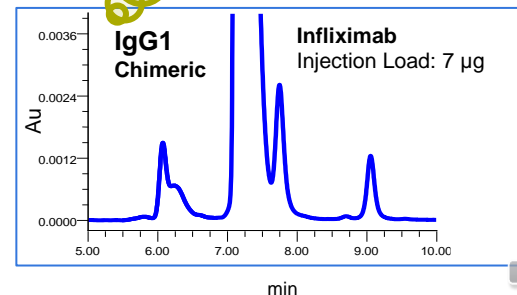
Mobile Phase: 2xPBS (20mM Phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)

Instrument: ACQUITY UPLC H-Class Bio

System Dispersion: **<10µL**

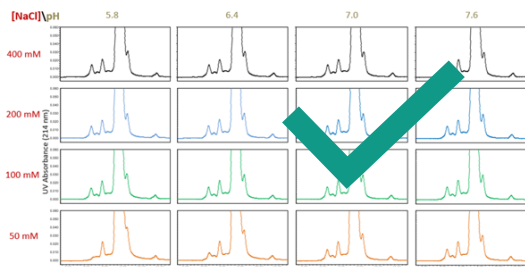


2X Phosphate Buffered Saline (PBS) shown here to give excellent resolutions and estimations of aggregates and fragments for several classes of proteins.

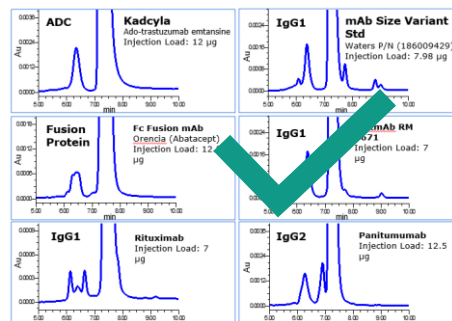


SEC Challenges for Reliable Biotherapeutic Protein Size Variant Analyses

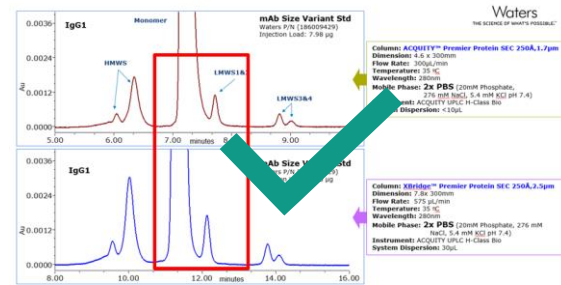
Long SEC Method Development Time (Desire to have Platform Method using PBS)



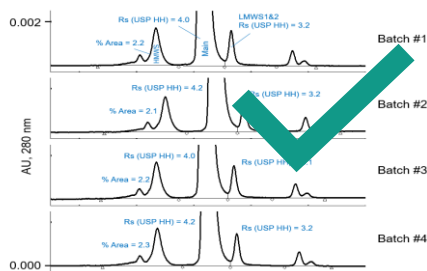
Out of the Box Performance for Protein Aggregate, Monomer, and Fragment SEC Analysis



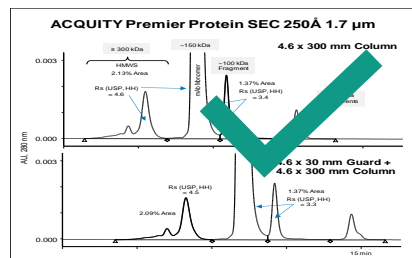
The option to have speed (1.7 μ m) and scalability (2.5 μ m)



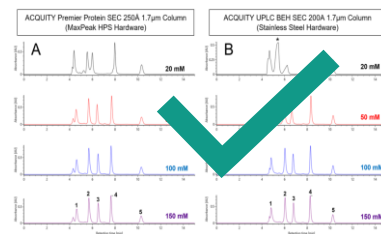
Batch to Batch and Consistency



Decrease Cost per Analyses with Guard



Improved SEC / MS Analysis of Native mAbs



QR Code Enabled for Column Specific and Authenticity Information

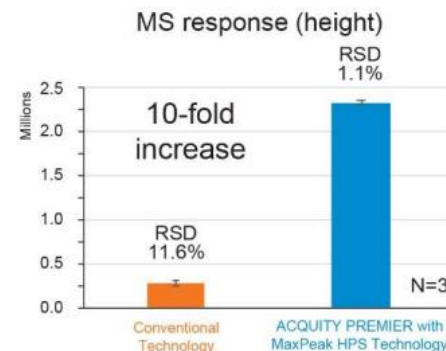
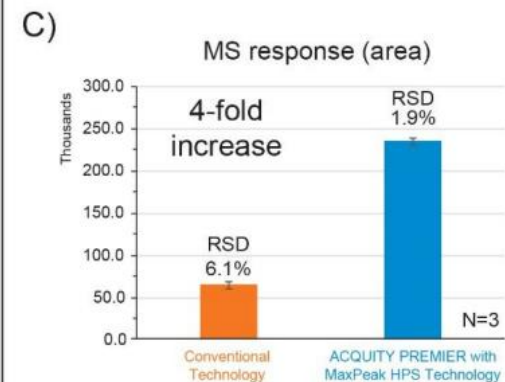
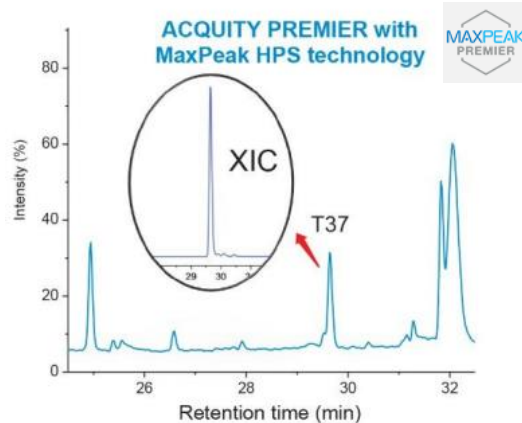
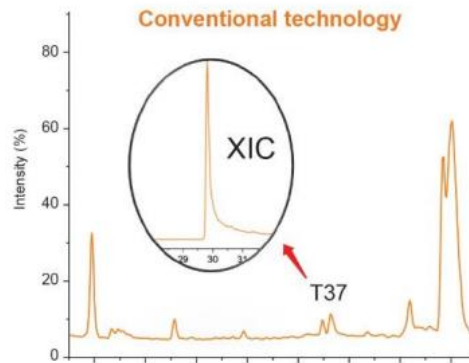
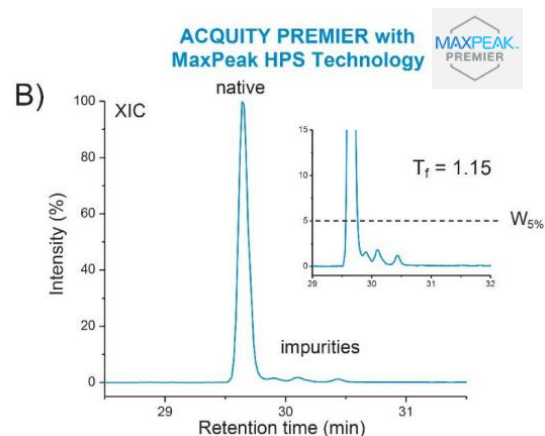
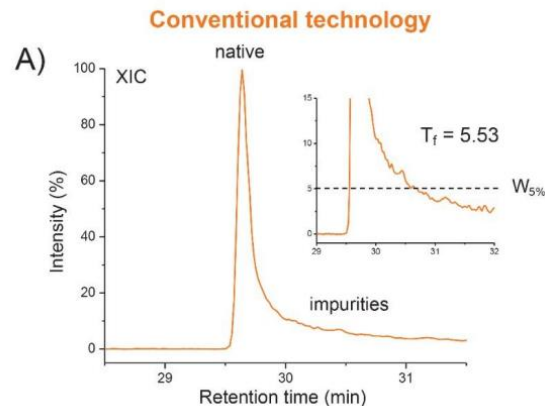




Peptides



MaxPeak Premier for Peptide Mapping



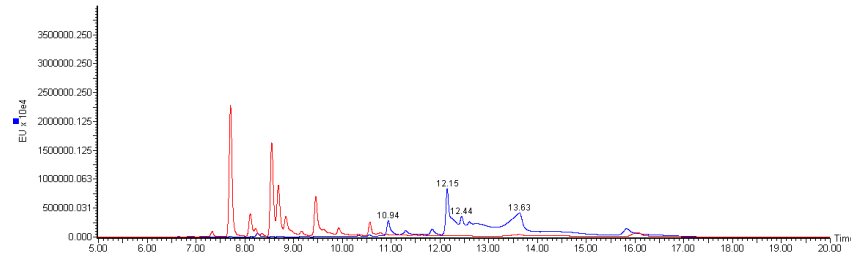


Glycans



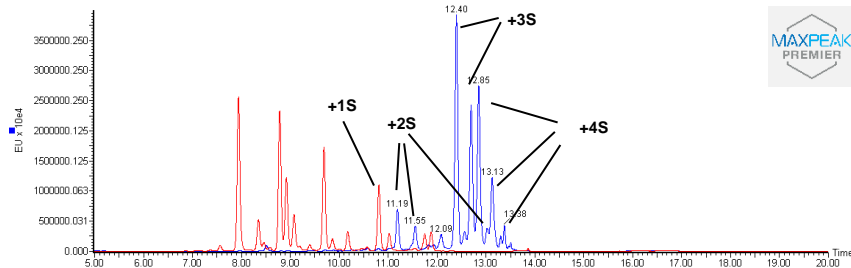
Representative Chromatogram from #1 Injection

Premier Glycan Amide and Glycan C18AX



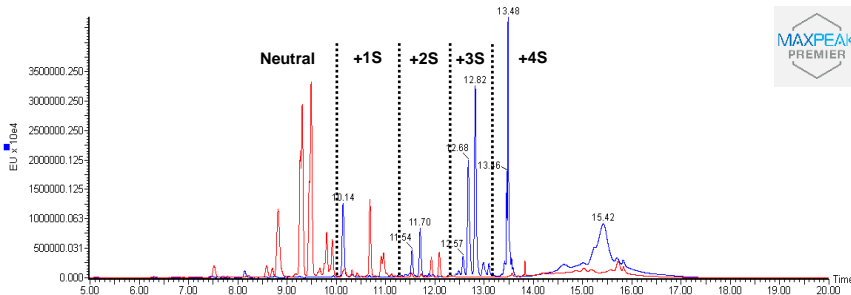
ACQUITY Glycan Amide

- Column conditioning is critical



ACQUITY PREMIER Glycan Amide

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



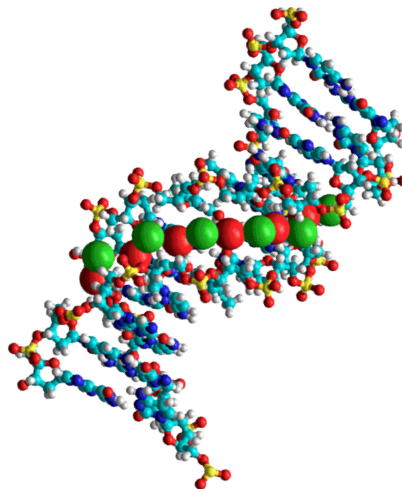
ACQUITY PREMIER Glycan C18 AX

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



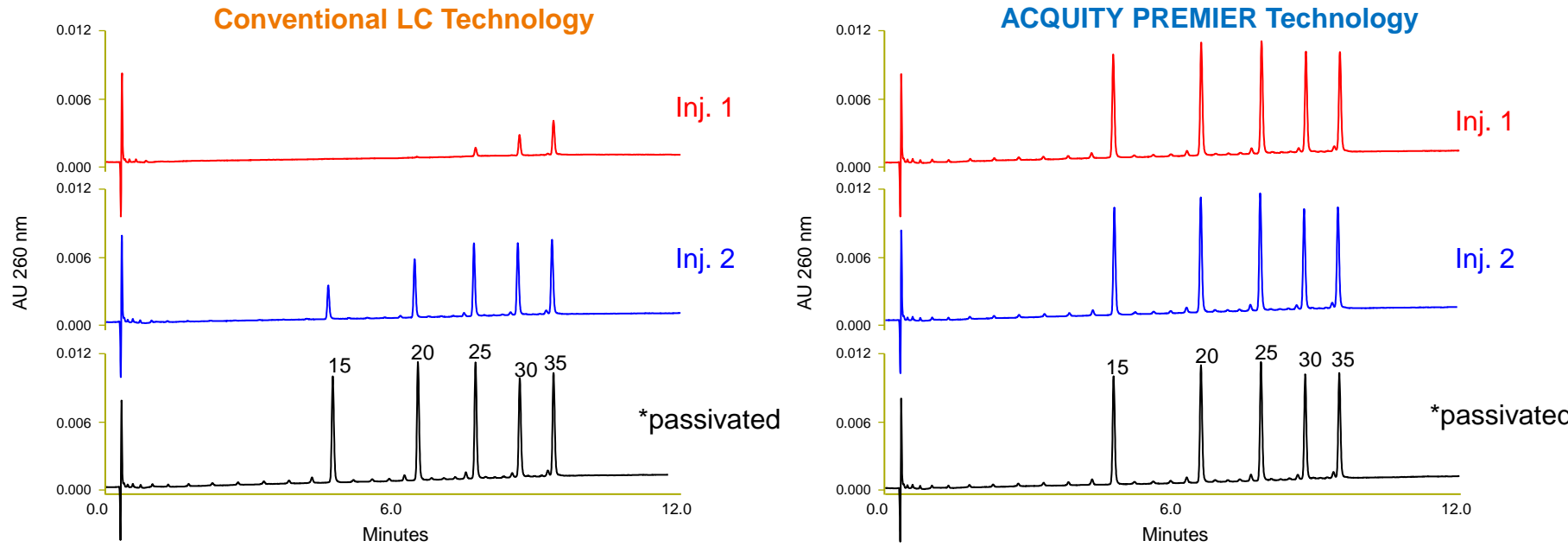


Oligonucleotides



ACQUITY PREMIER: *Performance from Injection #1*

15-35mer Oligonucleotide Standard (oligodeoxythymidines)



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

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

- 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 losses 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

What can MaxPeak Premier Columns do for your protein SEC analysis?
Ensure MaxPeak Premier performance for ALL separations

MAXPEAK PREMIER

Universal of Method
(NaCl) 400 mM, 200 mM, 100 mM, 50 mM

MaxPeak Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

MaxPeak Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

For Waters complete line of MaxPeak Premier Columns, visit waters.com/MaxPeakColumns

Peptide Premier

What can MaxPeak Premier Columns do for your peptide analysis?
Ensure MaxPeak Premier performance for ALL separations

MAXPEAK PREMIER

REDUCED TAILING
ACQUITY Premier Peptide C18 C₈ vs. C₁₈

54% reduction in tailing

INCREASED SENSITIVITY AND RECOVERY
ACQUITY Premier Peptide C18 C₈ vs. C₁₈

35-fold increase in sensitivity

MaxPeak Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

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MAXPEAK Premier White Paper

[WHITE PAPER]

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Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces

M. Lasker, T. K. Weller, M. G. S. M. de Almeida, C. Bessat, K. Smith, R. Strubel, P. Ravnkilde, J. Bojarski, and K. Wymann
Waters Corporation, Milford, MA, USA

CHALLENGES IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) SEPARATIONS OF METAL-SENSITIVE ANALYTES

Because of its manufacturability and pressure capabilities, stainless steel has long been the preferred material for constructing HPLC columns. It is susceptible to corrosion and can negatively impact the peak shape and recovery of some analytes.† Transition metal ions in this layer are electron deficient, which leads them to act as Lewis acids. Meanwhile, many analyte molecules contain moieties that are electron rich, such as phosphate and carboxylate groups. Being Lewis bases, these molecules can adsorb to transition metal ions on the surface of HPLC columns. This interaction becomes very significant if a molecule has a high affinity for the electron-deficient transition metal ions.

COMPARING MaxPeak HPS FOR MaxPeak Premier Columns AND PLATES

MaxPeak High Performance Surfaces are a collection of novel surfaces designed to address the shortcomings of materials traditionally used in chromatographic analyses. Reversed-phase and HPLC MaxPeak Premier Columns are constructed with a novel

Oligo Premier

What can MaxPeak Premier Columns do for your oligonucleotide analysis?
Ensure MaxPeak Premier performance for ALL separations

MAXPEAK PREMIER

OUT OF THE BOX PERFORMANCE
Oligonucleotide C₁₈, C₈, and C₄ Columns

HIGH RECOVERY AND REPEATABILITY
Oligonucleotide C₁₈, C₈, and C₄ Columns

MaxPeak Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

For Waters complete line of MaxPeak Premier Columns, visit waters.com/MaxPeakColumns

Glycan Premier

What can MaxPeak Premier do for your glycan analysis?
Ensure MaxPeak Premier performance for ALL separations

MAXPEAK PREMIER

OUT OF THE BOX PERFORMANCE
Glycan C₁₈, C₈, and C₄ Columns

MaxPeak Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

For Waters complete line of MaxPeak Premier Columns, visit waters.com/MaxPeakColumns

MAXPEAK Premier HPS Ordering Guide

[ORDERING INFORMATION]

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MaxPeak Premier Columns featuring MaxPeak High Performance Surfaces

Good chromatography is as much about preventing the detrimental interactions you don't want, as it is creating the ones you do.

MaxPeak Premier Columns provide:

- Reduced column conditioning and preservation times
- Improved sensitivity and peak shapes
- Simple mobile phases, without complex additives
- Time savings in method development
- Reduced risk and greater confidence in data and decision-making

Available with particle technologies and quality manufacturing you can trust: ultra-fine particles, porous, oligonucleotide, and glycan separations in both normal-phase and HPLC separation modes.

MAXPEAK PREMIER HPS FOR MAXPEAK PREMIER COLUMNS AND PLATES

MAXPEAK PREMIER HPS FOR MAXPEAK PREMIER COLUMNS AND PLATES

The background of the slide features a complex, abstract network of interconnected nodes and lines. The nodes are represented by small circles in various shades of blue and grey, while the lines are thin, light blue. This network structure is most prominent on the left side and fades into the background towards the right. A solid blue horizontal band spans the width of the image, serving as a backdrop for the text.

Waters

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Confidence

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



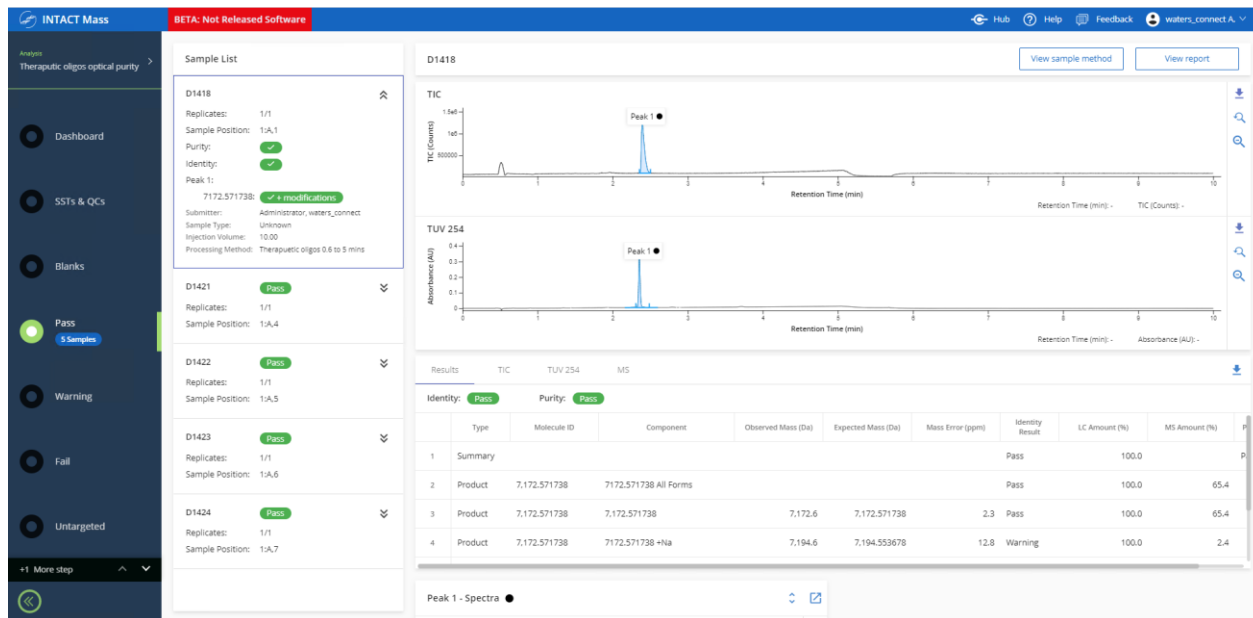
Productivity

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

