

Pushing the Edge... With All Deliberate Speed

Joe Fredette

Senior Marketing & Business Development Manager
Biopharmaceuticals Business

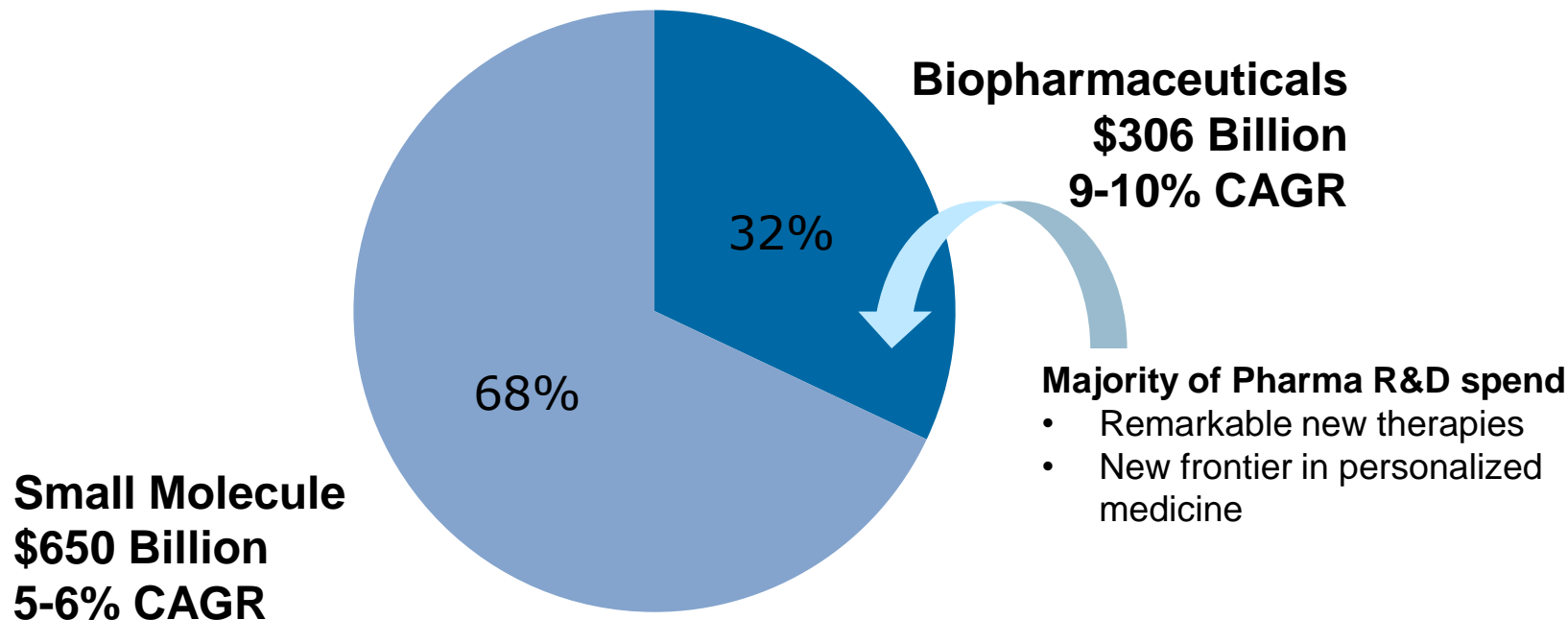
Presentation Outline

- Biopharma Industry Perspective
- How Waters is Responding
- Our Goal of Harmonization
- Conference Highlights



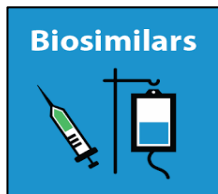
A Race for Productivity is Underway!

Pharma Industry Forecast - 2020



(*source: Global Industry Analysts, Inc.)

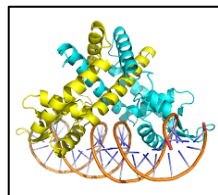
Background – Major Industry Trends



Biosimilars fueling a race for greater productivity



Increased outsourcing



Larger and more complex molecules



Evolving Regulatory Requirements / Drive for Harmonization

Current Efforts at Improving Productivity

Single-Use / Disposable Manufacturing

Quality by Design (QbD)

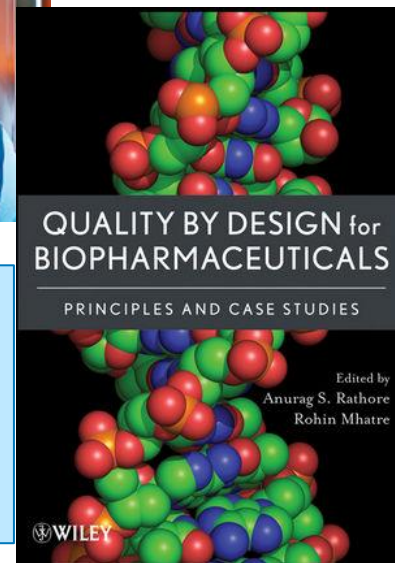
Continuous Manufacturing (CM)



Pharma Catches on to Continuous Manufacturing

By Stephanie Neil , Automation World - December 6, 2017

Support from the FDA, industry groups and automation suppliers is helping pharmaceutical companies break the batch habit in favor of a continuous approach to drug production.



Where Time is Lost Today

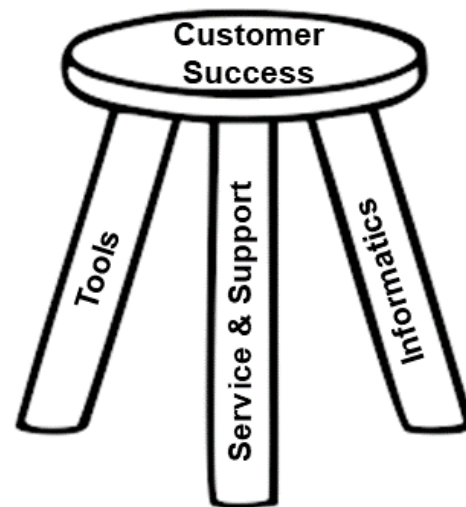


- Managing the hidden complexities and risks when transferring data & methods
- Ensuring data integrity and regulatory compliance across the organization
 - *2017 saw a doubling in the number of warning letters issued to drug product manufacturing sites**
- System validation, maintenance and record keeping for GLP / GMP compliance
- Troubleshooting inconsistent analytical performance

How is Waters Responding?

Our Vision

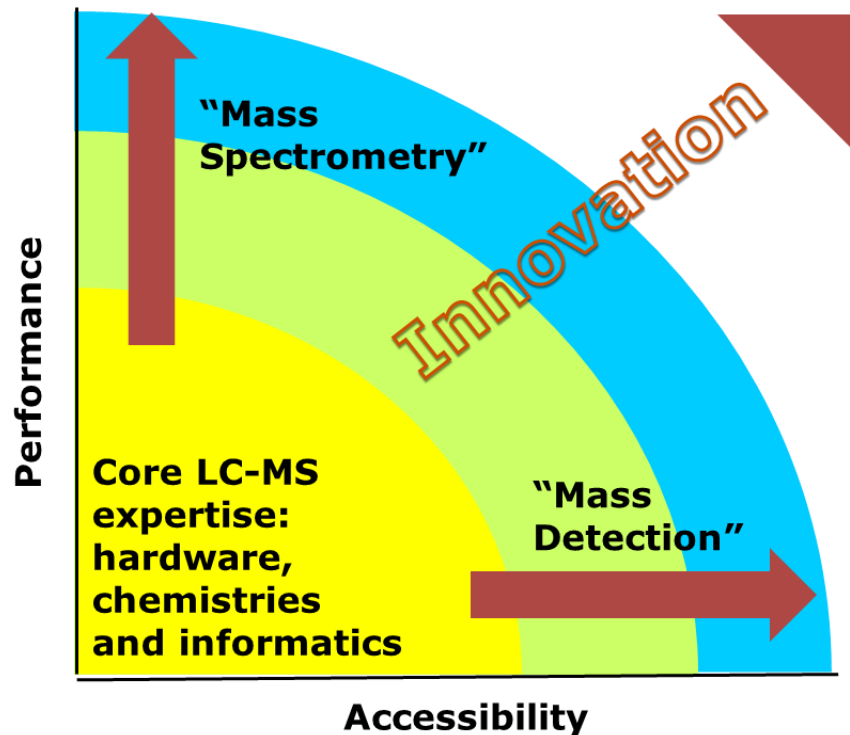
A comprehensive portfolio of fit-for-purpose analytical tools that all work together within a single compliance-ready informatics environment; one that facilitates method and data transfers while preserving data integrity, and that creates workflow efficiencies that fuel productivity gains across the drug discovery, development and manufacturing pipeline



Dimensions of Innovation

The Cutting Edge

Pushing the edge of capability and performance



Acquity QDa

Solutions for Routine Use

Focused on ruggedness, ease-of-use, size and affordability

Fit-for-Purpose Deployment of Mass Spectrometry



Non-Regulated

Regulated - GXP

Discovery & Characterization

Product & Process Monitoring / QC



Synapt G2 Si
& VION HDMS



Xevo G2 XS

“Mass Spectrometry”

- Most capable instruments
- Leading edge performance
- Expert level end users
- Large and not inexpensive



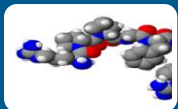
ACQUITY QDa

Future Offerings

“Mass Detection”

- Designed for max. accessibility
 - Ease-of use
 - Minimal training
 - Compact size
 - Affordable and Scalable
- Rugged, Robust Performance

QDa Biopharma Applications



Peptide-based Multi-Attribute-Monitoring

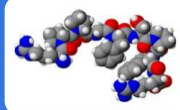
Confirm product identity (ID)

- Peptide Map / Profile
- CDR peptides

Post Translational
Modifications (PTM) analysis

- Oxidation
- Deamidation, et.al.
- Glycopeptide Analysis

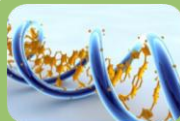
Sequence variants, ...more



Synthetic Peptides



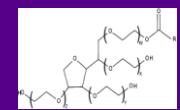
Released N-Glycans



Oligonucleotides



ADC Free Drug Analysis



Polysorbate 80 / 20
Stability Monitoring

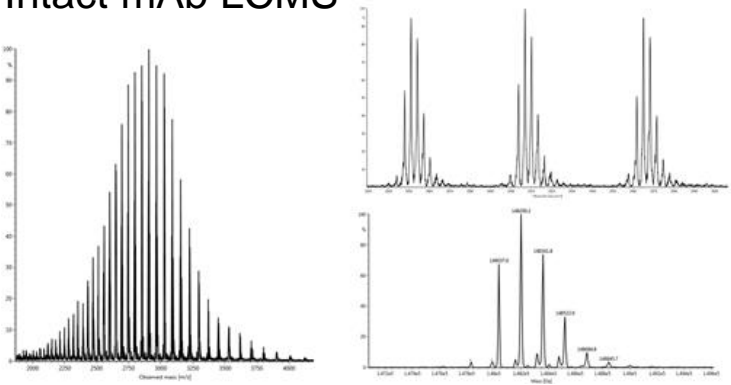
So What Does a High Resolution Mass Detector Look Like?

Fit for purpose attribute analysis for the most complex biotherapeutics

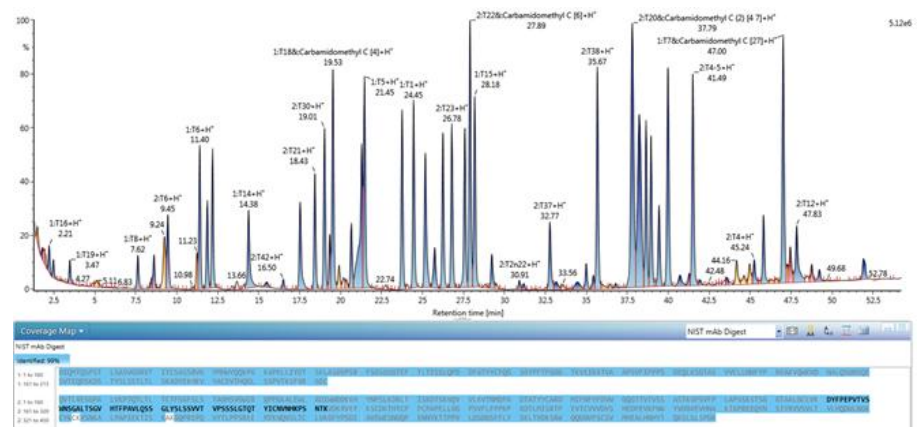
Visit Henry Shion at **Poster WP699** for more details



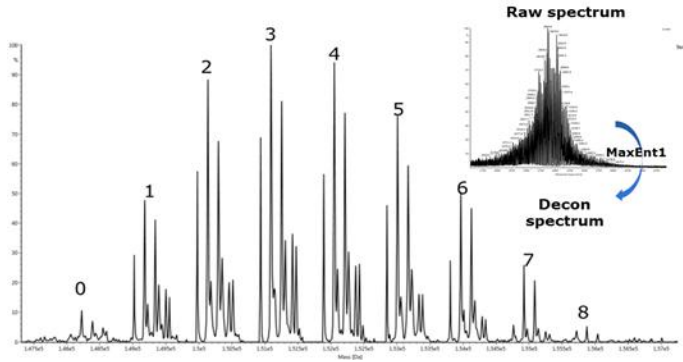
Intact mAb LCMS



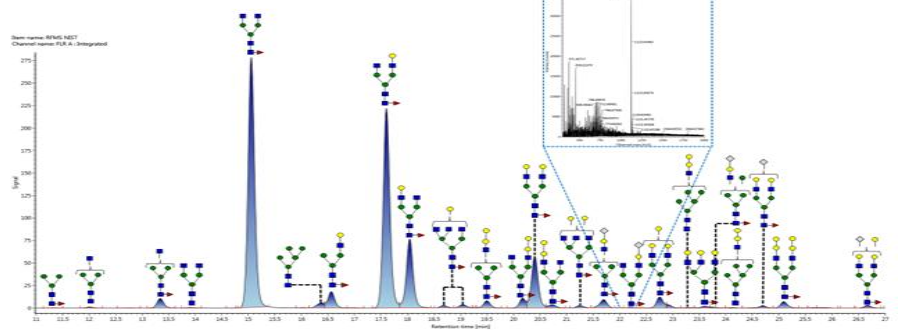
NIST mAb Peptide Map



Native SEC-MS of ADC



NIST mAb Released Glycan Profile





ASMS 2018: 66th Conference on Mass Spectrometry and Allied Topics

Waters Biopharmaceutical Activities



A Busy ASMS for the Waters Biopharm Team

Biopharmaceutical Presentations at ASMS 2018

BREAKFAST SEMINARS

7:00 am - 8:00 am

Waters Hospitality Suite, Hilton San Diego Bayfront

Space is limited. Register today
at www.waters.com/asms

Tuesday, June 5, 2018

The Benefits of Native Mass Spectrometry in Biotherapeutic Characterization

- Collision Induced Unfolding: A New Paradigm in Protein Stability Measurements

Presenter: Brandon T. Ruotolo, Ph.D, University of Michigan

- Hyphenation of Non-Denaturing Chromatographic Methods to Native Mass Spectrometry and Ion Mobility for Therapeutic Protein Characterization

Presenter: Mr. Anthony Ehkirch, University of Strasbourg

Wednesday, June 6, 2018

Get Automated: Achieve Your Automation Potential for Complex Large Molecule LC-MS Workflows

Presenter: Jennifer Fournier, Director of Product Marketing, Chemistry Group, Waters Corporation

ORAL PRESENTATIONS

Monday, June 4, 2018, 9:50 am

- MOC** Streamlining the Identification and Monitoring of Product and Process Attributes in Biopharmaceutical Development and QC with MAM-Based Workflows

Presenter: Welbin Chen, Waters Corporation

POSTER PRESENTATIONS

Monday, June 4, 2018

- MP 300** LC-MS Glycan Analysis of Fusion Proteins Facilitated by Rapid Glycosylamine Labeling and Site-Specific Profiling

- MP 430** Synthetic Peptide Impurity Analysis and Purification

- MP 725** Significant Improvements in Spectral Quality of Non-Covalent Protein Complexes using SEC-Native MS

Wednesday, June 6, 2018

- WP 042** Data Independent Acquisition Modes for Identification, Quantification and Monitoring of Low-Abundance Host Cell Proteins During Monoclonal Antibody Bioprocessing

- WP 058** Analytical Scale Native SEC-MS for Robust Biotherapeutic Characterization

- WP 171** Characterising the Catabolism of Peptides Using Ion Mobility Enabled High Resolution Mass Spectrometry

- WP 676** Monitoring Critical Quality Attributes (CQA): Core-Fucosylation of N-Glycans Using an Integrated Subunit LC-MS Workflow Method

- WP 699** Towards Overcoming the Challenges of Implementing Accurate Mass MS for Routine Biotherapeutic Analysis

Thursday, June 7, 2018

- ThP 5583** Bridging the Analytical Workflows for Characterizing and Monitoring Product Quality Attributes (PQAs) of Biotherapeutics by a Common Data Acquisition Mode

- ThP 683** Optimizing Data Processing Parameters for HRMS-based Intact Level mAb Quantification

- 2 Breakfast Seminars on
 - Native MS
 - Sample Prep Automation (Digestion and Glycans)
- Oral on Multiple Attribute Monitoring (MAM)
- 10 Posters
 - Released Glycan and Glycopeptide Analysis
 - Multiple Attribute Monitoring (MAM)
 - Native MS and Native SEC-MS
 - Synthetic Peptide Impurity and Catabolism
 - Host Cell Protein Analysis
 - DIA Peptide Mapping
 - Intact Protein LCMS Quan

Biopharmaceutical Breakfast Seminar

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You are invited

THE BENEFITS OF NATIVE MASS SPECTROMETRY
IN BIOTHERAPEUTIC CHARACTERIZATION

[When]

Tuesday, June 5, 2018

7:00 a.m. - 8:00 a.m.

Start ASMS off
on the right foot



[Where]

Waters Hospitality Suite
Hilton San Diego Bayfront
1 Park Blvd., San Diego, CA

Native MS approach is becoming increasingly important for the characterization of biotherapeutics. This breakfast seminar will highlight the importance, and applications, of native MS in the characterization of biotherapeutics. Presentations will showcase novel research on the use of ion mobility MS in structural characterization of proteins for improving protein drug discovery and development processes. Please join us for a light breakfast, great science, and engaging discussion of this exciting area of biopharmaceutical MS analysis.

Collision Induced Unfolding: A New Paradigm in Protein Stability Measurements

Presenter: Brandon T. Ruotolo, Ph.D, University of Michigan

Hyphenation of Non-Denaturing Chromatographic Methods to Native Mass Spectrometry and Ion Mobility for Therapeutic Protein Characterization

Presenter: Mr. Anthony Etkirch, University of Strasbourg

Register online at: www.waters.com/NativeMSbreakfast

Space is limited, pre-registration is required.

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BENEFITS OF NATIVE MASS SPECTROMETRY FOR BIOTHERAPEUTIC CHARACTERIZATION

Tue June 5th, 2018 7:00 – 8:00 AM

Waters Suite- Hilton San Diego Bayfront

- Collision Induced Unfolding: A New Paradigm in Protein Stability Measurements. [Brandon T. Ruotolo, Ph.D, Univ. of Michigan](#)
- Hyphenation of Chromatographic Methods to Native Mass Spectrometry and Ion Mobility for Therapeutic Protein Characterization. [Mr. Anthony Etkirch, Univ. of Strasbourg](#)
- Register at information desk or online at: www.waters.com/NativeMSbreakfast

Limited Spaces Remaining !!

MP 725 - Improvement in Spectral Quality of Non-Covalent Protein Complexes using SEC-Native MS

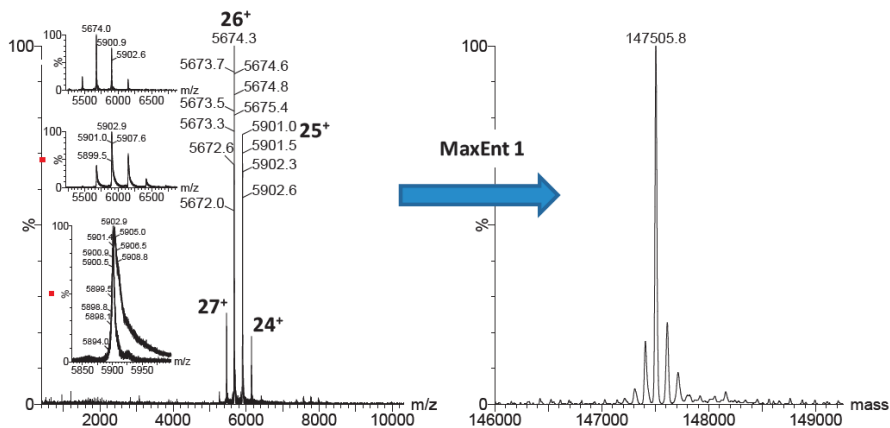


Figure 3: Improvement of spectral quality for tetrameric ADH (147kD) using SEC-Native MS over static nanospray

WP 058 - Analytical Scale Native SEC-MS for Robust Biotherapeutic Characterization

Trastuzumab emtansine (T-DM1), calculated DAR=3.52

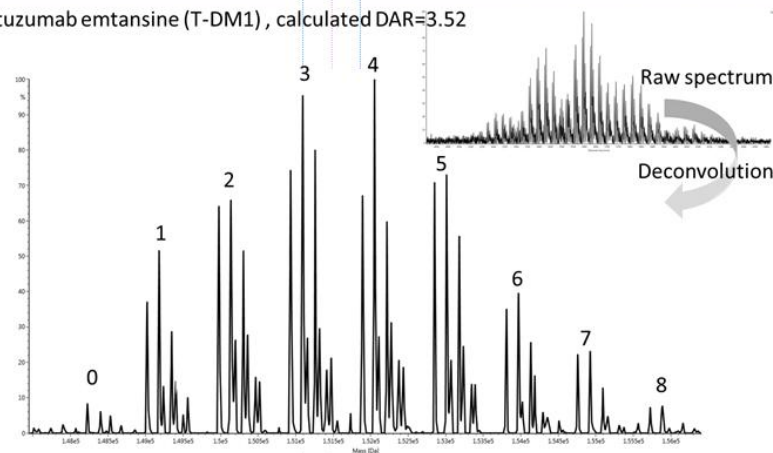


Figure 5: SEC-Native MS of Trastuzumab-DM1 ADC. Deglycosylation was not required for this DAR analysis.

Sample Preparation is no longer one dimensional *for Released N-Glycan Analysis with GlycoWorks RapiFluor-MS*



Manual Prep
8-96 samples



Low throughput
Semi-Automation
8-24 samples



Higher throughput
Automation
48-96 samples

- Platform scalability for **8 to 96 samples** at a time
- **Purposefully designed kits** for manual use and automated liquid handling platforms
- Available **base scripts** and **layouts** for simplified deployment on larger bed liquid handling platforms.

Automation Breakfast Seminar

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GET AUTOMATED: ACHIEVE YOUR AUTOMATION POTENTIAL FOR COMPLEX LARGE MOLECULE LC-MS WORKFLOWS

[When]

Wednesday, June 6, 2018

7:00 a.m. – 8:00 a.m.

[Where]

Waters Hospitality Suite
Hilton San Diego Bayfront
1 Park Blvd., San Diego, CA

Automation starts well before the robot. To truly obtain the benefits of automation your consumables need to be designed for automation platforms. Specially designed consumables - when coupled with a deep understanding of the method used - allow for a seamless translation of that information into your automation platform. Come see just how Waters is achieving this with the LC-MS workflows for protein quantification and released N-glycan analysis. Automation vendors, Andrew Alliance and Hamilton, are showcased in the Waters hospitality suite. Representatives will be there to answer any questions you have.

Presenter: Jennifer Fournier, Director of Product Marketing, Chemistry Group, Waters Corporation

Register online at: www.waters.com/AutomationBreakfast

Space is limited, pre-registration is required.

Start ASMS off
on the right foot



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GET AUTOMATED: ACHIEVE YOUR AUTOMATION POTENTIAL FOR COMPLEX LARGE MOLECULE LC-MS WORKFLOWS

Wed June 6th, 2018 7:00 – 8:00 AM

Waters Suite- Hilton San Diego Bayfront

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- Automation vendors, Andrew Alliance and Hamilton, are showcased in the Waters hospitality suite. Representatives will be there to answer questions.
- Presenter: Jennifer Fournier, Director of Product Marketing, Chemistry Group, Waters Corporation
- Register at information desk online at: www.waters.com/AutomationBreakfast

Limited Spaces Remaining !!

Oral Presentation MOC am 9:50 on Multi-Attribute Monitoring Analysis for Process Dev and QC

Streamlining Identification and Monitoring of Critical Quality Attributes in Biopharmaceutical Development and QC

Robert Birdsall, Ximo Zhang, Weibin Chen, and Ying Qing Yu

- AS-FTN
- Sample temp 10 °C
- Binary Pump
- Mixer volume = 380 uL
- MP A = H₂O, 0.1% FA
- MP B = MeCN, 0.1% FA



CM-A (column heater)

- Column temp 60 °C
- Column CSH 2.1 x 100 mm, 1.7 um

TUV

- 10mm analytical flow cell
- Sampling rate 10 Hz, $\lambda = 214\text{nm}$

QDa

- SIR acquisition
- 5 Hz sampling rate

Selected-Ion-Recording (SIR) for PQA/CQA acquisition

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Charge states to be monitored

Empower instrument control panel:

The screenshot shows the Empower software interface for the QDa™ Detector. The main window displays the 'QDa™ Detector' configuration with various tabs like 'Functions' and 'Events'. A table below shows the SIR (Selected Ion Recording) events for a 50.00 min run. The table includes columns for event number, polarity, start and stop times, and the number of masses recorded. The 'Run Time' is 50.00 min. Below the table, there are controls for 'Sampling Rate' (Target 5 points/sec, Actual 5.0 points/sec), 'Gain' (1), 'Pos' (1.5 kV), 'Capillary' (0.8 kV), and 'Probe' (600 °C). The interface is ready for use.

Event #	Polarity	Start (min)	Stop (min)	Run Time: 50.00 min	
1	SIR	Positive	0.00	11.70	SIR of 7 masses
2	SIR	Positive	0.00	11.70	SIR of 2 masses
3	SIR	Positive	11.71	15.70	SIR of 2 masses
4	SIR	Positive	11.71	15.70	SIR of 2 masses
5	SIR	Positive	15.71	17.70	SIR of 2 masses
6	SIR	Positive	15.71	17.70	SIR of 2 masses
7	SIR	Positive	17.71	20.70	SIR of 2 masses
8	SIR	Positive	17.71	20.70	SIR of 2 masses
9	SIR	Positive	20.71	25.70	SIR of 2 masses
10	SIR	Positive	20.71	25.70	SIR of 2 masses
11	SIR	Positive	25.71	33.70	SIR of 2 masses
12	SIR	Positive	25.71	33.70	SIR of 2 masses
13	SIR	Positive	33.71	38.70	SIR of 2 masses
14	SIR	Positive	33.71	38.70	SIR of 2 masses
15	SIR	Positive	38.71	50.00	SIR of 5 masses
16	SIR	Positive	38.71	50.00	SIR of 5 masses

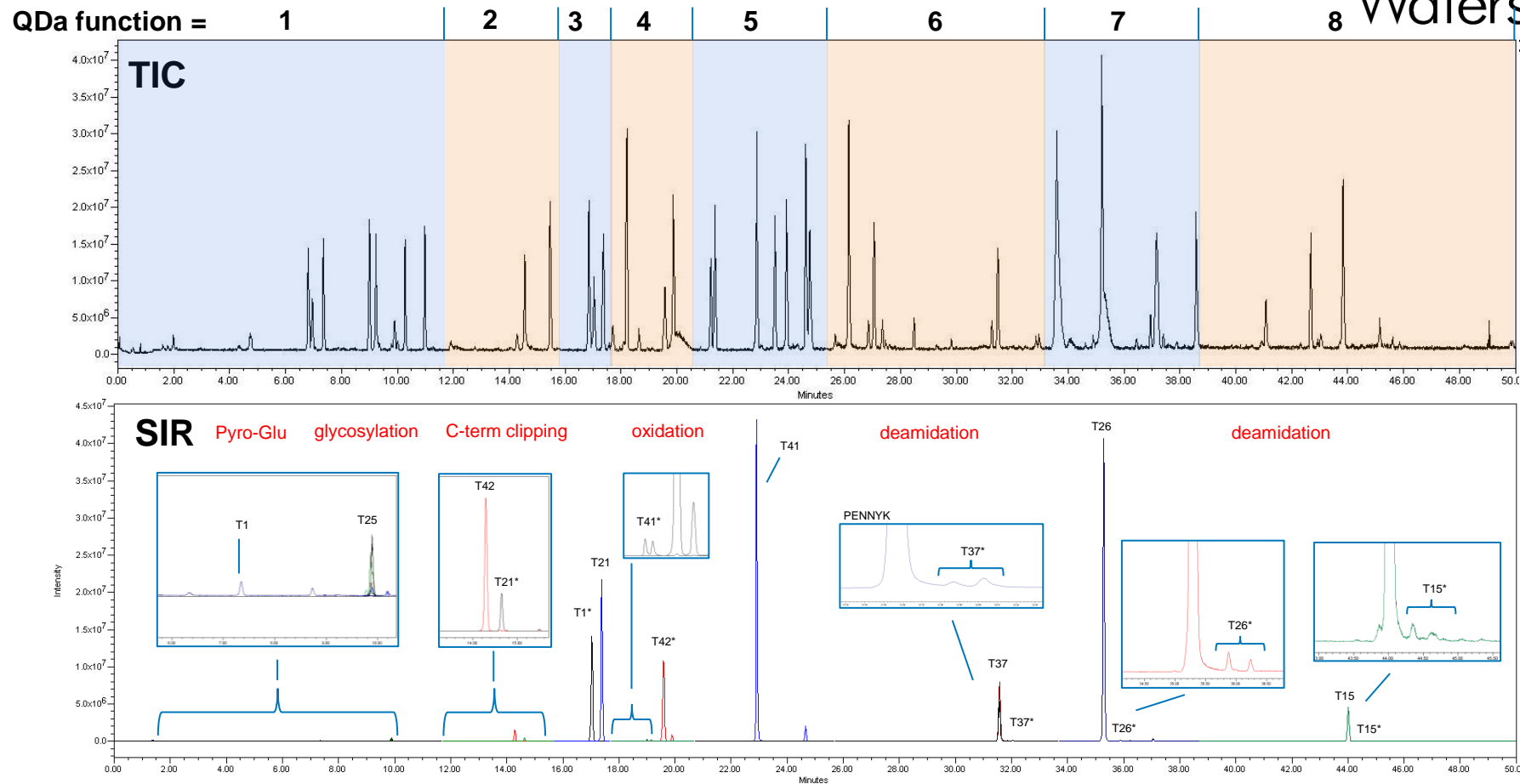
QDa Function table:

QDa	Frag	Modification	m/z
1	T25	Glycopeptides	FA2-879.2, FA2G1-933.2, FA2G2-987.3, A2-830.5, M5-803.1, FA1G1-865.5, FA1G1Gc1-726.2
2	T1	Pyro-Glu (native)	616.7, 308.9
3	T21	oxidized	852.0, 426.5
4	T42	C-term Lysine (native)	788.9, 395.0
5	T21	native	836.0, 418.5
6	T1	Pyro-Glu (mod)	599.7, 300.4
7	T42	C-term Lysine clip	660.7, 330.9
8	T41	oxidized	705.5, 564.6
9	T41	Native	836.0, 418.5
11	T37	PENNYK (deamidated)	849.6, 637.4
12	T37	PENNYK (Native)	849.2, 637.2
13	T26	Deamidated	905.5, 604.0
14	T26	Native	905.1, 603.7
15	T15	Deamidated	1120.6, 960.6, 840.7, 747.4, 672.7
16	T15	Native	1120.4, 960.5, 840.6, 747.3, 672.6

Increased throughput with MS-based workflows

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A Robust Methodology for Peptide Multi-Attribute Monitoring (MAM) Analysis

POSTER **Th583** Bridging the analytical workflows for characterizing and monitoring product quality attributes (PQAs) of biotherapeutics by a common data acquisition mode

Relative quantitation (Mod%) using MS^E across labs

Modification	Sites	Lab1		Lab2		
		Mod% (Mean)	RSD%	Mod% (Mean)	RSD%	
Oxidation	HC: DTLM(255)ISR	2.00	3.6	3.58	4.7	
	HC: DM(125)IFNFYFDVWGQGTTVTVSSASTK	2.24	3.0	5.63	3.8	
	LC: DIQM(4)TQSPSTLSASVGDR	0.90	2.28	1.39	3.3	
Deamidation	HC: FNWYVDGVEVHN(290)AK	0.17	1.3	0.19	2.9	
	HC: GFYPSDIAVEWESNGQPEN(392)NYK	3.35	3.6	3.08	3.11	
	HC: N(364)QVSLTCLVK	1.32	0.61	0.92	1.14	
Glycosylation	EEQYN(300)STYR	Aglycosylated	0.65	1.64	0.67	0.72
		G0F	42.05	2.28	39.26	0.45
		G1F	46.46	2.29	48.18	0.25
		G2F	9.95	0.94	11.02	0.70
		Man5	0.89	0.11	0.87	0.53

Comparable performance of MS and MS^E Data Acquisition

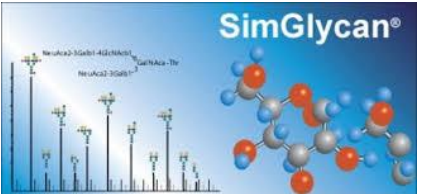
Modification	Sites	Modification%			
		MS only		MS ^E	
		Control	Degraded	Control	Degraded
Oxidation	HC: DTLM(255)ISR	1.36	5.14	1.27	4.87
	HC: WQQGNVFCSSVM(431)HEALHNHYTQK	1.19	2.56	0.86	2.45
	LC: DIQM(4)TQSPSTLSASVGDR	0.70	1.04	0.72	1.01
Deamidation	HC: WQQGN(424)VFSCVMHEALHN(437)HYTQK	0.13	1.11	0.04	1.05
	HC: GFYPSDIAVEWESN(387)GQPEN(392)N(393)YK	0.08	0.17	0.03	0.25
	HC: N(364)QVSLTCLVK	2.59	12.82	2.59	12.75
	HC: GFYPSDIAVEWESN(387)GQPEN(392)N(393)YK	2.16	32.36	2.19	32.08
	HC: N(364)QVSLTCLVK	0.15	1.12	0.15	1.12
		0.80	0.81	0.90	0.86

Harmonizing Workflows: UNIFI acquired biopharm data can be analyzed using the most common industry software tools

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ProSight PTM



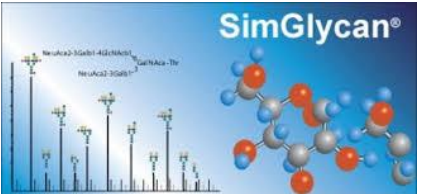
Harmonizing Workflows: UNIFI acquired biopharm data can be analyzed using the most common industry software tools

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ProSight Lite



Now you can access the suite of Protein Metrics capabilities with UNIFI acquired data



- Intact and Subunit Analysis
- PTM Analysis
- HCP Analysis
- Disulfide Bond Analysis
- Sequence Variant Analysis
- MAM Analysis
- Peptide Mapping
- Glycosylation profiling
- Etc...



WILL YOUR BIOPHARMA DATA LIVE IN CHAOS OR HARMONY?

Visit www.waters.com/tamethechaos to learn more.

(Play Video)

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