Waters lunchtime seminar, WCBP 2025

Partnering with Waters for Biopharmaceuticals analyses: Innovations supporting product development and release

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Waters™

Life saving therapies

Breast cancer patient survival after 4 years[1]

85%
with immunotherapy

67%
only chemotherapy

Crohn's disease patient remission after 1 year[2]

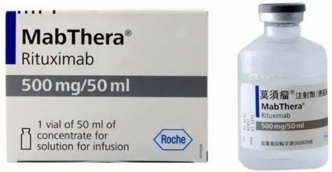
36%
with immunotherapy

12%
with placebo

Multiple Sclerosis patient free of lesion after 1 year[3]

93%
with immunotherapy

19%
with placebo



Sources: [1] Rodmon EH et al. N Engl J Med. 2005 ; Slamon DJ et al., N Engl J Med. 2001 ; [2] www.humira.com/crohns/about-humira/results-with-humira ; [3] Yamout et al., J. Imm. Res. 2018

Developing biopharmaceuticals is a tough business

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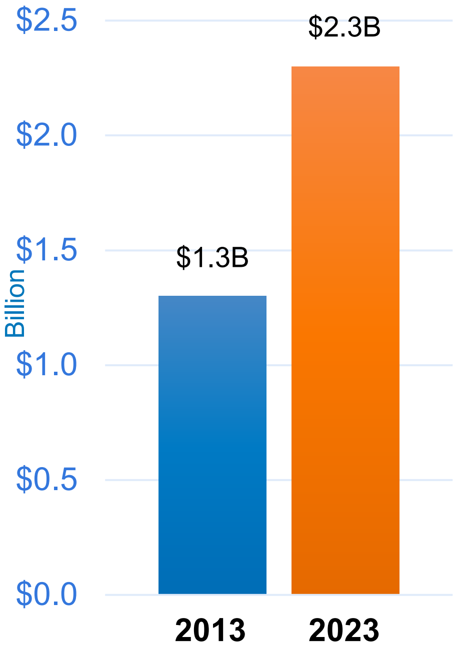
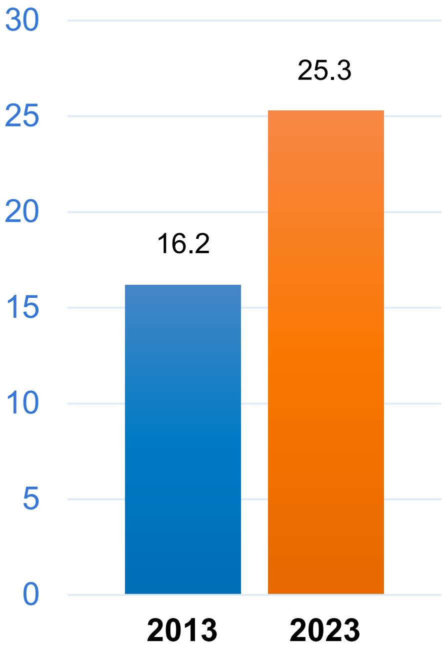
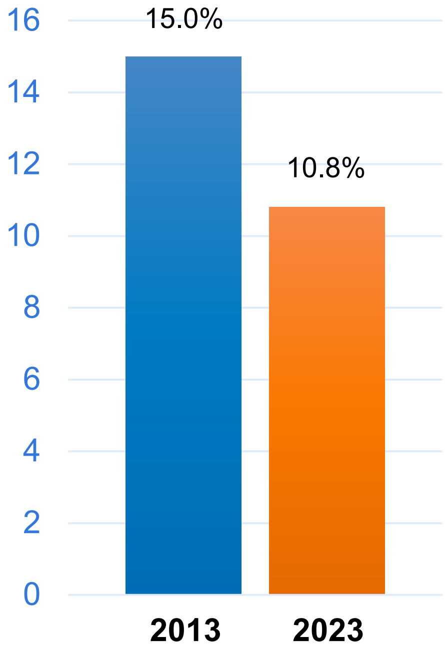
COST Average R&D cost to develop a therapy (Discovery to launch)



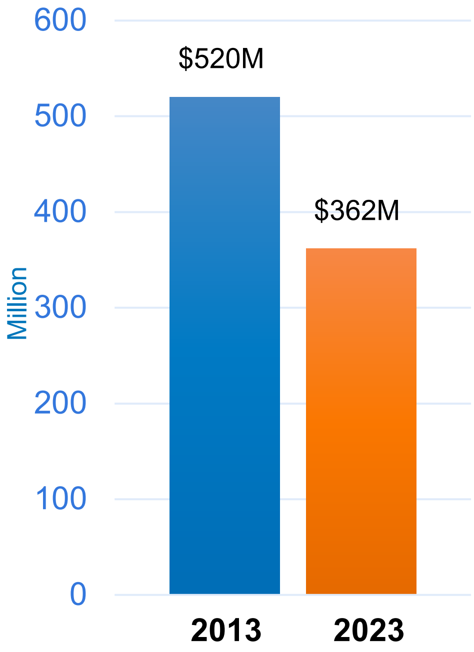
TIME Enrolment duration for industry sponsored clinical trials (months)



RISK Composite success rates (from IND to approval)



VALUE Average peak product sales (Per pipeline asset)



More efficient working practices are needed to mitigate these headwinds

Data source: Atlas Ventures year in review 2024, B Booth

How to foster access to life saving therapies to all those in need?

Faster and cost-effective development and production of biotherapeutics

Waters™

3 key industry drivers

COST

1 Confidence

Deliver the right results, first time. Meet requirements from Discovery, to Development & QA/QC

PRODUCTIVITY

2 Consistency

Continued technology evolution for the most effective & reliable separations & detection

COMPLIANCE

3 Connectivity

Address critical regulatory needs with seamless, streamlined LCMS & Light Scattering workflows

How to foster access to life saving therapies to all those in need?

Faster and cost-effective development and production of biotherapeutics

3 key industry drivers

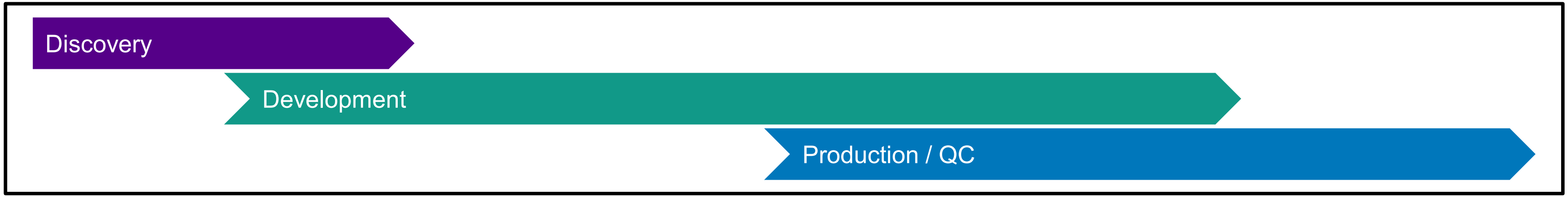
COST	PRODUCTIVITY	COMPLIANCE
$		
1 **Confidence**	2 **Consistency**	3 **Connectivity**
Deliver the right results, first time. Meet requirements from Discovery, to Development & QA/QC	Continued technology evolution for the most effective & reliable separations & detection	Address critical regulatory needs with seamless, streamlined LCMS & Light Scattering workflows

1

Confidence

Instrument solutions deliver results at point-of-need and meet specific requirements across the product lifecycle

Waters™



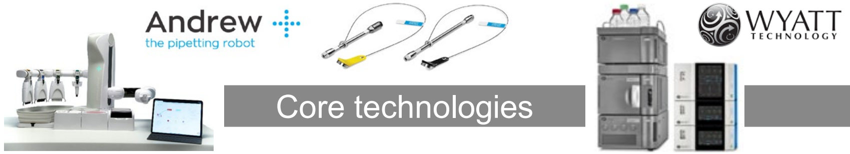
Non-regulated → **Regulated / GxP**

Little product knowledge: **Characterization**

- Leading edge analytical instruments
- Expert level

Good product knowledge: **Product and process monitoring / QC**

Accessible, rugged and robust -
Routine and throughput -

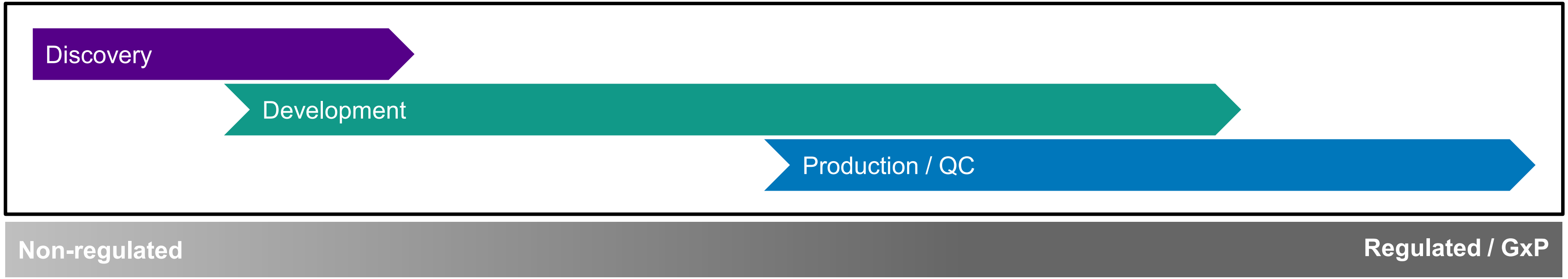


Core technologies

1

Confidence

2 New instrument solutions deliver results at point-of-need and meet specific requirements across the product lifecycle


Discovery

Development

Production / QC

Non-regulated

Regulated / GxP


Xevo™ | MRT

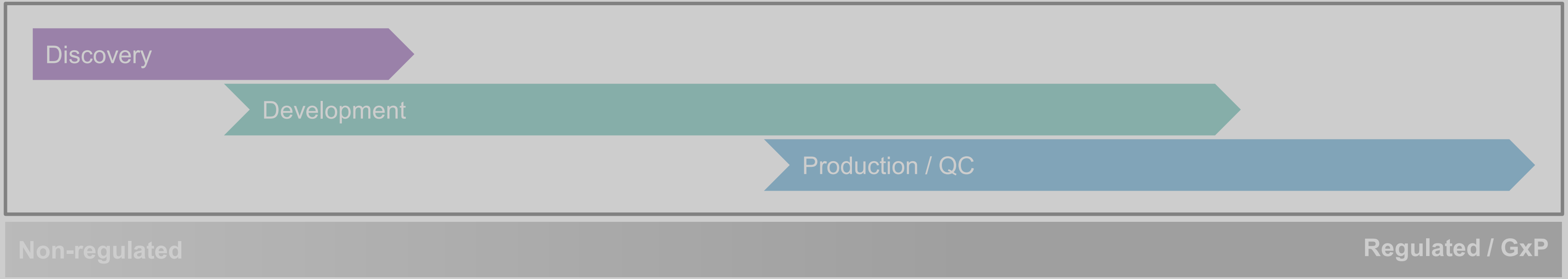

Acquity™ QDa II

1

Confidence

2 New instrument solutions deliver results at point-of-need and meet specific requirements across the product lifecycle

Waters™



Xevo™ | MRT

Acquity™ QDa II

1

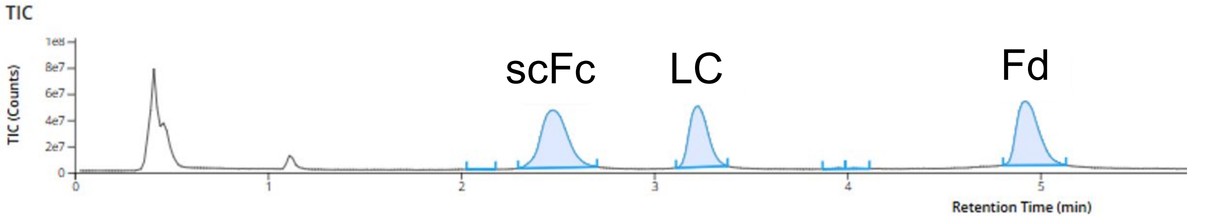
Confidence

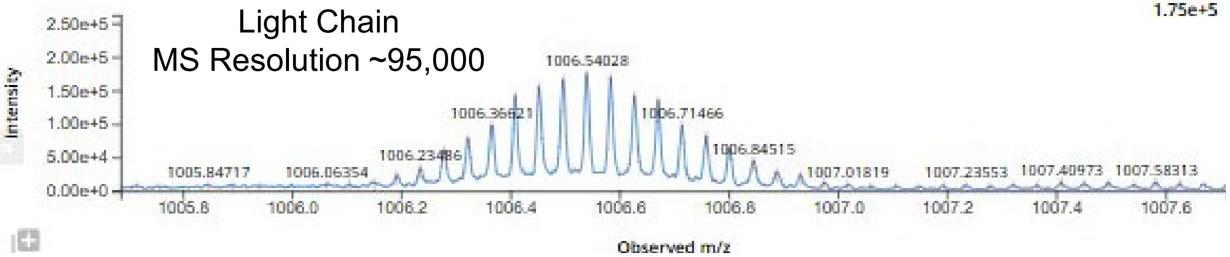
Xevo MRT: High sensitivity multi reflecting time-of-flight MS

Increased assurance of reproducibility and accurate assignments

Exceptional performance & speed without compromise

Resolution	Speed	Sensitivity	Mass Accuracy
100K (FWHM)	50 Hz MS	MS/MS	<500 ppb
@ *m/z* 956	100 Hz MS/MS	x10 increase*	RMS





Rep.	Cmpnt	Obs. mass (Da)	Mass error (ppm)	Cmpnt (Rel %)
1	Fd'	25688.8476	-0.58	76.78
2	Fd'	25688.8616	-0.04	77.31
3	Fd'	25688.8503	-0.48	77.69
4	Fd'	25688.8575	-0.20	77.27
5	Fd'	25688.8569	-0.22	75.10
6	Fd'	25688.8538	-0.34	77.03
7	Fd'	25688.8545	-0.31	75.86
8	Fd'	25688.8584	-0.16	74.97
9	Fd'	25688.8711	0.33	77.26
10	Fd'	25688.8574	-0.20	77.57
Avg		25688.86	**-0.22**	76.69
RSD		0.00		1.31
STD.dev		0.01	**0.25**	1.01

Reliable insight and confident data in early development can inform project decisions when change costs are low

Sample: Waters mAb Subunit Standard

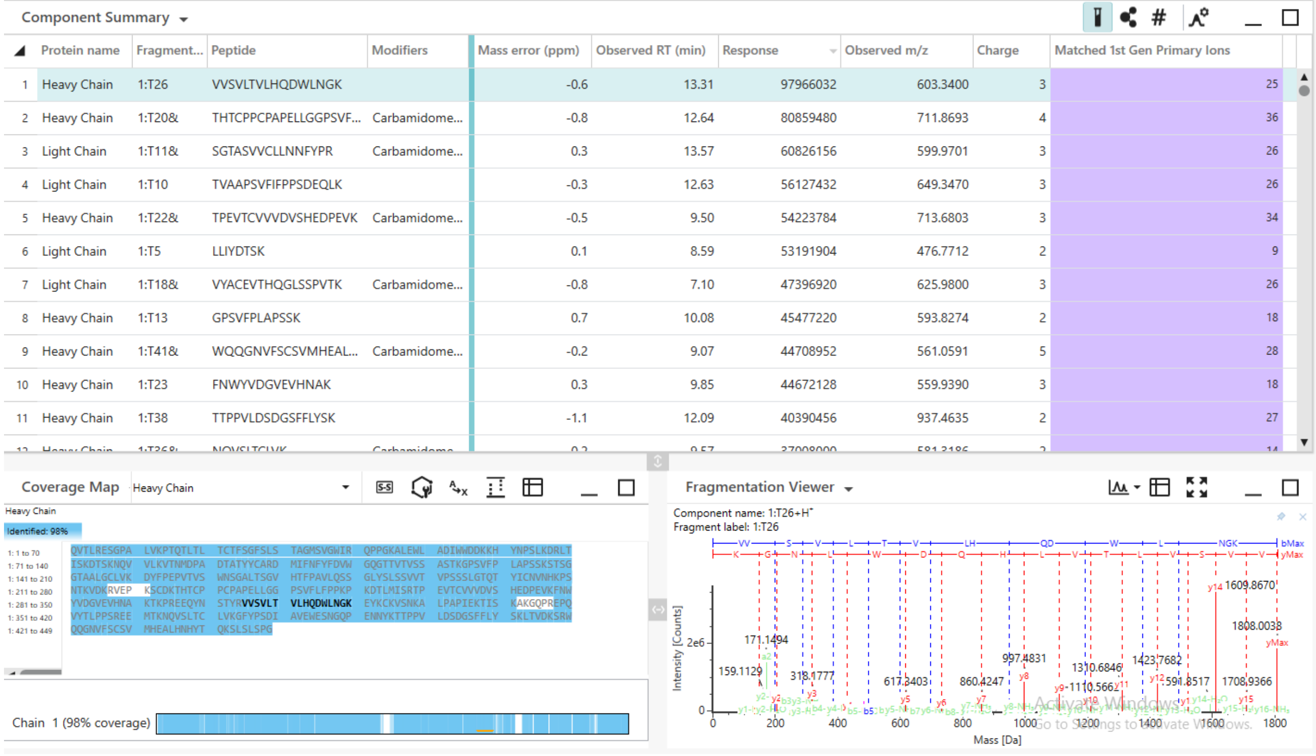
*compared to Xevo G3 Qtof @30 Hz

1
Confidence

Xevo MRT: peptide mapping data

Increased assurance of performance, reproducibility and assignments

Waters™



Component Summary

	Protein name	Fragment...	Peptide	Modifiers	Mass error (ppm)	Observed RT (min)	Response	Observed m/z	Charge	Matched 1st Gen Primary Ions
1	Heavy Chain	1:T26	VVSVLTVLHQDWLNGK		-0.6	13.31	97966032	603.3400	3	25
2	Heavy Chain	1:T20&	THTCPPCPAPELLGGPSVF...	Carbamidome...	-0.8	12.64	80859480	711.8693	4	36
3	Light Chain	1:T11&	SGTASVVCLLNNFYPR	Carbamidome...	0.3	13.57	60826156	599.9701	3	26
4	Light Chain	1:T10	TVAAPSVFIFPPSDEQLK		-0.3	12.63	56127432	649.3470	3	26
5	Heavy Chain	1:T22&	TPEVTCVVVDVSHEDPEVK	Carbamidome...	-0.5	9.50	54223784	713.6803	3	34
6	Light Chain	1:T5	LLIYDTSK		0.1	8.59	53191904	476.7712	2	9
7	Light Chain	1:T18&	VYACEVTHQGLSSPVTK	Carbamidome...	-0.8	7.10	47396920	625.9800	3	26
8	Heavy Chain	1:T13	GPSVFPLAPSSK		0.7	10.08	45477220	593.8274	2	18
9	Heavy Chain	1:T41&	WQQGNVFSCSVMHEAL...	Carbamidome...	-0.2	9.07	44708952	561.0591	5	28
10	Heavy Chain	1:T23	FNWYVDGVEVHNAK		0.3	9.85	44672128	559.9390	3	18
11	Heavy Chain	1:T38	TTPPVLDSDGSFFLYSK		-1.1	12.09	40390456	937.4635	2	27

Coverage Map Heavy Chain

Heavy Chain

Identified: 98%

1: 1 to 70 QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TAGMSVGWIR QPPGKALEWL ADIWWDDKKH YNPSLKDRLT
1: 71 to 140 ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG
1: 141 to 210 GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS
1: 211 to 280 NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
1: 281 to 350 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
1: 351 to 420 VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
1: 421 to 449 QQGNVFSCSV MHEALHNHYT QKSLSLSPG

Chain 1 (98% coverage)

Fragmentation Viewer

Component name: 1:T26+H⁺
Fragment label: 1:T26

98% sequence coverage

Peptides assigned using max 2 ppm mass error

Linearity: 2000 ng - 0.02 ng on column

High sensitivity & linear MS response enables confident tracking of low-level CQA's across samples

Sample: Waters mAb tryptic digest standard [Part 186009126]

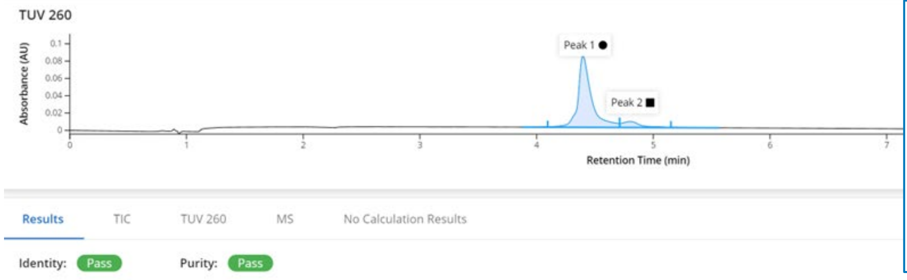
1

Confidence

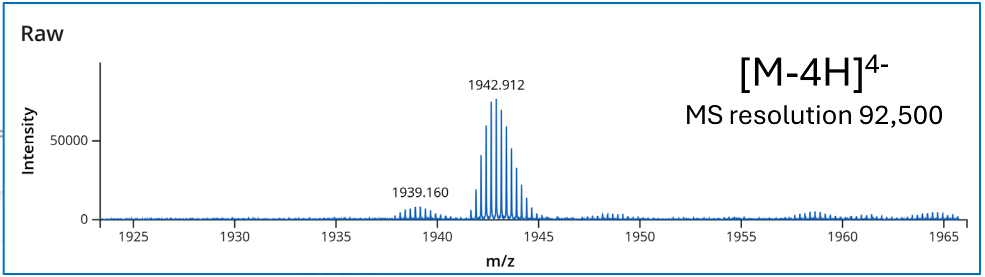
Xevo MRT: synthetic oligonucleotide data

Increased assurance of performance, reproducibility and assignments

Waters™



	Component	Observed mass (Da)	Expected mass (Da)	Mass error (ppm)	Observed UV RT (mins)	LC amount (%)	MS amount (%)	MSxLC (%) ▼
2	7,770.6809	7,770.687	7,770.681	0.8	4.40	89.8	70.0	62.9
3	7770.6809 PS to PO	7,754.673	7,754.704	-3.9	4.40	89.8	5.6	5.1
4	7770.6809 CNET	7,823.703	7,823.707	-0.6	4.80	10.2	42.3	4.3
5	7770.6809 +TEA	7,871.768	7,871.801	-4.3	4.40	89.8	4.4	4.0
6	7770.6809 +TEA(2)	7,972.897	7,972.922	-3.1	4.40	89.8	1.3	1.2
7	7770.6809 +TEA,Oxidation M	7,887.771	7,887.796	-3.2	4.40	89.8	0.7	0.6
8	7770.6809 +TEA,-CH3	7,856.786	7,856.778	1	4.40	89.8	0.6	0.5
9	7770.6809 +Na,+CH3	7,807.691	7,807.686	0.5	4.80	10.2	2.7	0.3
10	7770.6809 +TEA,CNET	7,924.805	7,924.828	-3	4.80	10.2	2.7	0.3
11	7770.6809 -CH3	7,755.668	7,755.657	1.3	4.80	10.2	2.4	0.2



$[M-4H]^{4-}$

MS resolution 92,500

INTACT Mass

Identity confirmed within a mass error of 1 ppm

Efficient MS^E fragmentation confirms sequence

Automatically confirm FLP sequence and confirm the presence and identity of impurities

Sample: 25'mer phosphorothioate antisense oligonucleotide (GEM 91)

1

Confidence

2 New instrument solutions deliver results at point-of-need and meet specific requirements across the product lifecycle

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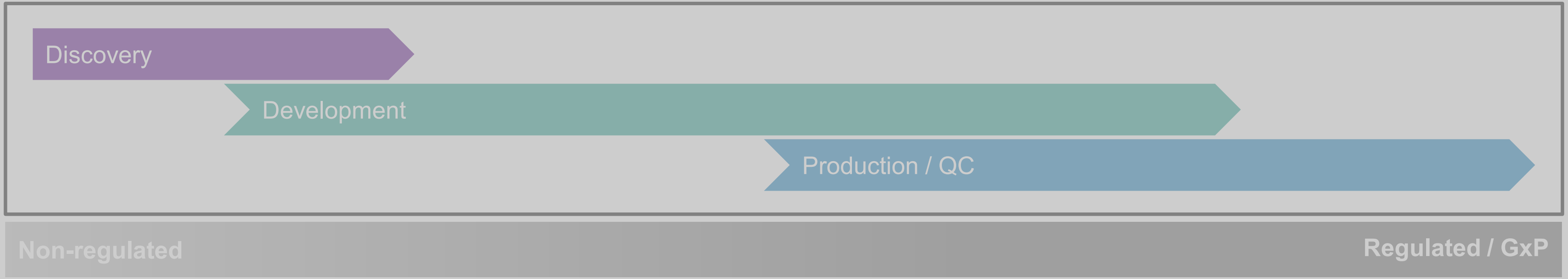

Discovery
Development
Production / QC
Non-regulated
Regulated / GxP


Xevo™ | MRT


Acquity™ QDa II

1

Confidence

Evolution of the ACQUITY QDa™ II

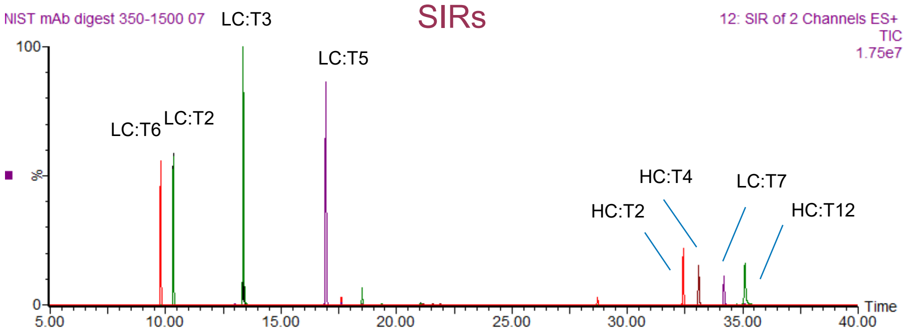
Smart Innovation for a solution widely deployed across development, manufacturing, and quality control

Extend dynamic range below UV baseline

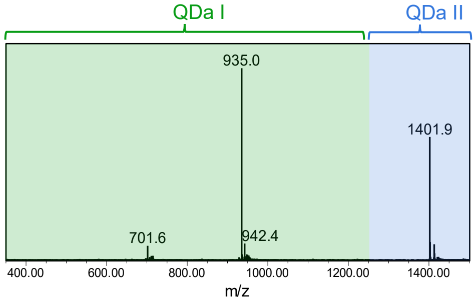
Follow molecules with no chromophores

Existing QDa methods directly transfer

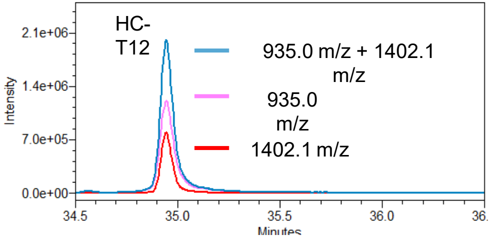
- ✓ Extended mass range to 1500 m/z
- ✓ Improved repeatability and robustness
- ✓ Up to 60% Reduction in Service Time
- ✓ Up to 70% Energy Savings vs. SQ systems



Wide Mass Range



+35% increase in MS response



All CDR peptides detected for successful ID test. Extended mass range enables increased MS response

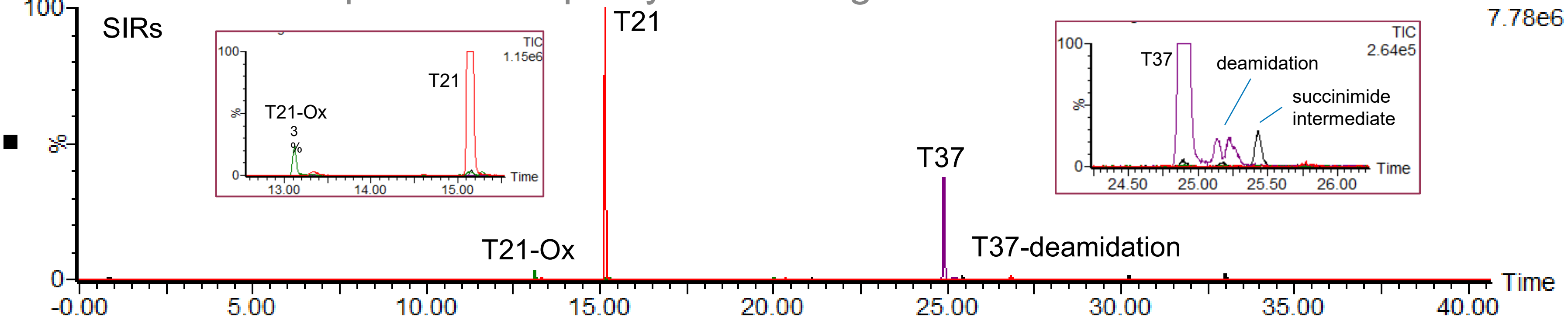
Sample: NIST mAb digest

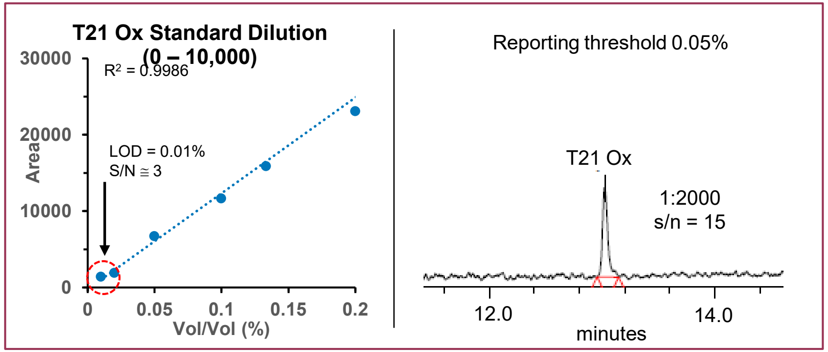
1

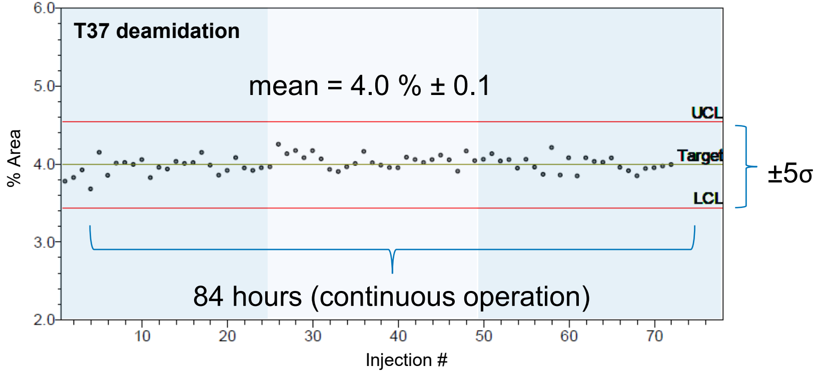
Confidence

Application example: monoclonal antibodies

Reproducible and consistent response at low level is required for impurity monitoring







Sensitivity and consistent peak area shows suitability for quantitative assays that demand precision and accuracy

Sample: NIST mAb digest

How to foster access to life saving therapies to all those in need?

Faster and cost-effective development and production of biotherapeutics

3 key industry drivers

COST	PRODUCTIVITY	COMPLIANCE
1 Confidence	**2 Consistency**	**3 Connectivity**
Deliver the right results, first time. Meet requirements from Discovery, to Development & QA/QC	Continued technology evolution for the most effective & reliable separations & detection	Address critical regulatory needs with seamless, streamlined LCMS & Light Scattering workflows

2

Consistency

Continued technology evolution

MaxPeak Premier family expands: Alliance™ iS Bio HPLC System

Waters™

Eliminate unpredictability of analyte losses due to metal interactions

Ultimate performance for more consistency & confidence in analytical data

- ✓ Increase sensitivity & sharpen peak shapes
- ✓ Minimize variability of results
- ✓ Reduce passivation & conditioning times

MAXPEAK PREMIER **LC technology** Acquity PREMIER Arc™ PREMIER alliance iS bio

Useability and transferability

- Reduce human errors up to 40%
- Up to 3X faster method migration and operator training

Improved, robust performance

- Lower carryover
- Increased injection precision
- Inert for Bioseparations

A routine system that introduces risk mitigation for enhanced productivity

2

Consistency

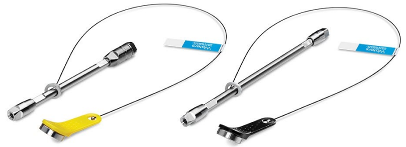
Continued technology evolution

Transforming SEC Analyses for Peptides & Proteins

Waters™

MAXPEAK PREMIER

Column hardware



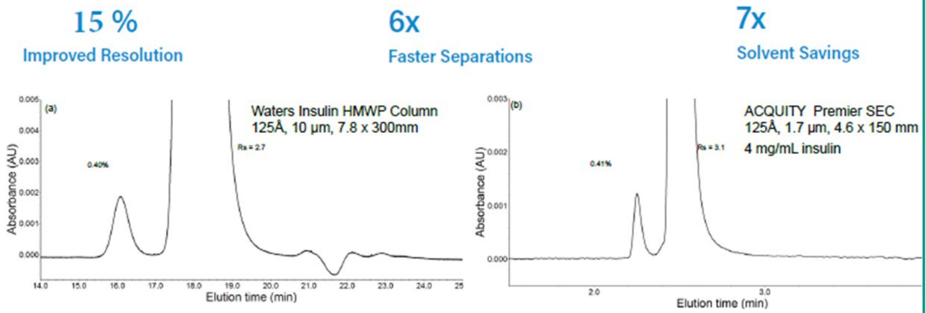
US and International Regulatory Agencies require submission of precise and accurate size-variant separations of biotherapeutic peptides (e.g,. GLP-1 receptor agonists) and small protein drugs

Why 125Å SEC MaxPeak Premier Columns?

Compared to USP Listed SEC Column for insulin analyses:

- **+15% Resolution** on HMWS vs Insulin Monomers
- **6x Faster** than Current HPLC-based Methods
- **7x** Organic Solvent **Savings**
- **Unmatched "Out of Box" Performance** for enhanced assurance for reliable data

1.7 and 2.5 µm particles for seamless scalability and method transfer



Consistent SEC separations help boost productivity & minimize risk on regulatory submissions & release testing

2

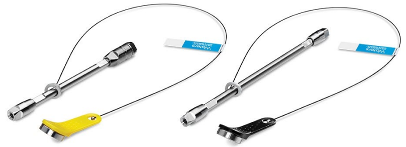
Consistency

Continued technology evolution

Advances for peptide and oligonucleotide purification

MAXPEAK PREMIER

Column hardware



Why MaxPeak Premier purification columns?

- **Predict Scale-Up:** Transition seamlessly from scouting methods to bench scale preparative columns, ensuring consistent scale up results
- **Protect Sensitive Compounds: Inert** preparative columns provide extra assurance for recovering metal-sensitive compounds such as peptides and oligos from complex mixtures
- **Boost Isolation Efficiency and Productivity:** Better resolution and/or reduced preparative separation times with sub-5 um sorbent packing

Enhanced productivity though seamless, scalable purification for challenging therapeutic compounds

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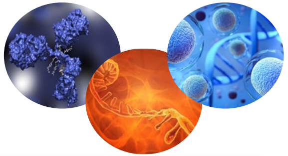
Address critical regulatory needs with seamless, streamlined LCMS & Light Scattering workflows

3

Connectivity

Evolution of Wyatt technology into Waters portfolio

Bringing connectivity and compliance for biologic modalities



✓ Adopt adjacent technology

The 2023 **acquisition of Wyatt Technology** has allowed Waters to build synergies to better support our customers

Digital connectivity through HPLC CONNECT with ASTRA™ software and HPLC + UPLC instruments

✓ Keep up with complexity

Innovation of biopharmaceutical tools for new modality analyses

ZetaStar DLS/ELS/SLS Instrument + Arc HPLC

GTxResolve Column Chemistries, 1000A, 125A verified by MALS and **application development**

Future: Focus on regulatory needs

Empower™

Demand for deeper characterization increasing with complexity

Migrate more analytical workflows into Empower to strengthen data integrity position

Streamlined analysis, data collaboration, enhanced data management

Deeply understand growing complexity of biologics with customers, delivering next generation connected, compliance ready solutions

3

Connectivity

Digested oligo mapping: enabling routine analysis

Simple workflow tools for confident sequence assignment

Digest sample with enzyme

Perform LC-MS analysis

Generate *in silico* digest products

Review accurate mass data

Calculate sequence coverage

*MS^e characterization with CONFIRM **Sequence** or third-party data connectivity

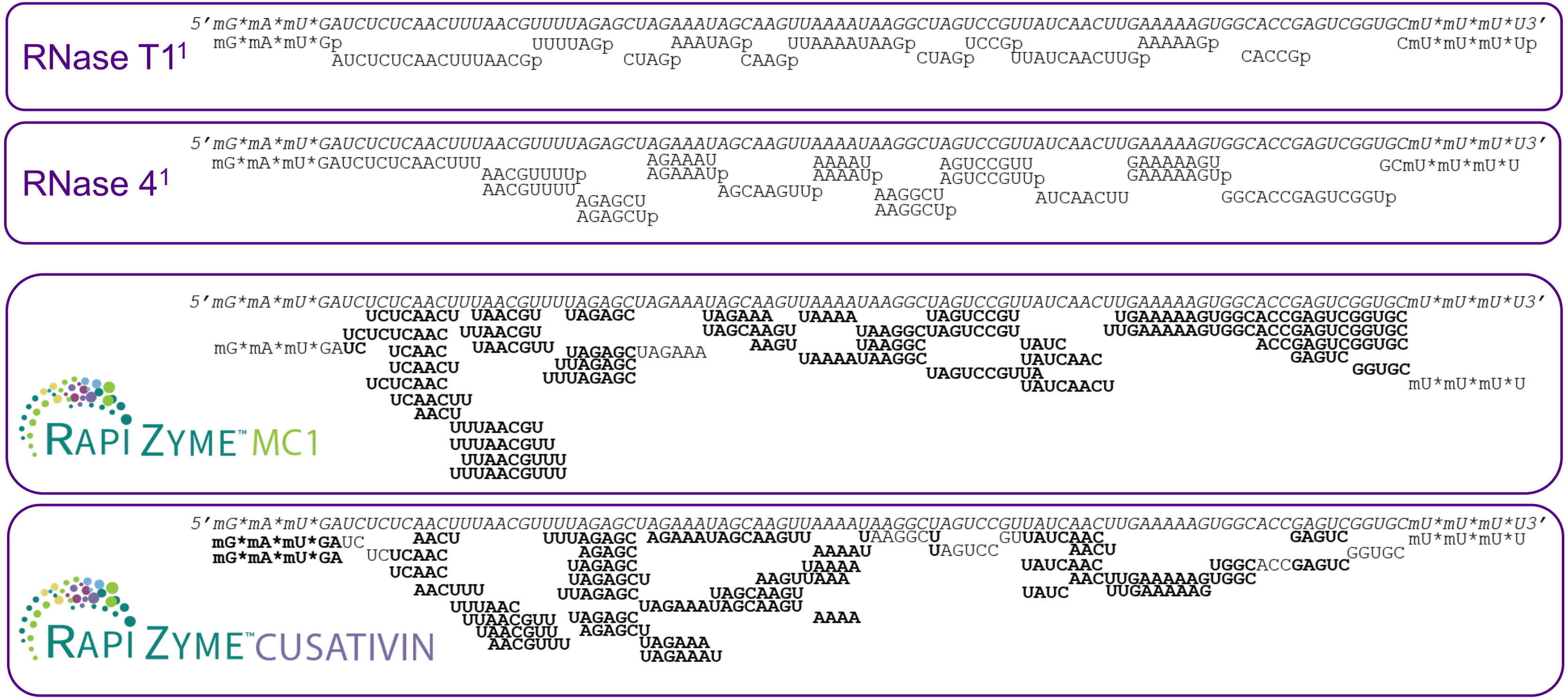
Compliance – ready workflows for monitoring of RNA sequence through development and manufacture

3

Connectivity

New enzymes for flexible and optimized RNA digestion

sgRNA digestion patterns for common RNases



RNase T1[1]

*5′mG*mA*mU*GAUCUCUCAACUUUAACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U3′*

mG*mA*mU*Gp UUUUAGp AAAUAGp UUAAAAUAAGp UCCGp AAAAAGp CmU*mU*mU*Up

AUCUCUCAACUUUAACGp CUAGp CAAGp CUAGp UUAUCAACUUGp CACCGp

RNase 4[1]

*5′mG*mA*mU*GAUCUCUCAACUUUAACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U3′*

mG*mA*mU*GAUCUCUCAACUUU AGAAAU AAAAU AGUCCGUU GAAAAAGU GCmU*mU*mU*U

AACGUUUUp AGAAAUp AAAAUp AGUCCGUUp GAAAAAGUp

AACGUUUU AGCAAGUUp AAGGCU AUCAACUU GGCACCGAGUCGGUp

AGAGCU AAGGCUp

AGAGCUp

RAPIZYME™ MC1

*5′mG*mA*mU*GAUCUCUCAACUUUAACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U3′*

UCUCAACU UAACGU UAGAGC UAGAAA UAAAA UAGUCCGU UGAAAAAGUGGCACCGAGUCGGUGC

UCUCUCAAC UUAACGU UAGCAAGU UAAGGCUAGUCCGU UUGAAAAAGUGGCACCGAGUCGGUGC

mG*mA*mU*GA**UC** **UCAAC UAACGUU** **UAGAGC**UAGAAA **AAGU UAAGGC UAUC ACCGAGUCGGUGC**

UCAACU UUAGAGC UAAAAUAAGGC UAUCAAC GAGUC

UCUCAAC UUUAGAGC UAGUCCGUUA GGUGC

UCAACUU UAUCAACU mU*mU*mU*U

AACU

UUUAACGU

UUUAACGUU

UUAACGUUU

UUUAACGUUU

RAPIZYME™ CUSATIVIN

*5′mG*mA*mU*GAUCUCUCAACUUUAACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U3′*

mG*mA*mU*GAUC **AACU UUUAGAGC AGAAAUAGCAAGUU** **U**AAGGC**U** GU**UAUCAAC** **GAGUC** mU*mU*mU*U

mG*mA*mU*GA UC**UCAAC** **AGAGC AAAAU** **U**AGUCC **AACU** GGUGC

UCAAC UAGAGC UAAAA UAUCAAC **UGGC**ACC**GAGUC**

UAGAGCU AAGUUAAA AACUUGAAAAAGUGGC

AACUUU UUAGAGC UAGCAAGU UAUC UUGAAAAAG

UUUAAC UAGAAAUAGCAAGU

UUAACGUU UAGAGC AAAA

UAACGUU AGAGCU

AACGUUU UAGAAA

UAGAAAU

Longer digestion products with greater sequence overlap and increased redundancy

Longer digestion products reduce ambiguity, allowing differentiation at MS1 level

[1] Digestion product ending in *p* denotes high abundance of linear phosphate form

How to foster access to life saving therapies to all those in need?

Faster and cost-effective development and production of biotherapeutics

Waters™

3 key industry drivers

COST

1 Confidence

Reduce costs with confident decision making

Xevo™ | MRT Acquity™ QDa II

PRODUCTIVITY

2 Consistency

Recuce troubleshooting and investigations

MAXPEAK™ PREMIER alliance™ iS | bio

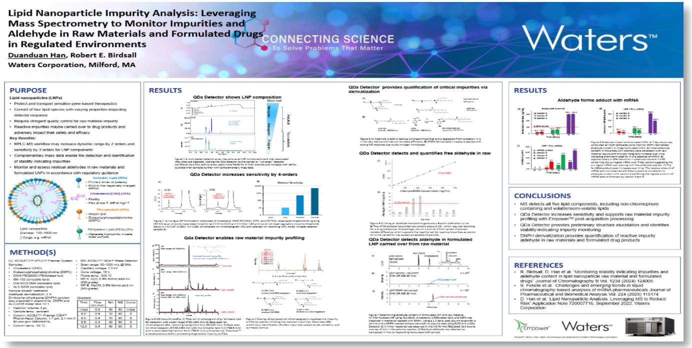
COMPLIANCE

3 Connectivity

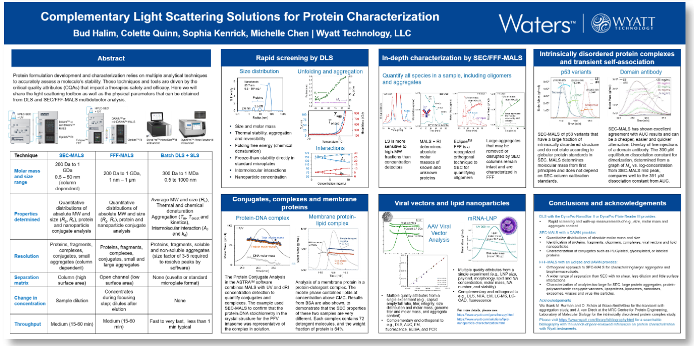
Seamless workflows to meet regulatory needs

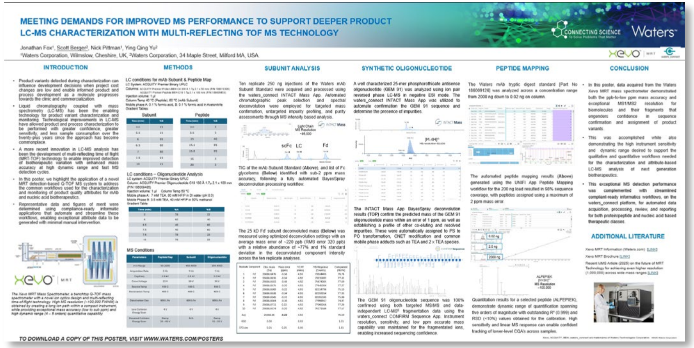
Waters™ | WYATT TECHNOLOGY waters_connect

Waters | Wyatt Technologies at WCBP 2025: Come & see us at **Booth #27**


Lipid Nanoparticle Impurity Analysis: Leveraging Mass Spectrometry to Monitor Impurities and Aldehyde in Raw Materials and Formulated Drugs in Regulated Environments
Duanduan Han, Robert E. Birdsall
Waters Corporation, Milford, MA

CONNECTING SCIENCE
To Solve Problems That Matter


Complementary Light Scattering Solutions for Protein Characterization
Bud Halim, Colette Quinn, Sophia Kenrick, Michelle Chen | Wyatt Technology, LLC


MEETING DEMANDS FOR IMPROVED MS PERFORMANCE TO SUPPORT DEEPER PRODUCT LC-MS CHARACTERIZATION WITH MULTI-REFLECTING TOF MS TECHNOLOGY
Jonathan Fox[1], Scott Berger[2], Nick Pittman[1], Ying Qing Yu[2]

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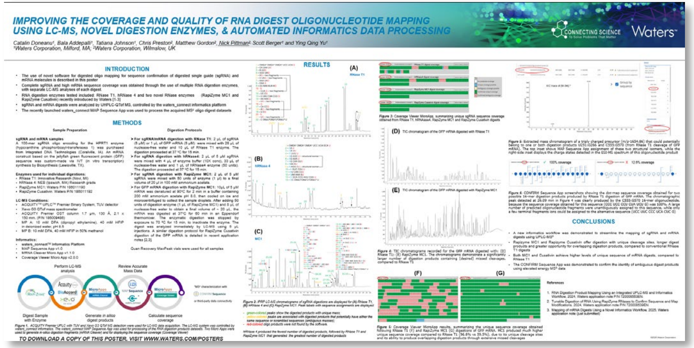
IMPROVING THE COVERAGE AND QUALITY OF RNA DIGEST OLIGONUCLEOTIDE MAPPING USING LC-MS, NOVEL DIGESTION ENZYMES, & AUTOMATED INFORMATICS DATA PROCESSING
Catalin Doneanu[1], Bala Addepalli[1], Tatiana Johnson[1], Chris Preston[1], Matthew Gordon[1], Nick Pittman[1], Scott Berger[1] and Ying Qing Yu[1]

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Virtual Posters:

#51 Lipid Nanoparticle Impurity Analysis

#54 Complementary Light Scattering Solutions for Protein Characterization

#61 Deeper product understanding with novel MRT-TOF technology

#62 sgRNA / mRNA oligo digest mapping

Pre-conference webinar available On Demand:

Light Scattering-Based Solutions for Excipients and Advanced Formulations

Luncheon technical presentation:

Integrating LC, MALS, and MS for Compliance and Connectivity in GMP Labs

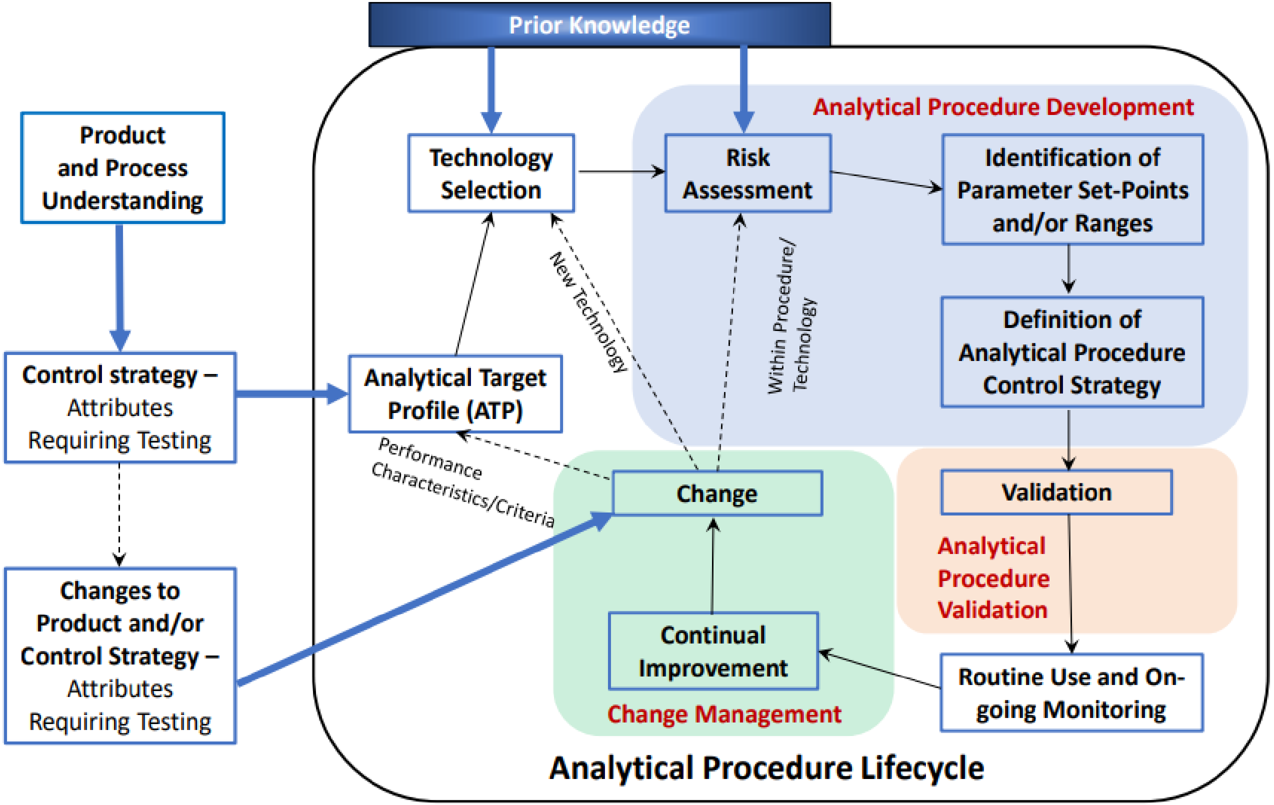
Serena Wu
Senior Analytical Specialist
Regeneron

Integrating LC, MALS, and MS for Compliance and Connectivity in GMP Labs

Serena (Yuwei) Wu

29Jan2025

REGENERON®



Prior Knowledge
Product and Process Understanding
Control strategy – Attributes Requiring Testing
Changes to Product and/or Control Strategy – Attributes Requiring Testing
Technology Selection
Analytical Target Profile (ATP)
Performance Characteristics/Criteria
New Technology
Analytical Procedure Development
Risk Assessment
Within Procedure/ Technology
Identification of Parameter Set-Points and/or Ranges
Definition of Analytical Procedure Control Strategy
Change
Continual Improvement
Change Management
Validation
Analytical Procedure Validation
Routine Use and On-going Monitoring
Analytical Procedure Lifecycle

ICH Q14

Agenda

- Transferring from Astra 6 and Astra 8: SEC-MALS for Reference Standard Characterization
    - Instrument/Software Comparison
    - Data Equivalency Study
- High Resolution Mass Spectrometer: routine release testing
    - Peptide Mapping ID Method
    - Alternative Format ID Method
    - MAM Capabilities

Agenda

- **Transferring from Astra 6 and Astra 8: SEC-MALS for Reference Standard Characterization**
    - **Instrument/Software Comparison**
    - **Data Equivalency Study**
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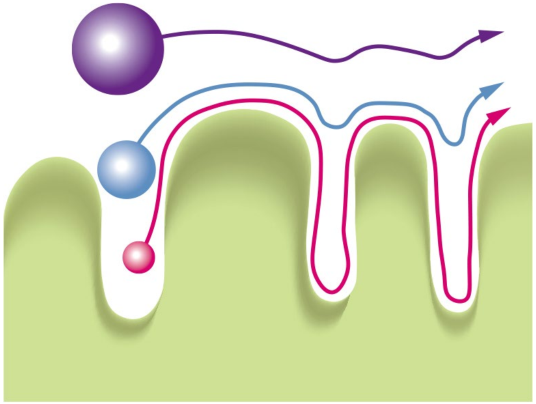
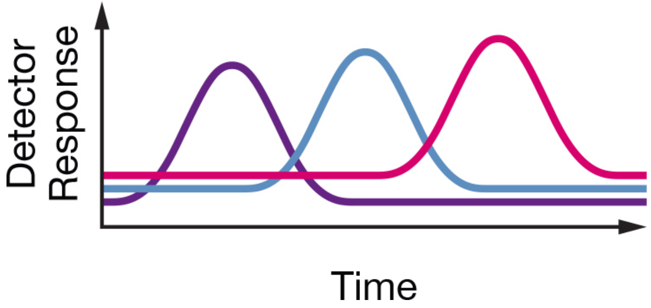
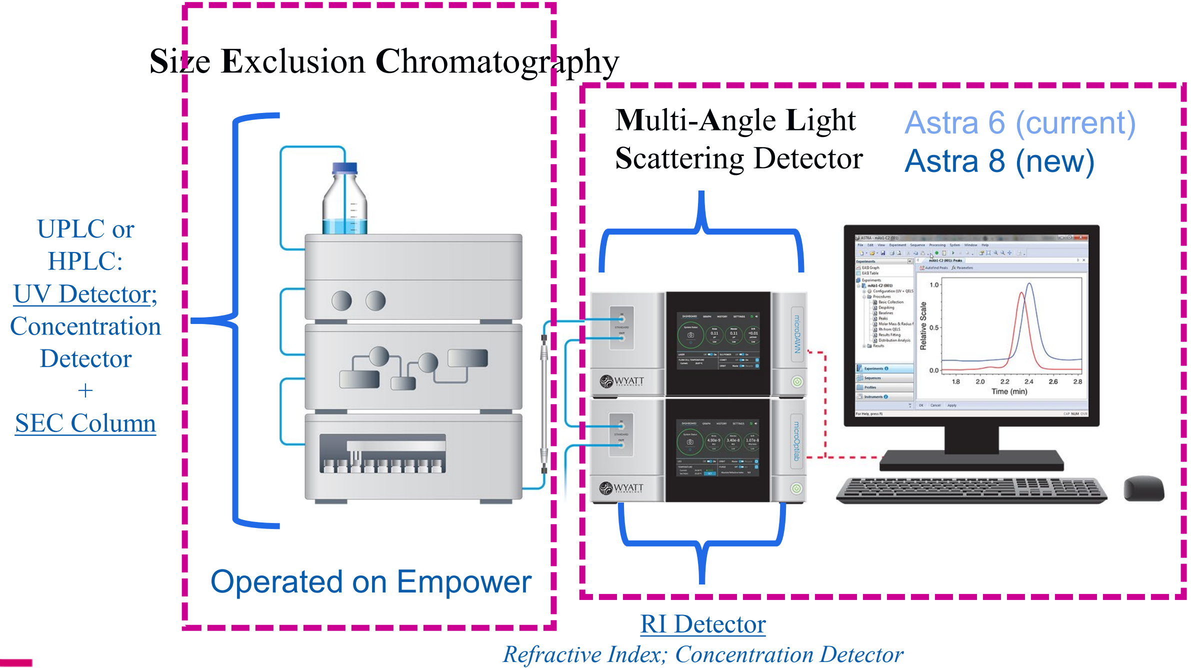
Background information on SEC-MALS

Molar mass of conjugated protein and modifier is measured based on data gathered from multiple detectors after separation





Chromatogram



Size Exclusion Chromatography

UPLC or
HPLC:
UV Detector;
Concentration
Detector
+
SEC Column

Operated on Empower

Multi-**A**ngle **L**ight
Scattering Detector

Astra 6 (current)
Astra 8 (new)

RI Detector
Refractive Index; Concentration Detector

Slides adopted from Wyatt LSU Training

RS Characterization Assay: SEC-MALS

- **What is Reference Standard (RS)?**
  - It is defined as a batch of drug substance or formulated drug substance selected and qualified to ensure accurate, reliable and consistent analytical measurement.
    - "A well-defined home-made ruler to measure the quality of Regeneron products"

- **Why perform extended characterization on RS?**
  - FDA and ICH guidelines requires additional characterization procedures beyond routine testing for reference material.
    - "We need to prove that our ruler is well-defined before measuring everything against it"

Characterization Method	SEC-MALS
Quality attributes	Molar mass of conjugated protein and modifier
Current platform	Astra 6 with Empower FR2
New platform	Astra 8 with Empower FR4

Comparison between Astra 6 vs. Astra 8 platforms

Updated instrument platform brought enhanced data integrity control

	Astra 6	Astra 8
1.	System approaching end-of-life and lacks continued vendor support by the end of 2024.	Continued vendor support under service plan.
2.	Runs on Empower FR2 which is being decommissioned.	Compatible with Empower FR4.
3.	Refractive index of mobile phase needs to be entered manually.	Refractive index model built in with instrument method.
4.	User permission levels not separated.	Different permission level for analyst and method developer.
5.	Detector front panel could be accessed during data collection.	Detector front panel is now locked during data collection.

Qualification Requirements

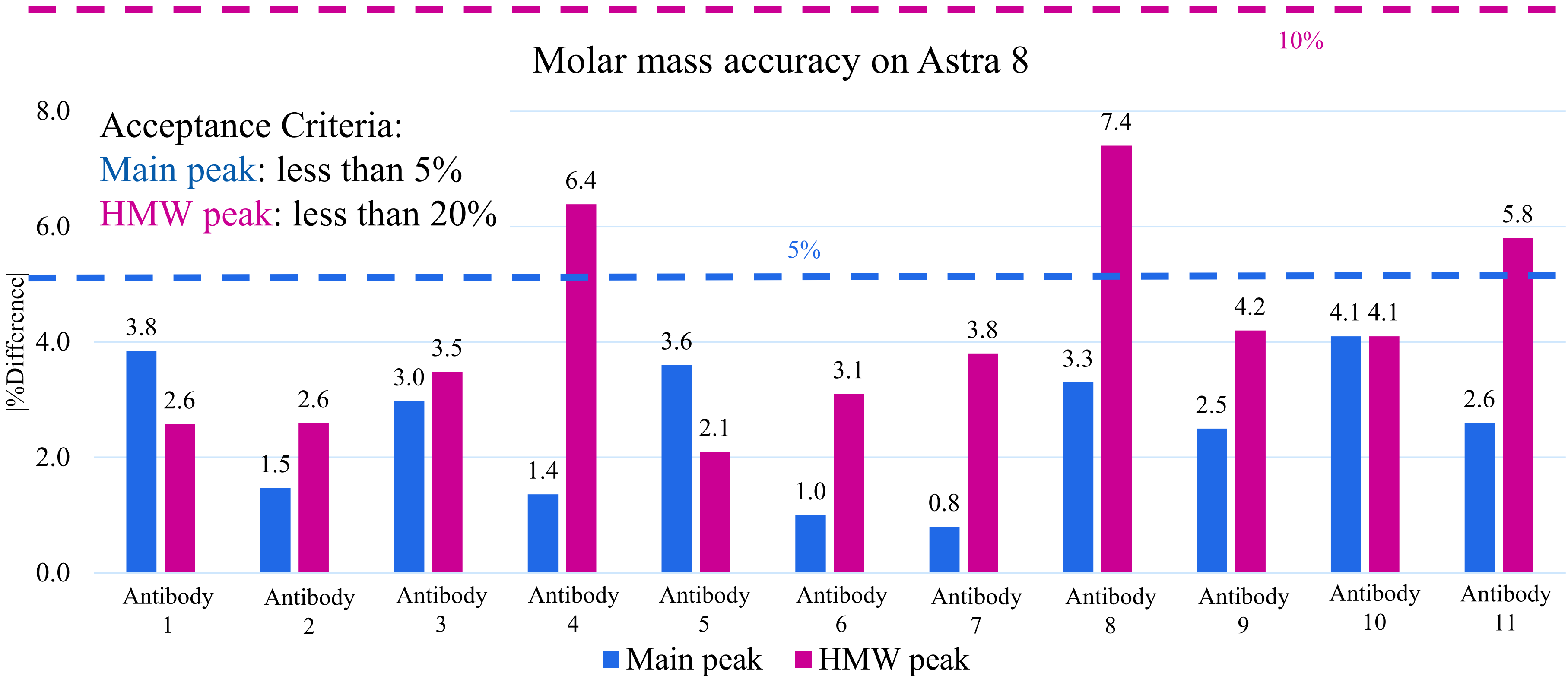
1. System qualification
    - Creation of operation and processing SOPs, data review WI
    - Assist with data integrity findings
2. Method qualifications
    - Transferring current CTPs to ASTRA 8
    - Method quality of life updates

System Comparisons

Equivalency Study between Astra 6 and Astra 8

Astra 8 Accuracy

Molar mass from Astra 8 was compared against target molar mass value

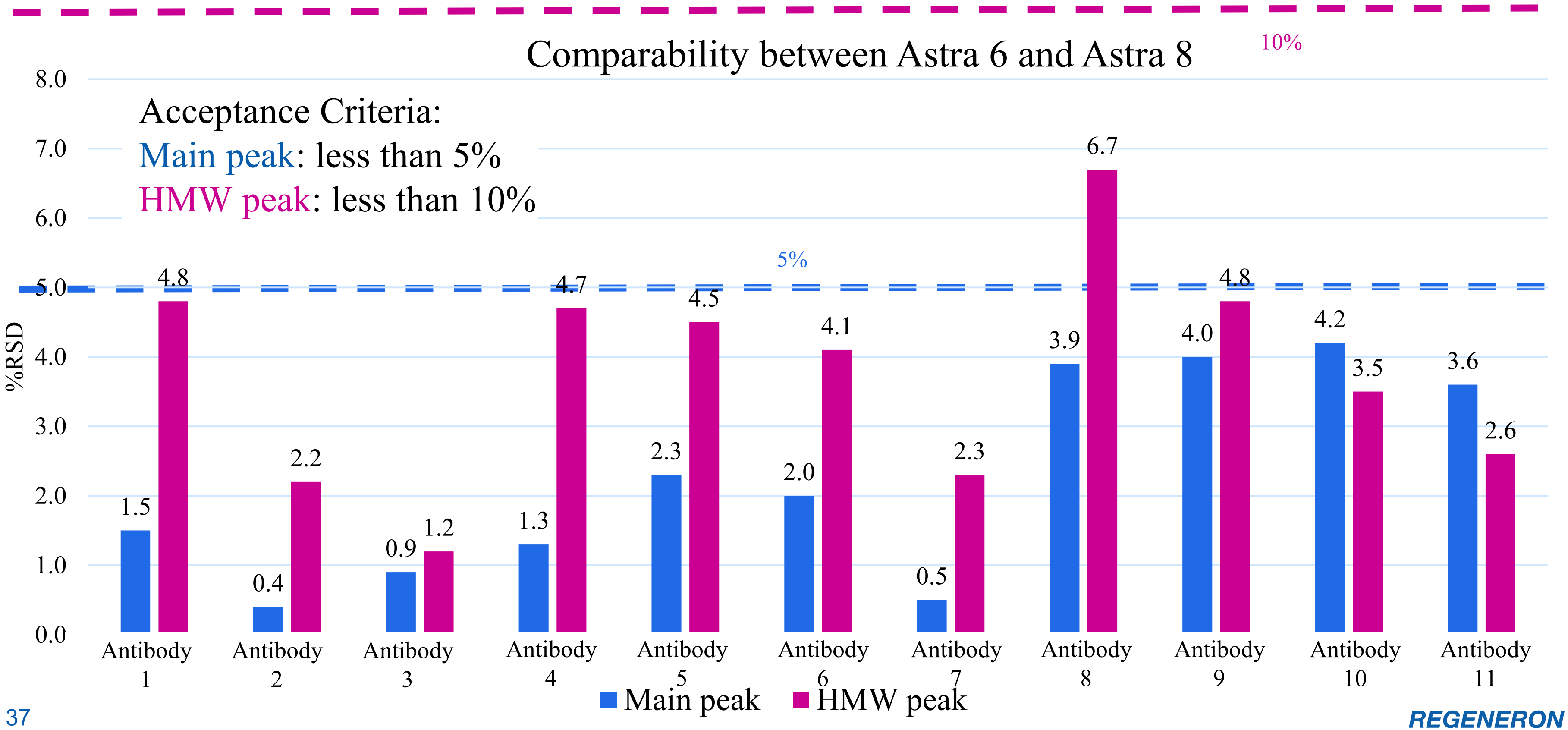


Molar mass accuracy on Astra 8

Acceptance Criteria:
Main peak: less than 5%
HMW peak: less than 20%

Data equivalency between Astra 6 and Astra 8

Molar mass from Astra 8 and historical qualification data from Astra 6 were compared for %RSD



Section Summary

ASTRA 6

- End of life
- Data integrity
- Elevated privileges for analysts
- Certain processing parameters need updating

ASTRA 8

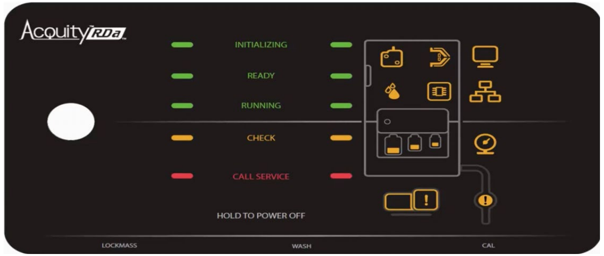
- Data integrity solutions
- Opportunity to update methods for best practices
- Opportunity to simplify procedures for analysts
- Proven comparability of molar mass data between systems

Agenda

- Transferring from Astra 6 and Astra 8: MALS for Reference Standard Characterization
  - Instrument/Software Comparison
  - Data Equivalency Study
- **High Resolution Mass Spectrometer: routine release testing**
  - **Peptide Mapping ID Method**
  - **Alternative Format ID Method**
  - **MAM Capabilities**

Implementing Mass Spec in QC Setting

Mass range:
50-7000 m/z for positive ions
50–5000 m/z for negative ions
Mass resolution: 10,000 FWHM



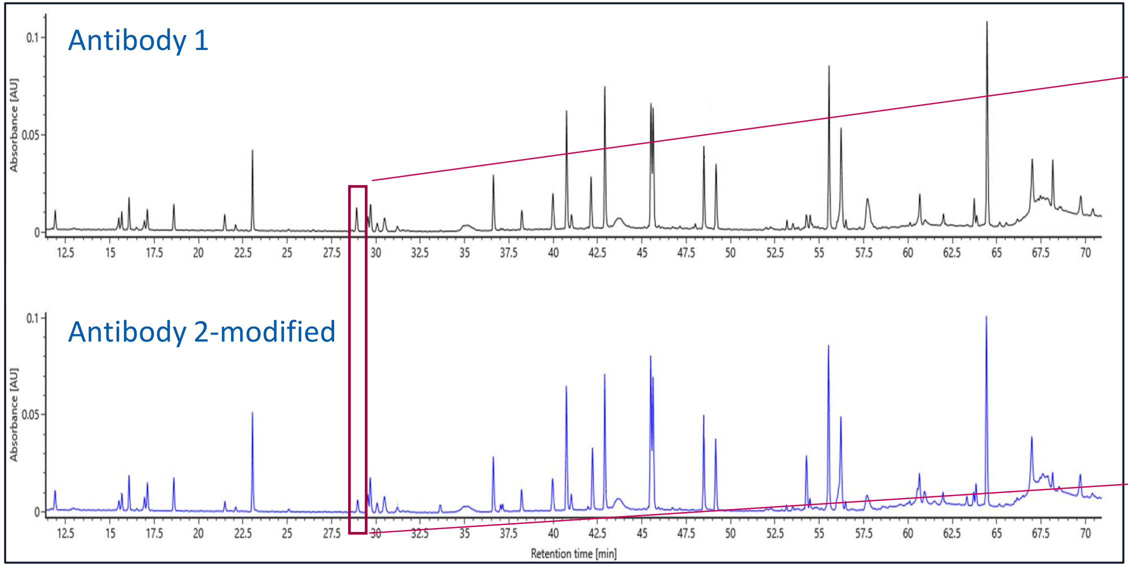
Mass range of up to 16,000 m/z
Mass resolution: 30,000 FWHM

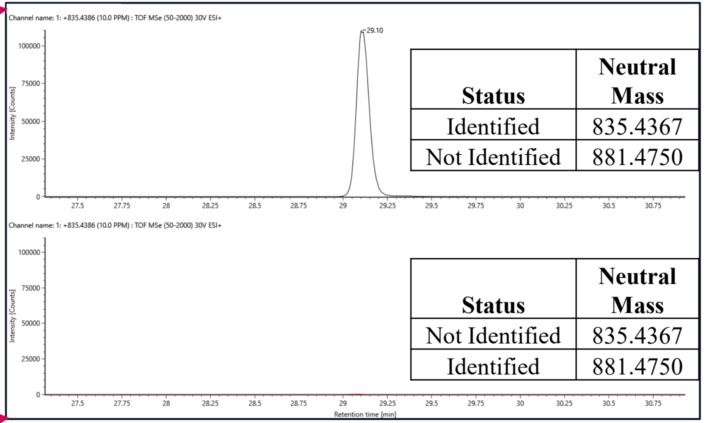
- Protein Identification
- Protein MAM
- Impurities
- Amino Acids
- Glycans
- Reference Standard Characterization
- Oligonucleotides Identification
- Oligonucleotide MAM
- Virus-like particles
- Adeno-associated virus

https://www.waters.com

Peptide Mapping Identification

- Per ICHQ6B, all identity tests of drug substances must be highly specific and should be based on unique aspects of its molecular structure and/or other specific properties
- Unique CDRs gives a unique UV profile used for identification
- New monoclonal antibodies with engineered modifications
  - 3 amino acid difference





Status	Neutral Mass
Identified	835.4367
Not Identified	881.4750

Status	Neutral Mass
Not Identified	835.4367
Identified	881.4750

Advantages of LC-MS for Peptide Map

LC-MS Peptide Map Method

Traditional UV Assay

- Long analysis times (2 hours/sample)
- Low throughput
- Ambiguity in data analysis

Challenges in robustness and repeatability:

- Changes in peak resolution
- Peak co-elution or splitting

BioAccord:10 min method

- Short analysis times (10 mins/sample)
- High throughput
- Simplified yes/no data analysis output

- Certain of peptide ID
- Changes to peak resolution and splitting have no impact

Demonstrating Specificity of the 10min peptide method

Testing scientific library of that's specific to mAb tryptic digest standard against a Regeneron antibody

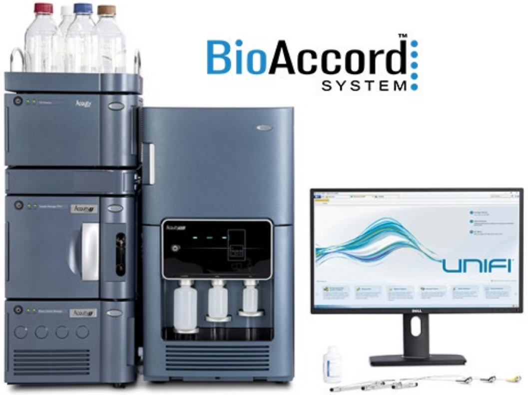
mAb tryptic digest standard: ID acceptance criteria met

Peptide Sequence	Chain	Expected mass (Da)	Identification Status
ALEWLADIWWDDK	HC	1660.8006	Identified
LTISK	HC	561.3606	Identified
VTNMDPADTATYYCAR	HC	1848.7891	Identified
DTLMISR	HC	835.4342	Identified
GFYPSDIAVEWESNGQPENNYK	HC	2544.1314	Identified
VTITCSASSR	LC	1081.5306	Identified
SGTASVVCLLNNFYPR	LC	1797.8952	Identified
VDNALQSGNSQESVTEQDSK	LC	2135.9687	Identified

Antibody 1: ID acceptance criteria not met

Peptide Sequence	Chain	Expected mass (Da)	Identification Status
ALEWLADIWWDDK	HC	1660.8006	Identified
LTISK	HC	561.3606	Not observed
VTNMDPADTATYYCAR	HC	1848.7891	Not observed
DTLMISR	HC	835.4342	Identified
GFYPSDIAVEWESNGQPENNYK	HC	2544.1314	Identified
VTITCSASSR	LC	1081.5306	Not observed
SGTASVVCLLNNFYPR	LC	1797.8952	Identified
VDNALQSGNSQESVTEQDSK	LC	2135.9687	Identified

Summary LC-MS Peptide Map Method



- Implements platform method
- Increases selectivity and specificity between nearly identical proteins
- Generates compliant-ready output
- Reduces ambiguity of data processing

- Automated sample preparation process greatly increases throughput
- Mitigates human error and reduce work-related injuries

https://www.andrewalliance.com/pipetting-robot/
https://www.waters.com/waters/en_US/BioAccord-LC-MS-System-for-Biopharmaceuticals/nav.htm?cid=135005818&locale=en_US

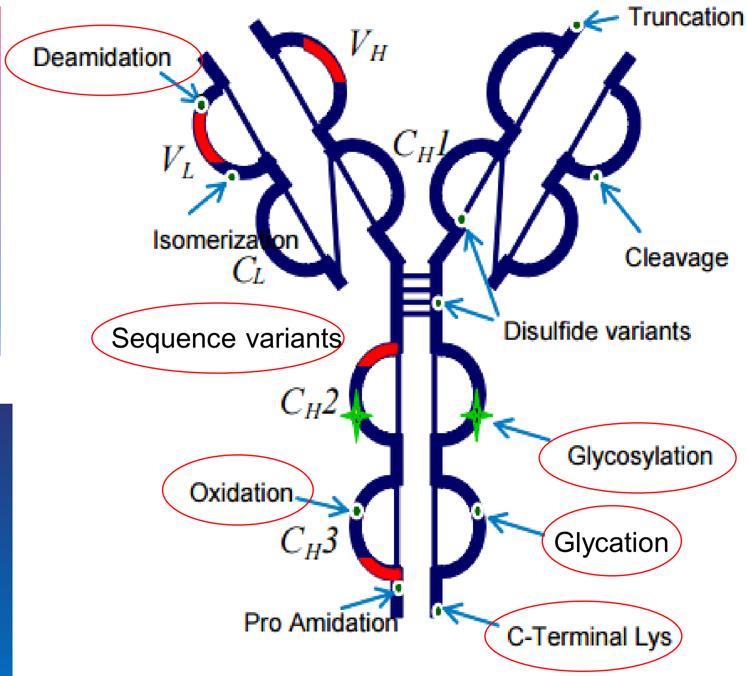
Implementing MAM for Improved Efficiency

Challenge

- Conventional techniques require several assays for PQAs
- Some assays require extensive prep and have long run times to ensure resolution

Goal

- Develop methods with improved efficiency using LC-MS capable of detecting and quantifying several PQAs
  - Shorten run times
  - Eliminate repeat testing



MAM Capabilities by 10 min LC-MS Peptide Mapping

Product identification by coverage map

↓

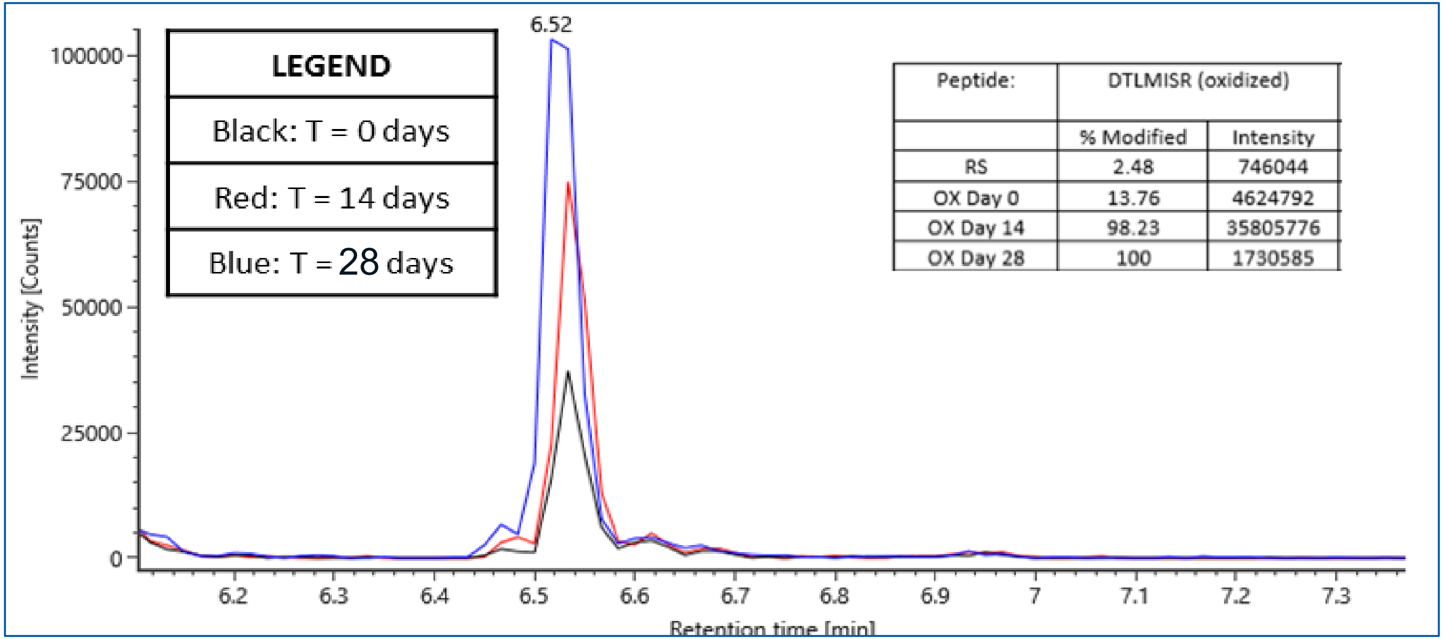
Create scientific library for peptides of interests

↓

Process by accurate mass screening

• FDA Information Request – Product oxidation study in FEP bag

Extracted ion chromatogram of Oxidized Met containing peptide



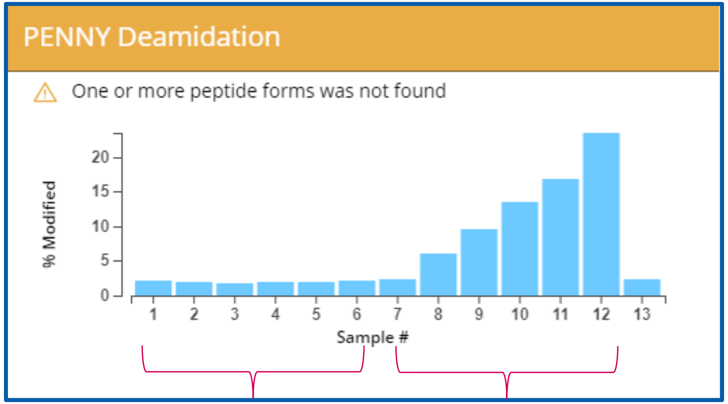
Peptide:	DTLMISR (oxidized)	
	% Modified	Intensity
RS	2.48	746044
OX Day 0	13.76	4624792
OX Day 14	98.23	35805776
OX Day 28	100	1730585

- Bioaccord Peptide MAM App

Peptide MAM

  - Monitors PTMs across different samples and timepoints
  - Calculates percent modification

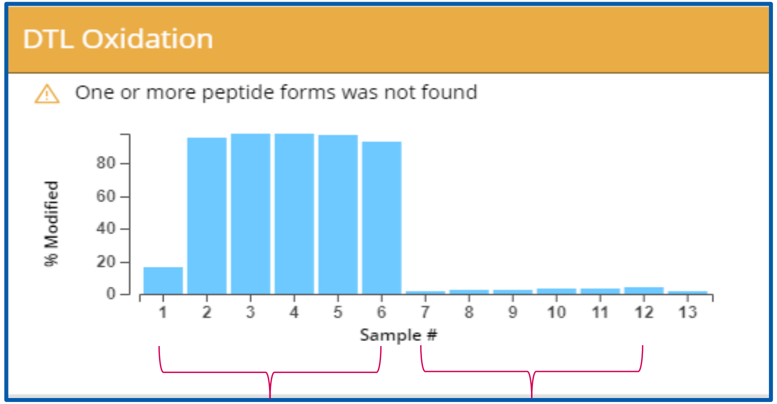
Sample #	Condition
1-6	Oxi day: 0, 7,14, 21, 28, 42
7-12	Dea day: 0, 7, 14, 21, 28, 42
13	Standard



PENNY Deamidation

⚠ One or more peptide forms was not found

Oxidation Deamidation



DTL Oxidation

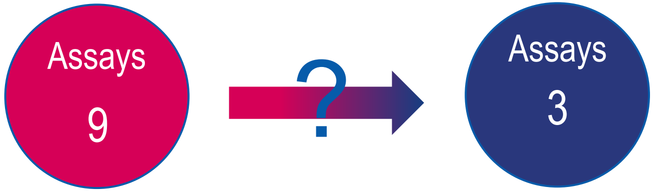
⚠ One or more peptide forms was not found

Oxidation Deamidation

Summary: Future of MAM

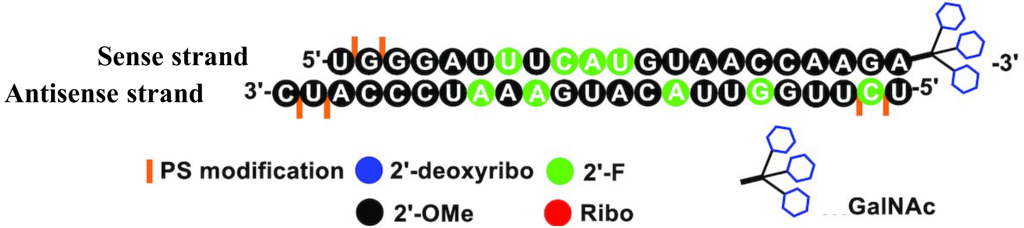
- More powerful detector gives potential for MAM
- Qualitatively and quantitively monitor product attributes
- Further work needed to evaluate potential reduction in release testing panels

Potential benefit of MAM



Potential Attributes for MAM
Oxidation
Deamidation
Glycation
Glycosylation
C-terminal Lys Clipping
Sequence Variants

siRNA Structure and Chemistry Method on Specification

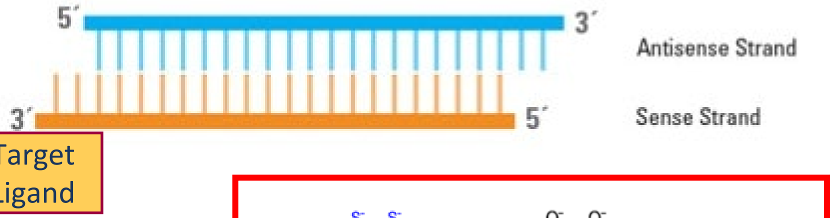


Method	Attribute
Non-denaturing-Ion Pair Reverse Phase by LC-UV	Duplex Purity
Denaturing-Ion Pair Reverse Phase by LC-UV	Single Strand Purity, GalNAc impurities
Denaturing-Anion Exchange by LC-UV	Single Strand Purity, PS to PO conversion
Non-denaturing-Ion Pair Reverse Phase by LC-MS	Identity

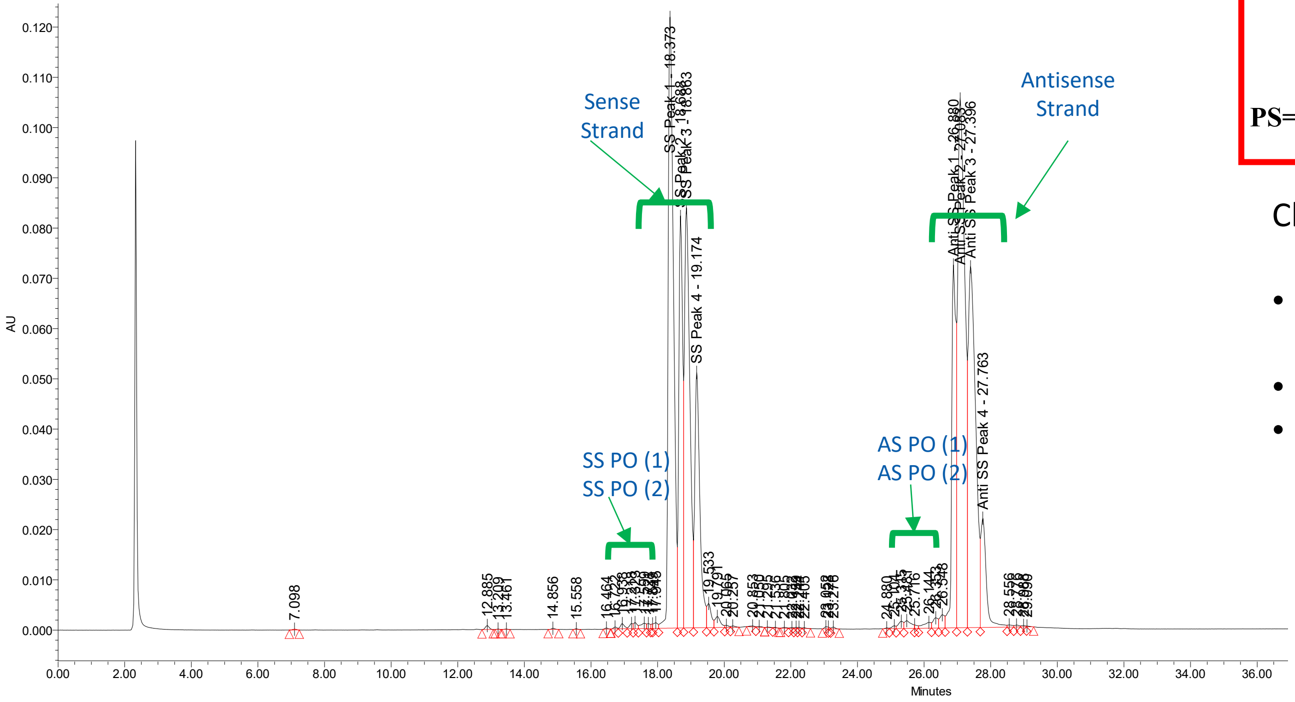
Nucleic Acids Research. ***2023****, 51*, 2529–2573

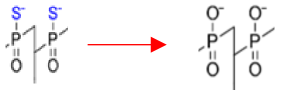
Denaturing AEX by LC-UV

Single Strand Purity



Integrated UV chromatogram





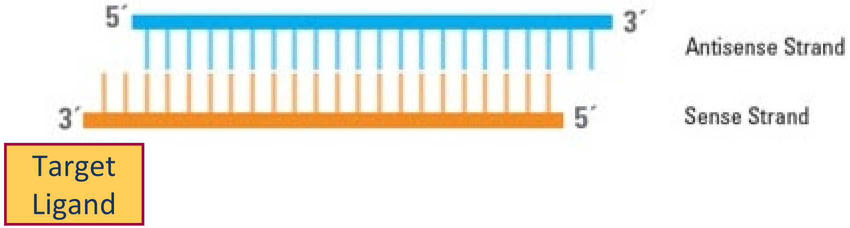
PS=phosphorothioate **PO=phosphorodiester**

Challenges:

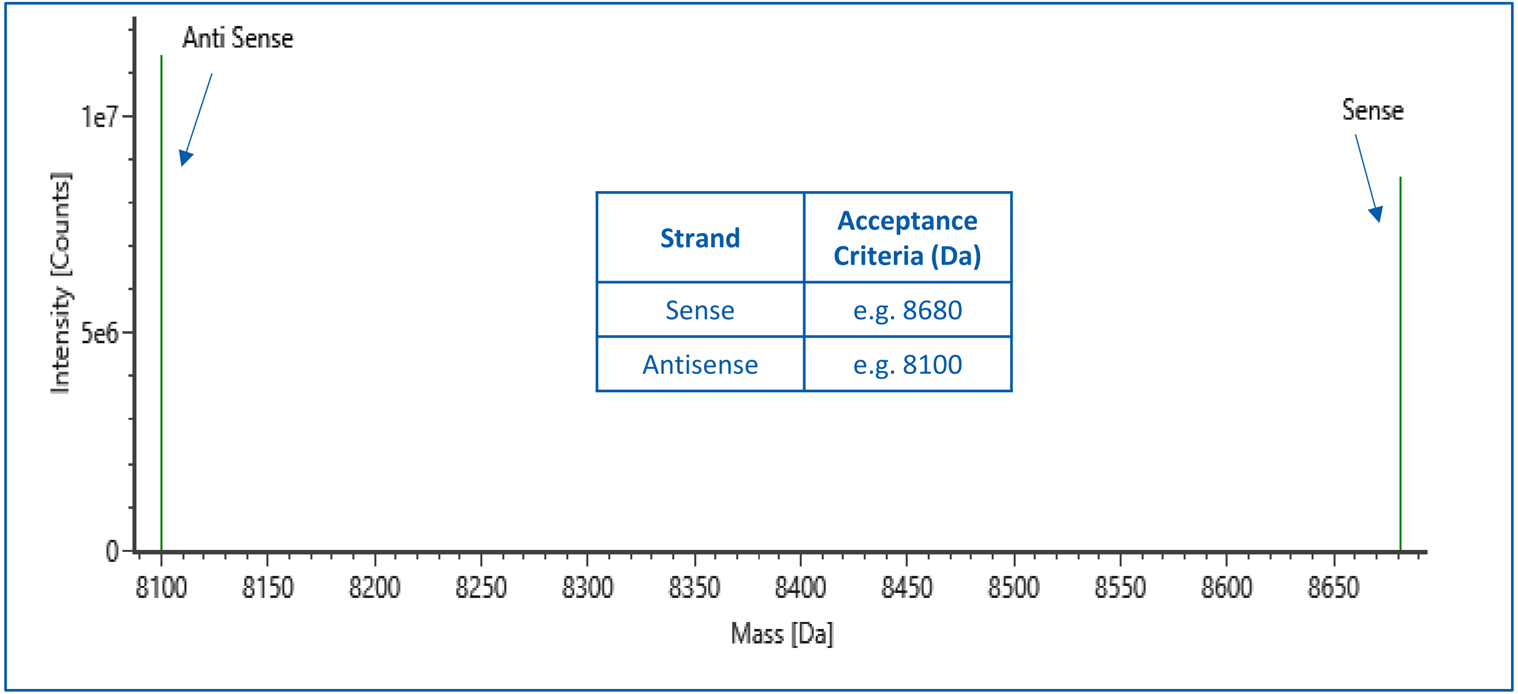
- Integrate entire UV chromatogram
- Report all impurities > 0.05% area
- Monitor new impurity not present in control

Alternative Format ID by LC-MS

siRNA Identification



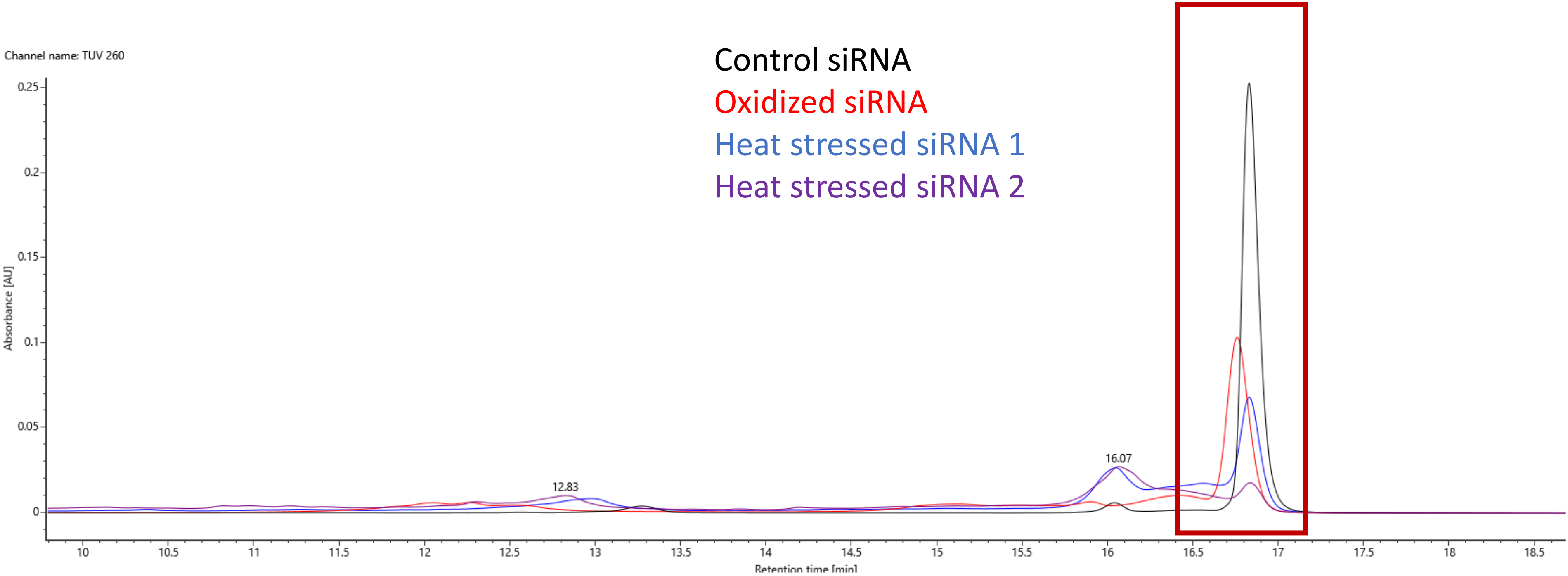
Target Ligand



Strand	Acceptance Criteria (Da)
Sense	e.g. 8680
Antisense	e.g. 8100

Oligo MAM by ND-IPRP-UV-MS

Mass spec compatible method



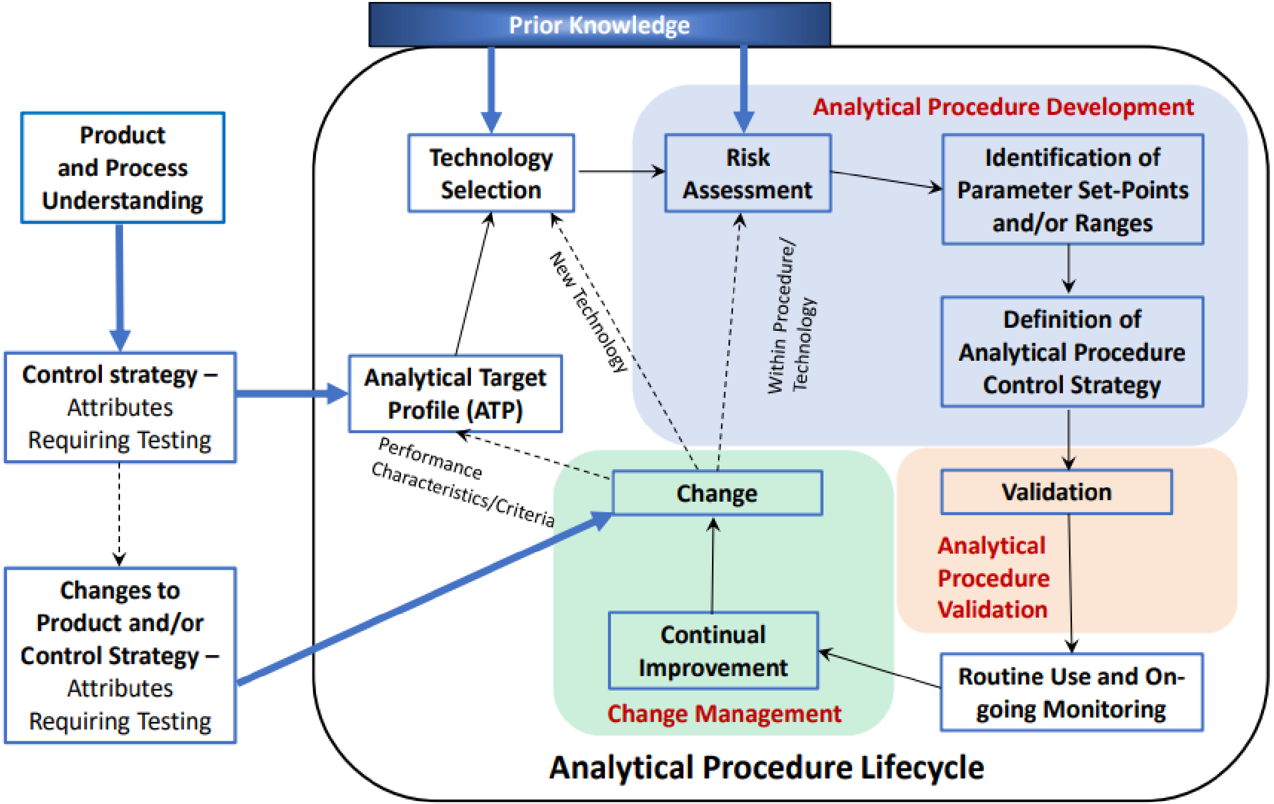
Quantification-%Purity

	% Purity		
	Control	**Oxidized**	**Heat**
Total Purity	95.46%	ND	52.64%
Loss of GalNacs	1.01% (Impurity loss of GalNac: 0.9-1.2%)	8.86%	37.86%
PS to PO	1.63%	80.73%	8.95%
Loss of nucleotides	1.32%	7.17%	ND

ND = Not Detected
% Purity=(Response/Total Response)*100
Total Purity=% Purity (Sense)+% Purity (Antisense)

Summary

- Established workflow for routine oligonucleotide ID test method
- Proof of Concept LCMS oligo MAM method to identify and quantify known impurities
- Further optimization on coeluting impurities and characterization of unknown impurities by MS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Validation
Analytical Procedure Validation
Change
Continual Improvement
Change Management
Routine Use and On-going Monitoring
Analytical Procedure Lifecycle

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Acknowledgement

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Rachel Mullen
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Seamus O'Connor
Paul Bigwarfe
Waters Team

Questions?