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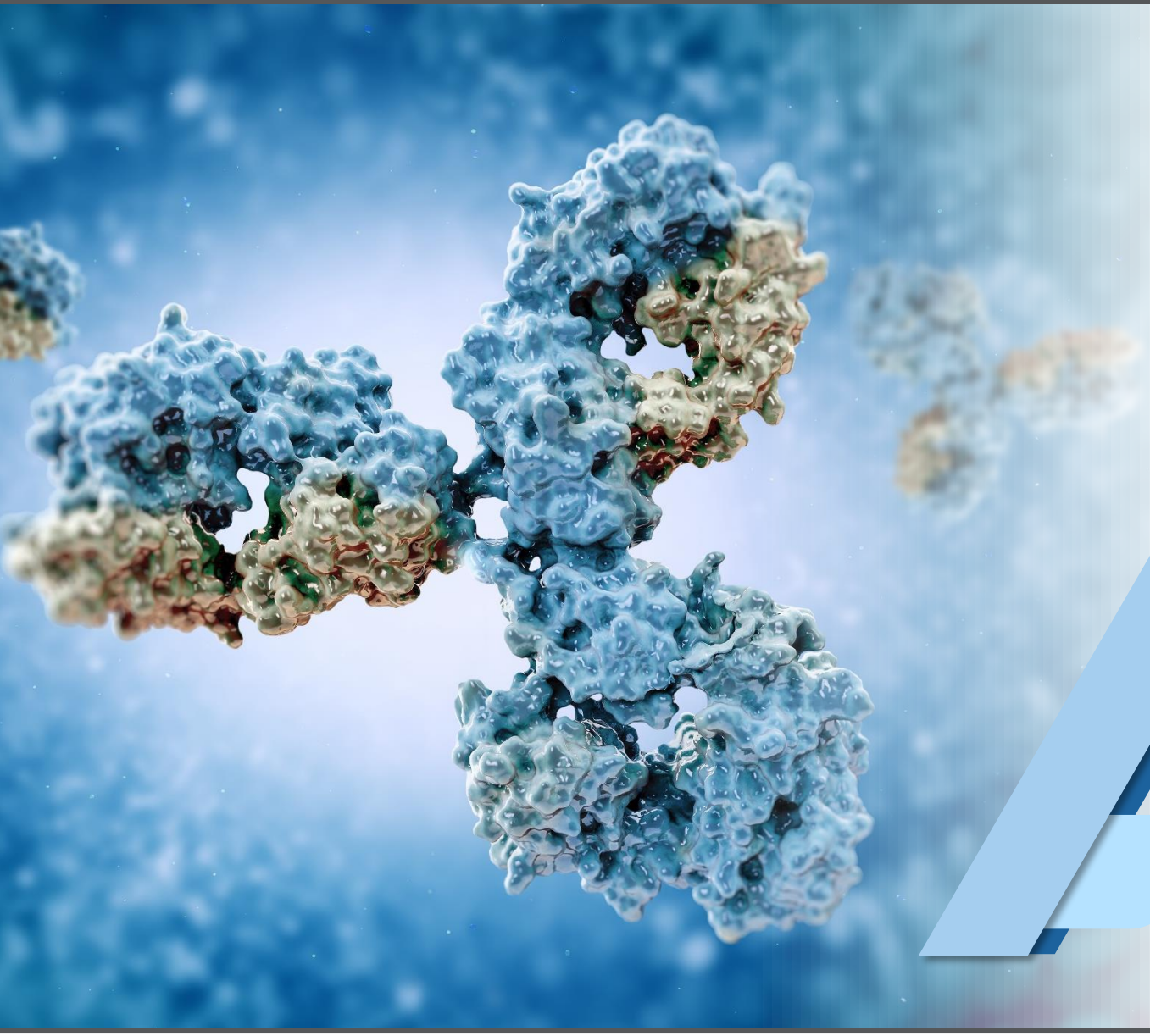
Advanced QA/QC characterization MS in QC : Multi Attribute Method

Global BioPharma Summit

A Complex Problem: Drug Safety and Quality



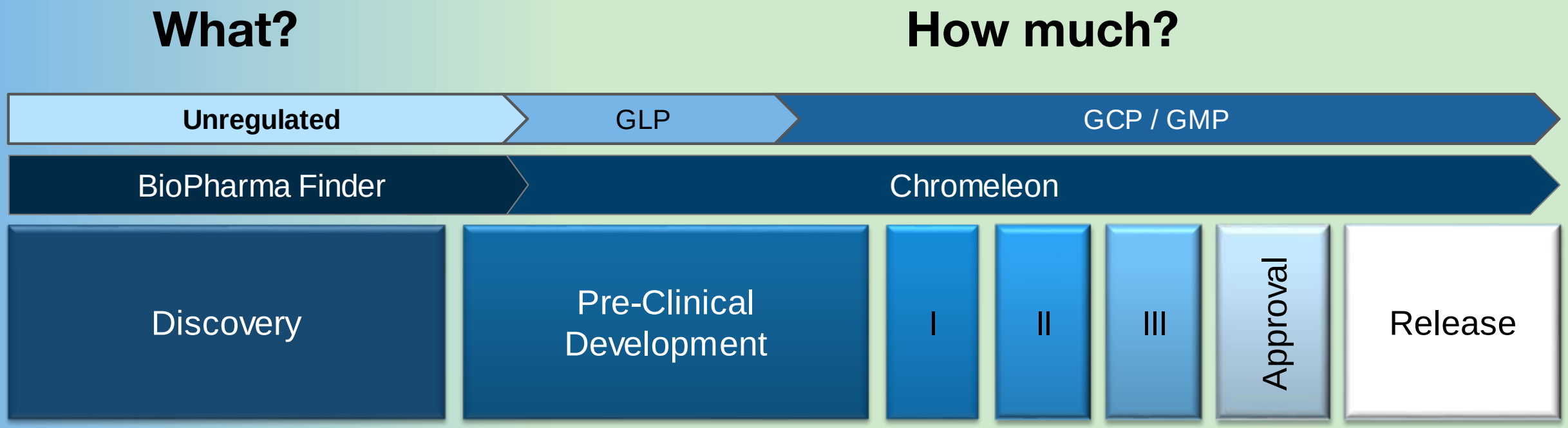
Safety
Is the product safe to use?
(e.g. Immunogenic effects?)

Potency
Does the drug have the expected effect?
(e.g. CDR complementation)

Knowledge
How do changes effect the therapeutic?
(e.g. Oxidation)

Quality
How do changes in process effect the product?
(e.g. Glucose concentration on glycoforms)

Drug Development Workflow: From Discovery to Production



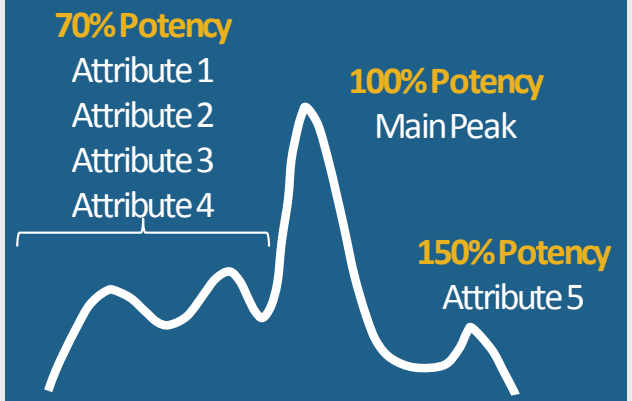
- Molecular Assessment
- Quality Control
- Attribute Science Groups
- Analytical and Automation Process Technologies
- Protein Attribute Chemistry
- Manufacturing

Advantages of MAM

Required Characterizations	MAM Method	Conventional Methods						
	Pep Map-MS	SEC	CEX	rCE-SDS	nrCE-SDS	HILIC	ID ELISA	HCP ELISA
Aggregate Assessment	No	Yes	Indirect	Yes	Yes	No	No	No
Deamidation (Isomerization) Assessment	Yes	No	Indirect	No	No	No	No	No
Disulfide Isoform Assessment	Maybe	No	Indirect	No	Yes	No	No	No
Glycation Assessment	Yes	No	No	Yes	Yes	No	No	No
High Mannose Assessment	Yes	No	No	No	No	Yes	No	No
Methionine Oxidation Assessment	Yes	No	No	No	No	No	No	No
Signal Peptide Assessment	Yes	No	No	No	No	No	No	No
Unusual Glycosylation Assessment	Yes	No	Indirect	Maybe	Maybe	Yes	No	No
CDR Tryptophan Degradation Assessment	Yes	Indirect	No	No	No	No	No	No
Non-consensus Glycosylation Assessment	Yes	No	No	Maybe	Maybe	No	No	No
N-terminal pyroGlutamate Assessment	Yes	No	Indirect	No	No	No	No	No
C-terminal Lysine Assessment	Yes	No	Yes	No	No	No	No	No
Galactosylation Assessment	Yes	No	No	No	No	No	No	No
Dimer Assessment	No	Yes	No	No	No	No	No	No
Fragmentation (peptide bond) Assessment	Maybe	Maybe	No	Yes	Yes	No	No	No
Disulfide Reduction (DS Fragmentation) Assessment	Maybe	No	No	No	Yes	No	No	No
Host Cell Protein Assessment	Yes	No	No	No	No	No	No	Yes
Mutations/Misincorporations Assessment	Yes	No	No	No	No	No	No	No
Hydroxylysine Assessment	Yes	No	No	No	No	No	No	No
Thioether Assessment	Yes	No	No	No	No	No	No	No
Trisulfide Assessment	Maybe	No	No	No	No	No	No	No
Non-glycosylated Heavy Chain	Yes	No	No	No	No	No	No	No
DNA Assessment	No	No	No	No	No	No	No	No
Cysteine Adducts Assessment	Maybe	No	Maybe	No	No	No	No	No
C-terminal Amidation Assessment	Yes	No	Indirect	No	No	No	No	No
CDR Conformers (HIC Isoform) Assessment	No	No	Indirect	No	No	No	No	No
O-linked Glycans Assessment	Maybe	No	No	No	No	No	No	No
Fucosylation Assessment	Yes	No	No	No	No	No	No	No
Residual Protein A	Yes	No	No	No	No	No	No	No
Identity	Yes	No	Yes	No	No	No	Yes	No

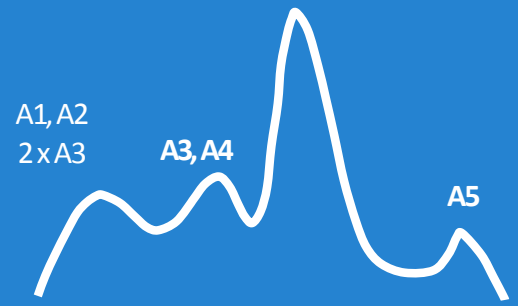
Multi-Attribute Method for QC

Current Release Method



CEX separation

Product Knowledge



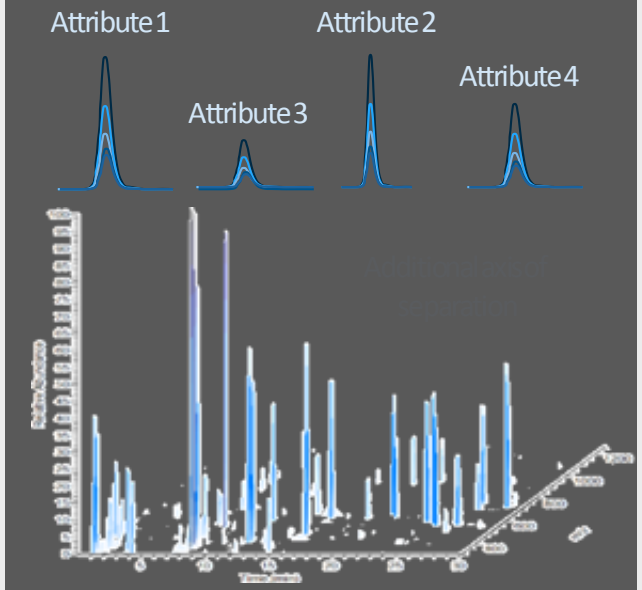
CEX separation

Sub-fraction Potency Assessment

Attribute	Potency
Main peak	100%
A1	50%
A2	110%
A3	95%
A4	102%
A5	150%

Multi Attribute Method

Replacing conventional CEX monitoring of pre-peaks with more specific method directly measuring attributes affecting safety and efficacy



High Res. Accurate Mass LCMS

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Key Tenets of MS in QC (aka MAM)

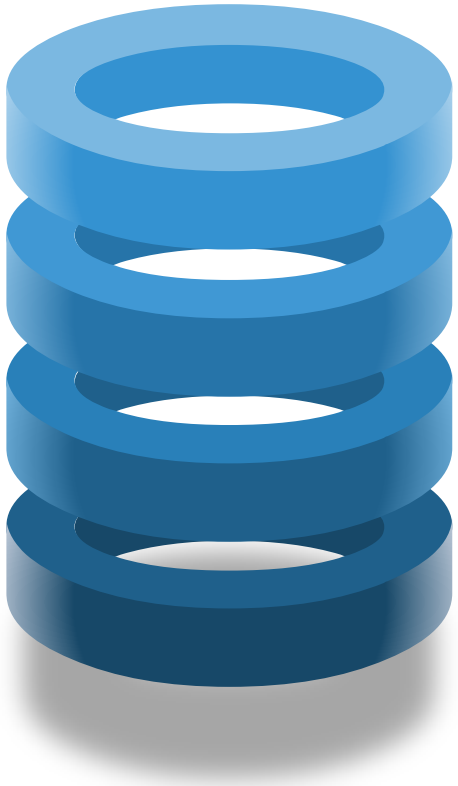
Multi Attribute Method

Confirmation

Confirm process by evaluating release product against a gold reference standard

High Resolution

Using High Resolution/Accurate Mass MS instrumentation to directly measure CQA



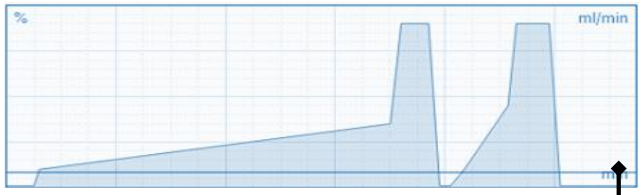
Quality by Design

Implement hybrid traditional (evaluation in QC) and QbD approach (monitoring of CQA and end-result of CPP space)

Knowledge

Eliminate the need of traditional lot release methodologies while increasing product knowledge

Materials



3 ug Trypsin Digested NIST mAb

Thermo Scientific™ Vanquish™ UHPLC
70 minute Gradient 250 uL/min
0.1% FA in H2O and MeCN
50° C Column Temp

Thermo Scientific™ Q Exactive™ HF
120k Resolution @ 200 m/z
300 to 1800 m/z

Thermo Scientific™ Chromeleon™ CDS 7.2 SR5
CFR 21 Part 11 Compliant Data Acquisition, Processing, and Reporting

Sample

Column

2.1 x 150 mm 1.5 uM
Thermo Scientific™ Accucore™ Vanquish C18+

uHPLC

MS

Processing

Discovery

Thermo Scientific™ BioPharma Finder™ 2.0
Component Detection, Peptide Mapping, and CQA Selection

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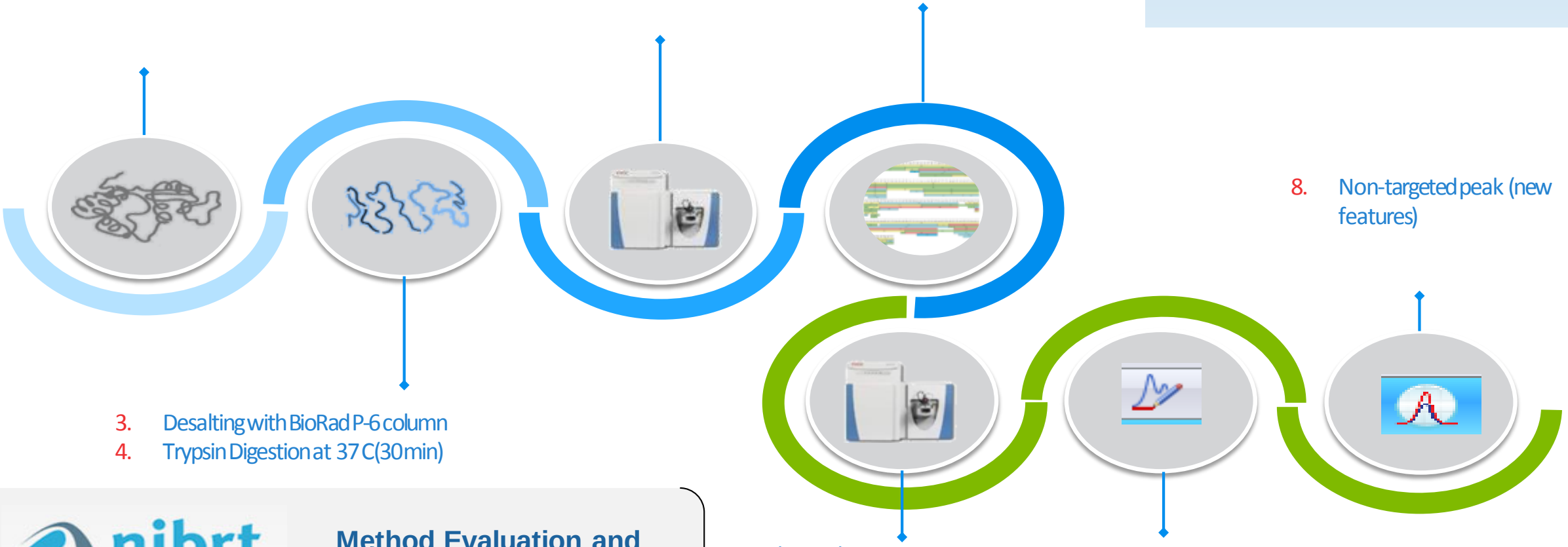
Methods

1. Product Denaturation with 7 M Guanidine HCl and reduction with 500 mM DTT (30 min)
2. Alkylation with 500 mM Iodoacetate (20 min)
3. Desalting with BioRad P-6 column
4. Trypsin Digestion at 37 C (30 min)
5. LC-MS/MS Analysis – Top 5
6. Peptide Mapping Selection of Attributes to Monitor

R. Rogers, et al. MAbs 2015, 7, 881-890



6. Chromeleon LC-MS Data Acquisition
7. Targeted peak detection
8. Non-targeted peak (new features)

nibrt National Institute for Bioprocessing Research and Training

Method Evaluation and Advancements

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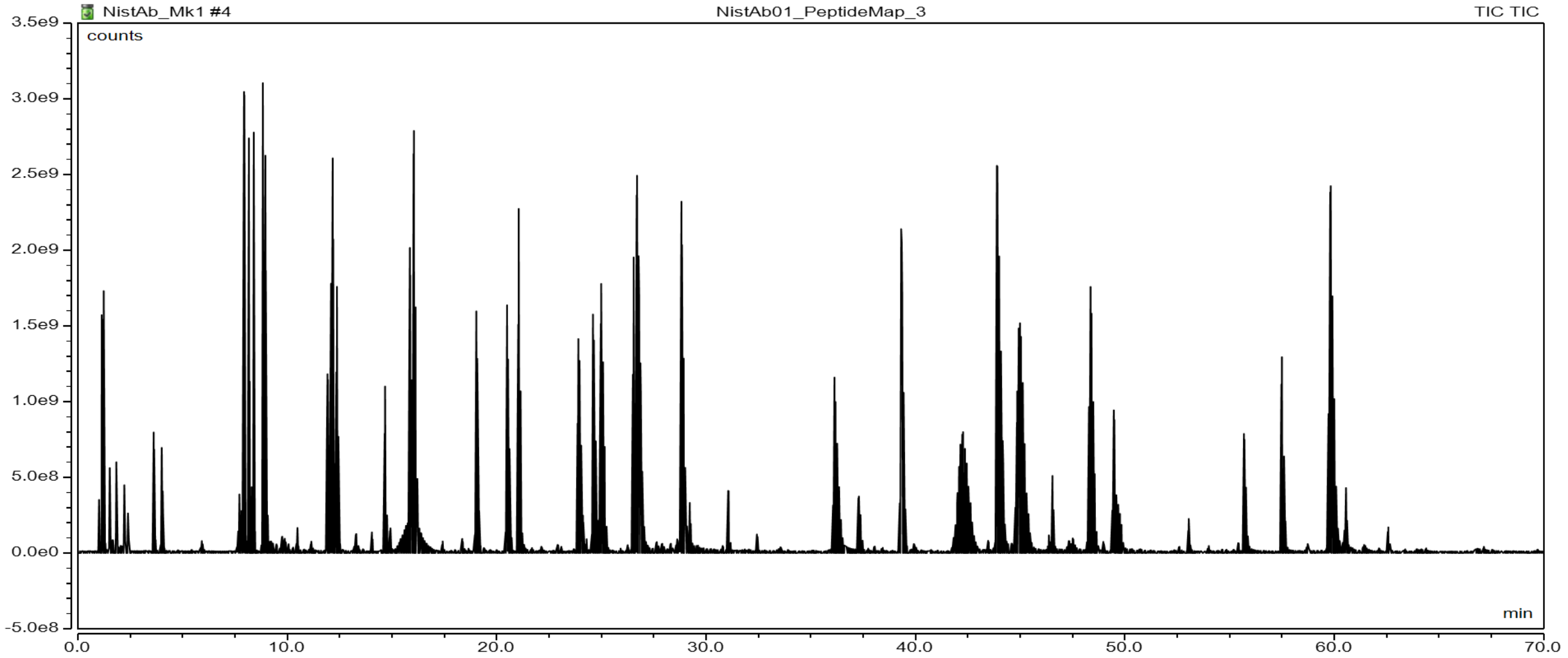
Peptide Mapping

TIC
Intensity 3e9

Basepeak
Intensity 1.6e9



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Peptide Mapping

TIC
Intensity 3e9

Basepeak
Intensity 1.6e9

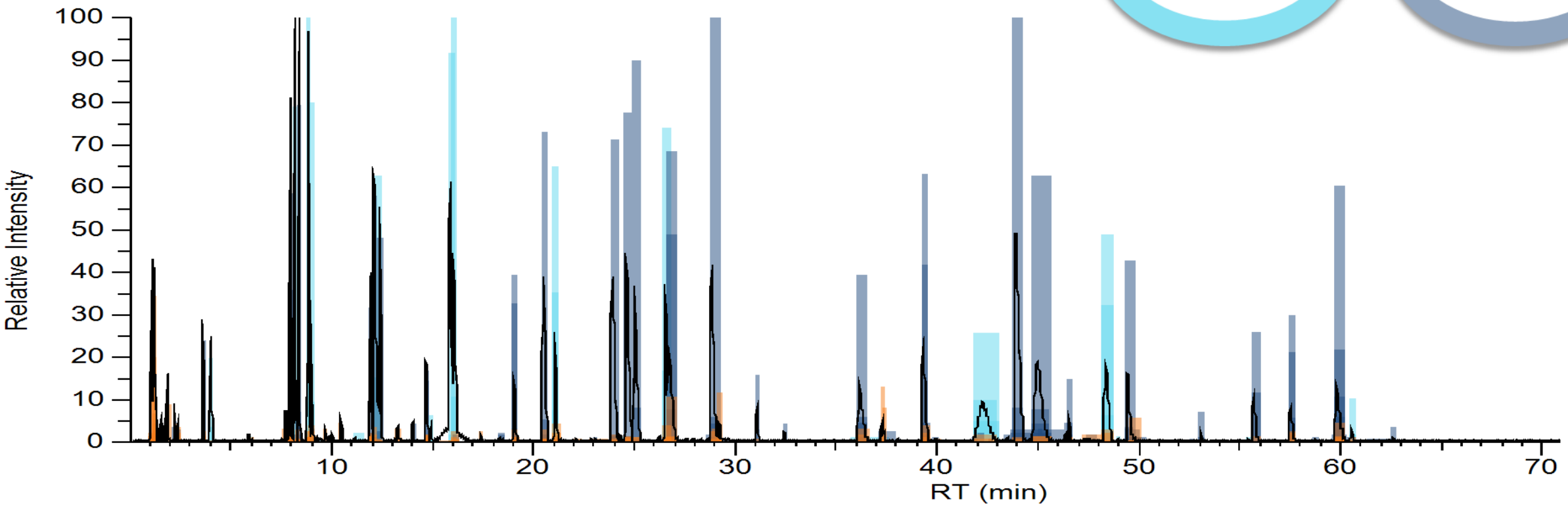
Light Chain

Heavy Chain

Unidentified

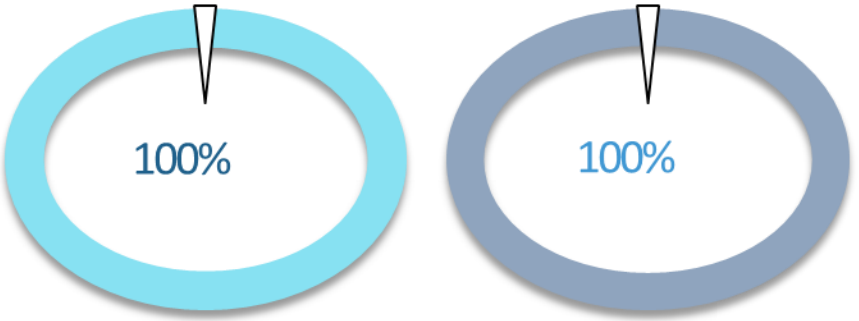
Feature Chromatogram

Detected features belonging to light chain, heavy chain, and unidentified species.



Sequence Coverage

Complete sequence coverage of heavy chain and light chain



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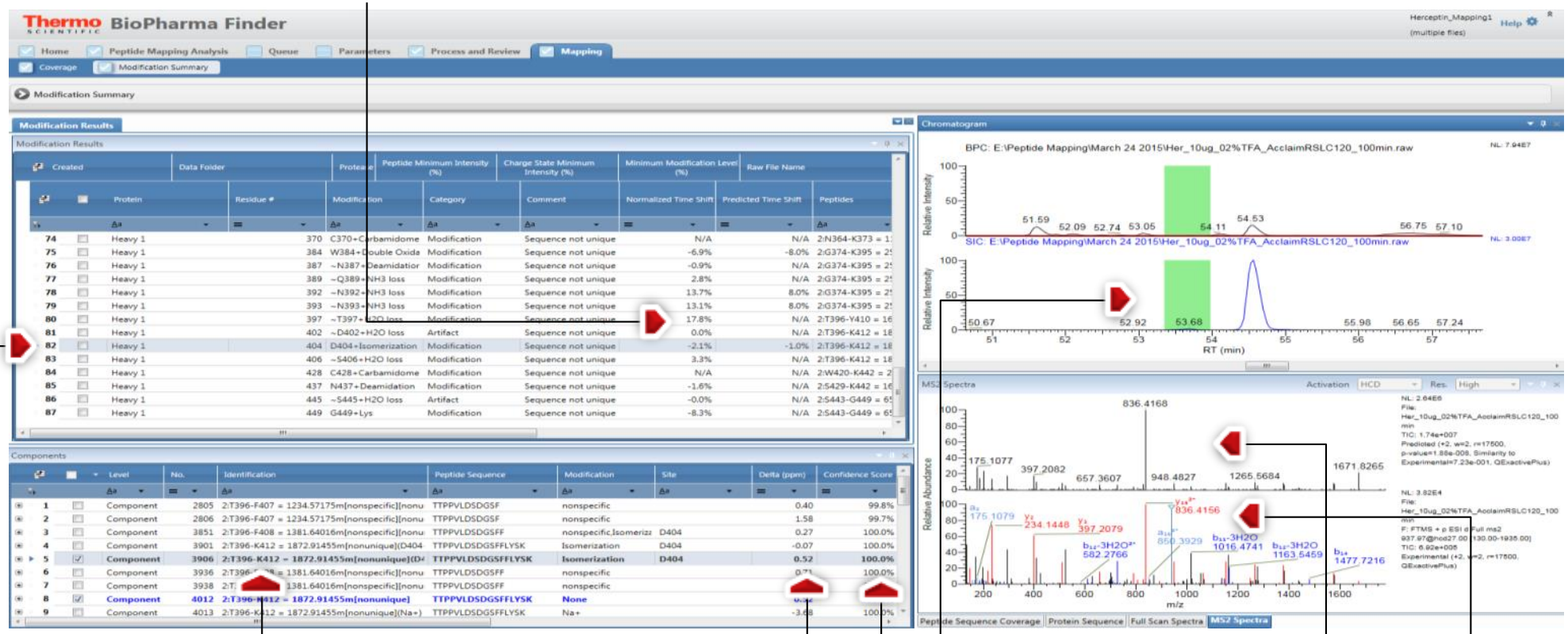
Establishing CQAs

BioPharma Finder™

Predicted/Observed RT shift



Modification Summary Chart

SELECT Component Used for Quantitation

<3 ppm mass error

Predicted

Component Extracted Ion Chromatogram

Observed Spect

Confidence

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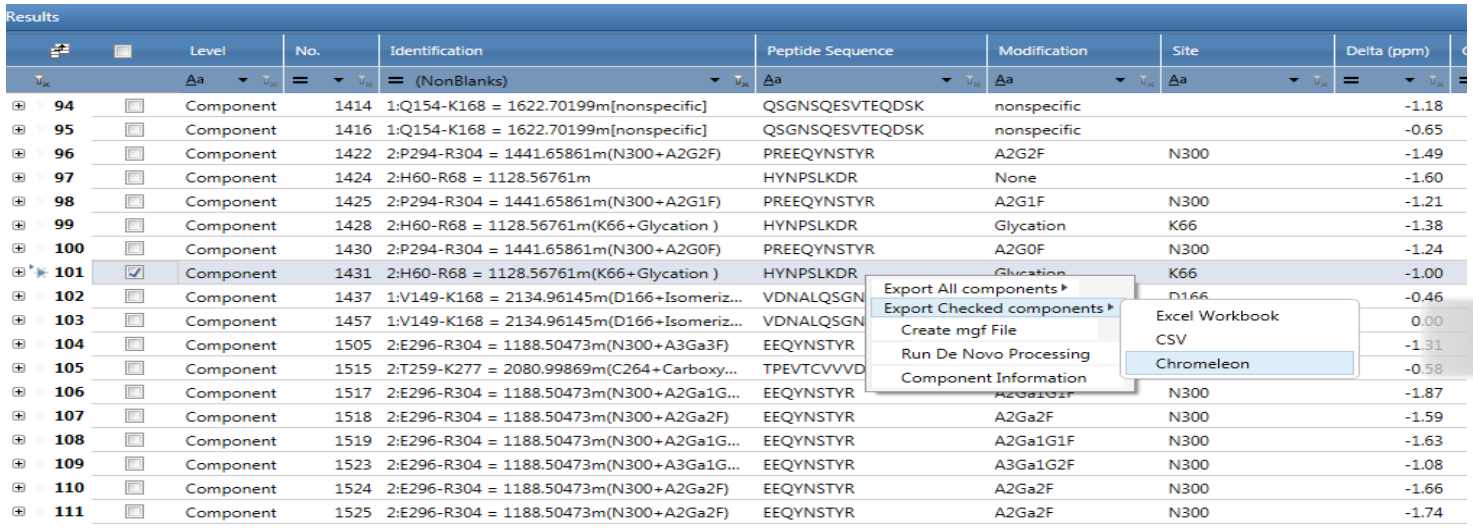
MS in QC – Late Stage Discovery to Process Dev / QC

- Analytical group is primary driver and is responsible for
  - Identification and selection of target peptides / modifications
  - Generation of targeted component list (HR/AM MS1) for QC
  - Complete analytical method (processing and instrument methods, designing report templates, eWorkflows)
  - Development of peptide mapping assay SOPs (for QC)
  - MS list is transferred directly from the discovery experiment in BioPharma Finder 2.0 to Chromeleon
  - Checkboxes enable easy selection of components to transfer.
  - More can be added at anytime in the future



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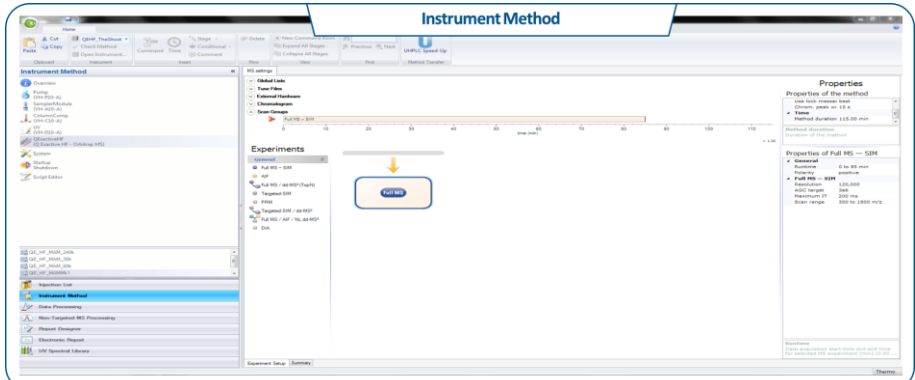
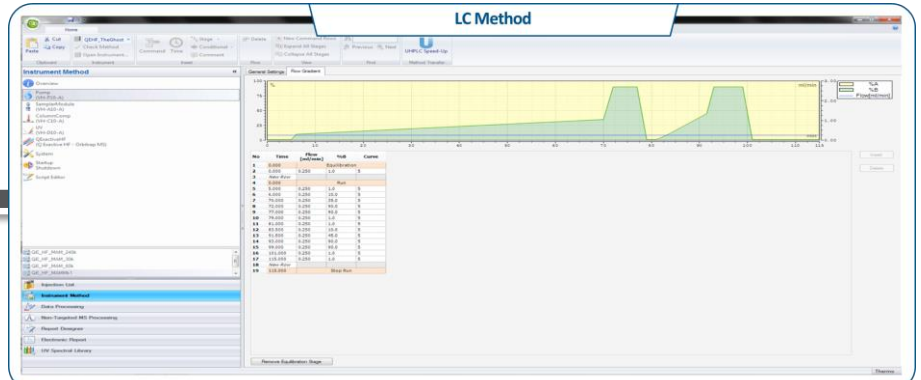
Acquiring Data

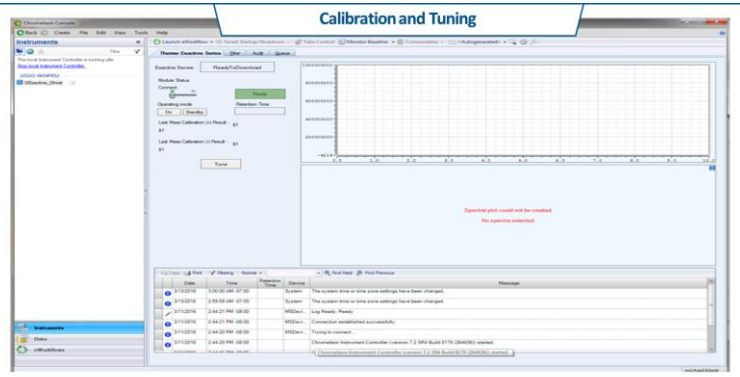
Instrument Method



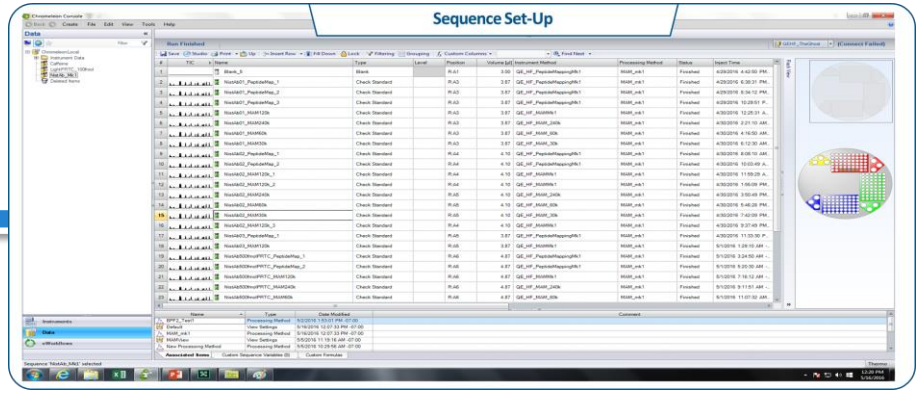
LC Method



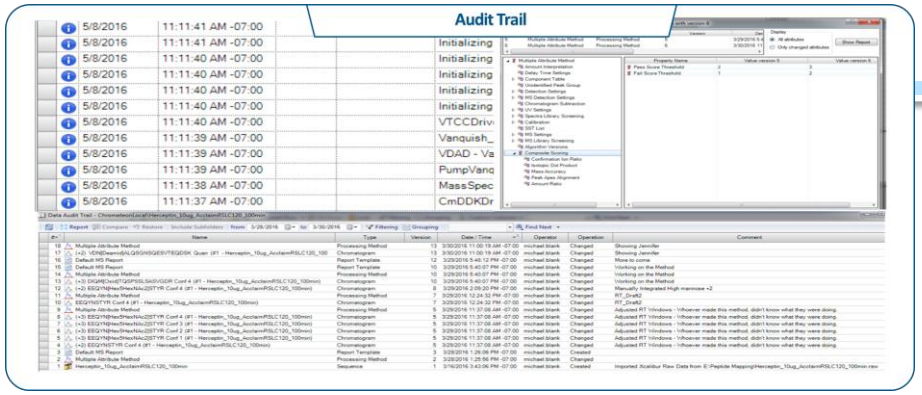
Calibration and Tuning



Sequence Set-Up



Audit Trail



Compliance Tools

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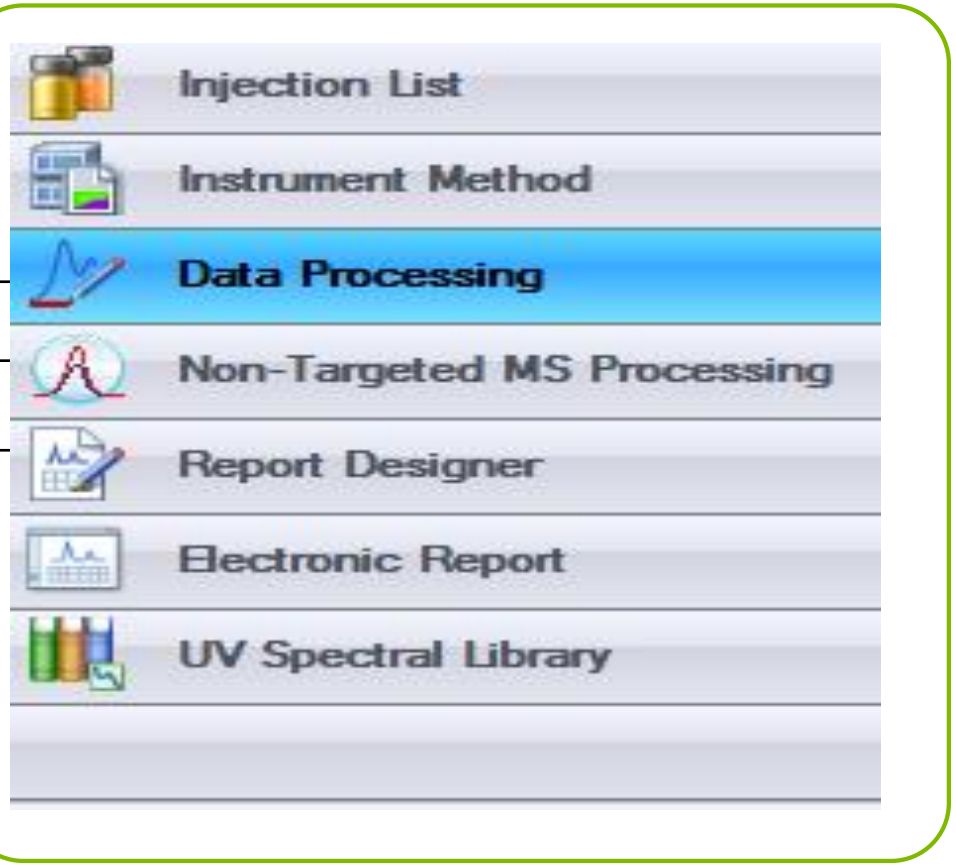
Data Analysis Processing Chromeleon

Chromeleon 7.2

- Set up injection sequence
- Build LC and MS methods
- Target. Confirm. Integrate.
- Look for new peptides/impurities/features
- Automatically generate reports outputs/formulas

Simple. Logical.

1. **Create Injection List** (eWorkflow)
2. **Define Instrument Method** (LC and MS, eWorkflow)
3. **Targeted MS Processing**
   Verify all expected peptides
4. **Non-Targeted MS Processing**
   Identify new peptides/impurities/modifications
   Comparison to reference standard (A to B)
5. **Reporting**



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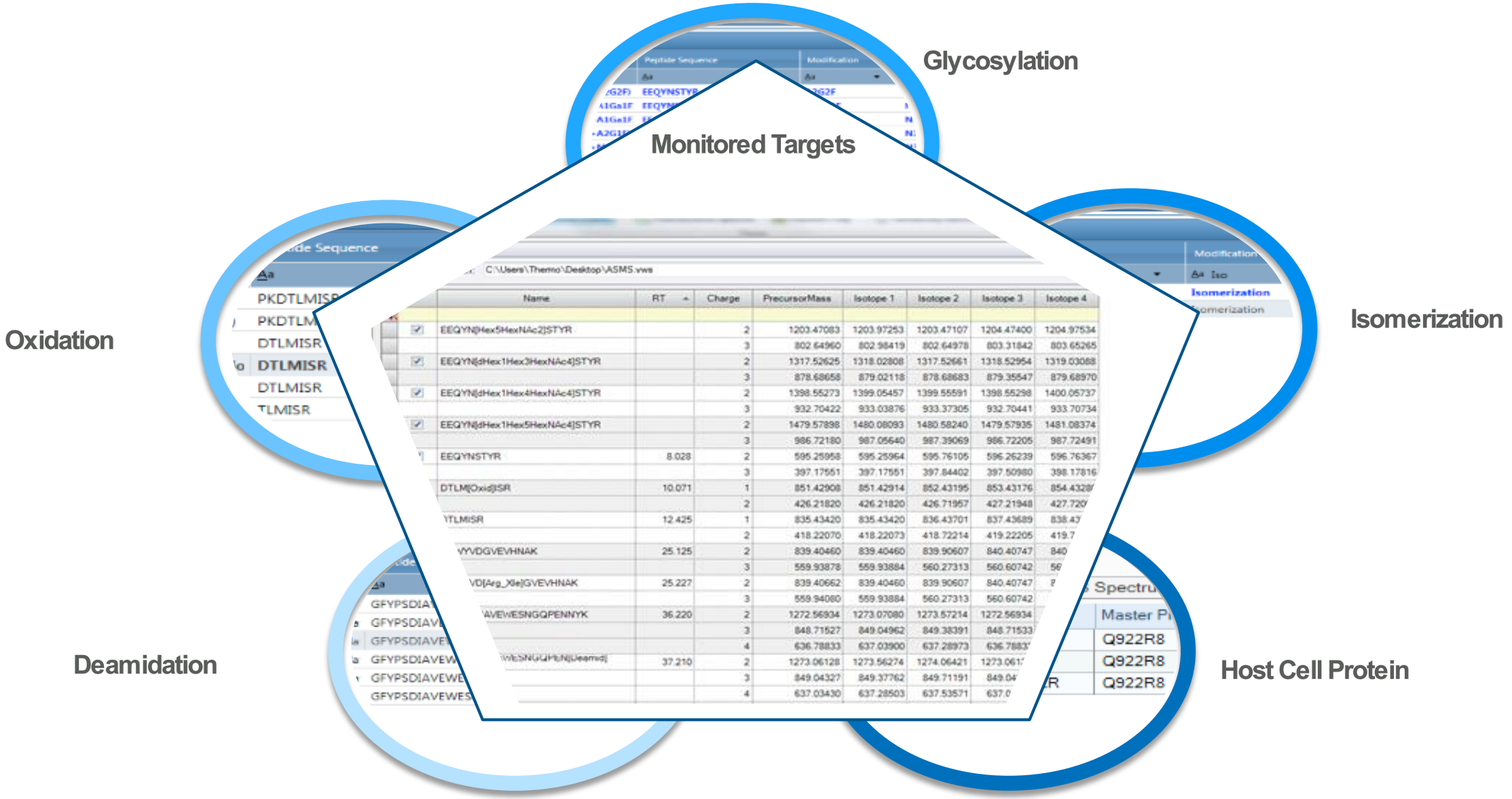
Building Targeted List of Critical Quality Attributes



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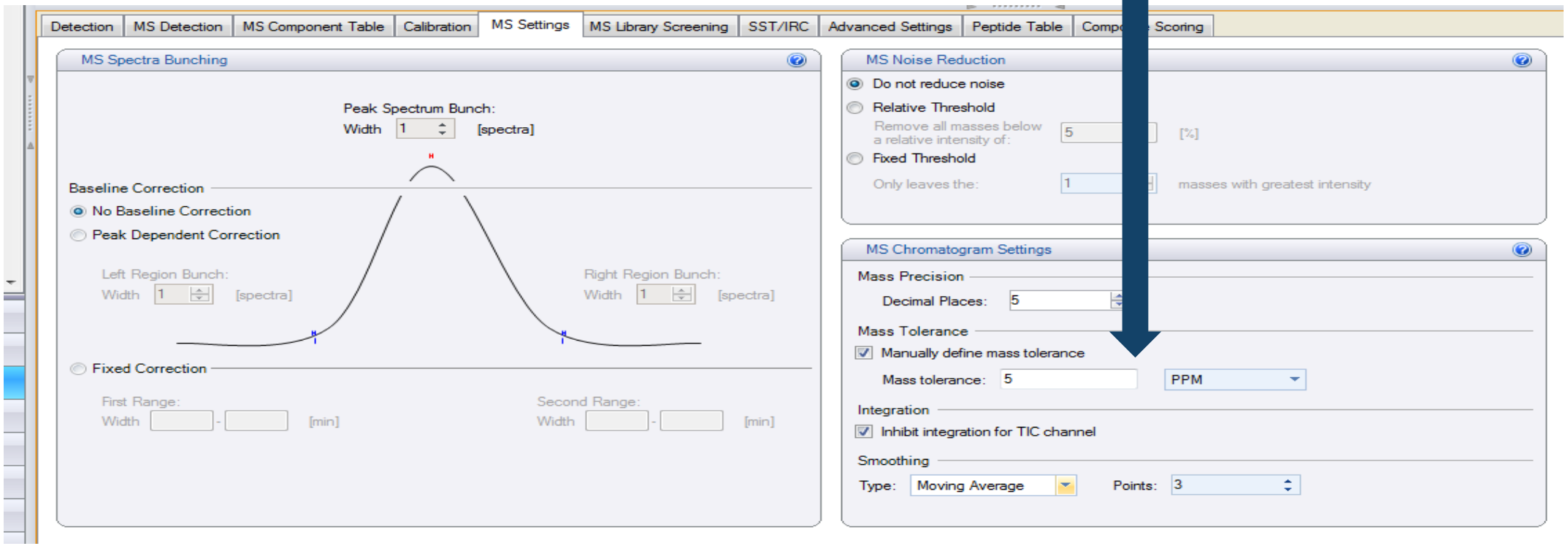
Adjust Processing Settings as Desired

Chromeleon 7.2

MS Settings

high resolution enables accurate extraction and quantitation



no offline recalibration necessary

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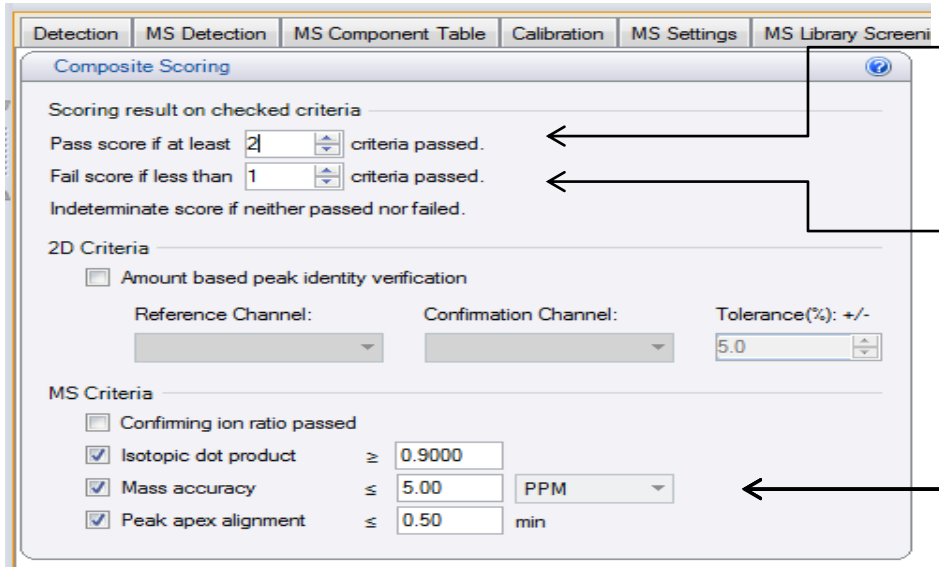
Adjust Processing Settings as Desired

Chromeleon 7.2

✓ MS Settings

Composite Scoring



No. of Conditions Satisfied for a Full Pass

No. of Conditions Satisfied for a Partial Pass

- Isotopic Ratios (Individual)
- Isotopic Correlation (Envelope)
- Mass Accuracy
- Retention Time Apex Alignment

Adjust Processing Settings as Desired

Chromeleon 7.2

- ✓ MS Settings
- ✓ Composite Scoring
- Integration Settings

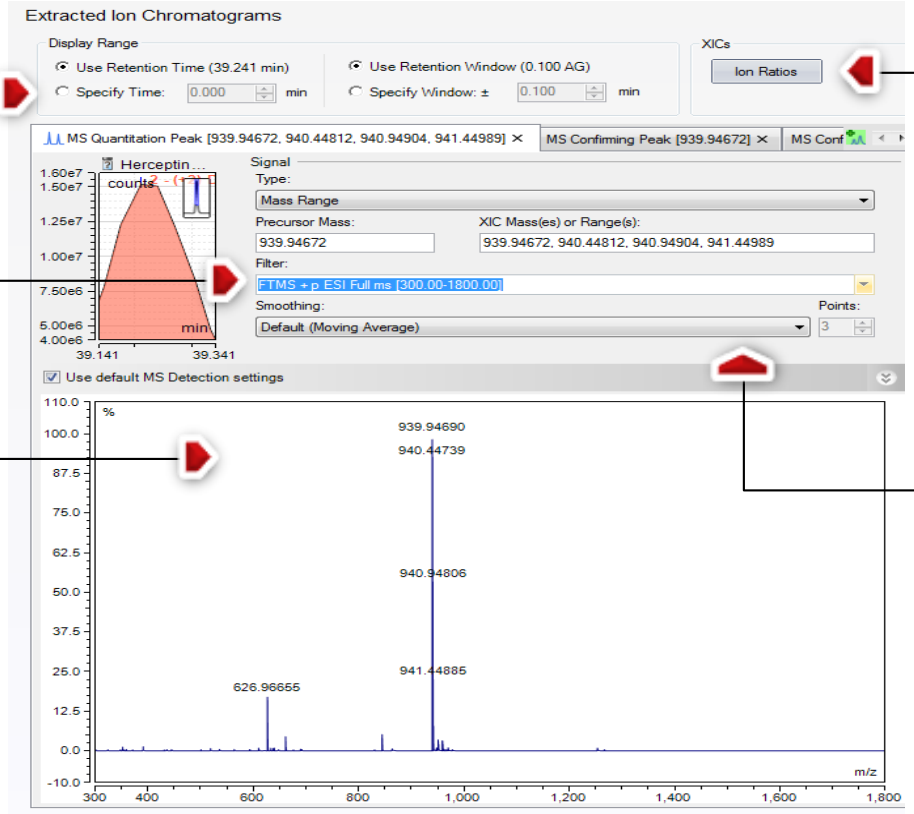
RT Extraction Settings

MS or MS/MS Filters

MS Spectra

Ion Ratio Settings

Smoothing



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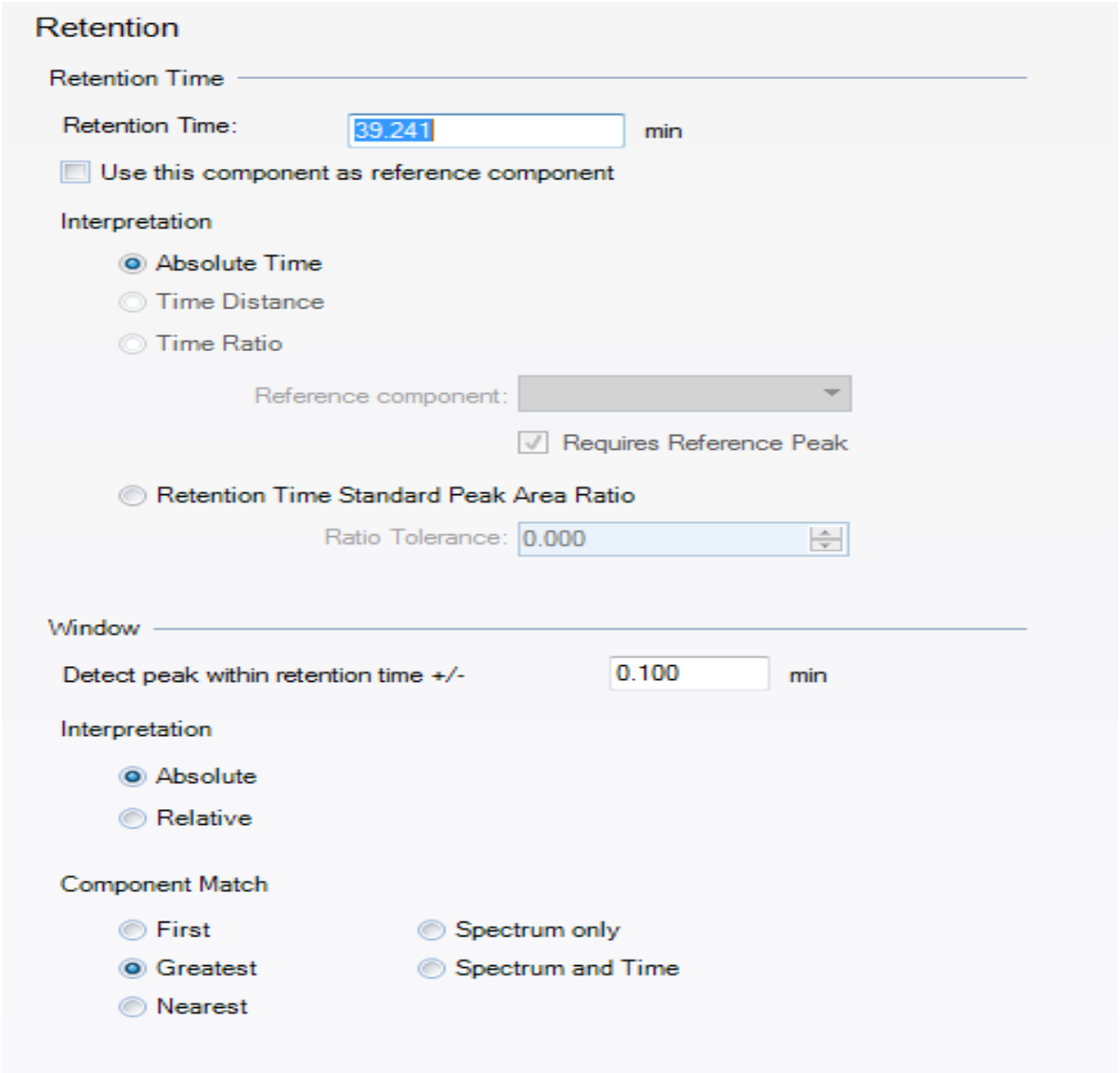
Adjust Processing Settings as Desired

Chromeleon 7.2

- ✓ MS Settings
- ✓ Composite Scoring
- ✓ Integration Settings
- ✓ Retention Time Settings

- Set absolute or relative retention time
- Set absolute or relative RT window
- Setting on specific to individual component
- First, greatest, nearest match within window



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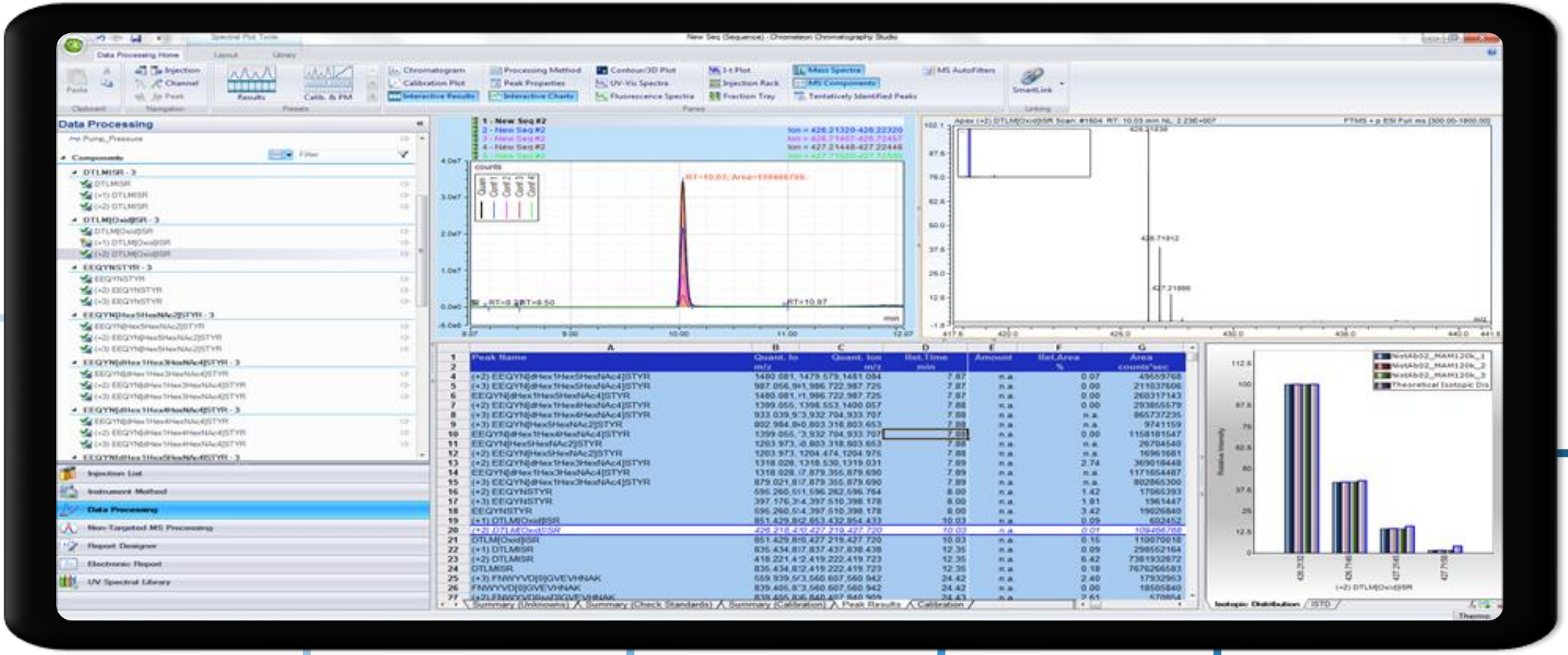
Quantification of CQA: M255 Oxidation



Components

Targeted components and their detection and quantitation status

Experimental Stage

Current state of experiment from injection to reporting

Ion Chromatogram

5 ppm extraction based on scan filters and optional smoothing

Interactive Results

Continually updated results during processing and acquisition

Mass Spectra

Visualize Full MS spectra for selected component

Isotopic Correlation

Validation of peptide identify based on theoretical elemental composition

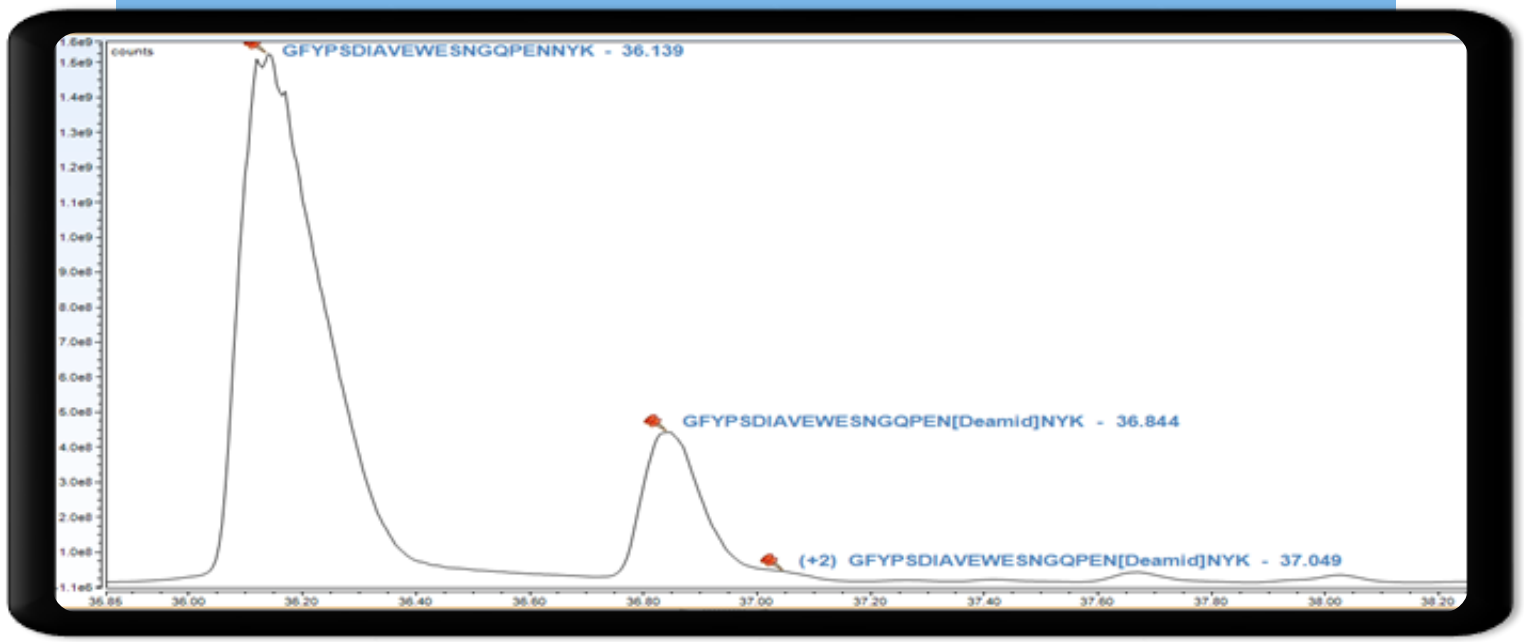
Separation by Resolution

Deamidation of N392

GFYPSDIAVEWESNGQPEN[Deamid]NYK

Chromatographic Resolution

Temporal separation of many deamidated species



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Separation by Resolution

Deamidation of N62

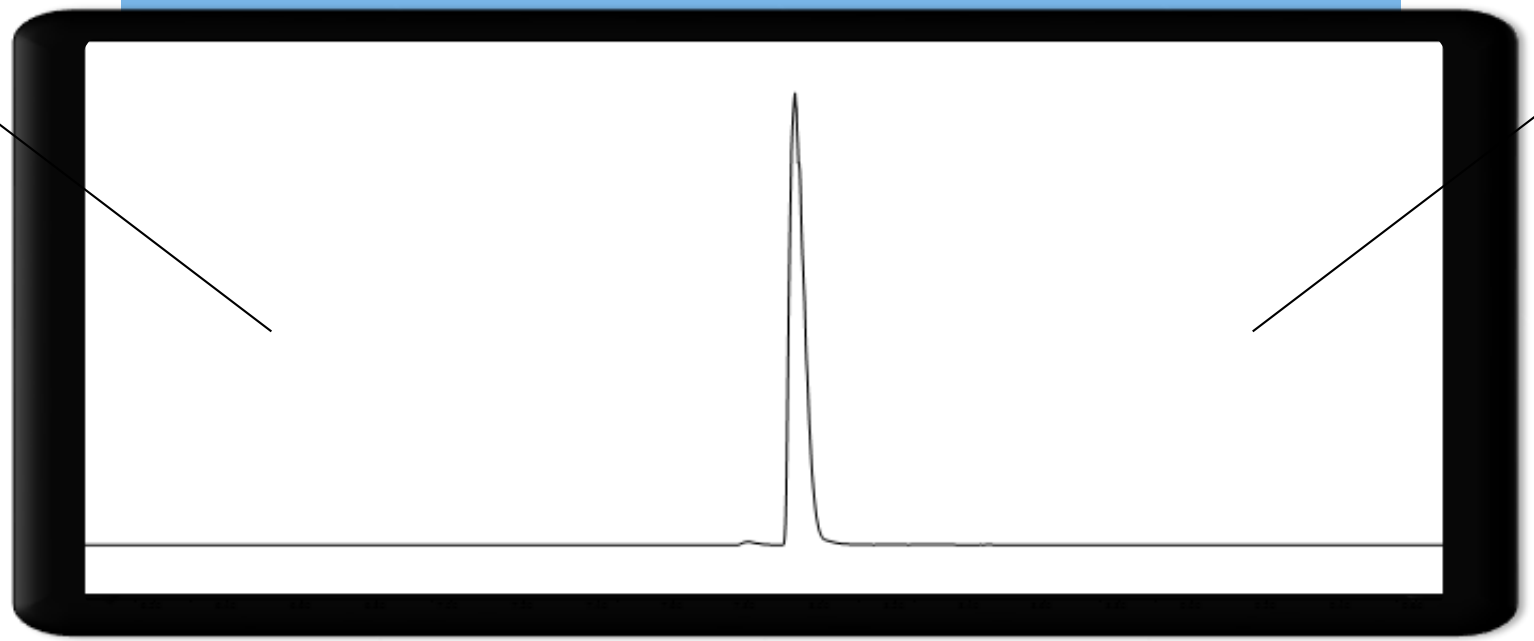
HYN[Deamid]PSLK

Not Chromatographically Resolved

No temporal separation of this deamidated species

Main Peak

Deamidation



Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

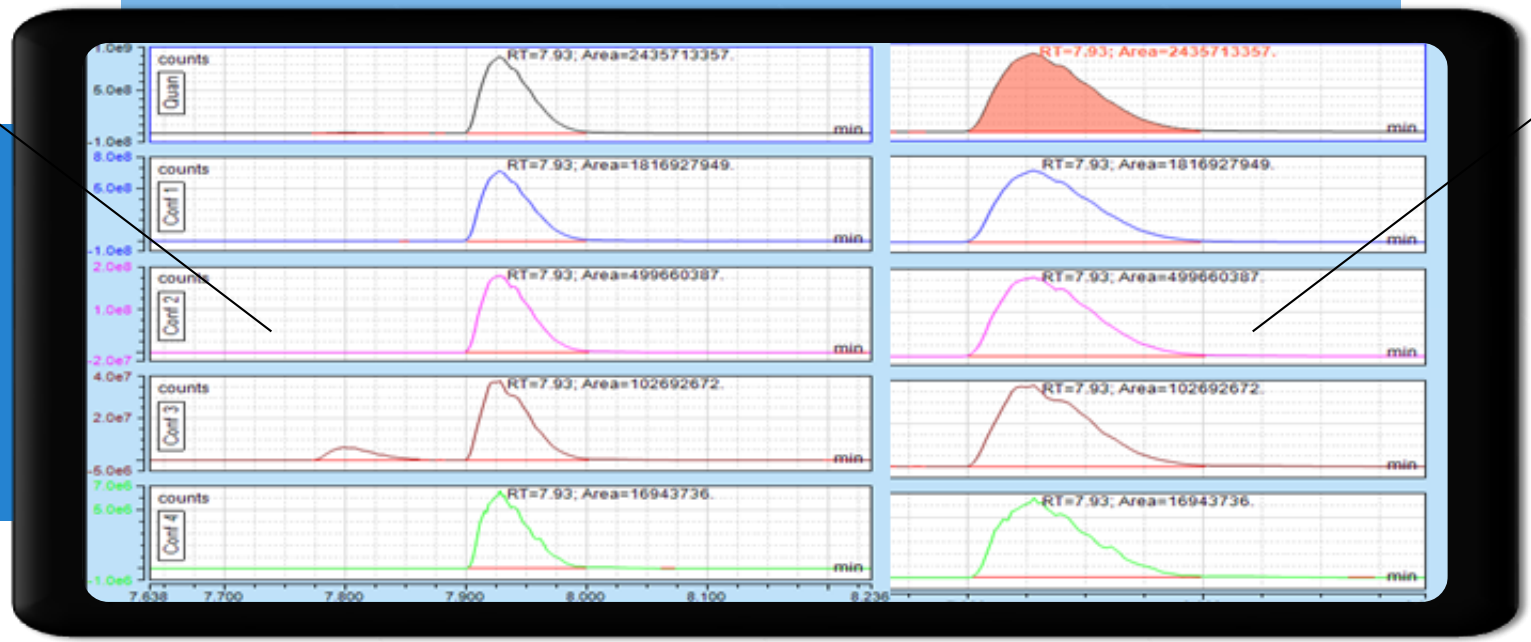
Not Chromatographically Resolved
No temporal separation of this deamidated species

Main Peak

Deamidation

30k Resolution
25 ppm Extraction

Completely incorrect quantitation



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Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved

No temporal separation of this deamidated species

Peak Name First Injection MS Quantitation Peak	Ret.Time min NistAb02_MAM30k MS Quantitation Peak	Area counts*min NistAb02_MAM30k MS_Quantitation
HYN[Deamid]PSLK	7.928	40595222.619
HYNPSLK	7.928	104371927.127
	TOTAL	144967149.7
	Rel Abundance	**72.00%**

30k Resolution
25 ppm Extraction

Completely incorrect quantitation

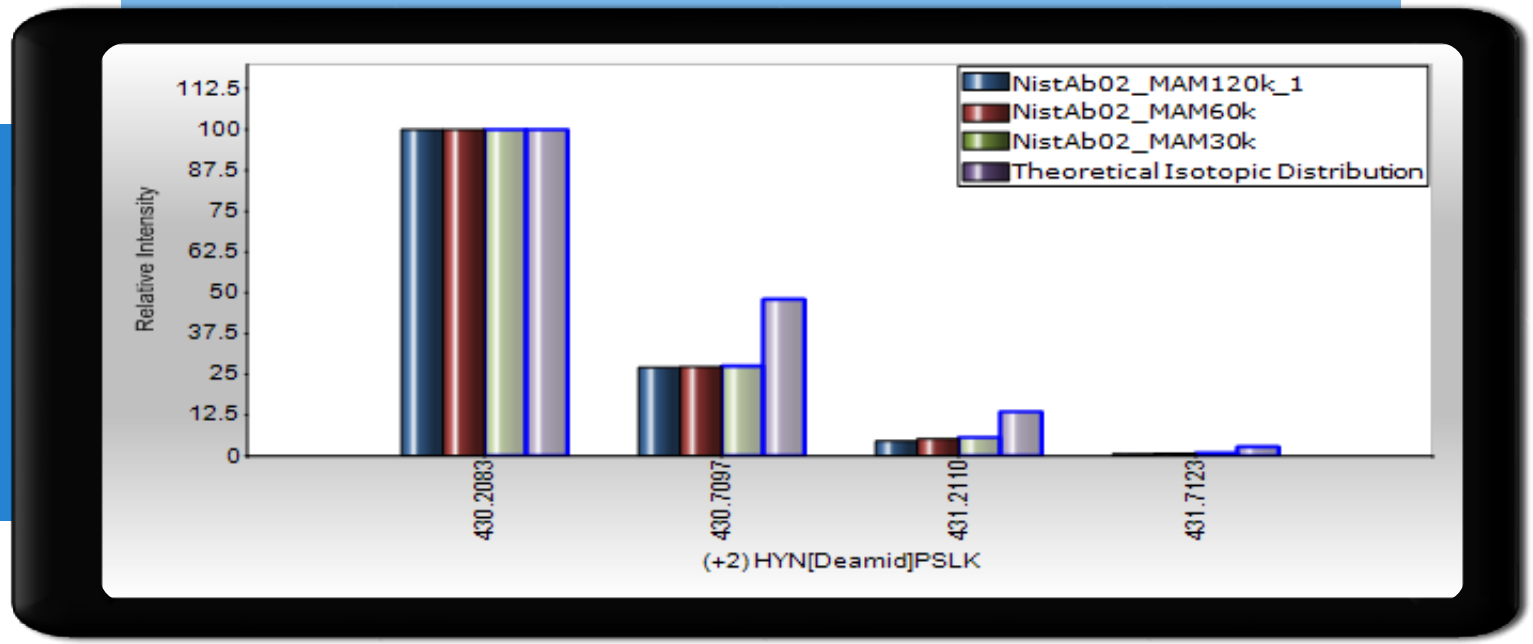
Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved

No temporal separation of this deamidated species



30k Resolution
25 ppm Extraction

Completely incorrect quantitation

Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved

No temporal separation of this deamidated species

Peak Name First Injection MS Quantitation Peak	Ret.Time min NistAb02_MAM30k MS Quantitation Peak	Area counts*min NistAb02_MAM30k MS_Quantitation
HYNPSLK	7.928	96322458.682
HYN[Deamid]PSLK	8.014	82470.684
	TOTAL	96404929.37
	Rel Abundance	**0.09%**

- **HYNPSLK - 2**
  - HYNPSLK
  - (+2) HYNPSLK
- **HYN[Deamid]PSLK - 2**
  - HYN[Deamid]PSLK
  - (+2) HYN[Deamid]PSLK

30k Resolution
5 ppm Extraction

Not enough true resolution, over extracts the data

Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

Main Peak

Deamidation

Not Chromatographically Resolved

No temporal separation of this deamidated species

30k Resolution
5 ppm Extraction

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Separation by Resolution

Deamidation of N62

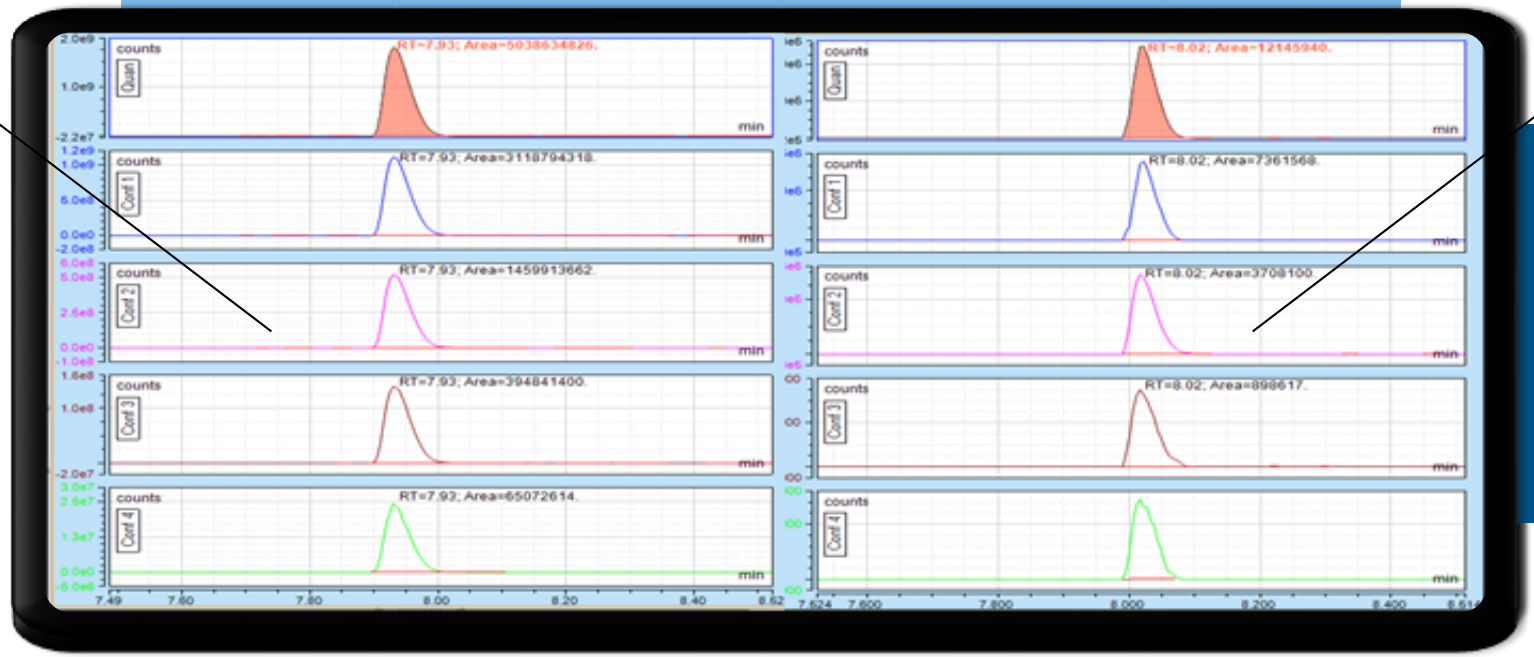
HYN[Deamid]PSLK

Main Peak

Deamidation

Not Chromatographically Resolved

No temporal separation of this deamidated species



120k Resolution
5 ppm Extraction

Spectrometrically resolve deamidated peptides

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Separation by Resolution

Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved

No temporal separation of this deamidated species

Peak Name First Injection MS Quantitation Peak	Ret.Time min NistAb02_MAM120k_1 MS Quantitation Peak	Peak Area counts*min NistAb02_MAM120k_1 MS Quantitation Peak
HYNPSLK	7.930	83977247.099
HYN[Deamid]PSLK	8.022	202432.341
	TOTAL	84179679.44
	Rel Abundance	**0.24%**

- **HYNPSLK - 2**
  - HYNPSLK
  - (+2) HYNPSLK
- **HYN[Deamid]PSLK - 2**
  - HYN[Deamid]PSLK
  - (+2) HYN[Deamid]PSLK

120k Resolution
5 ppm Extraction

Spectrometrically resolve deamidated peptides

Data Analysis Processing Chromeleon

Chromeleon 7.2

- Set up injection sequence
- Build LC and MS methods
- Target. Confirm. Integrate.
- Look for new peptides/impurities/features
- Automatically generate reports outputs/formulas

Simple. Logical.

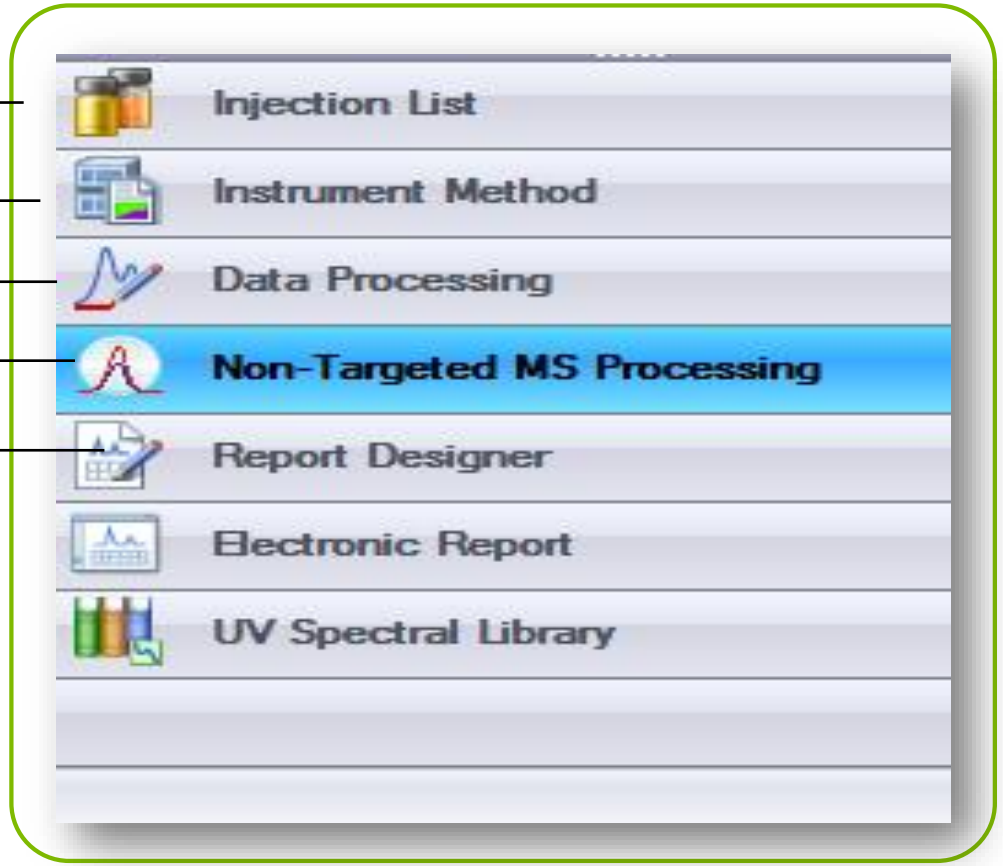
1. Create Injection List

2. Define Instrument Method (LC and MS)

3. Targeted MS Processing
Verify all expected peptides

4. Non-Targeted MS Processing
Identify new peptides/impurities/modifications
Comparison to reference standard (A to B)

5. Reporting



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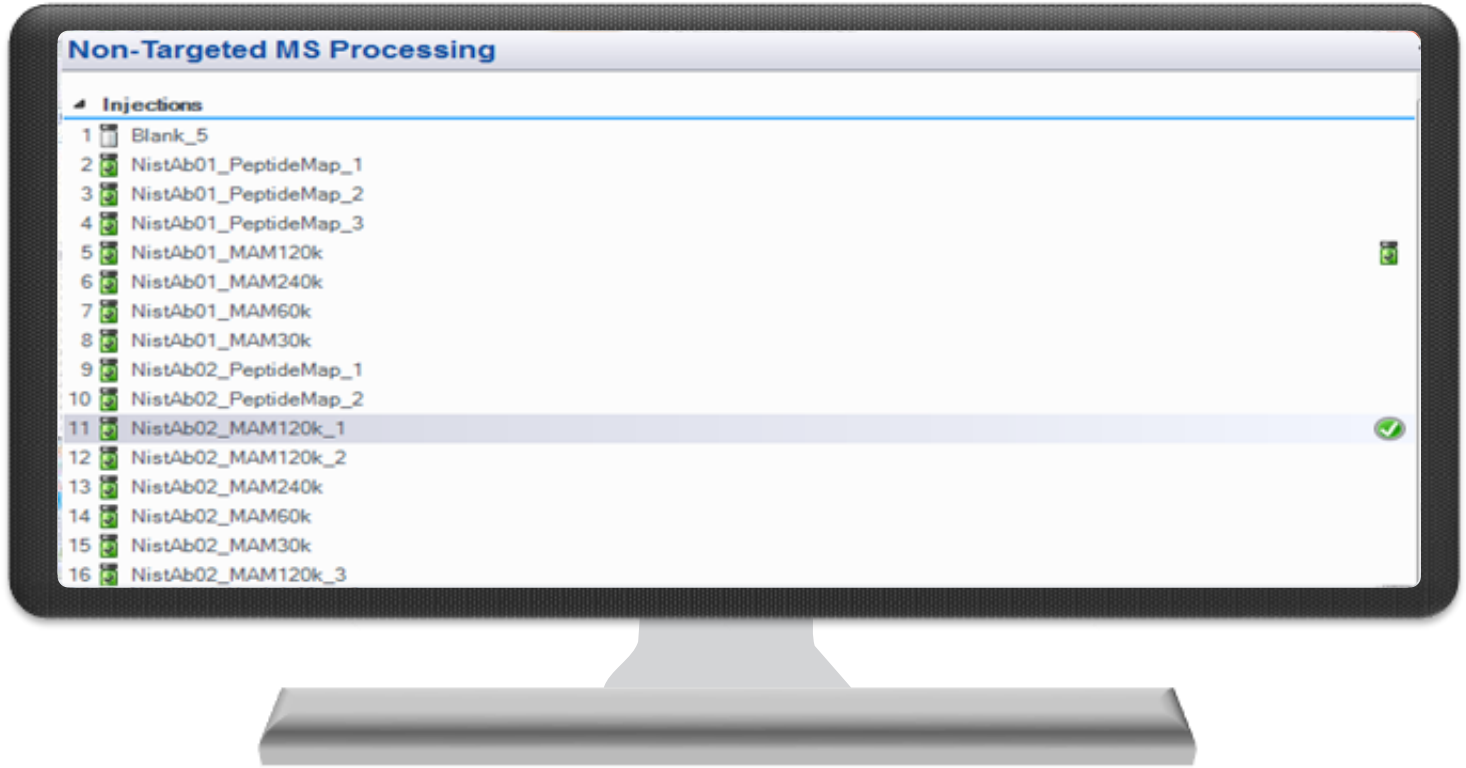
Detection of New Features: Batch to Batch

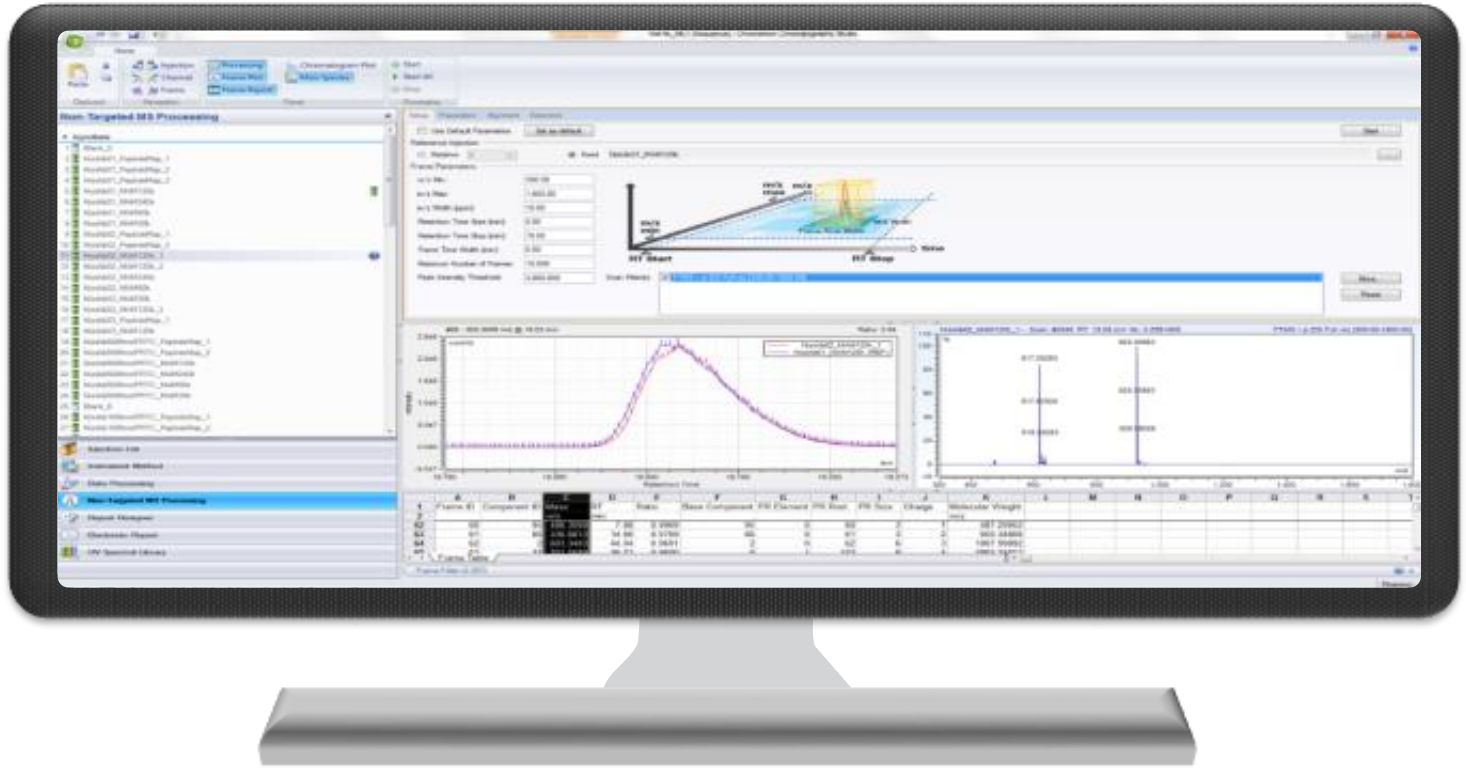
- **Assign a reference injection (A) and compare as many new injection (B) as desired**
  - First Sample Prep (A)
  - Second Sample Prep (B)
- **Set Alignment and Framing Settings**
  - 0.1% Base Peak 10 ppm 0.5 min
- **Automatic Alignment and Framing**
- **Reporting of Features**
- **Filtering of Detected Features**
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change



- **Validation of Results**

Detection of New Features: Batch to Batch

- **Assign a reference injection (A) and compare as many new injection (B) as desired**
  - First Sample Prep (A)
  - Second Sample Prep (B)
- **Set Alignment and Framing Settings**
  - 0.1% Base Peak 10 ppm 0.5 min
- **Automatic Alignment and Framing**
- **Reporting of Features**
- **Filtering of Detected Features**
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change



- **Validation of Results**

Detection of New Features: Batch to Batch

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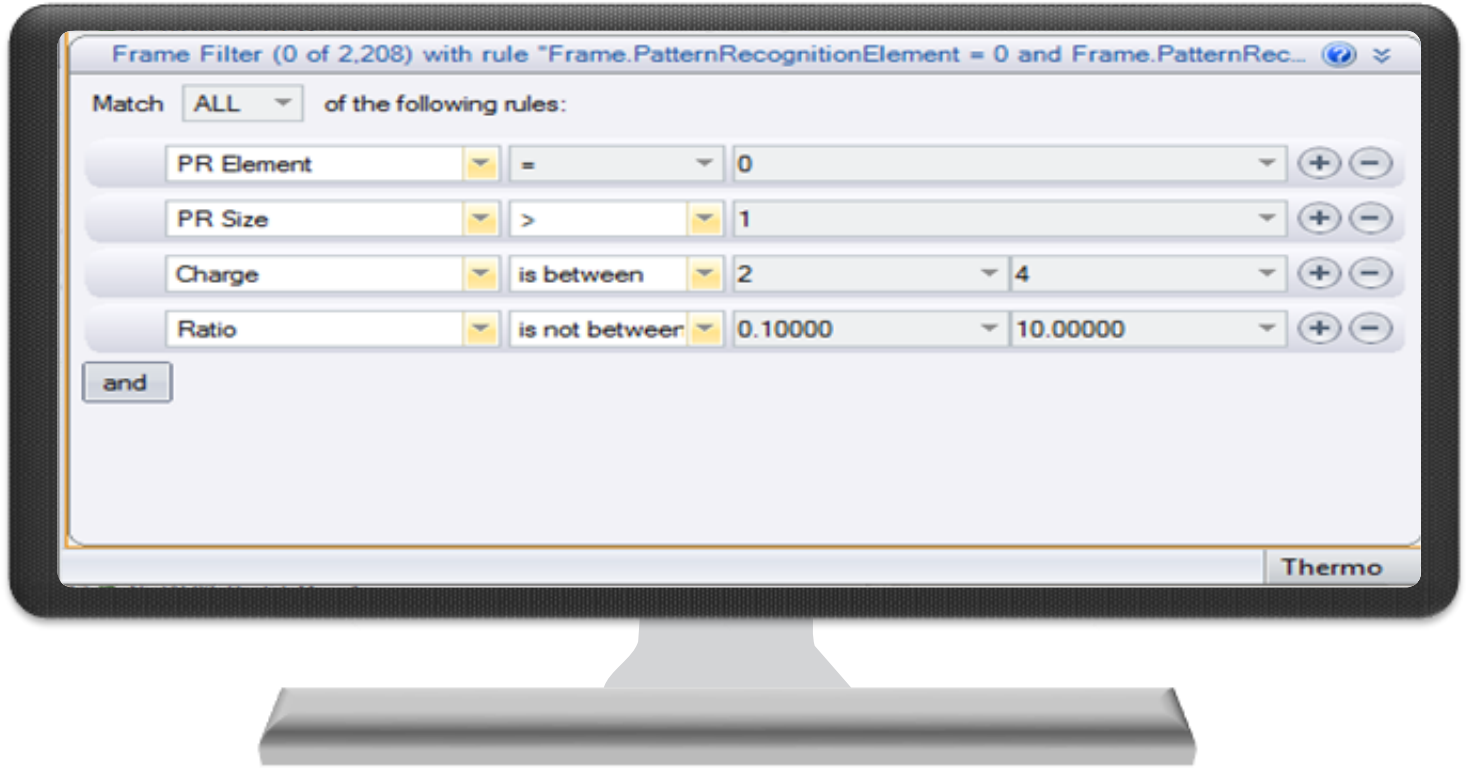

- **Validation of Results**

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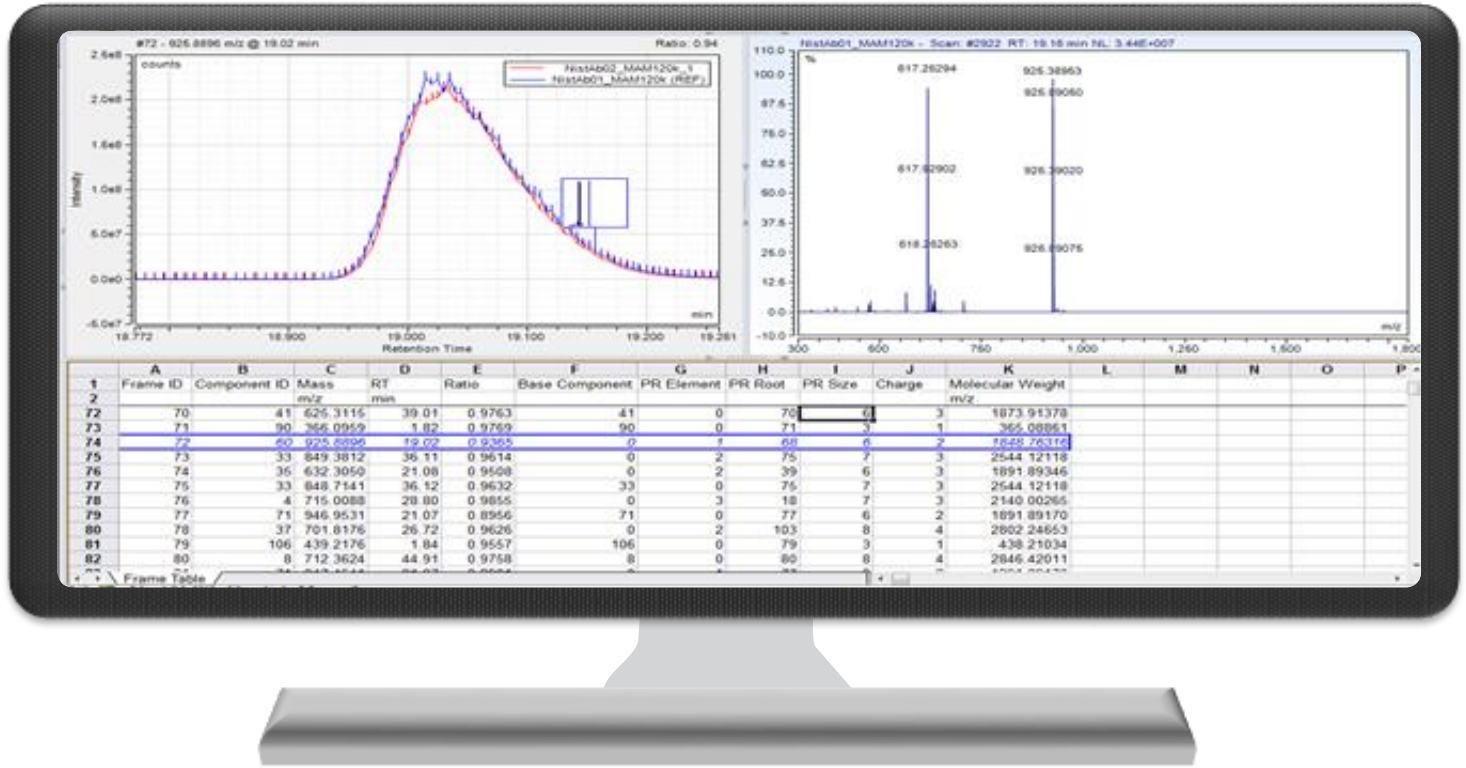
Detection of New Features: Batch to Batch

- **Assign a reference injection (A) and compare as many new injection (B) as desired**
  - First Sample Prep (A)
  - Second Sample Prep (B)
- **Set Alignment and Framing Settings**
  - 0.1% Base Peak 10 ppm 0.5 min
- **Automatic Alignment and Framing**
- **Reporting of Features**
- **Filtering of Detected Features**
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change



- **Validation of Results**

In Spec: No New Features

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Detection of New Features: PRTC Syn Peptide Kit

New Peak Detection

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Injection Details

Injection Name:	Acclaim15cm_PRTC	Run Time (min):	68.51
Vial Number:	R:C4	Injection Volume:	3.00
Injection Type:	Unknown		
Calibration Level:			
Instrument Method:	QEHF_MAM_MkV		
Processing Method:	SuperMAM	Dilution Factor:	1.0000
Injection Date/Time:	29/Mar/17 19:40	Sample Weight:	1.0000

Total Ion Chromatogram Table

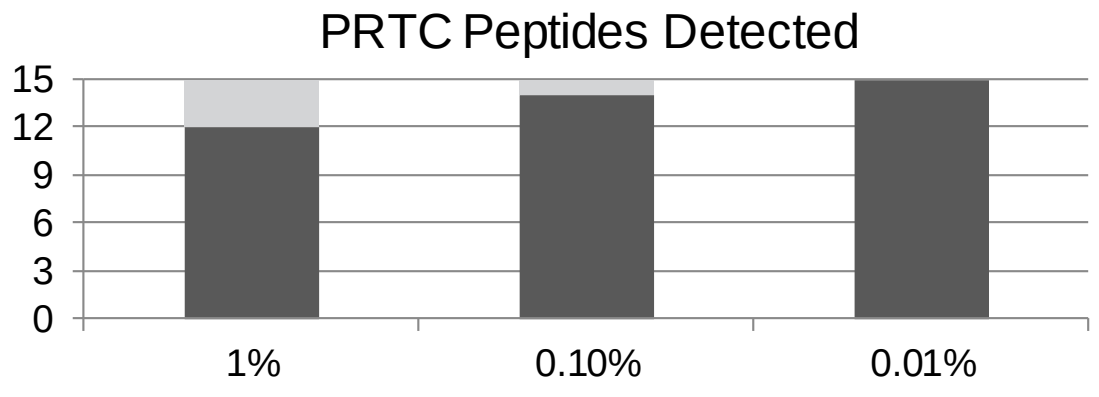
No.	Injection Name	Maximum Signal Value counts TIC	1% of TIC counts TIC	0.1% of TIC counts TIC
1	Acclaim15cm	1580663200	15806632	1580663
2	Acclaim15cm	1490999700	14909997	1491000
3	Acclaim15cm_PRTC	1438209500	14382095	1438210
4	Acclaim15cm_PRTC	1459710600	14597106	1459711

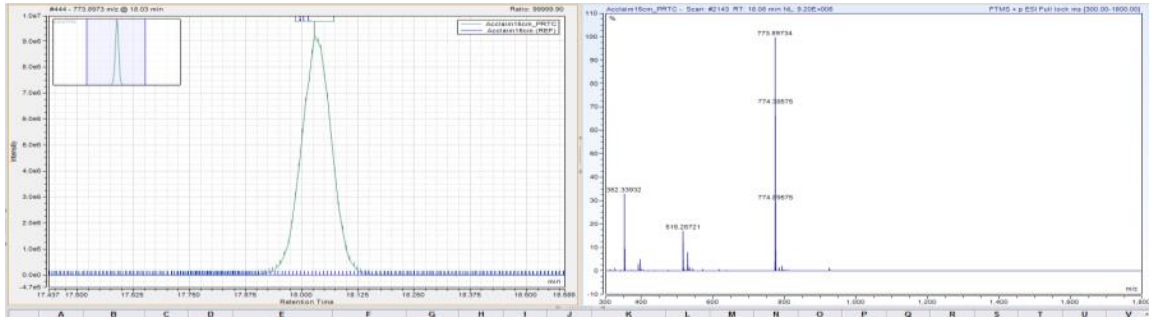
Detection Settings

Status: Not In Spec	m/z min	300.0000	Algorithm	FRAME
	m/z max	1800.0000	Alignment Bypass	False
	Frame Width [ppm]	10.0000	Alignment Min Intensity	1000.00
	Max Frames	5000	Alignment Bin Width	1.00
	Frame Width [ppm]	10.0000	Frame Filter	FTMS + p ESI Full lock ms [300.00-1800.00]:FTMS + p ESI Full ms [300.00-1800.00]
	Peak Intensity Threshold	1500000	Reference Injection No.	2
	RT Start [min]	2.00		
	RT Stop [min]	70.52		
	Frame Width [min]	2.50		
	Frame Width [ppm]	10.0000		

New Features

Frame ID	Component ID	Mass m/z	RT min	Ratio	Base Component	PR Element	PR Root	PR Size	Charge	Molecular Weight m/z
190	252	496.2873	8.79	99999.9000	252	0	190	3	2	990.55997
210	336	613.3156	8.14	99999.9000	336	0	210	3	2	1224.61655
286	299	422.7364	10.34	99999.9000	299	0	286	2	2	843.45817
316	357	493.7691	8.07	22834.7506	357	0	316	3	2	985.52372
331	219	558.3267	25.64	320623.5021	219	0	331	3	2	1114.63877
422	301	745.3943	26.10	99999.9000	301	0	422	3	2	1488.77402
444	330	773.8973	18.03	99999.9000	330	0	444	3	2	1545.78013
460	359	695.8323	12.55	5015.2316	359	0	460	3	2	1389.65012
548	317	301.1919	11.10	4675.0764	317	0	548	2	3	900.55376
551	334	801.4132	26.82	99999.9000	334	0	551	3	2	1600.81186
578	309	573.3038	34.85	111.9589	309	0	578	2	2	1144.59311
587	332	680.3748	38.89	99999.9000	332	0	587	3	2	1358.73496
655	358	498.8026	30.88	37933.1051	358	0	655	2	2	995.59073





Detection of new features in Nist Ab digest with 1% of Peptide RT Calibration peptides spiked in

Digest Only (A)

Digest + 500 fmol/uL PRTC (B)

Alignment and Framing Settings

1.0% Base Peak 10 ppm

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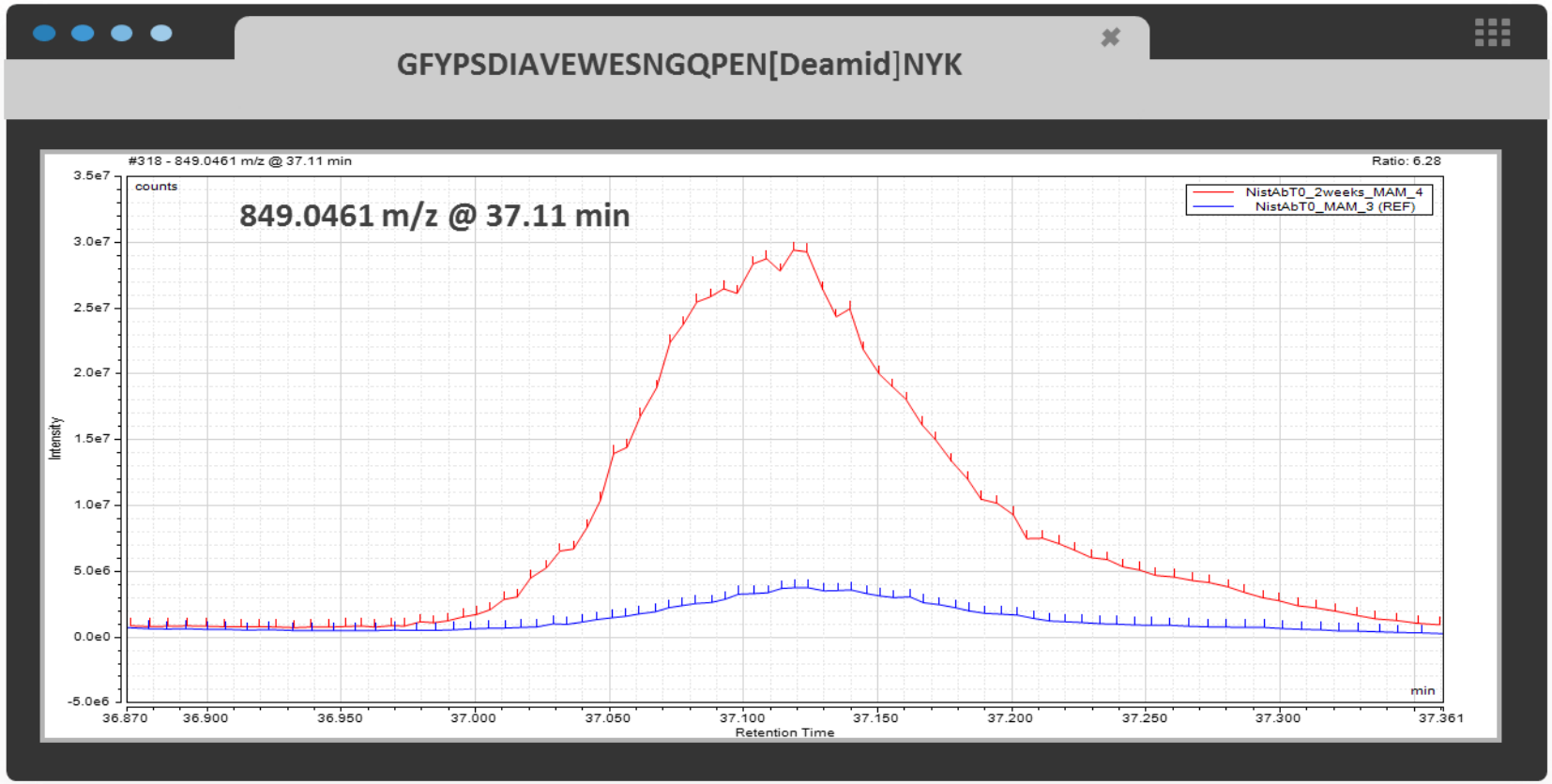
Detection of New Features: Stress Study

Two Weeks at pH 8

Forced stress study versus sample immediately after preparation



ThermoFisher SCIENTIFIC

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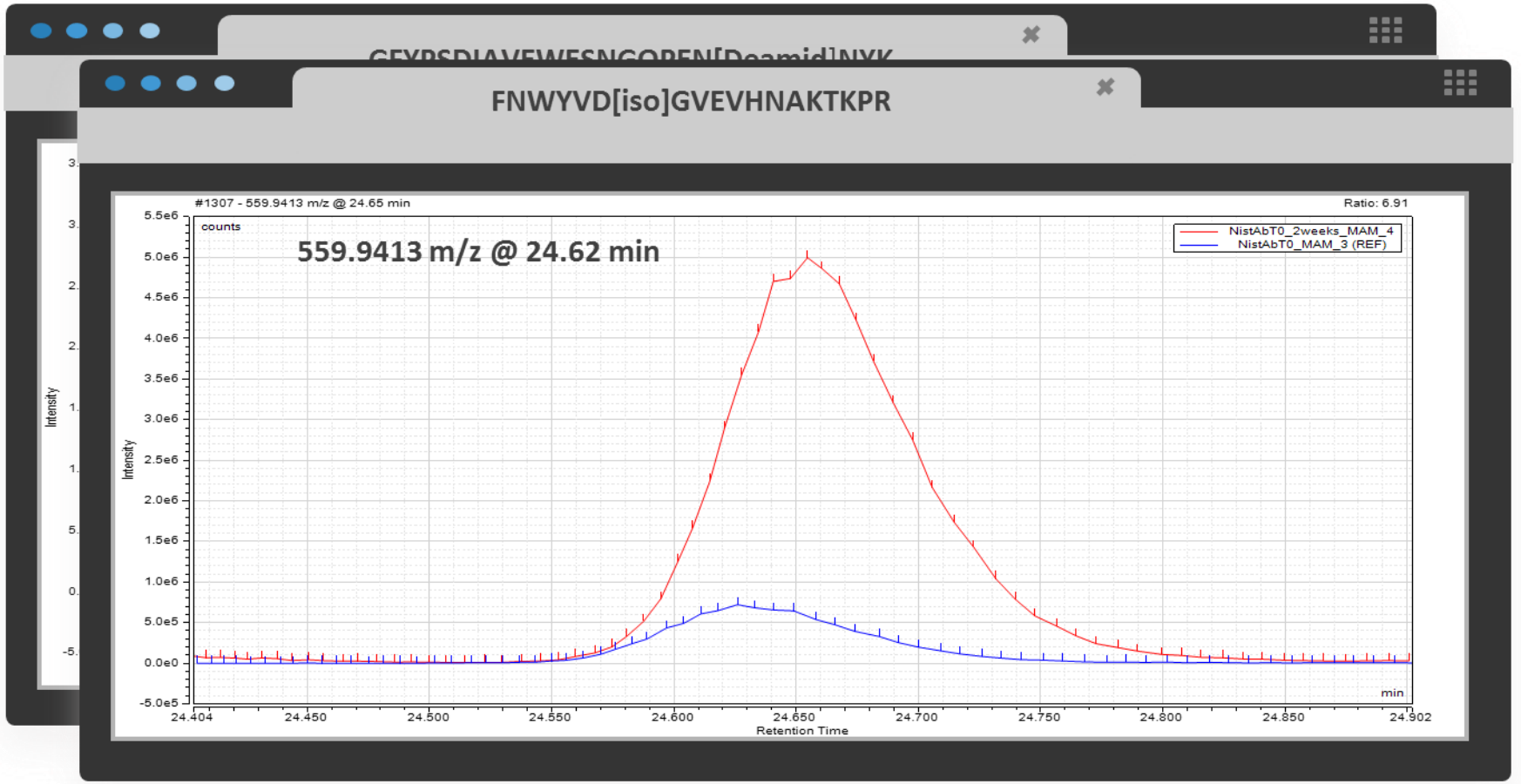


ThermoFisher SCIENTIFIC

System Suitability

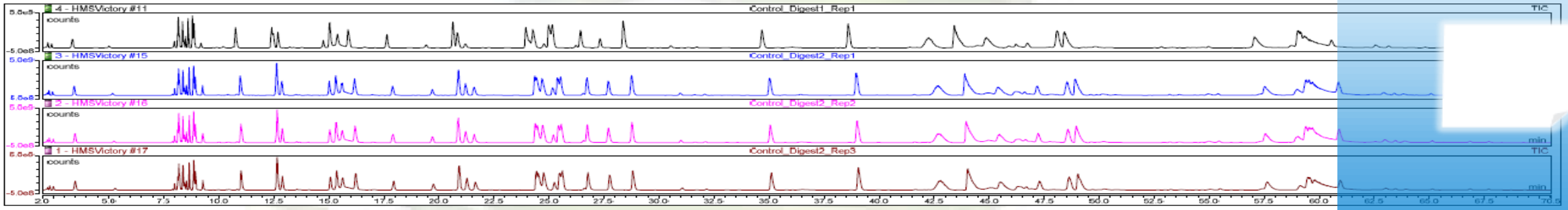
System Suitability

ThermoFisher SCIENTIFIC

Sequence Details

Name:	HMSVictory		
Instrument:	QEHF_TheGhost	Created On:	09/Nov/16 14:49:34
Imported Data:	False	Updated On:	02/Feb/17 11:08:45
First Injection:	NISTmAb_Control1_DDA_1		
Processing Method:	SuperMAM		
MS Acquisition Time [min]	70.52		
Method Length [min]:	115.00		
Total Time [hrs]	63.25		
Data Vault:	ChromeleonLocal	Created By:	Thermo
No. of Injections:	33	Updated By:	Thermo

Total Ion Chromatogram



Retention Time Stability

No.	Name	Retention (DETECTED) min EEQYN[dHex1Hex3HexNAc4]STYR	Retention (DETECTED) min DTLM[Oxid]SR	Retention (DETECTED) min DTLMISR	Retention (DETECTED) min DIQMTQSPSTLSASVGDR	Retention (DETECTED) min FNWYVDGVEVHNAK	Retention (DETECTED) min WQQGNVFSC[Carboxymethyl]SVMHEALHNHYTQK	Retention (DETECTED) min GFYPSDIAVEWESNGQPENNYK	Retention (DETECTED) min GFYPSDIAVEWESN
15	Control_Digest2_Rep1	8.14	10.33	12.87	20.89	24.37	25.40	35.03	35
16	Control_Digest2_Rep2	8.14	10.35	12.88	20.87	24.38	25.43	35.05	35
17	Control_Digest2_Rep3	8.14	10.34	12.89	20.93	24.43	25.50	35.11	35
	coefficient of variation	0.0%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%	0.
		PASS	PASS	PASS	PASS	PASS	PASS	PASS	PA

Peak Area Stability and Minimum Intensity

No.	Name	Area counts*min EEQYN[dHex1Hex3HexNAc4]STYR	Area counts*min DTLM[Oxid]SR	Area counts*min DTLMISR	Area counts*min DIQMTQSPSTLSASVGDR	Area counts*min FNWYVDGVEVHNAK	Area counts*min WQQGNVFSC[Carboxymethyl]SVMHEALHNHYTQK	Area counts*min GFYPSDIAVEWESNGQPENNYK	Area counts*min GFYPSDIAVEWESN
15	Control_Digest2_Rep1	1.68E+07	1.80E+06	1.39E+08	2.58E+08	3.10E+08	2.78E+08	2.00E+08	3.12
16	Control_Digest2_Rep2	1.64E+07	1.83E+06	1.38E+08	2.56E+08	3.08E+08	2.84E+08	2.01E+08	3.11
17	Control_Digest2_Rep3	1.64E+07	1.84E+06	1.38E+08	2.55E+08	3.07E+08	2.85E+08	2.00E+08	3.01
	coefficient of variation	1.3%	1.1%	0.7%	0.5%	0.4%	1.3%	0.4%	2.
		PASS	PASS	PASS	PASS	PASS	PASS	PASS	PA
	specified min area	5.00E+06	5.00E+05	5.00E+07	1.00E+08	1.00E+08	1.00E+08	1.00E+08	7.00
		PASS	PASS	PASS	PASS	PASS	PASS	PASS	PA

Mass Accuracy

No.	Name	Mass Accuracy ppm EEQYN[dHex1Hex3HexNAc4]STYR	Mass Accuracy ppm DTLM[Oxid]SR	Mass Accuracy ppm DTLMISR	Mass Accuracy ppm DIQMTQSPSTLSASVGDR	Mass Accuracy ppm FNWYVDGVEVHNAK	Mass Accuracy ppm WQQGNVFSC[Carboxymethyl]SVMHEALHNHYTQK	Mass Accuracy ppm GFYPSDIAVEWESNGQPENNYK	Mass A pp GFYPSDIAVEWESN
15	Control_Digest2_Rep1	-2.32	0.72	-1.17	-0.71	-1.09	-1.31	-2.01	1
16	Control_Digest2_Rep2	-1.94	0.79	-0.51	-0.45	-0.80	-0.46	-2.11	1
17	Control_Digest2_Rep3	-1.94	0.79	-0.88	-0.45	-1.16	-0.59	-1.92	1
	maximum mass error	2.32	0.79	1.17	0.71	1.16	1.31	2.11	1
		PASS	PASS	PASS	PASS	PASS	PASS	PASS	PA

Custom MS Report Template

ThermoFisher SCIENTIFIC

CQA Profiling

CQA Overview

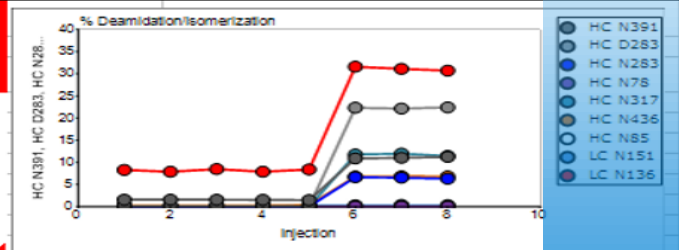
ThermoFisher SCIENTIFIC

Sequence Details

Name:	HMSVictory		
Instrument:	QEHF_TheGhost	Created On:	09/Nov/16 14:49:34
Imported Data:	False	Updated On:	20/Dec/16 17:07:26
First Injection:	NISTmAb_Control1_DDA_1		
Processing Method:	SuperMAM		
MS Acquisition Time [min]	70.51		
Method Length [min]:	115.00		
Total Time [hrs]	63.25		
Data Vault:	ChromeleonLocal	Created By:	Thermo
No. of Injections:	33	Updated By:	Thermo

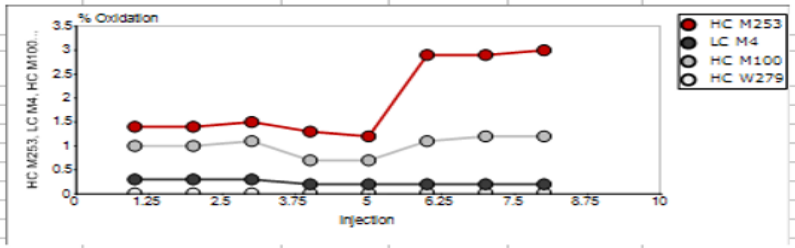
Deamidations and Isomerizations

No.	Name	PENNKY Deam HC N391 %	FNWY IsoD HC D283 %	FNWY Deam HC N283 %	NQVVLK Deam HC N78 %	VVSV Deam HC N317 %	WQQ Deam HC N436 %	YCAR Deam HC N85 %	VDNAL Deam LC N151 %	SGTAS Deam LC N136 %
11	Control_Digest1_Rep1	1.58	0.11	0.04	0.03	0.05	0.23	0.00	0.21	8.3
12	Control_Digest1_Rep2	1.59	0.11	0.04	0.03	0.05	0.24	0.00	0.30	7.9
13	Control_Digest1_Rep3	1.58	0.11	0.03	0.03	0.05	0.24	0.00	0.32	8.5
15	Control_Digest2_Rep1	1.54	0.11	0.04	0.04	0.05	0.23	0.00	0.40	7.9
18	Control_Digest3_Rep1	1.48	0.10	0.04	0.04	0.04	0.40	0.00	0.36	8.4
24	Stressed_Digest2_Rep1	10.89	22.34	6.66	0.04	11.78	6.80	0.12	1.94	31.6
25	Stressed_Digest2_Rep2	11.03	22.14	6.55	0.04	11.98	6.86	0.12	1.68	31.1
26	Stressed_Digest2_Rep3	11.25	22.41	6.31	0.04	11.45	6.84	0.12	1.65	30.7



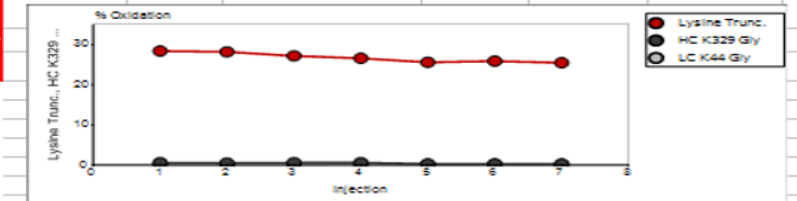
Oxidations

No.	Injection Name	DTLM Oxid HC M253 %	DIQM Oxid LC M4 %	DMIF Oxid HC M100 %	FNWY Oxid HC W279 %
11	Control_Digest1_Rep1	1.4	0.3	1.0	0.01
12	Control_Digest1_Rep2	1.4	0.3	1.0	0.01
13	Control_Digest1_Rep3	1.5	0.3	1.1	0.01
15	Control_Digest2_Rep1	1.3	0.2	0.7	0.01
18	Control_Digest3_Rep1	1.2	0.2	0.7	0.01
24	Stressed_Digest2_Rep1	2.9	0.2	1.1	0.02
25	Stressed_Digest2_Rep2	2.9	0.2	1.2	0.02
26	Stressed_Digest2_Rep3	3.0	0.2	1.2	0.02



Lysine Modifications

No.	Injection Name	SLSSPGK Lysine Trunc. %	VSNK HC K329 Gly %	APK LC K44 Gly %
11	Control_Digest1_Rep1	28.4	0.6	0.1
12	Control_Digest1_Rep2	28.2	0.5	0.1
13	Control_Digest1_Rep3	27.2	0.6	0.1
18	Control_Digest3_Rep1	26.6	0.6	0.2
24	Stressed_Digest2_Rep1	25.6	0.3	0.1
25	Stressed_Digest2_Rep2	25.9	0.3	0.1
26	Stressed_Digest2_Rep3	25.5	0.3	0.1



Sequence Overview | Glycan Profile | Sheet | Master CQA Table | Integration

Custom MS Report Template

ThermoFisher SCIENTIFIC

Glycan Profile

Glycan Profile

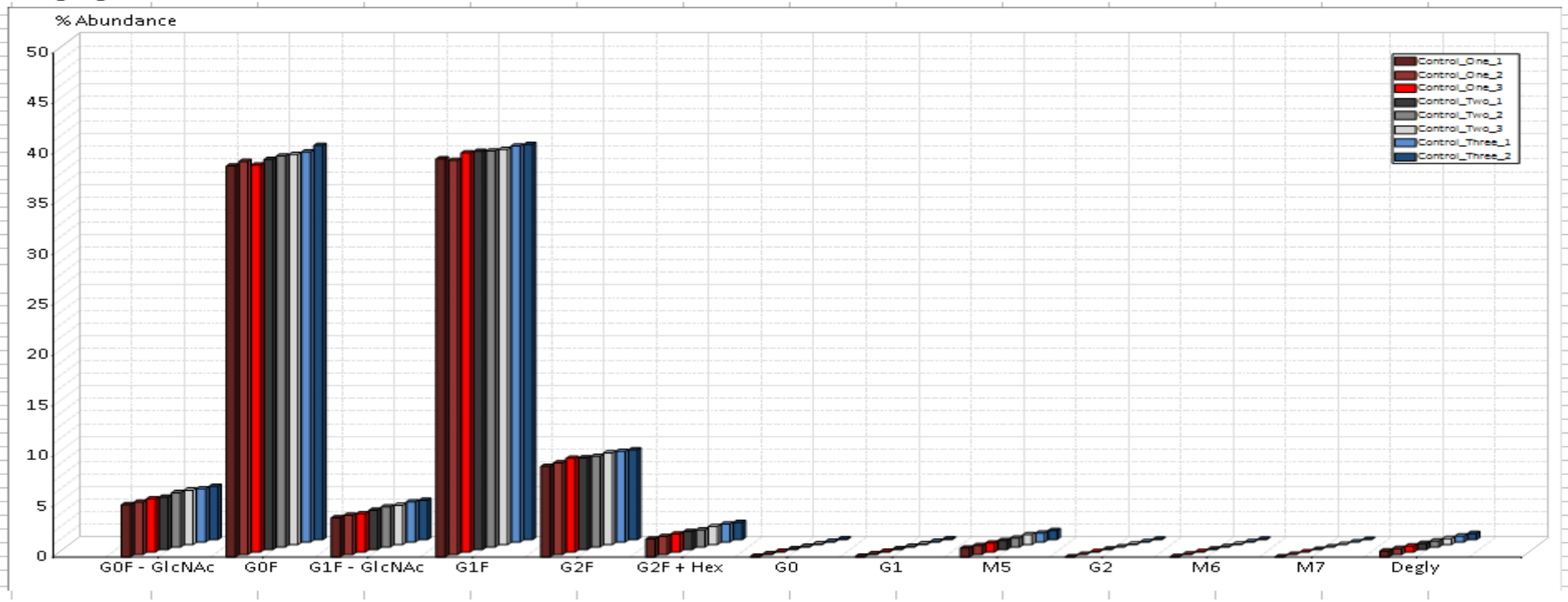
ThermoFisher SCIENTIFIC

Sequence Details

Name:	AppsNote8		
Instrument:	TheGhostandtheDarkness	Created On:	01/Feb/17 17:00:03
Imported Data:	False	Updated On:	03/Feb/17 15:17:33
First Injection:	Control_One_1		
Processing Method:	SuperMAM		
MS Acquisition Time [ml	70.52		
Method Length [min]:	115.00		
Total Time [hrs]	15.33		
Data Vault:	ChromeleonLocal	Created By:	Thermo
No. of Injections:	8	Updated By:	Thermo

EEQYNSTYR Glycopeptides

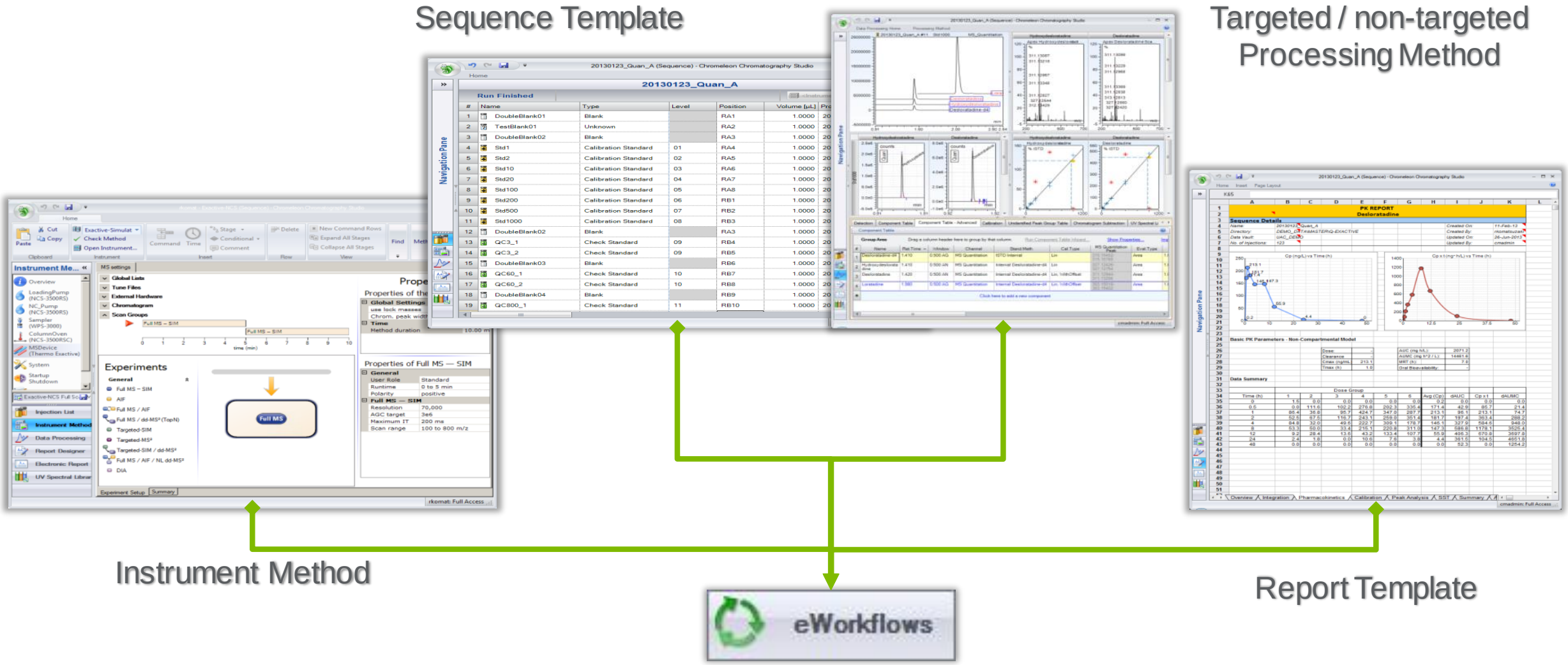
No.	Name	G0F - GlcNAc	G0F	G1F	G2F	G2F + Hex	G0	G1	M5	G2	M6	M7	Degly	Total
1	Control_One_1	5.2	38.8	39.5	9.0	1.8	0.1	0.1	0.9	0.04	0.05	0.02	0.6	100.0
2	Control_One_2	5.2	39.0	39.1	9.1	1.8	0.1	0.1	0.9	0.03	0.05	0.02	0.6	100.0
3	Control_One_3	5.3	38.4	39.6	9.3	1.8	0.1	0.1	0.9	0.05	0.05	0.02	0.6	100.0
4	Control_Two_1	5.2	38.7	39.5	9.1	1.8	0.1	0.1	0.9	0.05	0.06	0.03	0.6	100.0
5	Control_Two_2	5.4	38.8	39.3	9.0	1.7	0.1	0.1	0.9	0.04	0.05	0.02	0.6	100.0
6	Control_Two_3	5.4	38.7	39.2	9.1	1.8	0.1	0.1	1.0	0.05	0.06	0.02	0.6	100.0
7	Control_Three_1	5.3	38.7	39.3	9.0	1.8	0.1	0.1	0.9	0.05	0.06	0.03	0.6	100.0
8	Control_Three_2	5.3	39.1	39.2	8.9	1.7	0.1	0.1	0.9	0.05	0.05	0.02	0.6	100.0



Custom MS Report Template

ThermoFisher SCIENTIFIC

eWorkflows



- eWorkflow automates the acquisition, data processing, and reporting processes
- Ultimate goal of a QC implementation

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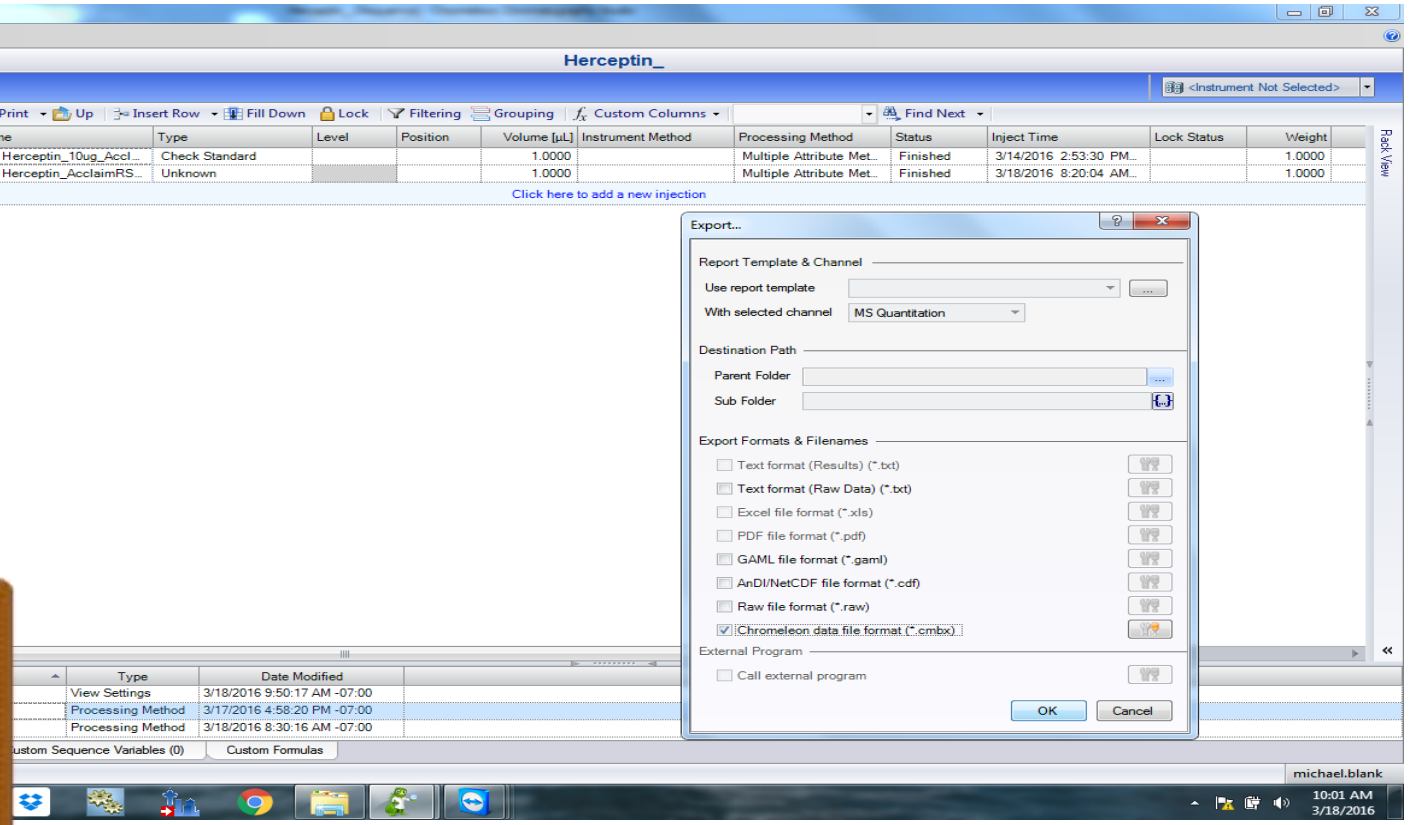
Pack Everything Up

Chromeleon 7.2

Contains:
Sequence
Methods (Instrument and processing)
Report Formats
Studio Layouts
Raw Data
And more…



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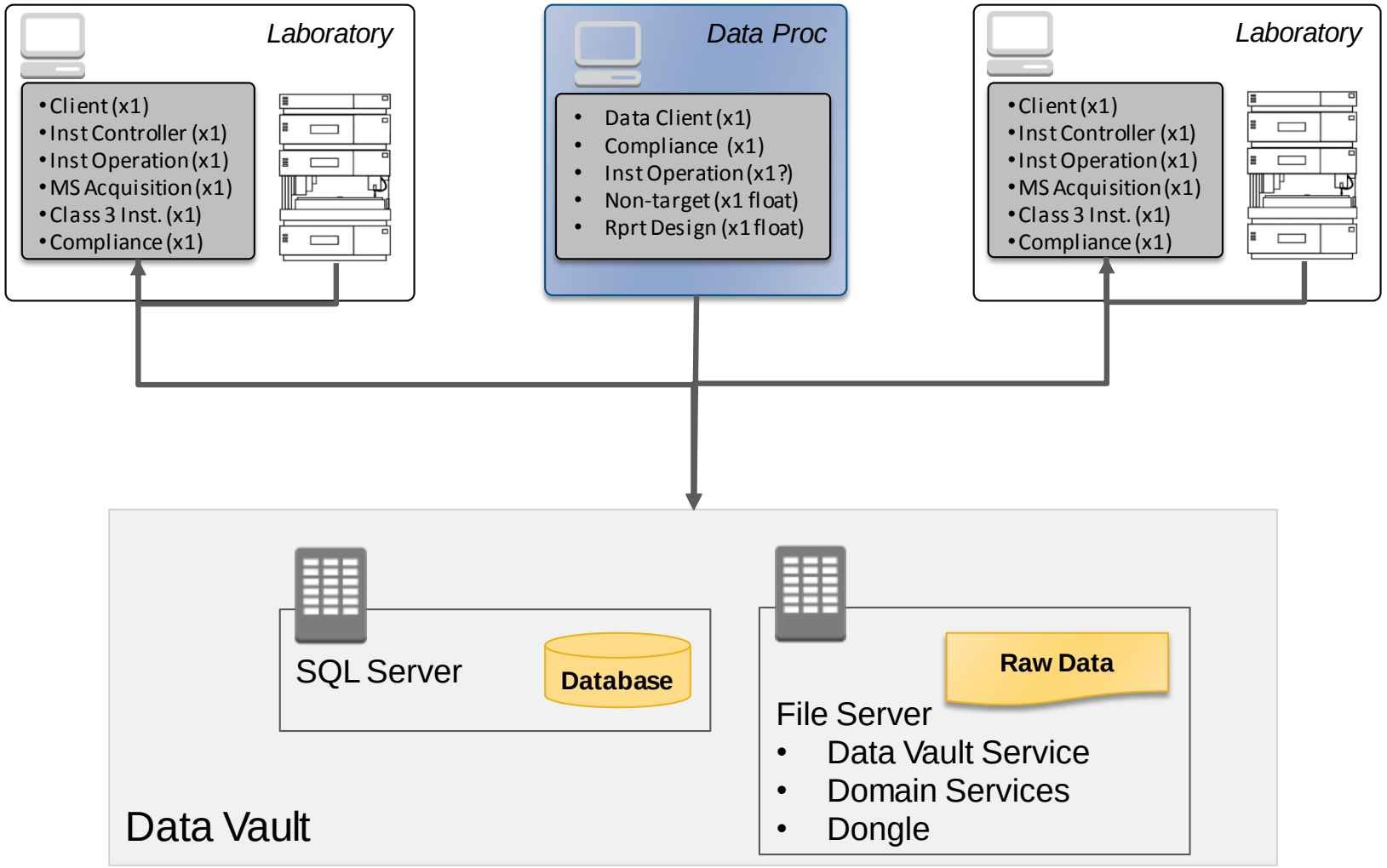
Enterprise Based Solution

Chromeleon 7.2

Remote:
Data Vaults
Data Processing
Licensing
Administration



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Conclusions

Multi-Attribute Method

Not just about reducing the number of tests, but a better way to validate purity

Insightful

Use high quality deeper knowledge of product to improve from development to production through QbD

Easy to Use

Simple LCMS methods without the need for advanced chromatography or gas phase separations

High Resolution

Take advantage of another dimension of separation to see what is missed.

Powerful

Replace numerous conventional lot release techniques while providing greater knowledge of product attributes

Previously Filed

Software/hardware already used for several filings to begin clinical trials with the FDA

Confident

Composite peptide scoring and separation of isotopes/charge states helps eliminate false quantitation