# Implementation of MAM for Quality Control of Monoclonal Antibody Therapeutics

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CASSS Mass Spec Virtual Symposium

Sept 15. 2020

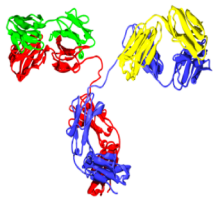
# Outline



- MAM overview
- Implementation of MAM in clinical QC
  - Method validation for clinical QC
  - Data processing
  - System suitability development
  - Data configuration and data integrity
- Summary

# Multi-Attribute Method (MAM) – from Development to QC

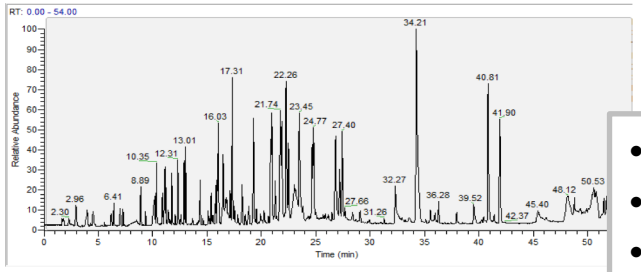




Proteolytic digestion

LC-MS/MS

HPLC
Mass Spec




- Chromatogram
- MS spectra
- MS/MS spectra

**QC Product Testing**

Non Targeted MS processing

Comparing files and detecting changes / new peaks

Targeted MS quantitation

Quantifying relative abundance of CQAs

Compliant reporting

**Characterization**

- Peptide sequence
- PTM (Oxidation, etc.)
- Sequence variant
- Clip
- other PQA
- Abundance of PQA

**CQAs to be monitored in QC**

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# MAM vs Conventional Product Testing Approaches.



A Example of Global vs Specific Attributes

| Global (Charge Assay) | Amino Acid Residue Specific (MAM) |
|---|---|
| Acidic variants | K65 Glycation |
| | K125 Glycation |
| | N320 Deamidation |
| | N389 Deamidation |
| | N394 Deamidation |
| | N141 Deamidation |
| | S26 Acidic glycan |

MAM offers

- Better understanding of products
- Detection and quantification of specific CQAs in release and stability
- No bridging between product understanding and testing
- The potential of replacing multiple conventional methods

# Implementation of MAM (LC-MS/MS based peptide mapping)



- We have been doing this (although we did not call it MAM) in ...
  - Molecule Assessment
  - Process Development
  - PV study
  - CQA assessments
  - etc.
- MAM has been an important method for product development and it is a natural progression that MAM is moving into QC.

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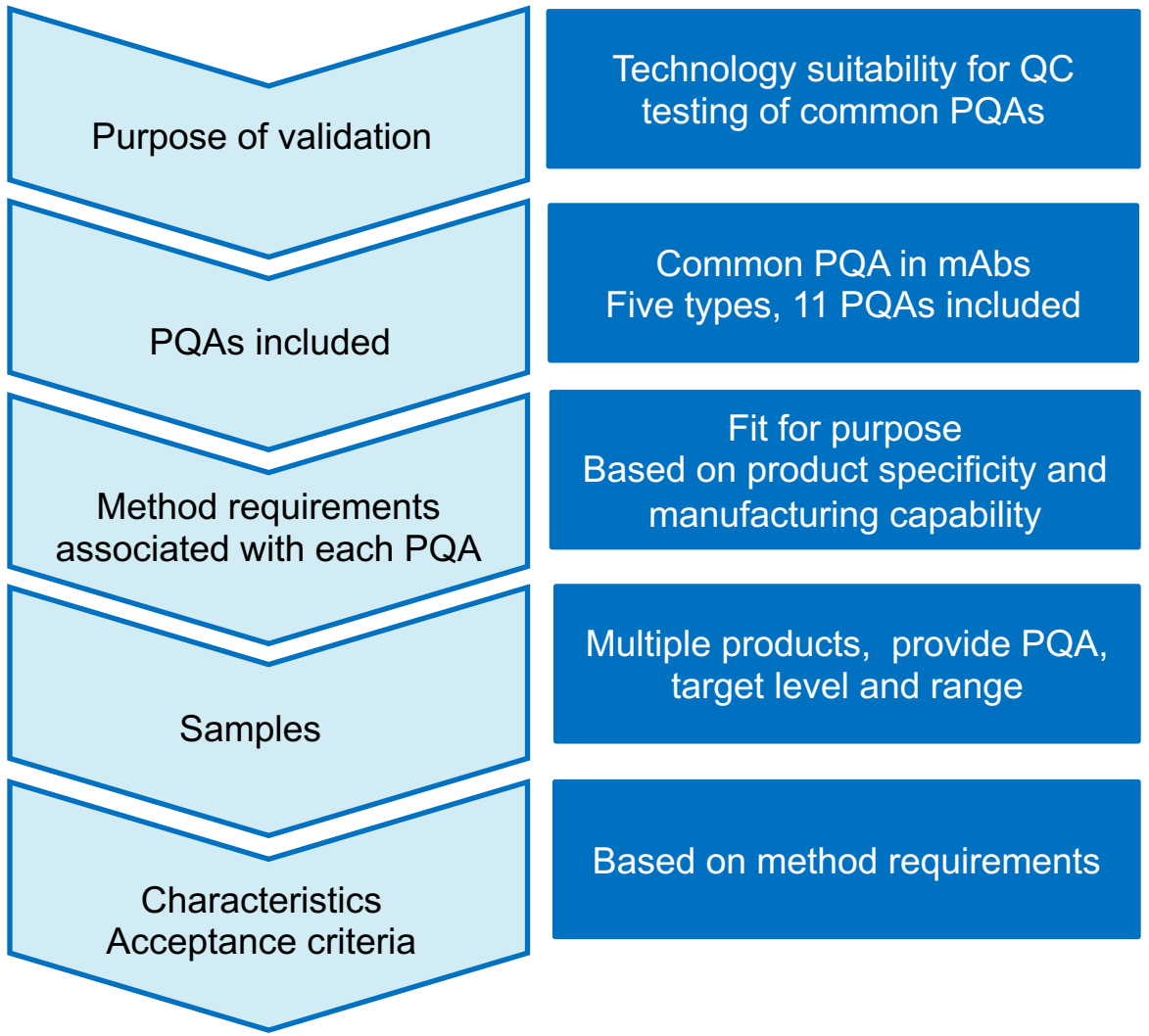
# Validation Strategy and Design





# Demonstrating Method Performance with Five Attribute Types / Four PQA Categories



| PQA Category | PQA | Molecule |
|---|---|---|
| Charge (acidic) | A LC N Deamidation | mAb1 |
| Charge (basic) | A HC D Isomerization | mAb1 |
| Oxidation | M Oxidation on DTLMISR | mAb2 |
| Oxidation | M Oxidation on MHEAL | mAb2 |
| Glycosylation | G0F | mAb3, mAb1 |
| Glycosylation | G1F | mAb3, mAb1 |
| Glycosylation | G2F | mAb3, mAb1 |
| Sequence Variant | Sequence variant | mAb2 |

# Performance Result Overview



| Characteristic* | Results |
|---|---|
| Specificity | Interference <0.1% |
| Accuracy: Recovery by Sample Admixture | 97-112% |
| Linearity | r =1.00 |
| Repeatability (n=6) | RSD < 5% |
| Intermediate Precision (n=12) | RSD < 7 % |
| Range | PQA specific |
| QL | PQA specific: 0.02 - 2.4% |
| Stability in autosampler (change in abundance at 24-, and 48- hour | Relative change from T0: -5.7% - 4.8% |

* Accuracy by sample loading was assessed during development
* Comprehensive robustness study was performed during qualification and result not included here

# Range Used for Each PQA



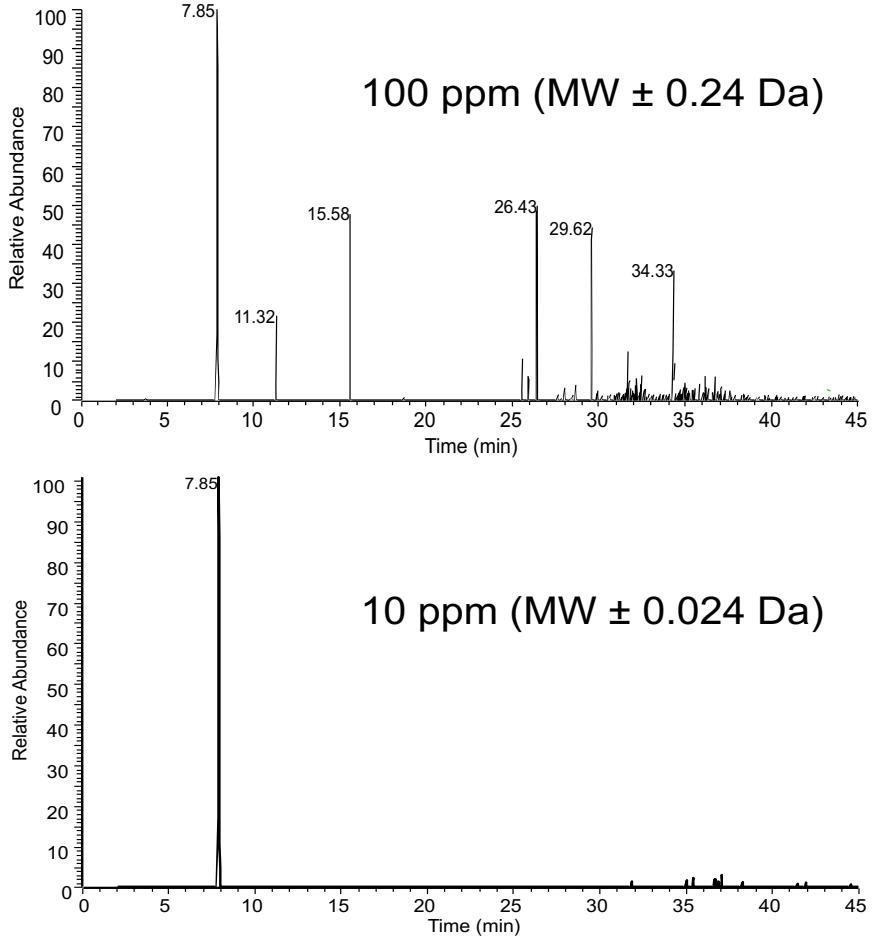
- Specific to product and PQA
- QC method requirement
  - Product specification
  - Manufacturing capability
- Method / technology capability
  - Recovery from sample prep
  - Recovery from column
  - MS ionization efficiency
  - Interference
- Range used for validation was bases on development experience
  - Level of CQA we need to measure

# Specificity- Ensured by High Res LC-MS



- 2D resolving power
  - Reverse-phase LC
  - High Res MS with 10 ppm mass tolerance
- Interference
  - Sample matrix and column carryover
  - Needs to be assessed for each PQA
  - Interference (specificity) = peak area in blank/peak area in sample
    - 9 /11 PQAs not detected in buffer blank
    - 2 /11 were detected at 0.05% and 0.01%

EEQYN[M5]STYR(+2) , m/z=1204.4740

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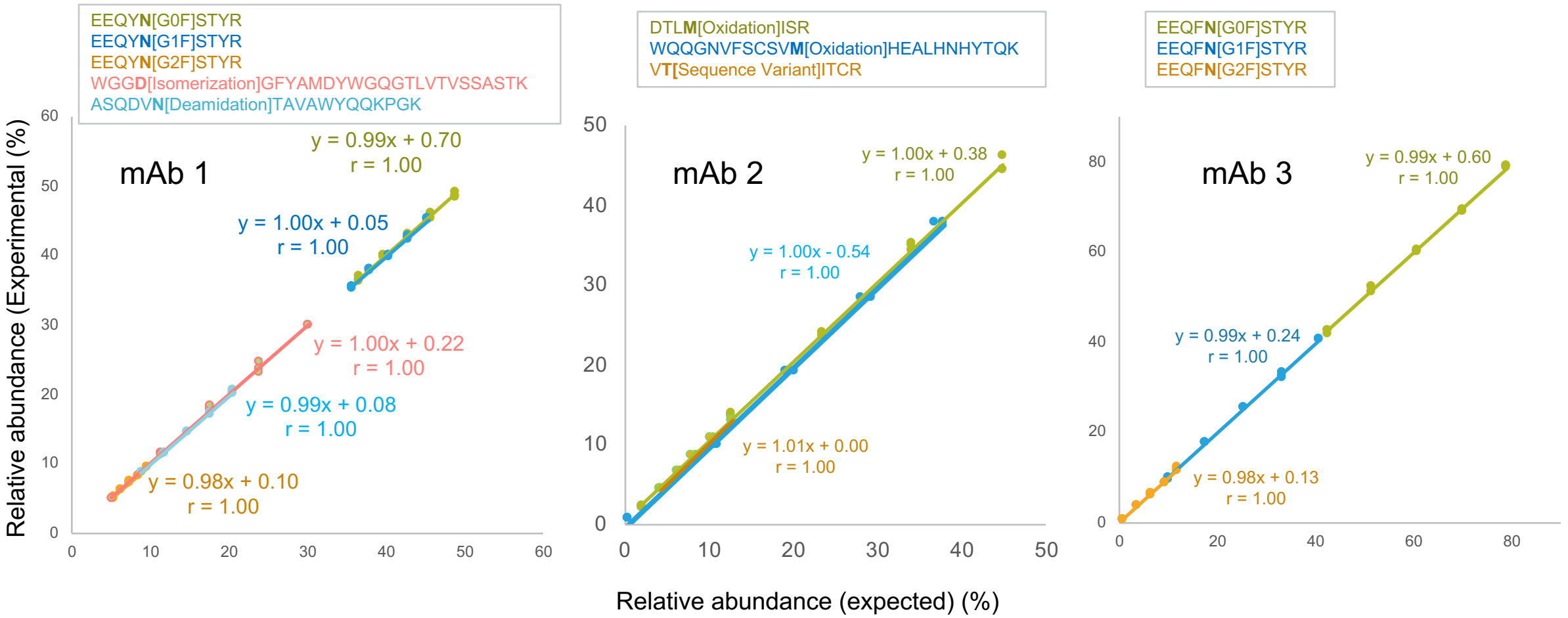
# Linearity, r = 1.00 for All PQAs



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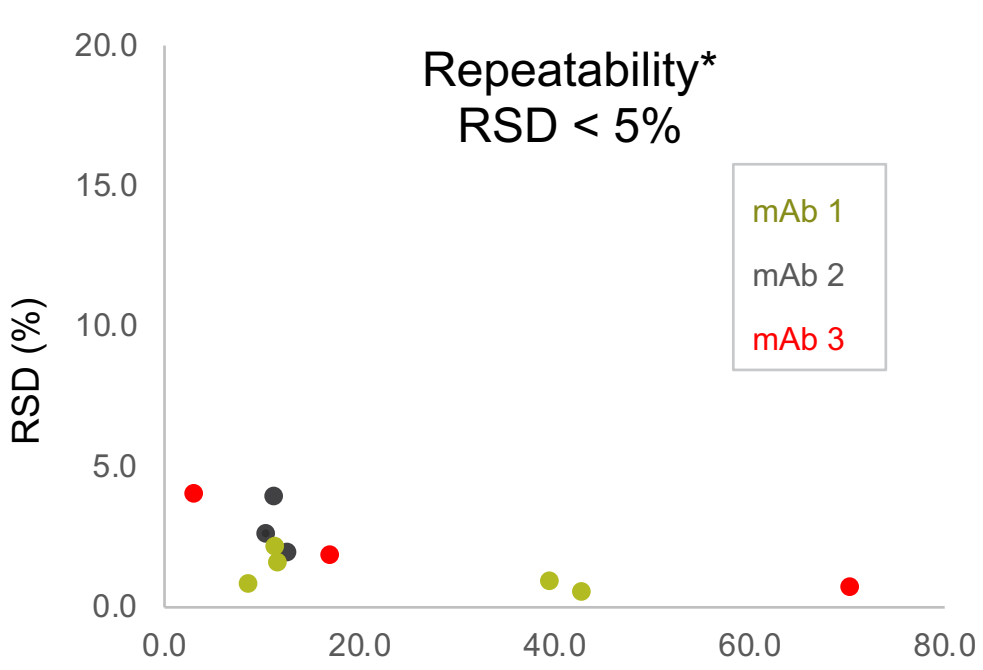
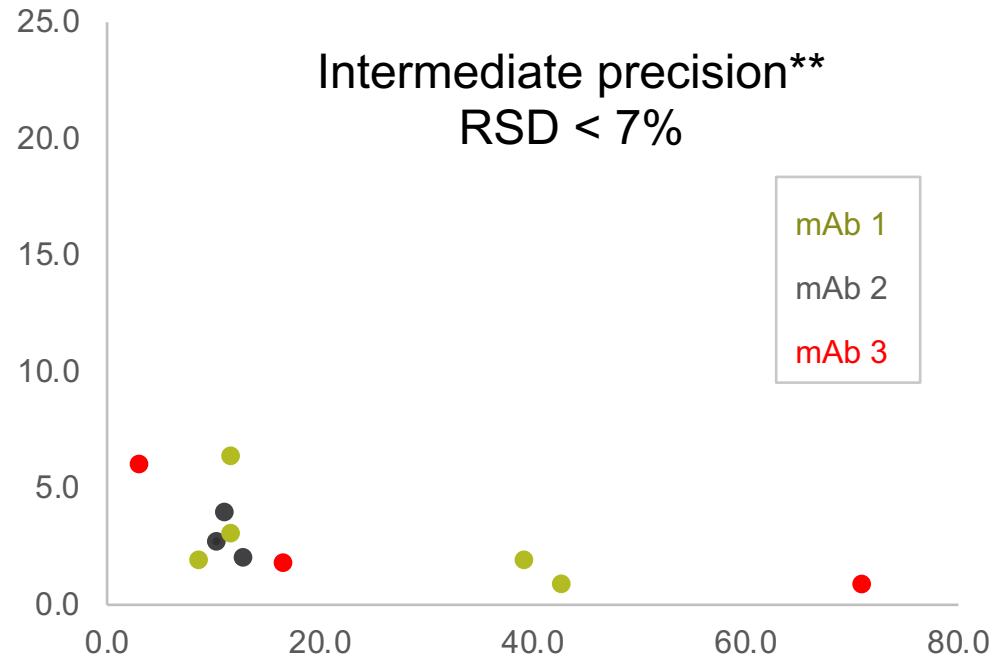
# Repeatability and Intermediate Precision: RSD < 7%






Mean of Relative abundance (%)

* 6 replication injections

** 6 analysts, 2 instruments, 3 column LOTs, 12 tests

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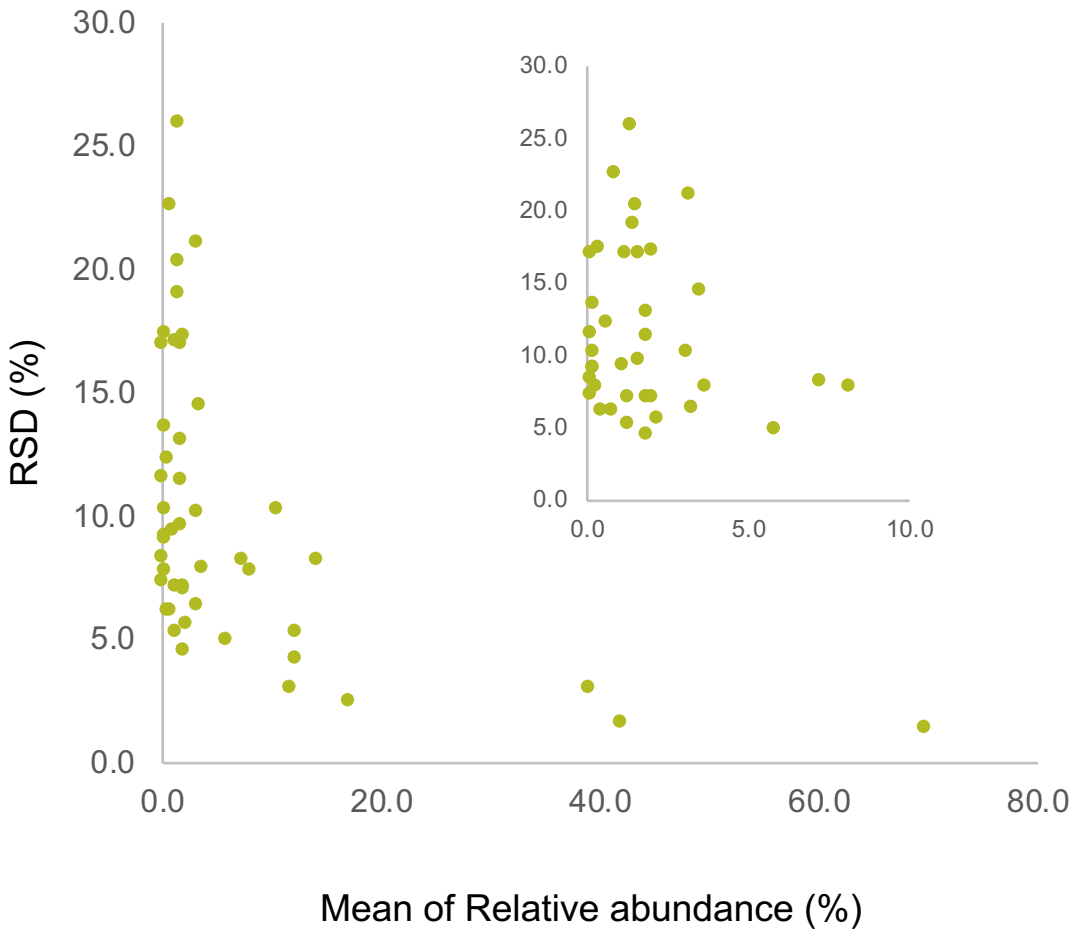
# Intermediate Precision: Extended Study



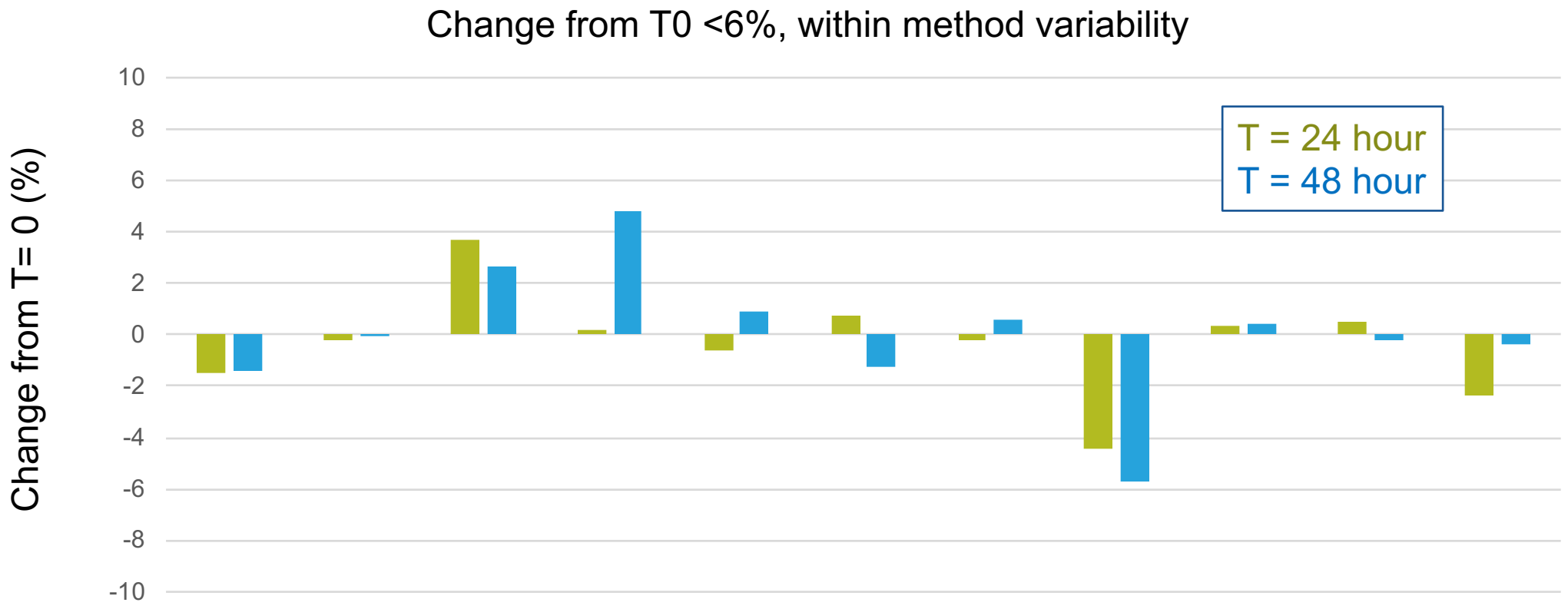



- Intermediate precision with 47 PQAs:
  - M and W Oxidation
  - Deamidation
  - Succinimide
  - Isomerization
  - Glycosylation
- RSDs of glycans are in general lower than that of other PQA types
- Higher RSD tends to be associated with low abundance (<5%)
- Further improvement may be needed based on method requirement

6 analysts, 2 instruments, 3 column LOTs, 12 tests

# Robustness, 48 hour Autosampler Stability Demonstrated





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# Targeted Quantitation Data Processing – Minimum Manual Adjustment



- Robust Data Processing Method
  - Low level Isotopic peaks excluded from quantitation
  - Retention time window covering expected variation
  - Use of relative retention time as needed
- Step-by-Step instruction for data processing
- Pre-saved view settings
- Well designed report template

# Challenges in Non-Targeted MS Processing (New Peak Detection, NPD)



- Optimization of parameter settings to minimize false positives
  - Retention time shift
  - Mass shift
- Method artifacts vs true “new peak” in products
  - Gas phase complex formed by different peptides
  - Overlapping isotope peaks from different peptides
  - Carryover
- MS expertise needed to identify method artifacts
  - Product specific exclusion list

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# Targeted Quantitation System Suitability - Strategy



- To assess the holistic performance of the workflow
  - Sample Prep
  - Chromatography
  - Mass spec
- Qualitative and quantitative characteristics
  - characterized attributes
  - other data quality characteristics
- Acceptance criteria
  - Expected performance
  - Fluctuations in instrument performance
  - Minor variations in sample preparation

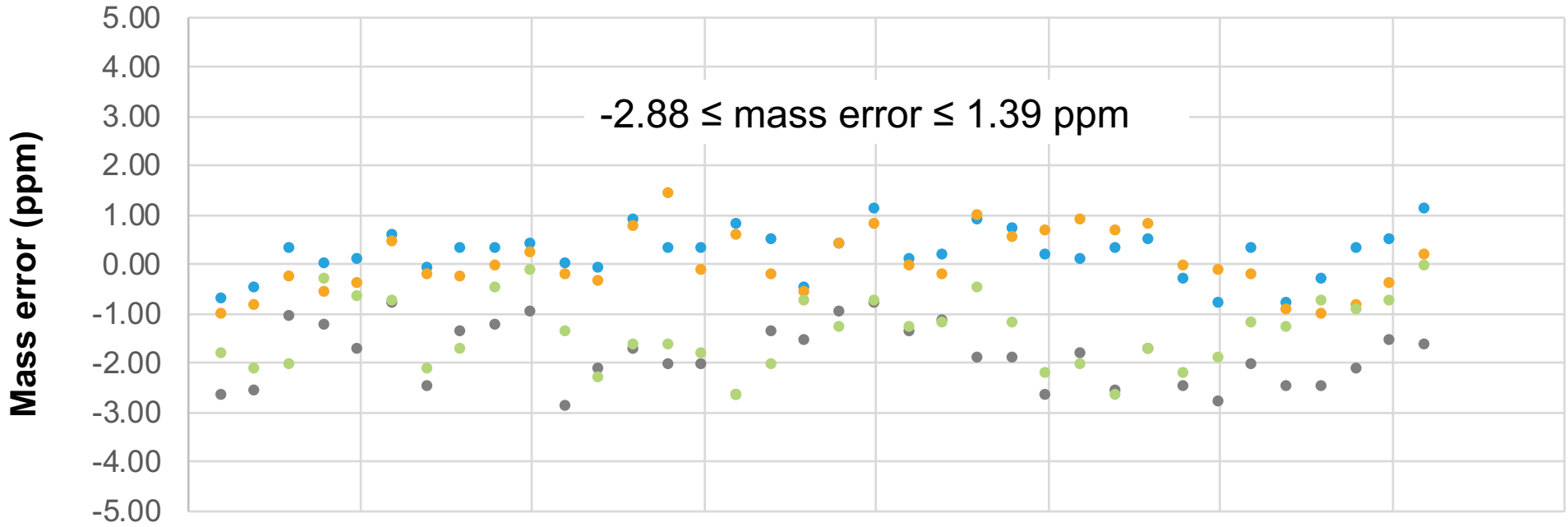
# Testing Parameters for Performance Demonstration



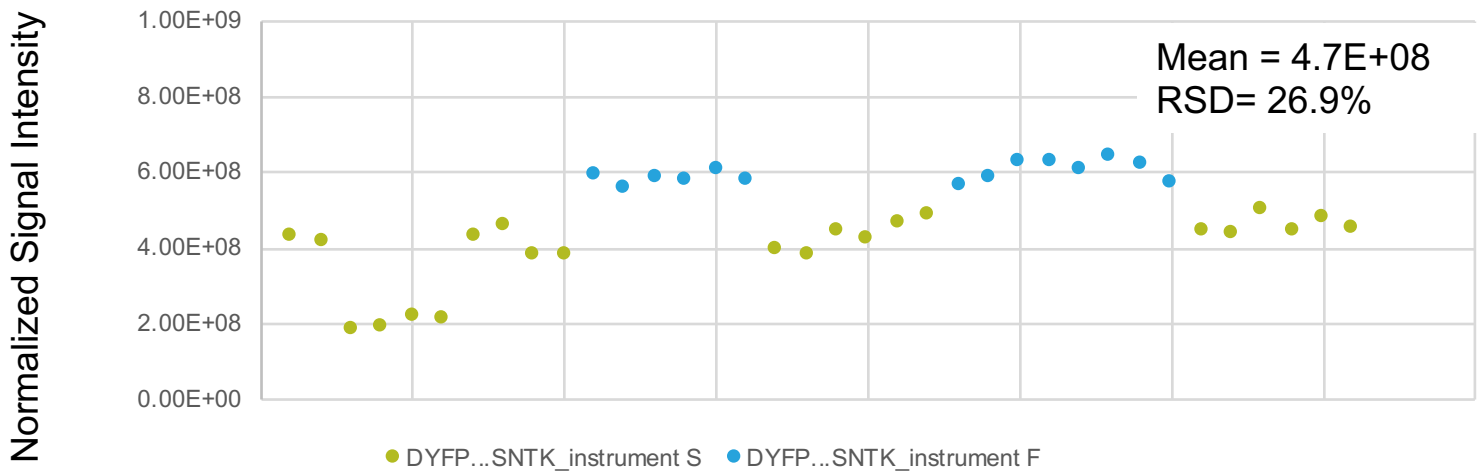
| Parameter | Performance |
|---|---|
| Mass accuracy of 4 selected peptides | • Mass accuracy |
| Signal intensity for 3 selected peptides eluted across run time | • MS instrument sensitivity<br>• Recovery from LC<br>• Recovery from sample preparation<br>• LC retention time |
| Relative abundance of an alkylated peptide | • Alkylation completeness<br>• MS relative quantitation |
| Relative abundance of a low-level, oxidized peptide | • MS instrument sensitivity<br>• MS relative quantitation<br>• Stability of oxidized peptides<br>• Column aging |
| Visual inspection of profile | • Entire workflow performance |

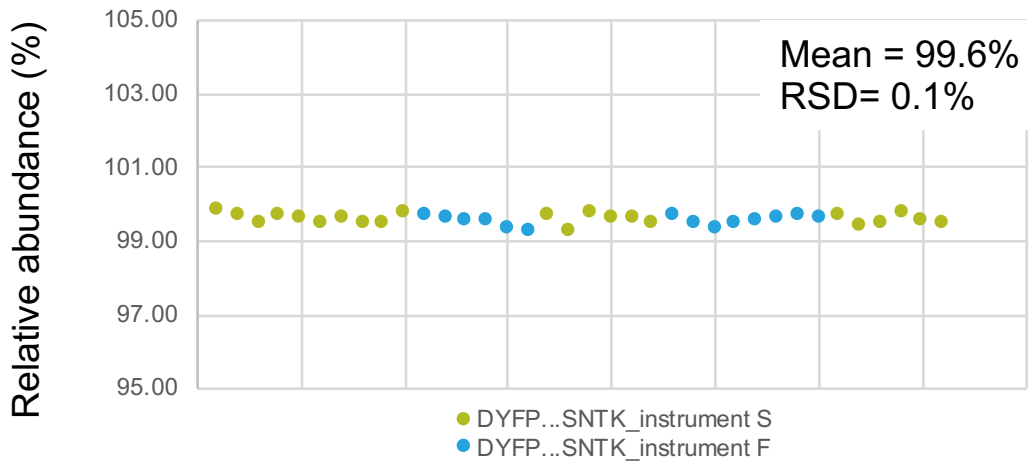
# Mass accuracy of 4 selected peptides (36 runs)

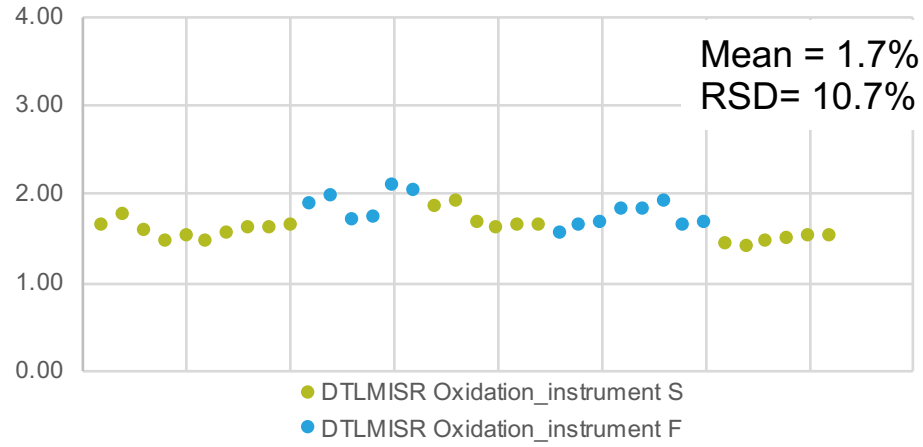




# Variability in Signal Intensity vs PQA Relative Abundance (36 runs)

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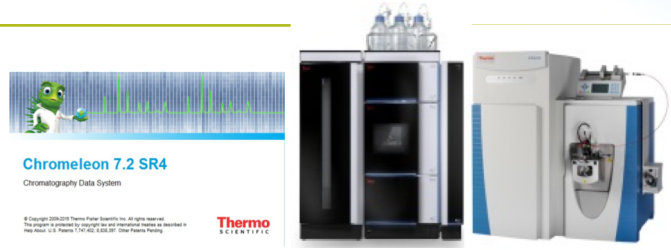
# Informatics with Data Integrity






- Chromeleon 7.2  for data acquisition, processing, reporting
  - Automated data acquisition
  - Semi automated data processing
  - Electronic reporting and reviewing
- 21 CFR Part 11 compliant in terms of electronic records
  - Complete electronic records with embedded system audit trails
  - Access privileges
  - Electronic signatures
- Internal qualification guided by QA to ensure a fully compliant assay including data acquisition, data processing, result reporting

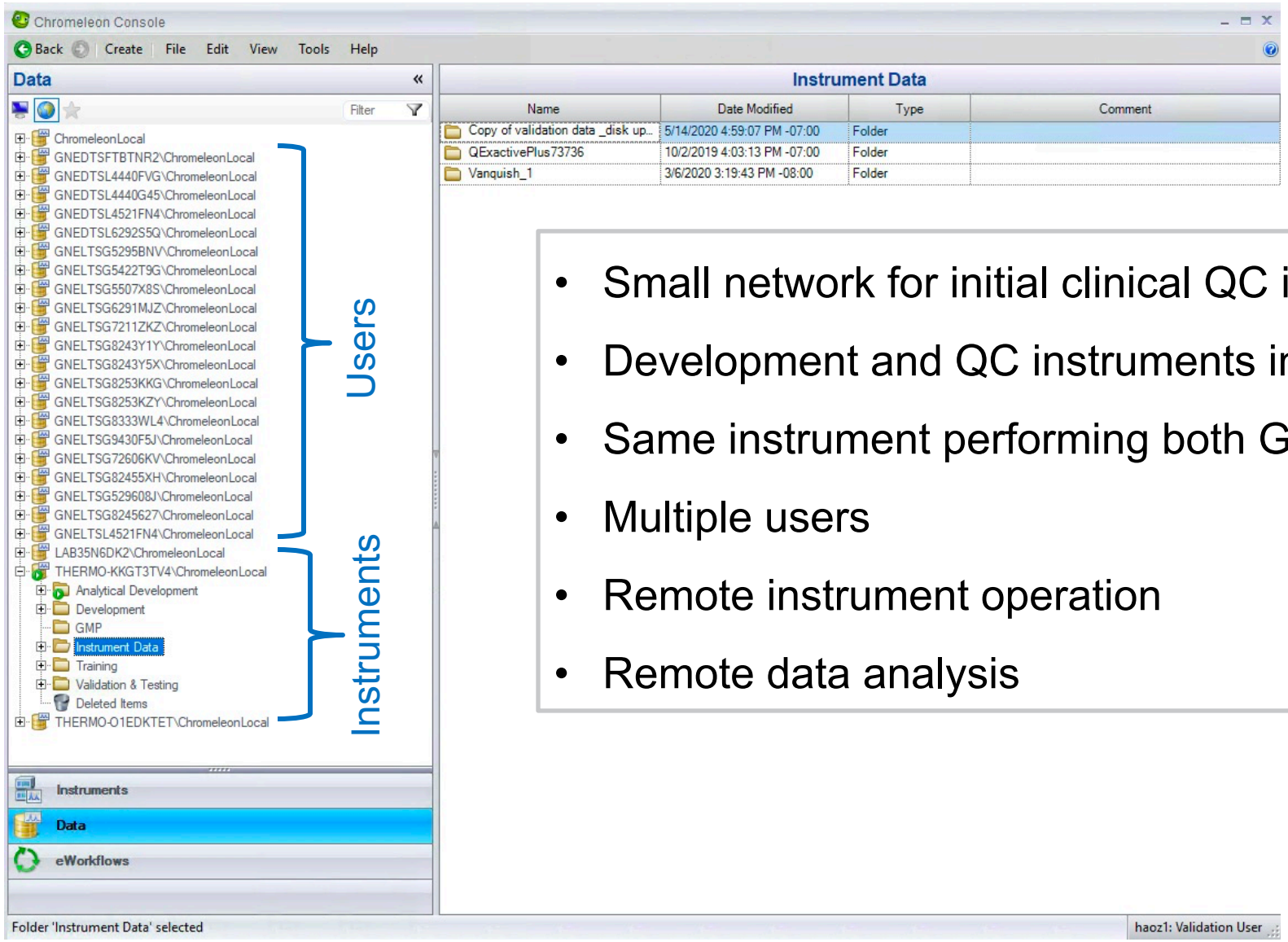
# GMP Compliance of Hardware and Software



| Component | Implementation |
|---|---|
| Equipment and software qualification | • Hardware and software IQ/OQ<br>• SOPs<br>• Data integrity and system risk assessment |
| User account administration | • Access for authorized users, user permissions specific to user role |
| Method validation | • Internal validation of method for clinical QC, analyst training |
| Electronic signature | • Chromeleon electronic signature for analyst, reviewer and approver |
| Audit trial | • Embedded system audit trail within Chromeloen software<br>• Internal audit trail review |
| Data backup | • Validated data archival and retrieval |

# Small Network Configuration Supporting Multiple Instruments and Users






- Small network for initial clinical QC implementation
- Development and QC instruments in the same network
- Same instrument performing both GMP and non-GMP work
- Multiple users
- Remote instrument operation
- Remote data analysis

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# Summary



- A MAM targeted quantitation workflow was implemented in clinical QC.
- The method was validated for monitoring of common PQAs and demonstrated acceptable performance for a quantitative, purity assay.
- The targeted MS data analysis requires minimum manual adjustment, while method artifact is one of the major challenges for NPD.
- A comprehensive system suitability was developed to ensure acceptable performance of sample prep, chromatography and mass spectrometry.
- The MAM targeted workflow is ready to be used for clinical product release and stability testing.

# Acknowledgements


**Development**

Benjamin Moore
Chengfeng Ren
Monica Sadek
Frank Macchi

**QC**

Lindsay Yang
Jack Harris
Jennifer Moore
John De Los Santos
Jack Yim
Emily Liu
Laura Yee
Vanessa Tran
Milady Ninonuevo
Louisette Basa

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Vikas Sharma
Guoying Jiang

**Thermo Fisher Scientific**

Royston Quintyn
Haichuan Liu
Lena Arthur
Kimy Young
Giulio Pe
Reiko Kiyonami
Yan Chen