Development and Assessment of the Multi-Attribute Method (MAM)

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Overview

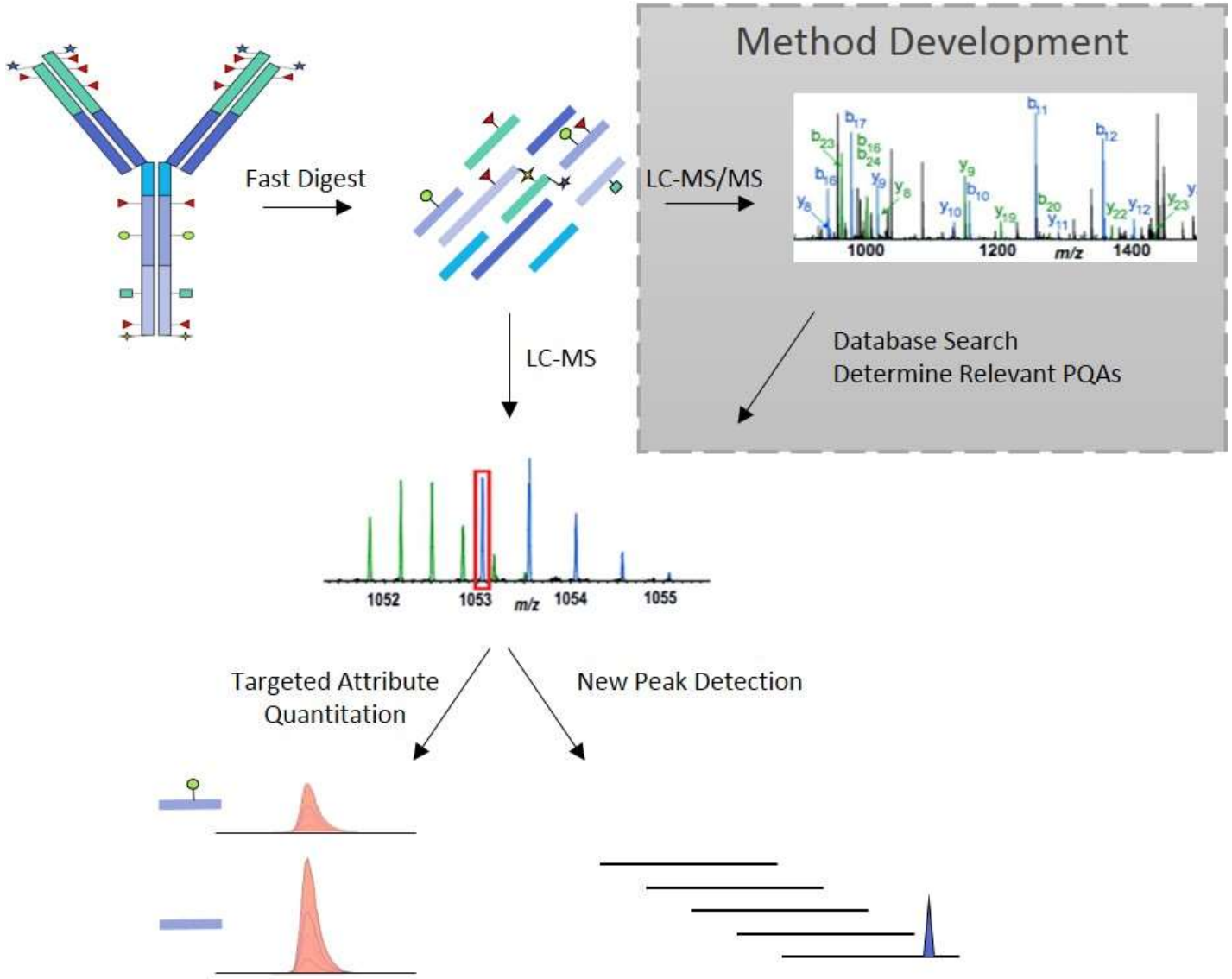
- Learning Objectives
- Multi-attribute method (MAM)
- MAM research at FDA
- Summary and future of MAM research

Learning Objectives

- Understand the basic workflow of MAM
- Understand general benefits and concerns of MAM
- Understand the four points to consider for MAM

Multi-Attribute Method

- LC-MS based peptide mapping method for control testing of therapeutic proteins
- Proposed to ETT as a replacement for conventional methods
- OPQ conducting lab-based assessment



MAM Properties

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

From Michael Blank, Thermo (Adapted from Rich Rogers, Just Therapeutics)

General Benefits of MAM

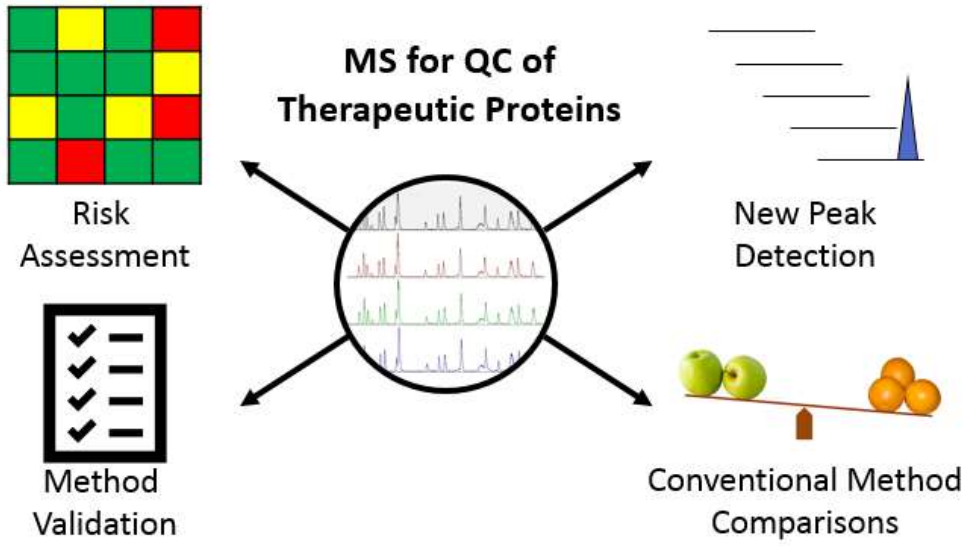
- Testing multiple attributes at once

  → Fewer instruments and assays
- More detailed information at the molecular level
  - Analysis of site-specific modifications can allow for tighter control
- Can differentiate between species that may overlap using chromatographic approaches
- New peak detection allows for control of unexpected new modifications

MAM Implementation

Four major points to consider:

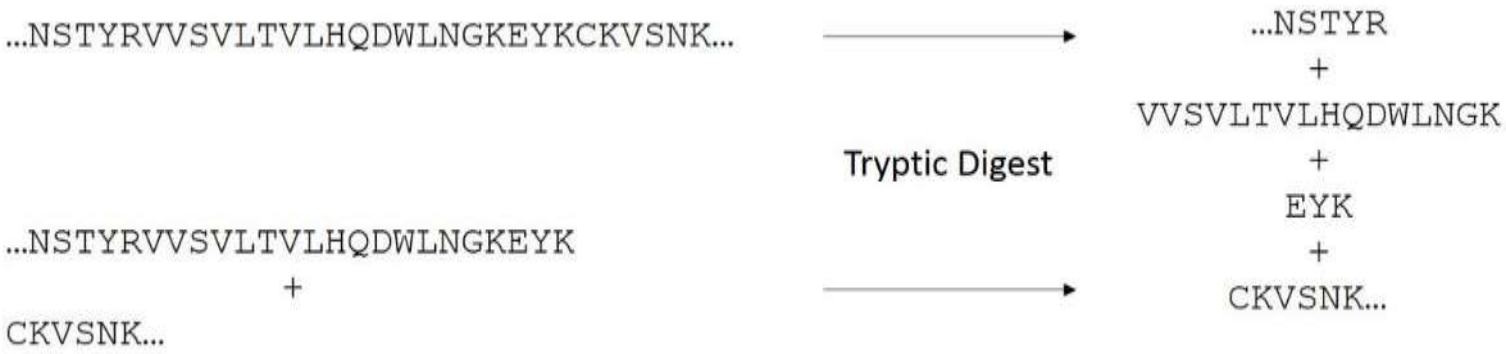
- Risk assessment
- Method validation
- Capabilities and specificities of new peak detection feature
- Comparison to conventional methods



Risk Assessment

- Should weigh benefits and risks for implementation
- Product and CQA specific

- Potential risk example:
  - Loss of clipped species information



Method Validation

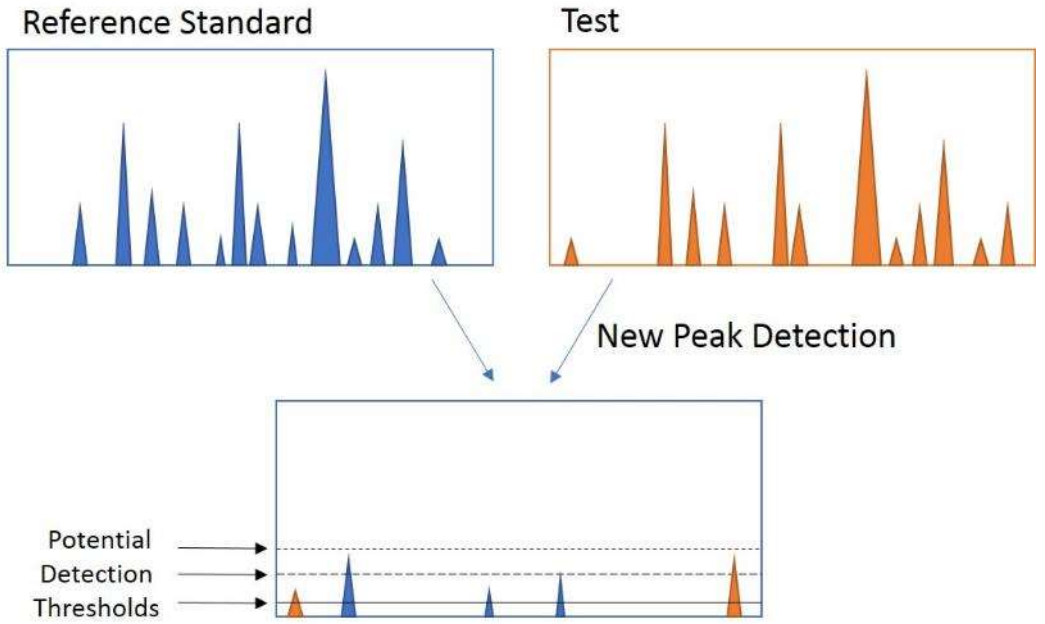
- As an analytical method, MAM needs to be validated
- Can base on ICH Guidelines and FDA Guidances
- More challenging aspects include:
  - Precision
  - LOD/LOQ
  - System suitability

Relevant Guidance Documents:

- ICH Q2 (R1) – Validation of Analytical Procedures
- ICH Q6B – Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
- FDA Guidance on Validation of Chromatographic Methods
- FDA Guidance on Analytical Procedures and Methods Validation for Drugs and Biologics

New Peak Detection

- Allows for detection of changes not directly monitored
- As a stability-indicating method, should detect unknown impurities
- Success highly dependent on software parameters:
  - Retention time window
  - Mass accuracy window
  - Peak detection threshold



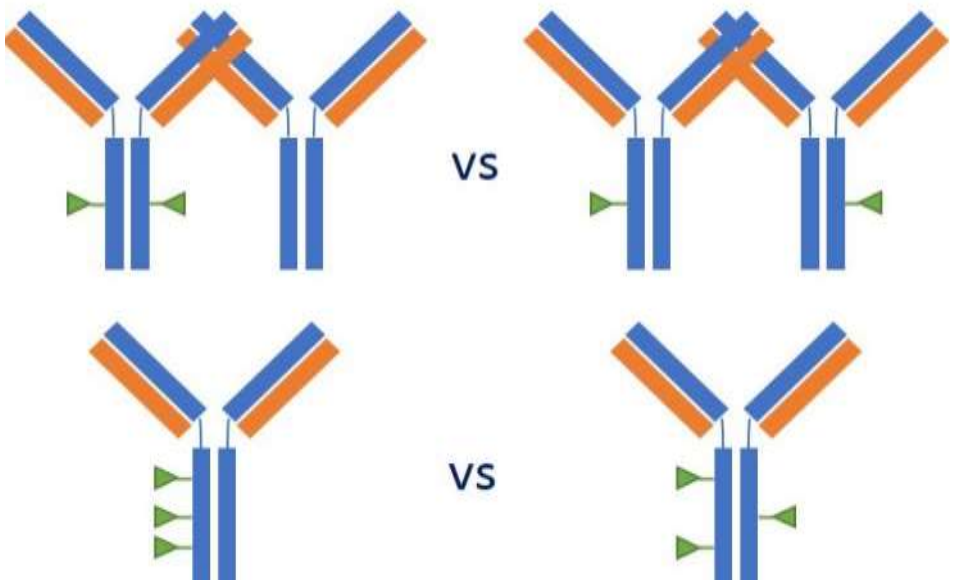
Conventional Method Comparisons

- Comparisons should be informed by risk assessment
- Help to better understand advantages and disadvantages
- Perform during method and product development
- Measurements may not correlate

Attribute by Conventional Method	Target by MAM
Released N-glycans by HILIC glycan profiling	Glycopeptides
Charged variants by CEX	Specific post-translational modifications, N- and C- terminal variants, sialylated species
Clipped species and other size impurities/variants by rCE-SDS	Specific clipped species

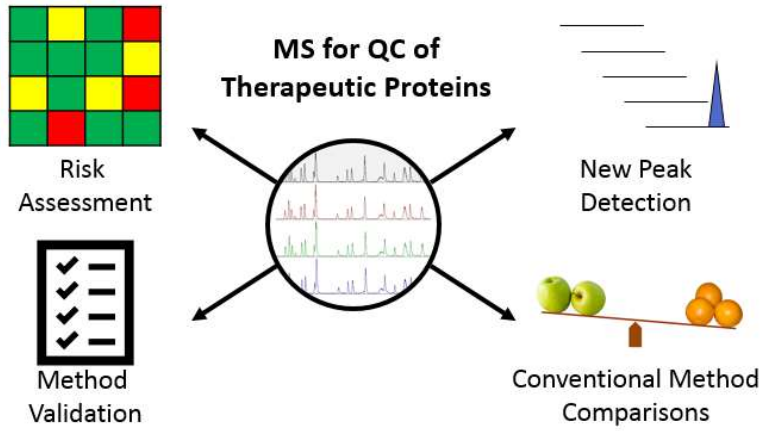
Additional Considerations

- May lose information at the protein level
  - Can’t tell distribution of modifications based on bottom up approaches
- Would a difference in distribution of a modification affect safety or efficacy?
  - Case by case based on risk assessment
- Fit for purpose
  - Demonstrate that new QC method is monitoring all relevant CQAs
  - Which PQAs are CQAs and need to be monitored is product specific



FDA Research Overview

- Established in-house MAM capabilities to explore and better evaluate usage of the approach
- Used rituximab (approved and unapproved) as a model protein



Method Validation

- System Suitability
- Precision
- LOD/LOQ

New Peak Detection

- User Comparisons
- Forced Degradation

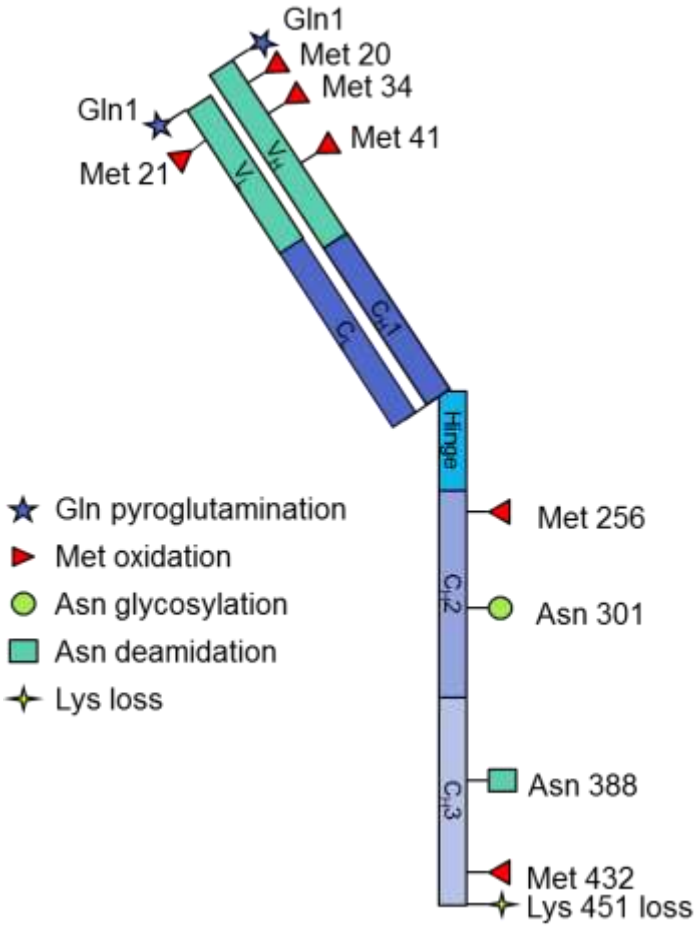
Conventional Method Comparisons

- Forced Degradation
- Glycan Profiling

Method Overview

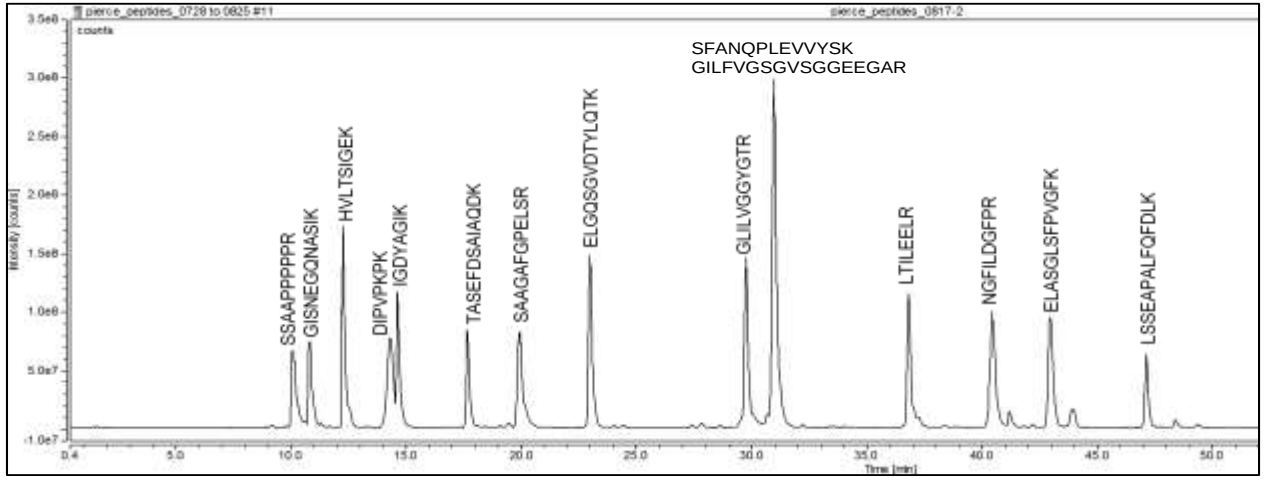
- Monitored the relative abundance levels of 21 product quality attributes (PQAs) across 11 sites

- Method was capable of distinguishing between approved and unapproved products for 10 of those PQAs



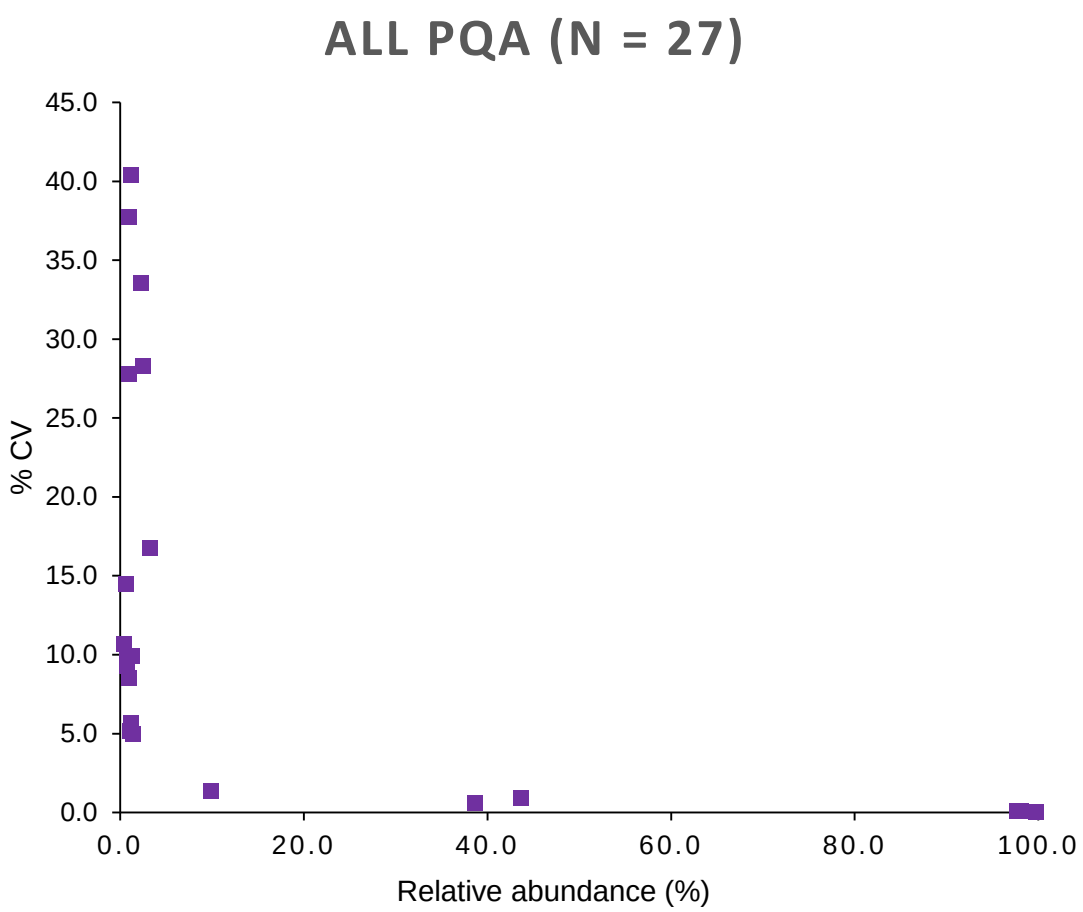
Method Validation: System Suitability

- Pierce Peptide Mix – 15 peptides
  - Use 12 for SST
- Set RT and Rel. Abundance limits based on historical data
- Additional Limits for RT and Rel. Abundance %CV
- Also assess mass accuracy, resolution, and signal:noise

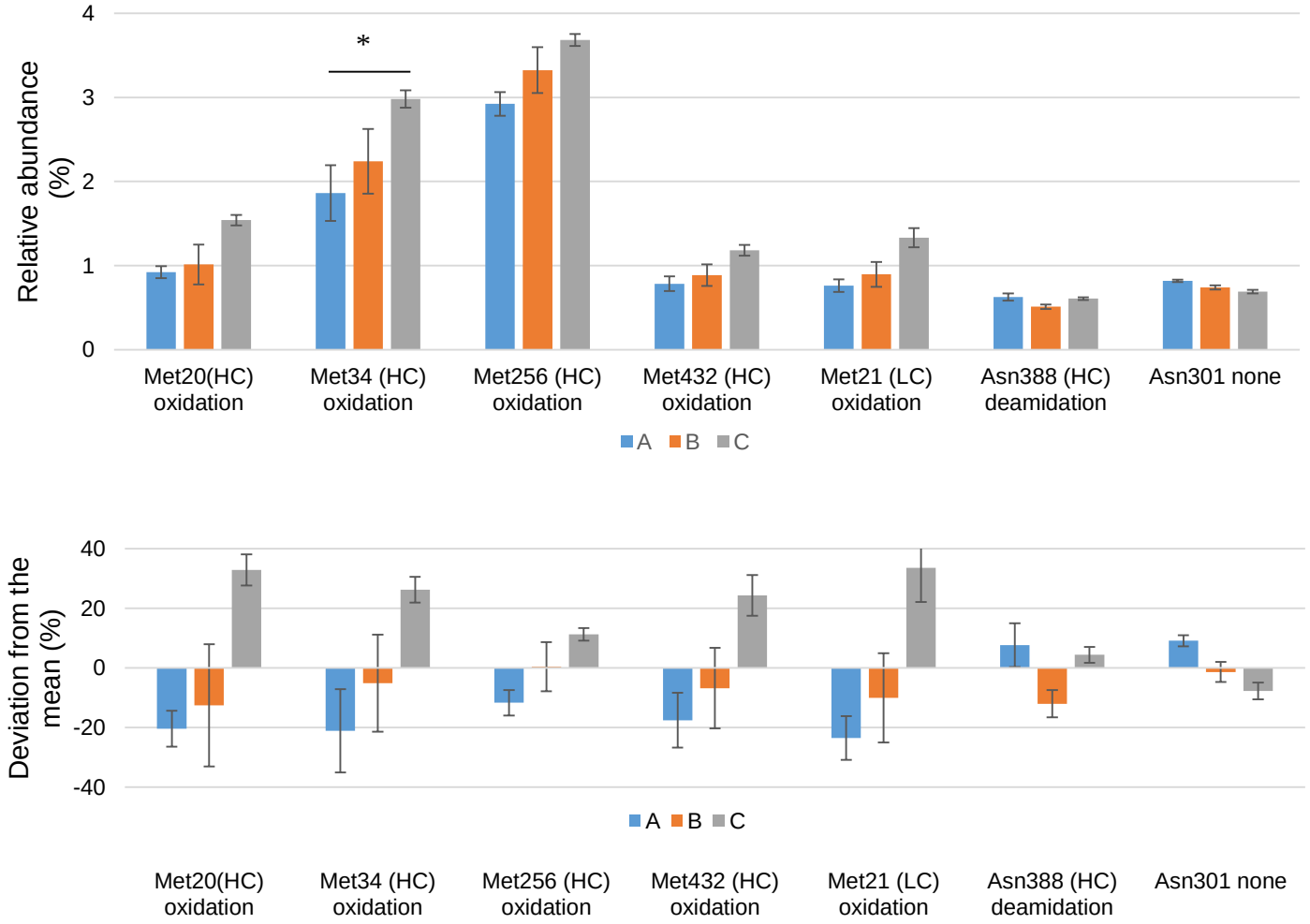


Method Validation: Reproducibility and Precision

- 3 users x 3 digests x 3 injections
- Results generally reproducible
- Highest variability for low abundance oxidation sites
- User experience correlated with variability

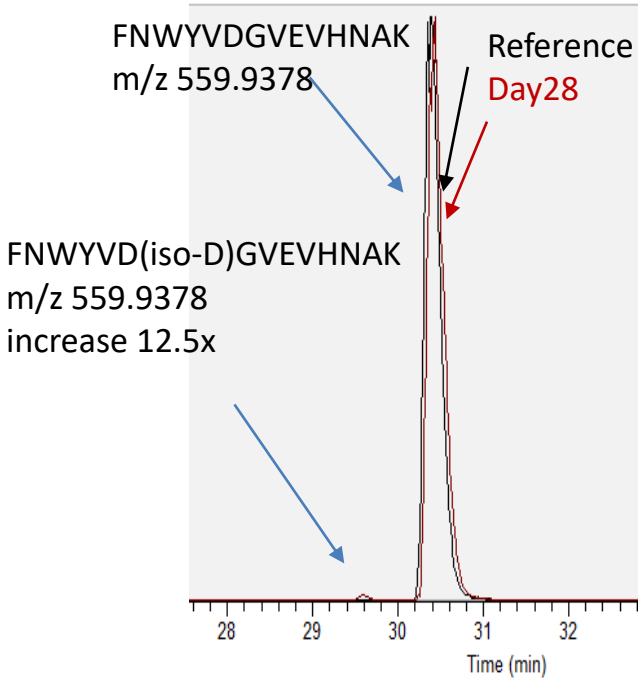


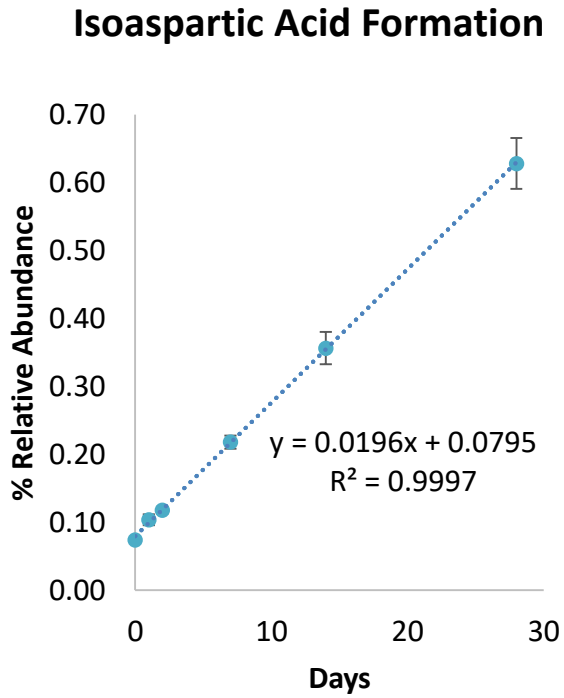
Method Validation: User Variation



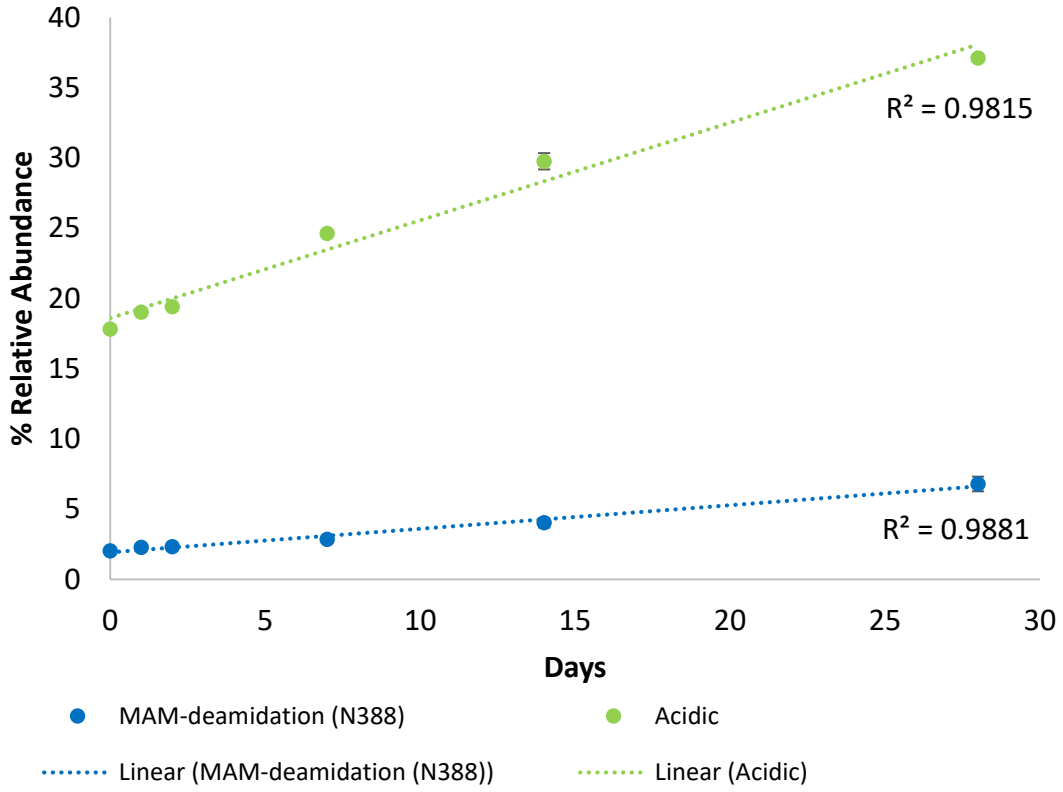
New Peak Detection: Forced Degradation

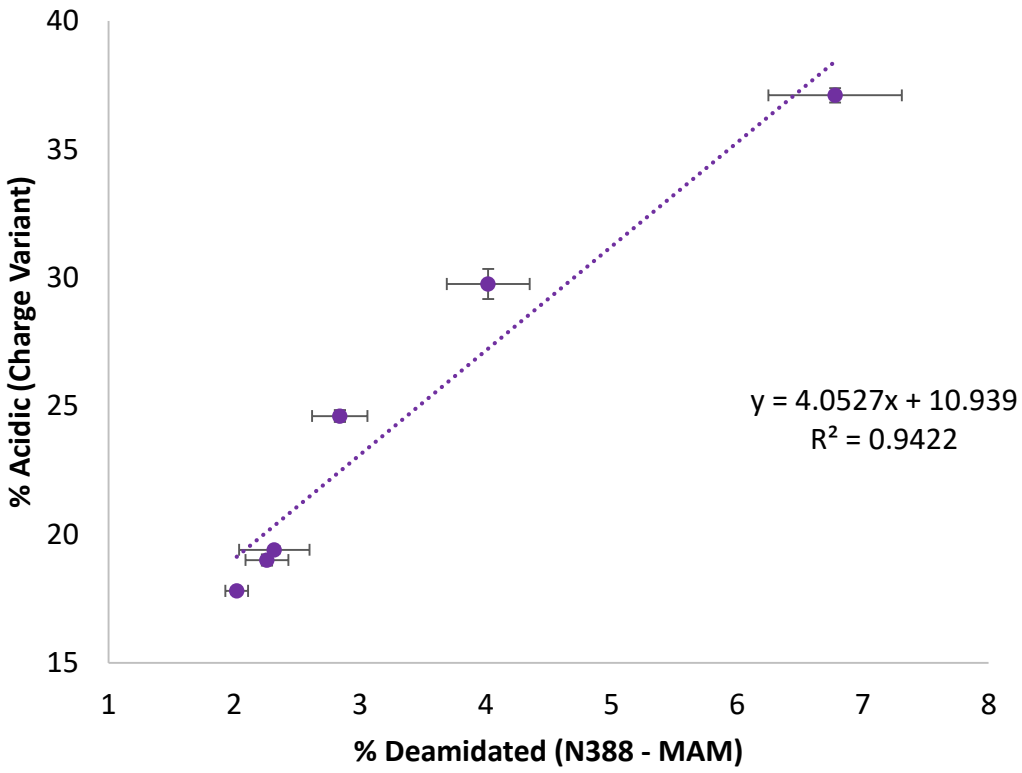
- Forced degradation – 28 days at 40 °C/75% RH
- Linear increases in oxidation and deamidation over time-course
- One new peak was detected
  - Aspartic Acid → Isoaspartic Acid
- > 12.5-fold increase over 28 days

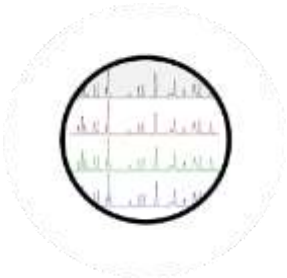




Method Comparisons: Forced Degradation

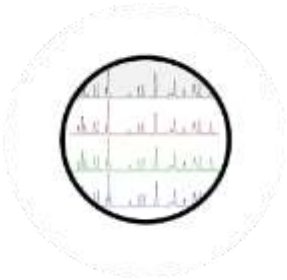






Summary

- Risk Assessment: should be considered when developing MAM
- Method Validation: established SST approach, assessed precision, reproducibility, LOD/LOQ, and more
- New Peak Detection: established NPD suggested parameters and used to test forced degradation samples
- Method Comparisons: compared forced degradation trends and glycan profile



Ongoing and Future Research

- Currently running and analyzing data from year-long stability and accelerated stability studies with MAM and conventional methods
- Conducting software comparison
- Site-to-site MAM comparison coming soon

Acknowledgements

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ETT

Challenge Questions

MAM stands for:

A) Multi-attribute monitoring

B) Multi-attribute method

C) Multi-analysis method

D) Multi-assessment monitoring

Challenge Questions

True or false:

Risk assessments for MAM should be product and method specific.

Challenge Questions

Which of the following is not one of FDA's points to consider for MAM?

A) Risk assessment

B) New peak detection

C) Software comparisons

D) Method validation

E) Method comparisons


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