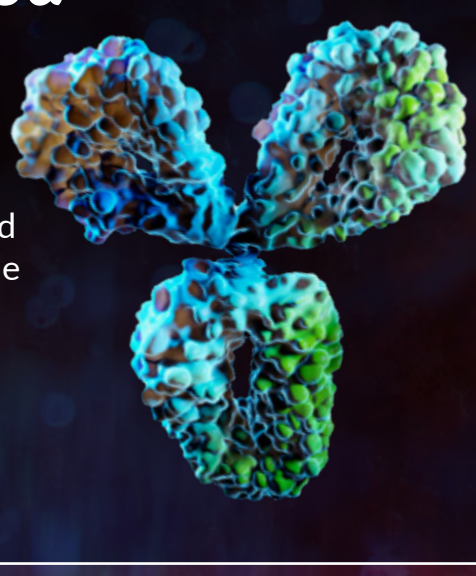
Using the Multi-Attribute Method for Antibody Drug Discovery



The multi-attribute method (MAM) is an LC/MS-based approach for assessing quality control at the amino acid level.[1] MAM has increased in popularity over the last several years and there is now considerable expert technical, compliance, and regulatory guidance available on applying MAM.[2,3] This infographic—in the context of MAM for monoclonal antibody (mAb) drug discovery—presents an overview, the general workflow, benefits and limitations, considerations for implementation, as well as recent examples of ongoing use.

Overview

First report on the use of MAM for mAbs: 2015; focused on glycan number and type, and amino acid modifications[4]

Essential features: Simultaneous detection, identification, quantitation, and quality control for meeting GMP requirements[5]

Workflow[6]

Step 1: Discovery and library creation based on peptide mapping

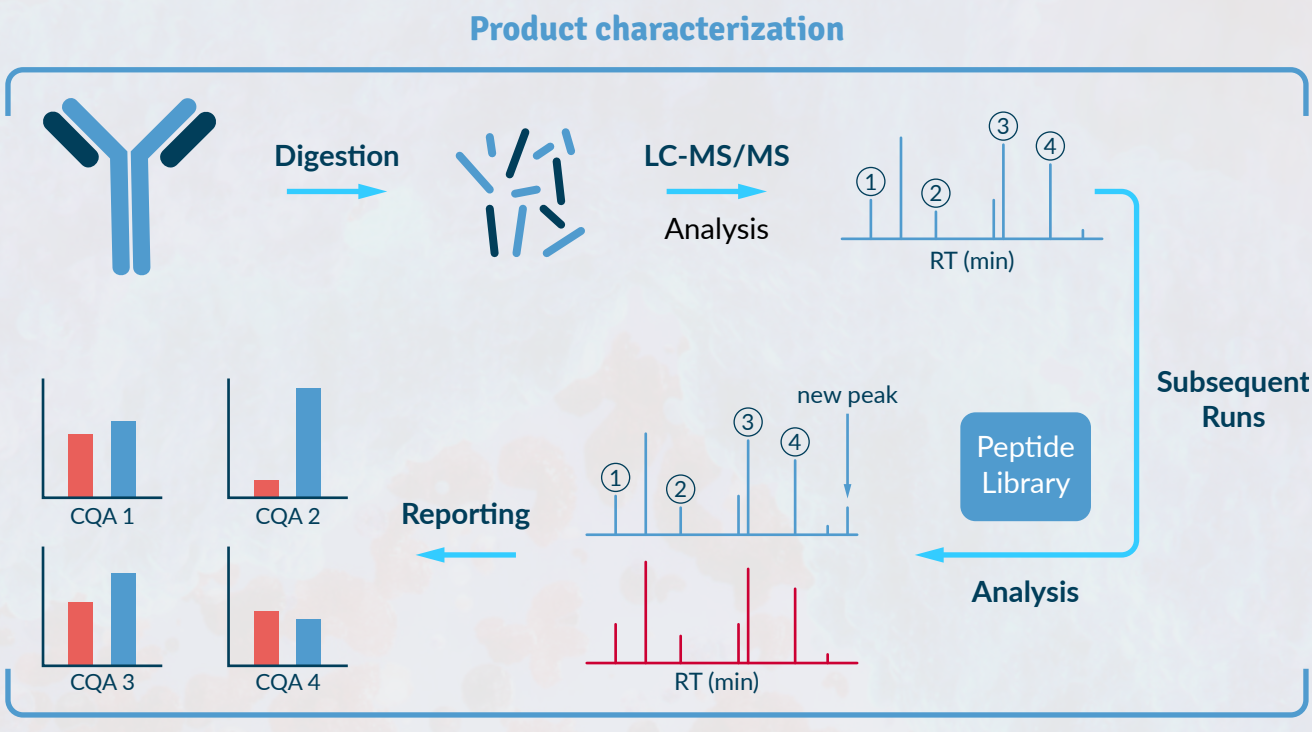
- **Purpose:** Identify pertinent product quality attributes (PQAs)
- **Requirement 1:** Complete and reproducible digestion
- **Requirement 2:** Few process-induced modifications

Readily detects non-consensus glycosylation, D-isomerization, and more

Step 2: Monitoring based on the library and new peak detection

- **Purpose:** Quantitate PQAs that might affect mAb stability or activity
- **Requirement 1:** Overwhelming majority of peaks have equivalent batch-to-batch abundance
- **Requirement 2:** Peaks with similar intensities have similar relative variability

Consistent with established quality-by-design principles for mAb production[7]



Benefits and Limitations[1,2]

Benefits

- **Site-specific information:** Occupancy of drug conjugation sites
- **High selectivity:** Missing amino acid residues
- **High sensitivity:** Isomerization sequence variants

Limitations

- High-order structure information such as aggregation state is inaccessible
- Artifacts attributable to ionization efficiencies, glycan degradation, and oxidation
- No well-established MAM protocol for mAbs

Implementation[2]

Sample preparation

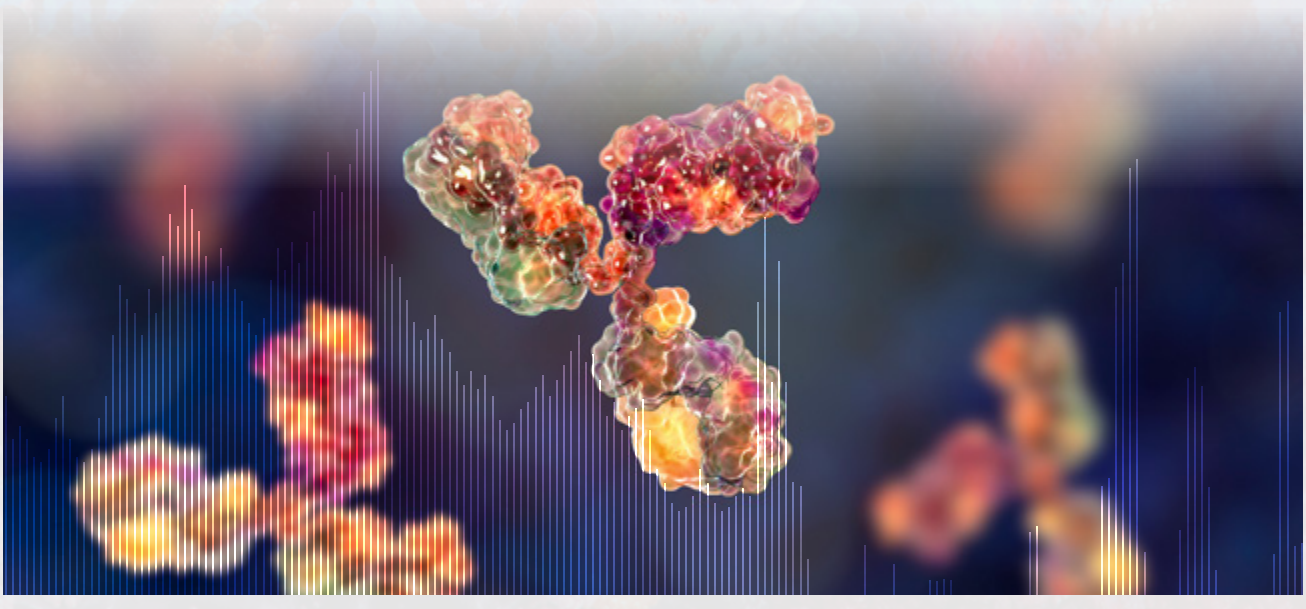
- **Protein denaturation:** Chaotrope might interfere with digestion, whereas detergent must be removed prior to MS analysis
- **Sulfide bond reduction:** Choice of agent depends on the solution pH, aqueous stability, and need to avoid oxidation artifacts
- **Alkylation of thiol groups:** Need to consider the solution pH, and must avoid exposure to light
- **Polypeptide digestion:** Trypsin endoprotease is the most common choice, followed by quenching with trifluoroacetic acid; optimization is a common pain point

Data analysis

- User-friendly software can facilitate cross-laboratory evaluations
- Common complications:
  - Same modification on more than one peptide
  - More than one modification on the same peptide
- Multiple reaction products
- New peak detection requires minimal false positives and false negatives; guidelines are available for setting appropriate thresholds[8]

LC/MS

- Micro-LC with a C18 reversed-phase column is most common
- High- and low-resolution MS instrumentation are common; choose to enable product-agnostic data acquisition and retrospective data analysis



Some Recent Applications

- Monitoring four distinct quality attributes simultaneously: variations in the thiol state of the inserted cysteines, N-linked glycosylation, reduction of interchain disulfide bonds, and polypeptide fragmentation[9]
- A comparison of seven adalimumab biosimilars with high sensitivity and specificity[10]
- A novel workflow was used to analyze size-related and charge-related variants of a mAb simultaneously in their native form[11]

Conclusion

MAM is becoming an established protocol for quality control of mAbs, which might have as many as 20–30 PQAs.[12] Understanding the basic science—encompassing the workflow, benefits, limitations, and implementation—will depend on cross-laboratory collaborations[13] that establish commonly accepted protocols, and ultimately enable consistent manufacturing of mAbs.

References

1 Millán–Martín S, et al. (2023). Comprehensive multi-attribute method workflow for biotherapeutic characterization and current good manufacturing practices testing. Nat. Protoc. 18(4):1056–1089.

2 Pohl T, et al. (2023). Technical considerations for the implementation of the multi-attribute-method by mass spectrometry in a quality control laboratory. Eur. J. Pharm. Biopharm. 188:231–242.

3 Gervais A, et al. (2023). Compliance and regulatory considerations for the implementation of the multi-attribute-method by mass spectrometry in a quality control laboratory. Eur. J. Pharm. Blopharm. 191:57–67.

4 Rogers RS, et al. (2015). Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics. mAbs 7(5):881–890.

5 Millán–Martín S, et al. (2024). Multi-attribute method (MAM): An emerging analytical workflow for biopharmaceutical characterization, batch release and cGMP purity testing at the peptide and intact protein level. Crit. Rev. Anal. Chem., in press.

6 Yang F, et al. (2023). Mass spectrometry-based multi-attribute method in protein therapeutics product quality monitoring and quality control. mAbs 15(1):2197668.

7 Finkler C, Krummen L. (2016). Introduction to the application of QbD principles for the development of monoclonal antibodies. Biologics 44(5):282–290.

8 Mouchahoir T, et al. (2021). New peak detection performance metrics from the MAM Consortium Interlaboratory Study. J. Am. Soc. Mass Spectrom. 32(4):913–928.

9 Hines AR, et al. (2023). Multi-attribute monitoring method for process development of engineered antibody for site-specific conjugation. J. Am. Soc. Mass Spectrom. 34(7):1330–1341.

10 Millán–Martín S, et al. (2023). Multi-attribute method (MAM) to assess analytical comparability of adalimumab biosimilars. J. Pharm. Biomed. Anal. 234:115543.

11 Kumar S. et al. (2023). Multiattribute monitoring of aggregates and charge variants of monoclonal antibody through native 2D-SEC-MS-WCX-MS. J. Am. Soc. Mass Spectrom. 34(7):1263–1271.

12 Rathore AS, et al. (2023). What should next-generation analytical platforms for biopharmaceutical production look like? Trends Biotechnol. 42(3):P282–P292.

13 Butré CI, et al. (2023). Interlaboratory evaluation of a user-friendly benchtop mass spectrometer for multiple-attribute monitoring studies of a monoclonal antibody. Molecules 28(6):2855.