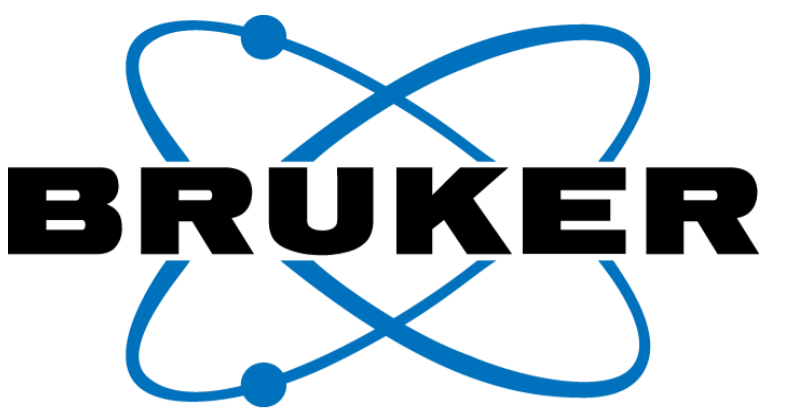


Integrated Characterization and Screening Workflows to Simplify the Design of MAM methods



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Introduction

With more and more products being licensed for commercial use or investigated in clinical trials, protein drugs are the fastest growing class of human therapeutics. In biotherapeutic process development, evaluating and tracking product quality attributes are crucial. Currently, multiple analytical assays are used for monitoring biologic development and production. Multi-attribute method (MAM) helps researchers to simultaneously measure and understand multiple quality attributes indicative of stability or quality. MAM combines accurate mass measurement and retention time to screen for known variants and contaminants as attributes that can be studied in a direct way. Here we characterize the NISTmAb digests to testify the reproducibility and accuracy of the peptide screening workflow.

Methods

Experimental

2 µg of a stressed NISTmAb digest was injected into the Bruker Elute UHPLC system coupled to the Bruker timsTOF Pro mass spectrometer,

with Waters CSH C18, 1.7µm, 2.1x150mm as the column. 2% to 35% in 30 min gradient was used and MS/MS acquisition was done in PASEF mode.

Data Analysis

Data was processed in BioPharma Compass 2021 (Bruker). Digested NISTmAb peptides were submitted to an in-silico MS² search to identify the peptides and associated modifications. The peptide mapping results were used to automatically create a list of the hotspots likely to undergo degradation on the NISTmAb. The list is then used as a MAM screening workflow (Figure 1). The defined method was performed in triplicate to determine its reproducibility and accuracy.

Results

Selected attributes representing typical heterogeneities were added to the screening list with accurate mass and retention time based on the peptide mapping analysis (Figure 2). The MAM peptide screening workflow then compared the reference peptide list against the LCMS data of 3 technical replicates. The selected NISTmAb attributes were reproducible across replicates (Figure 3). Oxidation and deamidation levels of peptides in the technical replicates are consistent with the reference. Individual extracted ion chromatograms can be visualized for manual verification (Figure 4).

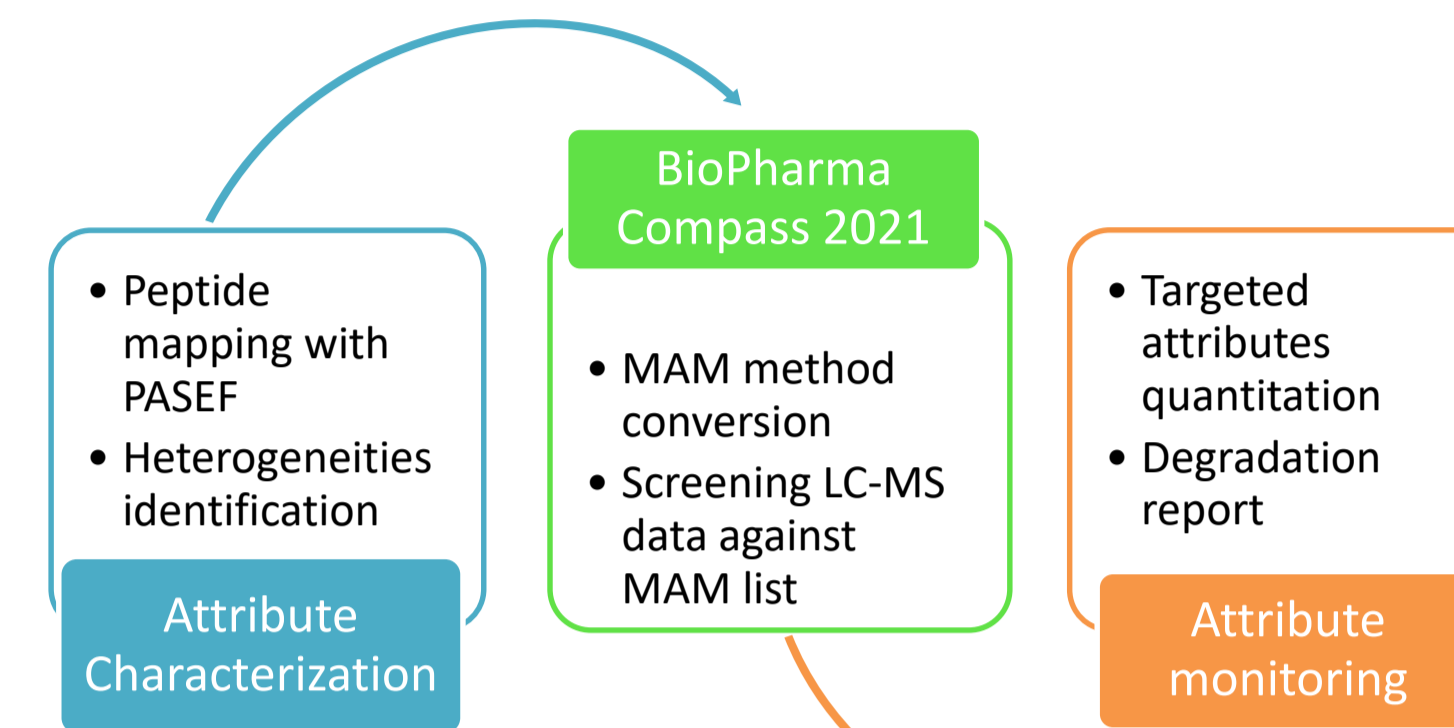


Figure 1. Workflow driven creation of a MAM peptide experiment in BPC 2021. The attribute characterization in the discovery phase and attribute monitoring in the routine analysis are fully integrated in a single interface.



Figure 2. Peptide mapping search result. Top shows the identified proteins with corresponding peptide details. Modifications and relative modification ratios can be obtained; Middle is the MS/MS spectrum and the corresponding chromatogram; Bottom show the protein sequence coverage.

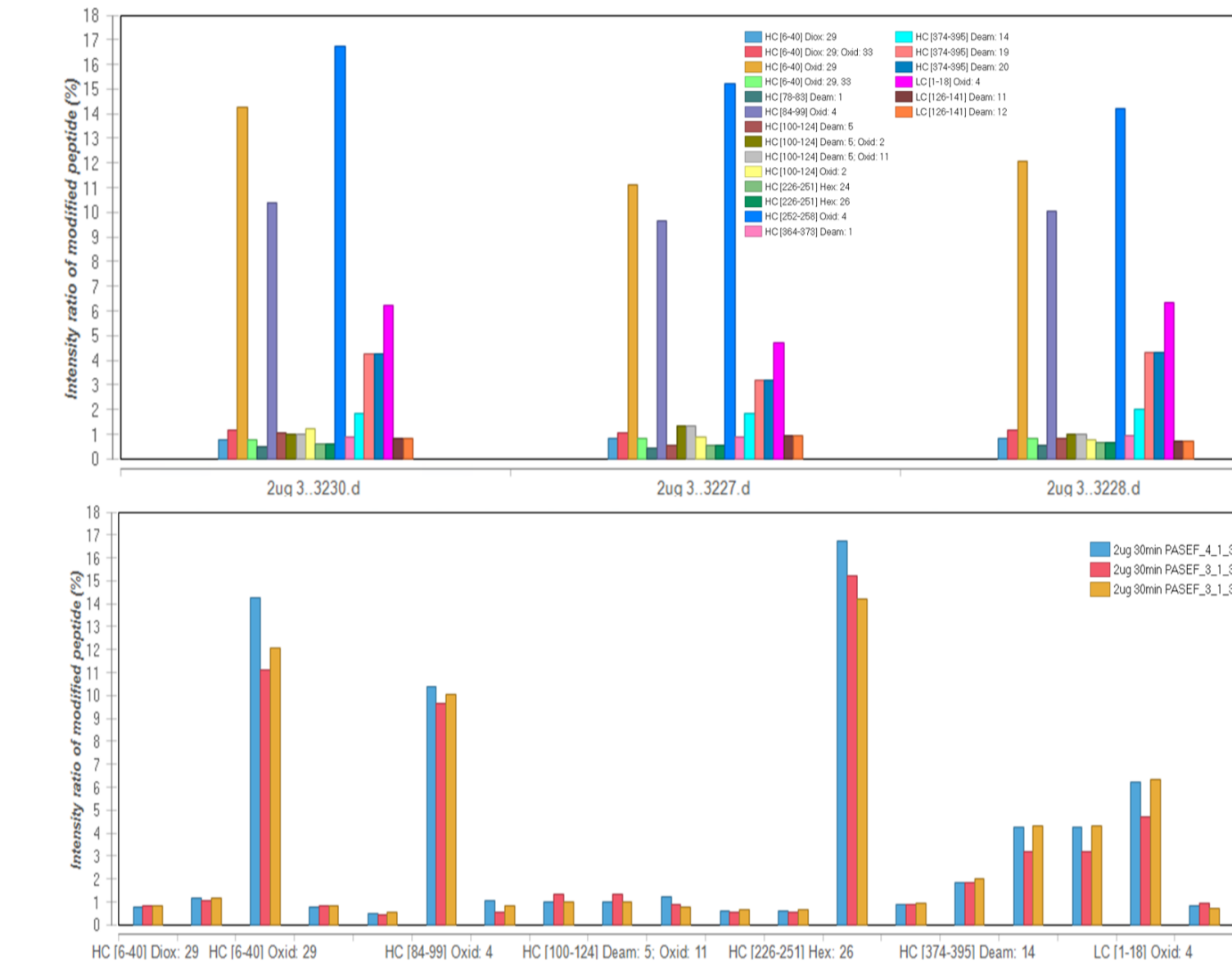


Figure 3. Comparison of selected NIST mAb attributes based on the ratio of modified to wildtype. Comparisons of modification ratios ordered by sample replicates (top) and modifications (bottom) are shown. Three technical replicates show a reproducible assessment of oxidation and deamidation across the samples.

Summary

Bruker BioPharma Compass 2021 provides a complete toolset for mAb heterogeneity identification, creation of attribute lists and MAM quantification based on area or intensity. This is integrated in an easy to learn interface that is highly automatable and can be used in regulated environments.

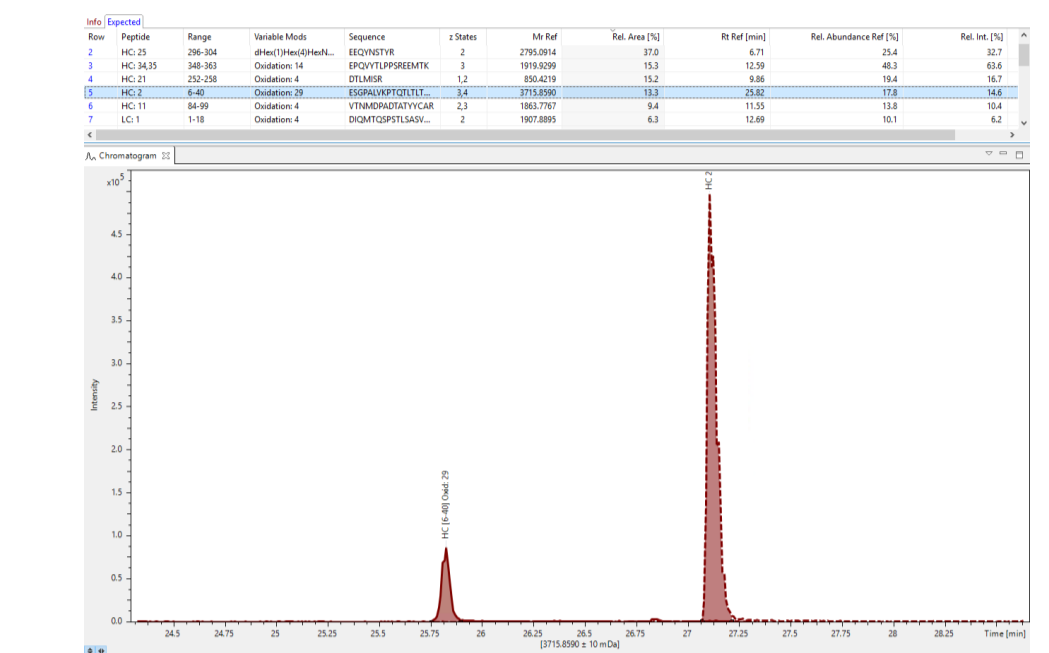


Figure 4. Example of the extracted ion chromatogram of an oxidized peptide and matching wildtype. Oxidation location and relative intensity ratio can be found, with comparison to the reference values obtained from peptide mapping experiment.

Conclusions

- BioPharma Compass 2021 offers rapid peptide mapping with accurate quantification of modification ratio and localization of modification sites.
- Seamless integration from peptide mapping to peptide screening provides efficient tools to develop MAM methods.
- Reporting tools comparing the attribute level variation between samples facilitate the data review for large batches.

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