thermo scientific

HR Multi-Attribute Method for biopharma analysis

Resolution matters from research to routine



DEAMIDATION - CQA



HR Multi-Attribute Method: a comprehensive workflow for the assessment of critical quality attributes

Complete characterization of biotherapeutic proteins is a mandatory requirement in reproducible and safe drug production. With the advent of ever-increasing drug complexity, in-depth knowledge gained early in drug discovery facilitates the development of improved quality control strategies.

Small modifications in protein sequence can have a measurable impact on the safety and biological activity of the drug product. By directly measuring potential critical quality attributes (CQAs) important information can be derived for the optimization of production processes ultimately affecting product quality.

Evaluating and tracking potential CQAs is crucial to ensure quality, safety, and efficacy. Potential CQAs are typically assessed using multiple chromatographic and electrophoretic methods, which is resource intensive. These methods are profile based and are often not capable of identifying and quantifying potential residue-specific CQAs.

High resolution mass spectrometry alone offers a viable alternative to the requirement for multiple methods and provides detail on a molecular level often not discernable with traditional techniques. This is in alignment with the Quality by Design (QbD) approach to the development of pharmaceuticals, which is advocated by the regulatory agencies and is being adopted by biopharmaceutical companies.

Multi-Attribute Method (MAM) is a mass spectrometry-based peptide mapping method used to quantify multiple potential CQAs simultaneously. The number of individual tests (CEX, CE-SDS, HILIC, ELISA) for specific potential CQAs can be decreased considerably using an appropriately developed MAM, as it is shown in the adjacent table.

Required CQA				Analytica	al Technique	s		
Characterizations	MAM	SEC	CEX	rCE-SDS	nrCE-SDS	HILIC	ID ELISA	HCP ELISA
Aggregation	\bigcirc		Indirect			\bigcirc	\bigcirc	\bigcirc
CDR Tryptophan Degradation		Indirect	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
C-terminal Amidation		\bigcirc	Indirect	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
C-terminal Lysine		\bigcirc		\bigcirc	\bigcirc	\bigcirc	\bigcirc	0
Cysteine Adducts		\bigcirc		\bigcirc	\bigcirc	\bigcirc	\bigcirc	0
Deamidation		\bigcirc	Indirect	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Disulfide Isoforms		\bigcirc	Indirect	\bigcirc		\bigcirc		\bigcirc
Disulfide Reduction		\bigcirc	\bigcirc	\bigcirc		\bigcirc	\bigcirc	\bigcirc
Fragmentation (Peptide Bond)			\bigcirc			\bigcirc	\bigcirc	\bigcirc
Fucosylation		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Galactosylation		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	0
Glycation		\bigcirc	\bigcirc			\bigcirc	\bigcirc	\bigcirc
HCP		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	
High Mannose		\bigcirc	\bigcirc	\bigcirc	\bigcirc		\bigcirc	\bigcirc
Hydroxylysine		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Identity		\bigcirc		\bigcirc	\bigcirc	\bigcirc		\bigcirc
Methionine Oxidation		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Mutations & Misincorporations		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Non-concensus Glycosylation		\bigcirc	\bigcirc			\bigcirc	\bigcirc	\bigcirc
Non-glycosylated Heavy Chain		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
N-terminal pyroGlutamate		\bigcirc	Indirect	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
O-linked Glycans		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Residual Protein A		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Signal Peptide		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Thioether		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Trisulfide		\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Unusual Glycosylation		0	Indirect				0	0

Quality attribute characterization analyses covered by the MAM.

Yes = 🔵 Maybe = 🌒 No = 🗌



"Orbitrap technology and great search algorithms, like the one implemented in the BioPharma Finder software, allow us to fully leverage Multi-Attribute Method for characterizing our biotherapeutics. One key component of MAM is New Peak Detection. New Peak Detection is absolutely essential for using MAM as a QC release method." – Dr. Richard Rogers, Just Biotherapeutics, Inc.

A verified analytical workflow supported by a single vendor

The Thermo Scientific[™] HR Multi-Attribute Method (HR MAM) is a powerful high resolution mass spectrometry-based integrated hardware-software workflow, delivering comprehensive characterization and monitoring from research to Quality Control (QC). Built within a safe, compliant environment, the HR MAM enables characterization of biologics with ease and allows potential CQAs to be monitored throughout every stage of the drug development process, while also providing purity testing with the feature of New Peak Detection (NPD).

The high quality data generated by combining high resolution liquid chromatography and high resolution accurate mass (HRAM) mass spectrometry is seamlessly interrogated by a powerful software workstream to provide the maximum confidence in securing product quality.





HR Multi-Attribute Method workflow simplicity with the right tools



Verifying your system

The Thermo Scientific[™] Pierce[™] BSA Protein Digest Standard is used to verify the suitability of the LC/MS system for MAM with defined acceptance criteria.

ATTRIBUTE CHARACTERIZATION



Data Acquisition

in DDA MS/MS

Attribute Minina

with Peptide Mapping

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BioPharma Finder

Drug Product

Tryptic Digest



Productivity through separation and speed

The Thermo Scientific[™] Vanquish[™] Horizon UHPLC system offers exceptional robustness, high gradient precision, low dispersion, improved reproducibility and peak efficiency for every targeted quantitation workflow with ease and confidence.

The Thermo Scientific[™] Accucore[™] Vanquish[™] C18+ UHPLC column coupled with the Vanquish UHPLC system takes advantage of the extended pressure capabilities of the instrumentation and utilizes the system to its full potential. Robust 1.5 µm solid core particles deliver maximal peak capacities allowing reproducible LC-MS analysis.

Remove doubt with resolution and data quality

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The Thermo Scientific[™] Q Exactive[™] Plus Hybrid Quadrupole-Orbitrap[™] mass spectrometer is a powerful benchtop peptide mapping instrument with high resolution accurate mass capabilities. It offers sensitive, accurate and confident peptide mapping of therapeutic proteins with unparalleled acquisition speed, mass accuracy and spectral quality.

Exceptional confidence in identification of peptides and modifications enables accurate targeted quantitation and fast batch interrogation.





Confident discovery data interrogation COMBINED with targeted compliance-ready CQA analysis

The Thermo Scientific[™] BioPharma Finder[™] software is the perfect software for the discovery step of the HR MAM with high confidence peptide identification and sequence mapping.

Discovery and early development laboratories operating outside the compliance requirements benefit from straightforward and automated relative quantitation for targeted peaks defined by the **peptide workbook**. It allows fast screening of large datasets for potential CQA identification, which is verified by a unique MS2 spectra prediction algorithm, isotope distribution and accurate mass information.

Seamless connectivity to Thermo Scientific[™] Chromeleon[™] Chromatography Data System (CDS) for compliant workflows.

Chromeleon CDS was designed with compliance in mind. It has successfully passed hundreds of customer audits by meeting regulatory requirements for Data Integrity in GLP and GMP environment.

Chromeleon software delivers superior networking capabilities, advanced LC and MS instrument and data acquisition control, and automated, compliance-ready data processing and reporting; making this CDS ideal for routine monitoring of CQAs and NPD at any phase of the drug development.

Run your analyses compliantly in an enterprise environmentfrom method creation to final reporting.



CQA Selection and Workbook Transfer for Method Generation

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Seamless transition from discovery to routine securing biopharmaceutical quality control

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Data acquisition

- Peptides are analyzed under the control of Chromeleon CDS using a Data Dependent Acquisition (DDA) to produce MS/MS spectra for comprehensive peptide mapping of the protein under the control and stressed conditions
- For routine monitoring samples are analyzed in MS1 only under the control of **Chromeleon CDS**

Data processing and peptide identification

• The MS/MS data are processed with **BioPharma Finder** software to identify peptides with high confidence

BioPharma Finder

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- The acquired spectrum is compared to a predicted (mobile proton model) spectrum based on the FASTA sequence
- Accurate mass, retention time shifts, charge state, fragmentation spectra (MS/MS) are all used to produce a confident identification

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Peptide Workbook

- Quality attributes of interest are identified, and peptide specific information is saved to a Target peptide workbook within BioPharma Finder software
- Target peptide workbook allows targeted searching and quantitation within BioPharma Finder software as well as exporting information to Chromeleon CDS for routine compliant monitoring



ATTRIBUTE CHARACTERIZATION



Targeted CQA peak quantitation

- The **peptide workbook** is imported into **Chromeleon CDS**, as part of the MS Component Table in the data processing method
- Standardized processing method can be locked immune to further modification in a GMP environment
- Consistent, confident peak integration and quantitation is achieved using extracted ion chromatograms with optimized parameters for each potential CQA
- Isotopic distribution chart assures confident confirmation of a component

New peak detection

- Peak alignment and frame parameters can be pre-set
- Filtering for level of ratio enables screening for significant intensity changes
- Peak intensity threshold and ratio carefully set to avoid false positives
- Maximum number of potential new features is 16,000
- Ideal for purity testing and lot release
- Single or batch run for NPD

Automated report generation

- Spreadsheet based reporting with extensive options for customization offering flexibility to choose the right report template during setup
- Pass/fail criteria can be defined for CQAs and NPD



ATTRIBUTE MONITORING AND PURITY TESTING



RESOLUTION MATTERS FROM RESEARCH TO ROUTINE

Confidence from discovery to routine High resolution always on!









Accurate peak extraction and integration

The HRAM capability of the Q Exactive Plus mass spectrometer is essential to resolve overlapping peaks, ensure correct peak integration, and obtain accurate CQA quantitation using the MS full scan data.

B2 43

431 20

35,000

Correct identification and quantitation of deamidated peptide HYNPSLK is achieved with high resolution

A2

m/z 430.80

m/z

B1

430.60



20.0

15.0

430.10

430 10 430 20

430 20

8.100 8.159

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Isotope peaks of the deamidated peptide (B0-B2) are resolved from the wild type form (A1-A3) using an Orbitrap resolution setting of 140,000 (at *m/z* 200).

At a resolution setting of 70,000 (at *m/z* 200) the monoisotopic peak of the deamidated form (B0) is completely overlapping with the C13 peak of the wild type form (A1), resulting in the absence of this isotope in peak integration.

With a lower resolution setting of 35,000 (at m/z 200) all three isotope peaks of the deamidated form (B0-B2) are overlapping with the isotopes of the wild type form (A1-A3).

A resolution setting of 140,000 or higher (at *m/z* 200) is needed to detect significant abundance increase of the deamidated form (B0-B2) in the thermally stressed sample.

High mass accuracy improves peak extraction and integration

XIC of the deamidated "PENNYK" peptide with 10 ppm mass tolerance the wild type form (peak A) dominates the XIC of the deamidated form (peak B), which leads to wrong peak integration.



Extraction with 5 ppm mass tolerance correct peak (peak B) was integrated in the absence of the wild type form (peak A).





430 40

A1 + B0

Robust and reproducible quantitation



Excellent reproducibility of ratio measurement across ten technical replicates.



Consistent results for common CQAs on three different HR MAM instruments.

Reliable new peak detection

NISTmAb spiked with Pierce peptide retention time calibration mixture (PRTC). New peak (HVLTSIGEK in PRTC) reported by the Chromeleon CDS is shown in the figure below. All 15 PRTC peptides were detected as new peaks by the non-targeted MS processing feature in Chromeleon CDS, when compared with the NISTmAb control.



A: Injections, Channels and Frames information; B: XICs of the selected component in the PRTC spiked (red) and reference samples (blue); C: new peaks detected; D: filtering rules.

Low-level host cell proteins can be reproducibly detected and quantified. Peptide TFTTQETITNAETAK from glucose-6-phosphate isomerase was detected at lower than 15 ppm abundance level relative to the drug substance.



XIC (left panel) and mass spectrum (right panel) of peptide TFTTQETITNAETAK.



"The HR Multi-Attribute Method contains all of the software components for characterizing and releasing biotherapeutics from QC." - Dr. Richard Rogers, Just Biotherapeutics, Inc.

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HR Multi-Attribute Method



Verified—Pierce BSA Protein Digest Standard

BSA Digest Standard, LC-MS Grade, is used as a system suitability test standard to verify the performance readiness of the LC/MS system for HR MAM with defined acceptance criteria.



Reproducibility—Accucore Vanquish C18+ UHPLC Column

Accucore Vanquish C18+ UHPLC columns have ultra-short diffusion paths that result in extremely efficient separations reproducibly. The 1.5 µm particles enable fast separation and the resolution of complex mixtures, while enhancing workflows and productivity.

Speed–Vanquish Horizon UHPLC

Fully integrated and biocompatible system features high sample capacity for high-throughput workflows, industry-leading pumping performance, outstanding S/N and linearity, SmartInject and SmartFlow technology, and more. Making this a powerful system providing robust and reproducible separation of complex mixtures.



Confidence-Q Exactive Plus Mass Spectrometer

Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer provides the capabilities of high resolution accurate mass necessary for confident peptide identification, accurate CQA quantitation, and false positive free New Peak Detection. It is extremely accurate, identifying component peptides to within \pm 3 ppm. It is linear across 5 orders of magnitude, proving ideal for complex and varied peptide mixtures.

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Coverage-BioPharma Finder Software

BioPharma Finder software allows you to harness the power of the industry leading Thermo Scientific[™] Orbitrap[™] technology. Novel MS2 prediction algorithm increases the confidence in peptide sequence assignments, identification of the site and type of expected and unknown PTMs, detection of low-level impurities and sequence variants.



Compliance-ready-Chromeleon CDS Software

Chromeleon CDS delivers superior instrument control of high resolution MS instruments with CFR 21 Part 11 compliance-ready data acquisition, automation, and data processing as well as reporting for compliant GxP biopharmaceutical manufacturing and QA/QC environments.

Run your routine LC-MS peptide mapping analyses in a network deployable enterprise environment—from method creation to final reporting.



Find out more at thermofisher.com/mam



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