A regulatory Perspective on the Use of Mass Spectrometry for QC Testing of Therapeutic Proteins

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The views and opinions expressed here should not be used in place of regulations, published FDA guidances, or discussions with the Agency

Outline

- Use of MS for Biotechnology Product Analysis
- Examples of MS in BLA Applications
- MS in QC testing of Therapeutic Proteins
  - General Regulatory Considerations and Expectations
  - Examples of MS-method specific considerations
  - Case studies of MS in QC testing of Therapeutic Proteins

Use of MS for Biotechnology Product Analysis

- Identification
- Characterization
- Comparability (process change by same manufacturer)
- Comparative Analytical Assessment (biosimilar vs reference product)
- Surveillance for Adulteration
- Process Improvement
- PK/PD measurement

Rathore, D. et al., Expert Review of Proteomics, DOI: 10.1080/14789450.2018.1469982

MS in BLAs: Characterization

J. Am. Soc. Mass Spectrom. (2017) 28:786–794
DOI: 10.1007/s13361-016-1531-9

FOCUS: 28th SANIBEL CONFERENCE, CHARACTERIZATION OF PROTEIN THERAPEUTICS BY MS: RESEARCH ARTICLE

A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications

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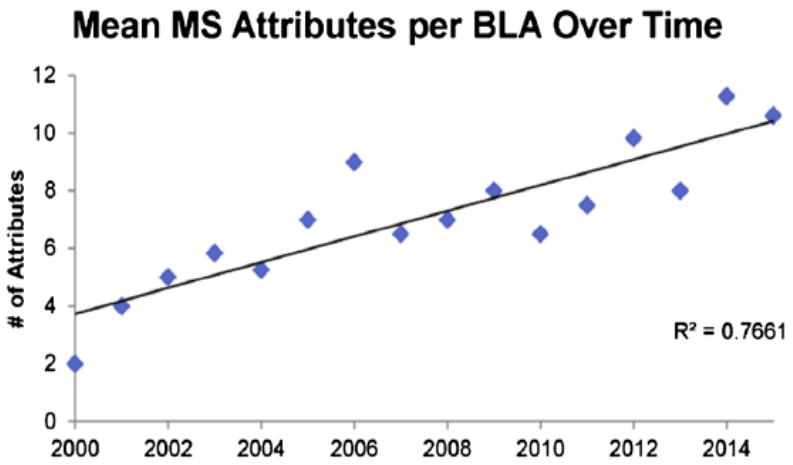


- 79 of 80* BLAs approved between 2000 - 2015 used MS in DS characterization
- # attributes analyzed increased over time
- Overall changes in types of MS assays used & types of characterization done over time are consistent with improvements in technology

Rogstad, S. et al., JASMS. 2016

***electronically submitted**

Attributes Analyzed

32 Specific MS Attributes found to be analyzed at varying levels across BLAs including structure, PTM*, product & process related impurities

MS attribute	% of MS BLAs	MS attribute	% of MS BLAs
Amino acid sequence analysis	97.5	Sequence variants (amino acid substitutions	8.9
Molecular mass	92.4	Covalent dimers	7.6
Disulfide bonds	77.2	Methionine/cysteine formylation	7.6
Glycosylation	70.9	Phosphorylation	5.1
Sequence variants (C-term)	64.6	Truncation	5.1
Sequence variants (N-term)	64.6	Acetylation	3.8
Deamidation	58.2	Aggregation	3.8
Oxidation	57.0	Folding/HOS	3.8
Size variants	27.8	Host cell proteins (HCPs)	3.8
Free thiols	25.3	Partial reduction	3.8
Glycation	22.8	PEGylation	3.8
Charge variants	19.0	Translucent particles	3.8
Other impurities	17.7	Zinc	3.8
Proteolysis/fragmentation	13.9	Glutathionylation	1.3
Succinimidation	12.7	Methylation	1.3
Isomerization	10.1	Norleucine incorporation	1.3
Other	10.1	Phosphogluconylation	1.3

Data source: Rogstad, S. et al., JASMS. 2016

***PTM = post translational modification**

Comparative Analytical Assessment

As of 07/23/2019, 23 biosimilar BLAs approved across 9 product classes

MS Usage	MS Workflow/ Instrumentation/Ionization	Attributes Analyzed
100% (12 out of 12 approved 351(k) BLAs reviewed spanning all product classes)	• Peptide mapping • Intact mass • Subunit analysis • Glycan profiling o LC-MS o LC-MS/MS o MALDI-TOF o HDX-MS ➢ ESI, LC-ESI, MALDI, nanoESI	• Amino acid sequence • Molecular weight • Disulfide bonds • PTM • Product related impurities • Process related impurities • HOS

https://www.fda.gov/drugs/biosimilars/biosimilar-product-information

MS for QC Testing

Use of MS in QC Testing

- MS is less commonly used in QC testing of therapeutic proteins due to complexity of therapeutic proteins and MS-method related considerations

MS Usage (As of 2017)	Protein BLAs	Peptide NDAs
Characterization	100%	100%
Control	0	65%

- Advances in technology (e.g. high resolution and high mass accuracy instruments) have led to increased use

Rogstad, S. et al., JASMS. 2016 and unpublished data

Regulatory Considerations for QC

- General regulatory expectations and considerations for MS are not different from other methods
- The principal expectation is to demonstrate that the method is fit for intended purpose
  - 21 CFR 211.165(e) and 211.194(a)(2)
- MS method specific challenges should also be addressed.
- Amount of information on method procedure and suitability typically varies with phase of development

General Regulatory Considerations/Expectations

Examples

- Method validation and system suitability
  - ICH Q2(R1)
  - Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics
- Comparison to conventional methods
  - Method bridging studies to support MS as a replacement for another method
  - To support method's ability to assess and monitor relevant quality attribute
- Assess impact on specifications acceptance criteria
  - ICH Q6B- "Specifications are linked to analytical procedure"

Cont'd

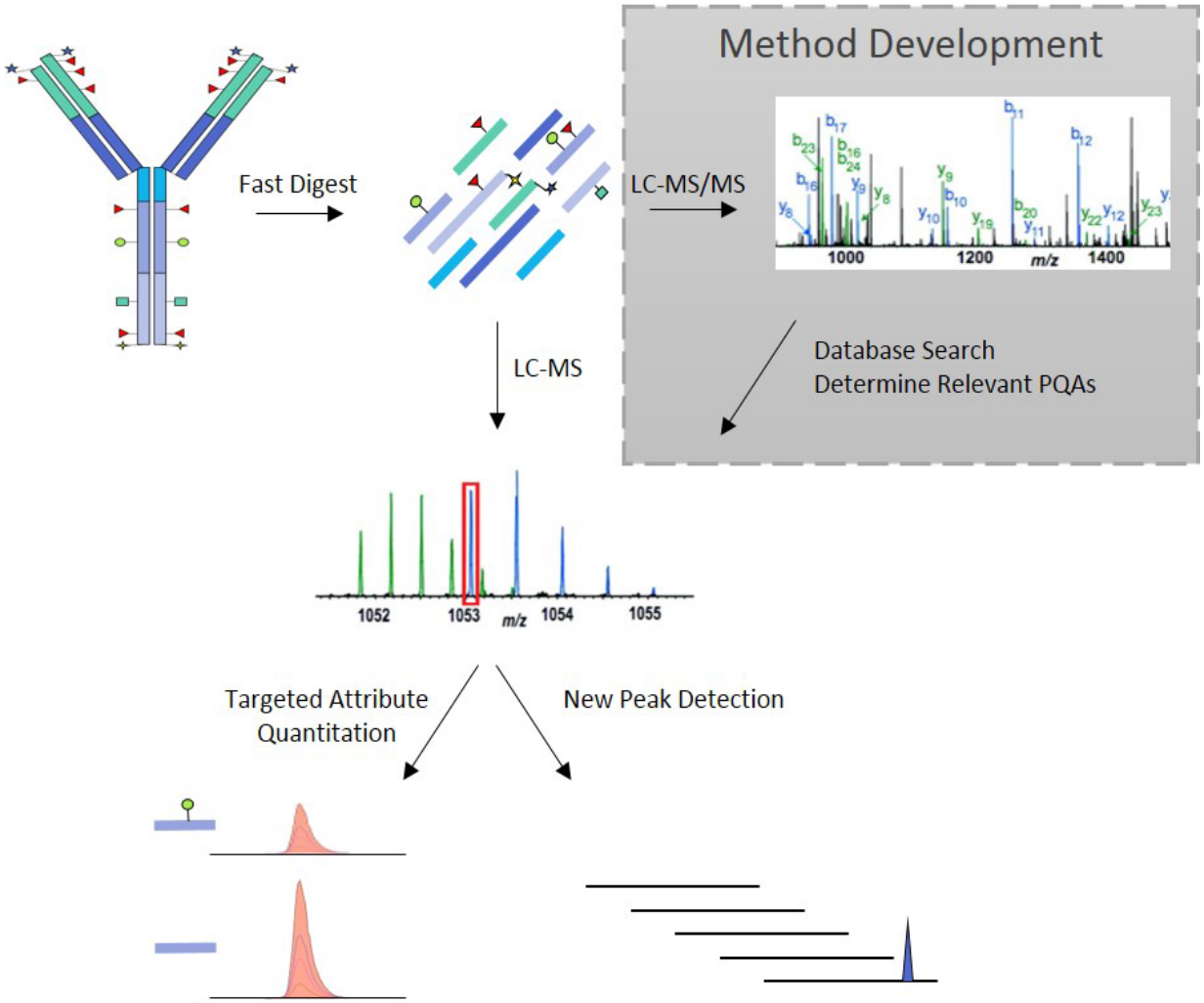
- Lifecycle management
  - Need for revalidation and/or method comparability studies?
    - Changes in method (reagents, instruments, software, e.t.c)
    - Method Transfer
    - Unexplained changes in method performance
      - e.g. method can only meet established system suitability criteria with repeated adjustment to operating conditions stated in the analytical procedure
  - Reserve samples and adequately qualified, properly stored reference standards are critical

Other considerations

- Discuss proposal with Agency
  - Review discipline
  - FDA Emerging Technology Team for novel technologies

Regulatory Considerations for MAM

Generic MAM workflow for a monoclonal antibody



Rogstad, S. et al., Analytical Chemistry. 2019 *Submitted*

Four Major Points to Consider for MAM

Risk Assessment

- Risks /benefits inherent to the method e.g. amino acid specific detection and control vs whole/intact molecule
- Should be product and CQA specific
- Commensurate with phase of devt.
- Assess relevance/criticality of the information lost and gained
- Alternative sources of lost information

Comparison to Conventional Methods

- Comparative testing between MS and conventional methods, address differences with respect to impact on product quality
- Understand correlation between MS and conventional methods
- Relevant metrics and statistical tests for comparing method performance
- Define plan for introducing MS

Method Validation

- General considerations for method validation based on intended use
- Additional considerations for **Precision, LOQ & LOD** and **system suitability**
- Rapidly evolving MS field
- Training

New Peak Detection

- Determine optimal parameters for NPD. Change in retention time window, mass accuracy, peak detection threshold can affect NPD
- Selection of appropriate peak detection threshold is critical

Rogstad, S. et al., Analytical Chemistry. 2019 *Submitted*

System Suitability Study for LC-MS

REPORT

mAbs 7:6, 1104–1117; November/December 2015

Performance metrics for evaluating system suitability in liquid chromatography—Mass spectrometry peptide mass mapping of protein therapeutics and monoclonal antibodies

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Instruments	Q-Exactive & Q-TOF
Samples	BSA sequence coverage standard spiked with peptides to simulate: 1. detection of one species in presence of a strong interference 2. Quantitation of sequence variants at different concentrations
Method Variables	Source voltage, MS1 and MS2 scan time, selection threshold

BSA sequence coverage did not effectively identify settings that led to limited dynamic range or poorer absolute mass accuracy with the two systems. Additional metrics determined to be necessary to establish system suitability for protein therapeutic characterization by LC-MS.

Model system in the study is not intended to reflect regulatory requirements, which are application and product specific.

Zhou, M. et al., MAbs. 2015

Table 2. Summary of the proposed metrics for system suitability test, with proposed acceptance criteria and possible troubleshooting targets. The thresholds of 15% for CV and +/− 20% for relative quantitation are adapted from current FDA recommendations[34]

Metric	Purpose	Definition	Recommended Threshold	Possible Cause for Failure
CV of Peak Area	Check signal stability	%CV of peak area of EIC at 100%	<15%	Instable signal, likely an issue at the electrospray
Protein Sequence Coverage	Check method settings for identification capability	Determined from search algorithm based on the confidence level chosen	Near complete is ideal	Acquisition setting, intrinsic sensitivity, LC separation
Average Mass Error	Report overall mass accuracy	Average of absolute ppm error of all identified peptides with high confidence (or known peptides)	From system specification <5 ppm or lower (High resolution)	Temperature change, invalid calibration, incorrect method settings (e.g., lock mass, AGC)
Mass Resolution	Check instrument resolution	Full width at half maximum mass peak resolution at 100% concentration	Within instrument specification (instrument and operation mode dependent)	Invalid instrument tuning/ qualification, instrument malfunctioning
Ion Source Settings	Minimize non-native species generated in source	Monitor the relative abundance of a fragile peptide in the standard	Maintain sensitivity while minimize excess activation of fragile species	Source tuning, source acceleration voltage too high
S/N of EIC	Defining LOD	S/N of target peptide EIC	> 3	Bad instrument tuning, bad EIC peak shape, bad spraying condition, intrinsic sensitivity limit of system
MS^2 Identification Score	Check MS^2 quality at different concentrations	Target peptide score/cutoff threshold for intra and inter-scan	≥1	Acquisition setting, intrinsic sensitivity, LC separation
Mass Accuracy/Resolution Change	Evaluate confidence of mass measurement at low concentrations	Compare mass error/ resolution at low and 100% concentration	Ideally minimal change at low concentrations	Instrument specific and can be normal to see mass error/resolution change at different signal intensities. Need to adjust data analysis procedure accordingly.
Accuracy of Relative Quantitation	Check accuracy and dynamic range of quantitation	Percent accuracy of the experimental result relative to the expected result	80% –120%	Bad EIC extraction method, co-eluting species, instrument intrinsic limit
CV of Relative Quantitation Result	Check precision of quantitation	%CV of peak area ratios of a target peptide pair	<15% unless only intended for semi-quantitation	Low sensitivity/bad peak shape at the concentration, incorrect data processing, interfering co-eluting species
Differential Peak Sampling	Check potential error in quantitation due to stochastic sampling across EIC peak	Ratio of relative quantitation results between the proposed method and the MS^1 only method	0.8~1.2 Use the MS^1 only method for quantitation if exceeds the range	Improper MS^2 settings such as the selection threshold, MS^1 and MS^2 scan rate, etc.

Proposed metrics not intended to reflect regulatory requirements, which are application and product specific.

Zhou, M. et al., MAbs. 2015

Case studies of MS in QC

Case Study 1: MS for Control of a Product Related Impurity

- Post approval change from conventional method to MS proposed for control of a specific impurity
- Impurity is a CQA for the product
- Monitored at DS release & DP release and stability testing
- Current method quantitates modification at one predominant site
- MS quantitates modification at multiple sites, including predominant site
- Specifications acceptance criteria for current method also proposed for MS

Case Study 1 : Supporting Studies

- Method validation
  - MS showed improved sensitivity (LOQ) and precision
- Method bridging studies
  - Comparison of impurity levels measured by MS and conventional method
    - multiple, different types of samples (release, stability, forced degradation)
    - both methods equally effective for detecting changes in impurity under routine and relevant stress conditions
    - Comparable mean values observed; however MS data had lower variability and lower max values compared to conventional method
  - Structure-Function studies to support MS specification acceptance criteria
    - Similar correlation of impurity levels with biological activity using both MS and conventional method

Case Study 1 : Regulatory Considerations

- Risk assessment: Knowledge of the impact of the manufacturing process on the CQA
- Method validation adequate
- System suitability criteria adequately defined
- MS showed improved sensitivity and precision (likely due to automation and lower method-induced artifacts)
- Proposed specifications acceptance criteria for MS (same as current acceptance criteria) not supported by MS method capability and clinical experience.
  - Sponsor asked to revise acceptance criteria based on sufficient number of commercial lots, appropriate statistical analysis, and justification (e.g. clinical experience)

Method change accepted with revised specifications acceptance criteria

Case Study 2: MS for Control of Multiple Attributes

- MAM proposed as a DS/DP release/stability specification assay for control of multiple attributes/product related impurities

- Proposed attributes/Impurities are generated by different PTM/ chemical modifications/ degradation pathways

- Change proposed during product development; method qualification provided to support use of MAM

Case Study 2: Regulatory Considerations

- Insufficient information provided to understand whether the method can adequately assess and monitor relevant quality attributes and its comparability to conventional assays

- Agency requested use of MAM as a supplement to conventional assays for release and stability testing until sufficient information is provided to support replacement of conventional methods with MAM

Case Study 2: Additional Supporting Studies

- Method validation
- Comparison of MAM to conventional methods
  - Extensive characterization of product by conventional methods to understand the specific attributes present and detected
  - Multiple lots, different types of samples (release, stability, forced degradation) tested by both methods
- New Peak Detection (NPD)
  - LOD/LOQ for NPD determined
  - NPD function and NPD LOQ/LOD verified
  - Stability indicating capability of NPD function verified

Case Study 2: Regulatory Considerations

- Sufficient additional supporting data/information provided to support replacement of conventional assays with MAM over time.

- Agency agreed to the Sponsor's proposed sunset strategy for phasing out conventional methods over time

Case Study 3: MS as a Complimentary Assay to a QC method

- Attribute controlled as part of in-process testing and at DS release using same conventional method

Proposed change (post approval)

- Eliminate the DS specification
- Retain in-process testing with current assay but test further downstream with revised (higher) IPC action limit
- Add MS for investigation of lots that exceed the new IPC action limit
  - MS used as an essential tool for making lot disposition decisions during OOS investigations

Case Study 3: Review challenges with the proposal

- Revised IPC action limit higher than current DS specification criteria and IPC limits
  - higher limit is based on small scale process characterization studies
  - not consistent with the current specifications and IPC limits, which are based on pilot scale, clinical and commercial experience
  - no additional downstream purification/attribute refining step
- No direct correlation between attribute type and levels measured by conventional method and MS
  - Attribute levels by MS are significantly lower than the levels by the conventional method; levels are not reduced by a similar factor from lot to lot

Case Study 3: Challenges cont'd

- No data to confirm that species identified by MS are the same as those identified by conventional method
- Concern that MS could be detecting different aspects of attribute profile than the conventional method and possibility that lots found to be OOS by conventional method could be released after MS-based investigation without considering full attribute profile

- Data for attribute coverage by MS compared to conventional method coverage not provided
  - Quantitative read outs of methods not comparable due to differences in methodology

Case Study 3 cont'd

Available Information & Additional Supporting Data Provided

- Product and process knowledge
- Safety and product quality risk assessment
- Qualification of MS method for non-routine testing
- Additional information on possible attribute coverage by MS
- Identification, quantitation, and safety risk assessment of some abundant specific attribute species detected by MS
- Investigation procedure for OOS results

Regulatory Decision

- Sponsor allowed to make change but asked to lower the new IPC action limit to be consistent with clinical & commercial manufacturing experience & the eliminated DS specification acceptance criteria

Summary

- Use of MS for characterization and QC testing of therapeutic proteins is expected to increase due to advances in technology
- The primary regulatory expectation is to demonstrate that the method is fit for intended use
- The general regulatory expectations for MS are not different from other assays; however, MS method specific challenges and attributes should also be addressed.
- Reserve samples and adequately qualified, properly stored reference standards are critical

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Thank you!