

Roundtable Session 1 – Table 5 – Best Practices of Extended Characterization Mass Spec Methods

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Abstract:

Mass spectrometry (MS) is widely used in the extended characterization of biologic therapeutics to support primary structure confirmation, characterization of product- and process-related impurities, process characterization, comparability assessments, and regulatory submissions. Although most MS methods are not routinely employed as GMP release assays, the data generated from these studies can have substantial regulatory impact, particularly in support of BLAs and Prior Approval Supplements (PAS). As MS applications move from exploratory characterization toward BLA/PAS-enabling studies, expectations for method performance, qualification, documentation, data integrity, and long-term lifecycle management become increasingly important. MS methods used in these contexts must be scientifically sound, fit for purpose, and appropriately qualified according to their intended use.

This roundtable will discuss best practices for qualification and application of common MS-based extended characterization methods (intact, peptide mapping, disulfide mapping) in late-stage CMC programs. Key topics include defining fit-for-purpose qualification, managing MS signal variability, regulatory expectations for reporting MS data, as well as considerations for method transfer, data integrity, automation. The goal is to share practical experiences and lessons learned across the industry to enable generation of robust, reliable, and regulatorily meaningful MS data while balancing scientific rigor, development timelines, and operational feasibility.

Discussion Questions:

1. Fit-for-Purpose Qualification Strategy: When should platform MS methods be sufficient, and when should molecule-specific MS methods be developed and qualified for late-stage programs? Which activities, such as PRS characterization or PPQ-batch comparability, should require a qualified MS method?
2. Method Performance and Acceptance: Criteria What method performance characteristics should be evaluated for different MS workflows (e.g., intact mass, peptide mapping, disulfide mapping), and how should acceptance criteria be established and justified?

3. Managing Method Variability: How can MS method qualification account for inherent signal variability and complexity in sample preparation, instrumentation, data acquisition, data processing, and analyst-to-analyst differences?

4. Lifecycle Management and Method Transferability: What lifecycle management strategies are most effective for maintaining qualified extended-characterization MS methods across instrument platforms, LC configurations, software versions, analysts, laboratories, and development phases? How do organizations trend method performance and establish action limits over time without triggering continual requalification activities?

5. Regulatory Reporting of MS Data: What are current best practices for presenting MS characterization data in regulatory submissions? In late-phase comparability/similarity studies, how should teams decide which PTMs, sequence variants, or unknown peaks (NPD) require identification, quantitation, trending, or reporting? how do you set and justify qualitative vs quantitative acceptance criteria for MS? How much raw data, processed data, sequence coverage information, PTM characterization, and method detail should be included in BLA/PAS filings versus retained as supporting documentation?

6. Auditability, Data Integrity, and AI-assisted Automation Consideration: Even when MS methods are used for characterization rather than release, late-stage and commercial applications may require stronger expectations for data integrity, software validation, audit trails, system suitability, analyst training, method transfer, troubleshooting, and lifecycle management. What are your best practices for ensuring MS data integrity and auditability (raw data, processing history, controlled parameters, cloud storage), and how do you validate automation/AI-assisted evaluation and reporting without undermining compliance?

Notes:

The conversation centered on how organizations balance scientific rigor, regulatory expectations, and operational practicality when using mass spectrometry for extended characterization of biologics. Participants generally viewed peptide mapping, intact mass, and related workflows as platform-based methods that can be used broadly in early development. Most agreed that these methods should already be scientifically robust and demonstrate adequate sequence coverage and performance before IND-enabling work. However, practices diverged on when a platform method must become product-specific and how formally that transition should be documented. Some participants reported completing full or fit-for-purpose qualification before Phase 3 or PPQ, with product-specific methods typically established to support commercial programs. Others used a qualified platform method followed by product-specific verification for late-stage activities,

particularly when the underlying procedure remained unchanged. However, the acceptability of platform methods for commercial programs was not confirmed.

There was no clear consensus on whether regulators explicitly require formal qualification of characterization methods for BLA submissions. Several participants considered qualification a regulatory expectation and noted that inspectors may request supporting documentation, particularly for comparability and PPQ-related data. Others had not encountered a specific written requirement or had seen characterization activities remain outside the scope of audits. Despite these differing views, the group broadly supported maintaining sufficient documentation—either a qualification report or a method development report—to demonstrate that a method is fit for its intended purpose. The practical distinction often appeared to be less about changing the analytical procedure and more about establishing product-specific records, acceptance criteria, and evidence that the method performs appropriately for the molecule.

The discussion also highlighted different philosophies for the scope of MS monitoring. Some organizations move from broad exploratory peptide mapping in early development to more focused MAM workflows in late-stage programs, emphasizing predefined CQAs and PTMs. Others monitor a wider set of modifications and incorporate them into new-peak detection lists. Participants noted that MAM adoption remains uneven and that NPD is even less widely used, in part because defining a meaningful “new peak” and setting appropriate thresholds can be difficult. Forced-degradation studies were identified as one useful way to determine which modifications matter and should be reported for late stage characterization and comparability study. Reporting thresholds varied by attribute and organization: many participants described a practical range of approximately 0.5–1% for PTMs, often favoring reporting above 1% to keep submissions focused, while sequence variants were treated more conservatively because numerous low-level variants may be significant cumulatively. Some groups also use NGS or multi-enzyme digestion to strengthen sequence-variant assessment and sequence coverage.

Managing method variability emerged as a major practical challenge. Participants acknowledged that signal intensity can change across instruments, analysts, sample preparations, software configurations, and time, even when mass accuracy remains acceptable. Reference standards, system-suitability tests, empirical operating ranges, and selected PTMs such as oxidation were commonly used to detect unacceptable performance. Some groups apply statistical limits such as three standard deviations, but participants cautioned that these limits can become impractical for low-abundance attributes or over a long method lifecycle. The group generally supported stopping and investigating a run when a meaningful system-suitability measure falls outside its range,

while recognizing that absolute intensity is often too unstable to serve as the sole criterion. Preventive maintenance, regular calibration, and analyst training were also emphasized, particularly because a calibration can pass while signal intensity is deteriorating.

Finally, participants described different expectations for method transfer, audit trails, and data integrity. Characterization groups may operate under more flexible controls than QC laboratories, but methods intended to support commercial products may eventually require formal transfer and molecule-specific documentation. Organizations also differ in whether QA applies audit-trail requirements to characterization data used for PPQ or BLA support. The overall direction of the discussion was that traceability will become increasingly important, whether it is provided through instrument software, chromatography data systems, or third-party IT solutions. Taken together, the conversation suggested that there is no single industry model: organizations are converging on fit-for-purpose, well-documented methods supported by meaningful controls, but the degree of formal qualification, breadth of reporting, and level of auditability still depend heavily on intended use, company practice, and regulatory experience.