Roundtable Session 1 - Table 5 - Expectations for the Development and Application of Extended Characterization Methods

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Abstract

Extended characterization methods are essential to provide deeper insights into product structure, function, and quality attributes of biotherapeutics and advanced modalities. This round table will discuss expectations for the development and application of such advanced analytical approaches, focusing on their role in product development, comparability assessments, and regulatory submissions. Key discussion points will include method development, feasibility and/or qualification, emerging technologies, and the balance between innovation and practicality in routine use. Participants from industry, academia, and regulatory backgrounds will share perspectives on how extended characterization can enhance product understanding, support risk-based control strategies, and drive analytical innovation.

Questions:

1. How should extended characterization be integrated into different stages of biologics development ? (Eg. type of data to be included in IND through BLA)

2. What are the expectations or requirements for development, feasibility and qualification of extended characterization methods at different stages of development? What are the parameters evaluated?

3. Is there growing expectation to include advanced extended characterization methods (eg. HDXMS, FPOP, x-ray crystallography, Cryo-EM, cell based potency) in regulatory submission?

4. What are the challenges in applying extended characterization methods on new modalities?

Notes:

Introduction and Participants

This roundtable discussion brought together analytical characterization experts from various sectors of the biopharmaceutical industry, including large pharmaceutical companies, contract development and manufacturing organizations (CDMOs), and biotechnology companies. Participants represented diverse areas of expertise including mass spectrometry, biophysical analysis, cell and gene therapy, and traditional biologics development. The discussion focused on practical experiences and industry practices for extended characterization methods across different stages of biologics development.

Key Discussion Topics and Questions Addressed

Integration of Extended Characterization Across Development Stages

The discussion centered on how extended characterization should be strategically implemented from IND through BLA submissions. Participants explored the evolution of analytical packages and the rationale for method selection at different development phases.

Method Development and Qualification Requirements

A significant portion of the discussion addressed expectations for method development, feasibility studies, and qualification requirements. The conversation covered the debate around full qualification versus fit-for-purpose approaches for different types of analytical methods.

Sequence Confirmation and Coverage Requirements

Detailed discussion occurred around peptide mapping strategies, with focus on achieving complete sequence coverage and the use of multiple proteases. Participants shared varying industry practices for sequence variant analysis and acceptable levels.

Charge Variant Characterization

The group discussed approaches to charge variant analysis, including fractionation strategies and the evolution from traditional ion exchange methods to newer techniques. The challenge of isolating sufficient material for potency testing was highlighted.

Advanced and Emerging Analytical Techniques

Participants explored the role of advanced methods such as hydrogen-deuterium exchange (HDX), covalent labeling, X-ray crystallography, and cryo-electron microscopy in regulatory submissions.

Main Insights and Practices Shared

Early Stage (IND) Characterization Strategy

- **Primary Structure Confirmation**: Intact mass analysis combined with reduced mass for multi-chain proteins, supplemented by tryptic peptide mapping
- **Initial CQA Assessment**: First-pass evaluation of critical quality attributes without extensive stress studies
- **Cell Line Selection Support**: Characterization data used to guide cell line development decisions
- **Sequence Coverage**: Most participants aim for 100% sequence coverage, typically achievable with trypsin alone, though some companies require multiple proteases

Late Stage (BLA) Characterization Expansion

- **Enhanced Analytical Package**: Addition of higher-order structure methods (AUC, DSC, CD/FTIR)
- **Detailed Impurity Characterization**: Individual peak isolation and identification for charge variants
- **Comprehensive PTM Analysis**: Detailed post-translational modification mapping
- **Method Qualification**: More extensive qualification studies, though approaches vary significantly

Sequence Variant Management

- **Acceptable Levels**: Industry practices range from 0.1% to 0.5% for individual sequence variants
- **Process Control**: Most companies engineer processes to minimize variants rather than characterize them extensively
- **Microbial vs. Mammalian**: Different acceptance criteria, with microbial systems having higher tolerance (1-2%) due to inherent characteristics

Challenges and Solutions Discussed

Method Qualification Dilemmas

Participants identified a fundamental challenge in qualifying characterization methods, particularly for mass spectrometry-based techniques that are "qualified by design" due to their inherent specificity. The group discussed the tension between regulatory expectations and scientific meaningfulness of qualification parameters.

Cross-linking Characterization

A significant challenge highlighted was the identification and characterization of protein cross-links, particularly distinguishing between authentic covalent cross-links and artifacts of analytical conditions. Participants shared experiences with apparent cross-links visible by CE-SDS that couldn't be confirmed by other methods.

Material Limitations for Fractionation

The discussion revealed ongoing challenges with charge variant characterization, particularly the need for sufficient material to perform potency testing on isolated fractions, limiting the depth of characterization possible.

Regulatory Agency Variability

Participants noted differences in regulatory expectations, with some agencies requesting more extensive characterization data than others, and the importance of precedent in shaping expectations.

Emerging Technologies and New Modalities

Bispecific and Multi-specific Antibodies

The discussion highlighted new critical quality attributes for bispecific molecules, including homodimer formation and chain mispairing. Participants shared challenges in detecting and quantifying these species, particularly when separation methods have limited resolution.

Cell and Gene Therapy Products

For AAV-based gene therapies, participants discussed:

- **Capsid Protein Analysis**: VP1, VP2, VP3 ratio determination and modification assessment
- **Genome Integrity**: Use of both gel-based and NGS approaches
- **Advanced Techniques**: Evaluation of charge detection mass spectrometry (CDMS) for particle characterization

Conjugated Products

Challenges with PEGylated and other conjugated products were discussed, including:

- **Limited Structural Analysis**: Loss of ability to perform detailed molecular characterization post-conjugation

- **Before/After Strategy**: Comprehensive characterization of unconjugated material followed by limited analysis of conjugated product
- **Novel Detection Methods**: Use of charge detection mass spectrometry and mass photometry for large conjugates

mRNA and Lipid Nanoparticle Products

Brief discussion of analytical approaches for mRNA vaccines, including NGS for sequence confirmation and HPLC methods for lipid ratio determination in LNPs.

Key Takeaways and Conclusions

Strategic Approach to Method Selection

The discussion reinforced that extended characterization should be implemented strategically, with IND-stage methods focused on confirming "you have what you think you have" and BLA-stage methods providing comprehensive documentation and comparability support.

Qualification Philosophy

There was strong consensus that qualification approaches should be fit-for-purpose and scientifically meaningful. Over-qualification of inherently specific methods (like mass spectrometry) provides little value and can create unrealistic expectations for method performance.

Technology Evolution

The industry continues to evolve toward more sophisticated analytical approaches, but practical considerations around cost, throughput, and regulatory acceptance remain important factors in method selection.

Regulatory Pragmatism

Successful regulatory interactions often involve demonstrating that comprehensive characterization has been performed while focusing on scientifically relevant parameters rather than pursuing exhaustive qualification of every method.

Future Directions

The discussion highlighted the need for continued development of analytical approaches for emerging modalities, with particular emphasis on methods that can handle the unique challenges of large, complex, or conjugated therapeutic molecules.

The roundtable concluded with recognition that while analytical capabilities continue to advance rapidly, the fundamental principles of fit-for-purpose method selection and scientifically sound characterization strategies remain central to successful biologics development.