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LEVERAGING ADVANCED ANALYTICAL TECHNOLOGIES TO STREAMLINE BIOSIMILAR DEVELOPMENT

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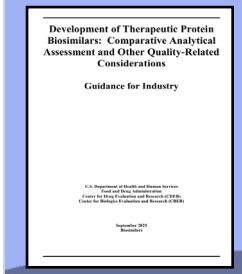
Agenda

- The Evolving Landscape of Biosimilar Development
- Critical Quality Attributes (CQAs)
- Multi-Attribute Monitoring
- Process Analytical Technology
- Functional Characterization
- Reference Product Variability
- Addressing Analytical Differences

THE EVOLVING LANDSCAPE FOR BIOSIMILAR DEVELOPMENT

Analytical Similarity: foundation for totality-of-evidence approach

FDA Sept 2025 (CAA)

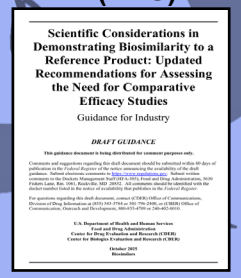

Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations

Guidance for Industry

EMA Mar 2026 (Tailored clinical)


Reflection paper on a tailored clinical approach in biosimilar development

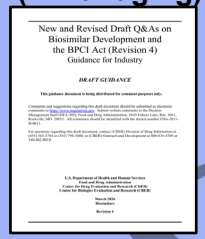
FDA Oct 2025 (CES)


Scientific Considerations in Demonstrating Biosimilarity to a Reference Product: Updated Recommendations for Assessing the Need for Comparative Efficacy Studies

Guidance for Industry

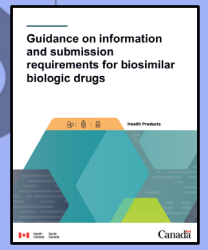
DRAFT GUIDANCE

FDA Mar 2026 Q&A Rev4 draft (PK bridging)


New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 4)
Guidance for Industry

DRAFT GUIDANCE

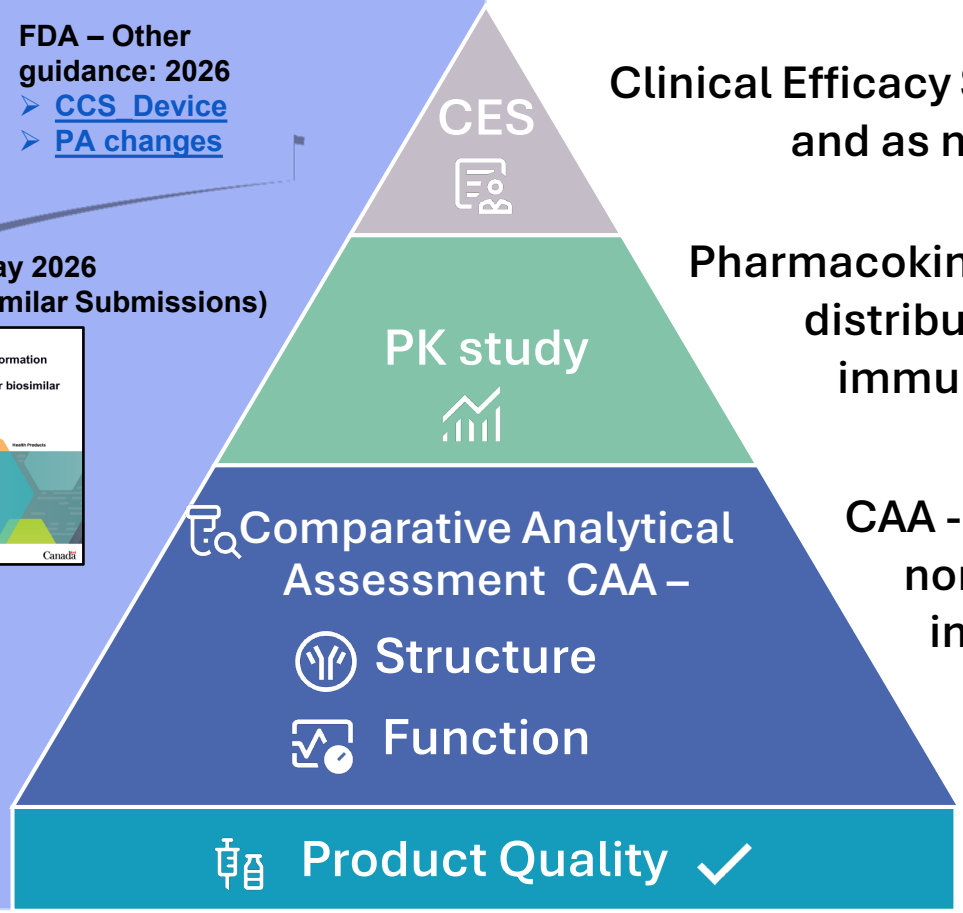
HC May 2026 (Biosimilar Submissions)


Guidance on information and submission requirements for biosimilar biologic drugs

Canada

FDA – Other guidance: 2026

- CCS Device
- PA changes



Clinical Efficacy Study – Tailored and as needed

Pharmacokinetic study – Drug distribution, safety, immunogenicity

CAA - Structure, function, non-clinical : similarity in efficacy, safety and immunogenicity

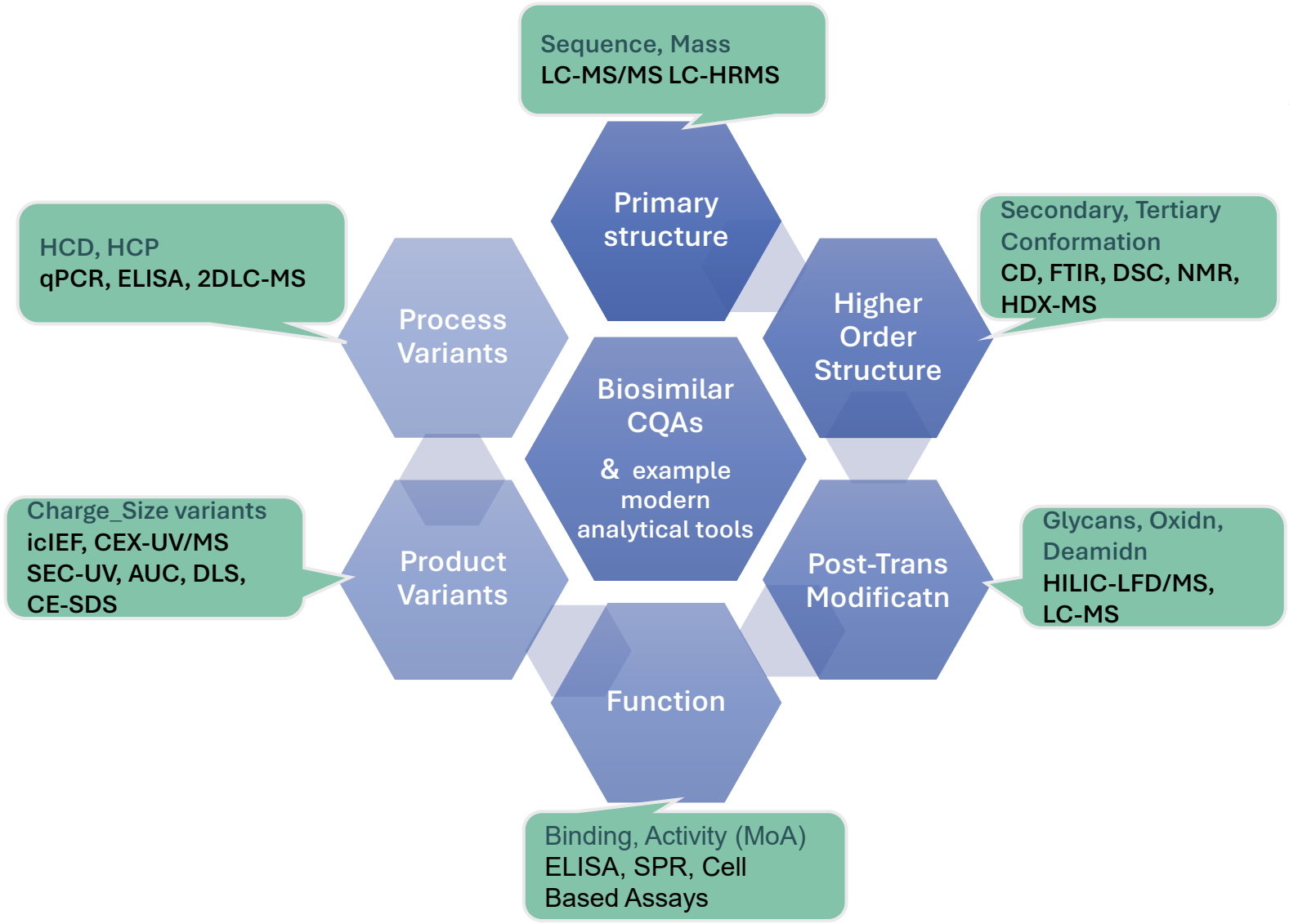
Consistent & robust manufacturing

REFERENCES:1-5

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CRITICAL QUALITY ATTRIBUTES (CQAs)

Modern analytical tools for molecular-level understanding of biosimilar CQAs



Advanced Mass Spectrometry: Transforming characterization and similarity assessment

- High-Resolution Mass Spectrometry (HRMS)
- Native MS, top-down and middle-down characterization approaches.
- Multi-attribute methods (MAM) for simultaneous monitoring of quality attributes.
- Increased sensitivity and throughput compared with traditional techniques.

Biosimilar development benefits

✅ Exceptional sensitivity
✅ Molecular-level characterization
✅ Detection of low-abundance variants
✅ Strong evidence for similarity assessment (sequence, variants, PTMs, molecule integrity)

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MULTI-ATTRIBUTE MONITORING (MAM)

Emerging analytical platform for simultaneous assessment of several Biosimilar CQAs

MULTI-ATTRIBUTE MONITORING (MAM)

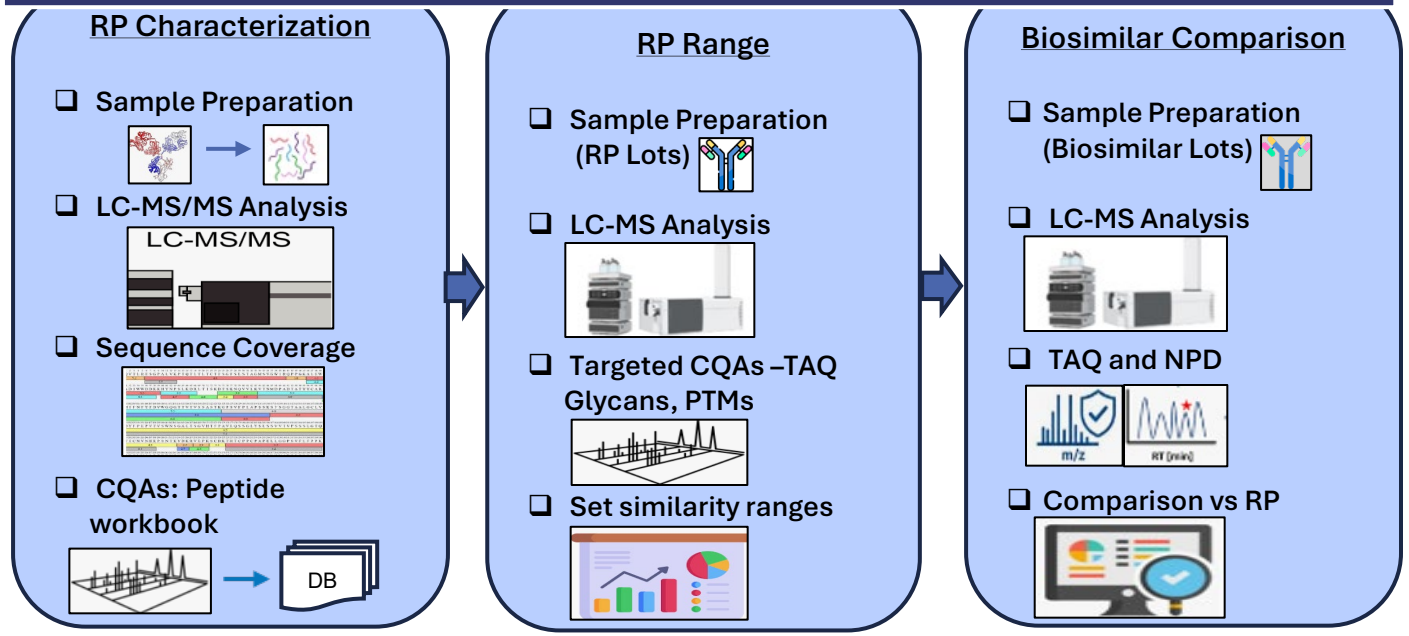
- ❖ **MAM is an LC-HRMS based analytical platform enabling simultaneous monitoring of multiple CQAs in a single automated peptide mapping workflow.**
- ❖ **Integrated target attribute quantification (TAQ) for Sequence variants, Glycans, Oxidation, Deamidation, charge variants and for New Peak Detection (NPD).**
- ❖ **Powered by High-Resolution, Accurate-Mass (HRAM) - UHPLC (RP) and advanced informatics, providing comprehensive, data rich product characterization**

POTENTIAL TO SUPPORT ACCELERATED BIOSIMILAR DEVELOPMENT

- ➢ **Reference Product (RP) Characterization –** deep molecular understanding of RP CQAs for QTPP establishment
- ➢ **Cell Line Development –** early CQA monitoring and clone selection
- ➢ **Process Development –** enhanced process optimization strategies with intact / subunit MAM
- ➢ **Comparative studies –** sensitive and comprehensive comparability and analytical similarity assessment
- ➢ **Regulatory agencies (FDA –** BsUFA research pilot program**, EMA and others) –** knowledge base creation and support for adoption
- ➢ **Commercial manufacturing –** Potential application in QC testing

REFERENCES: 6-8

MAM WORKFLOW FOR BIOSIMILAR COMPARATIVE ANALYTICAL ASSESSMENT



ADVANTAGES, LIMITATIONS OF MAM AND CONSIDERATIONS FOR QC TESTING

- ○ **Sensitive and accurate, simultaneous assessment –** well established for characterization, potential to support PAT and RTRT
- ○ **Initial expense to establish instrumentation, advanced informatics software –** but Instrument and software solutions well developed and available from Vendors
- ○ **Sample preparation artifacts, system drifts -** possible mitigation with automation and SS checks and standards (USP <1060>)
- ○ **Adoption under cGMP requires risk assessment,** method validation and transfer, understanding of NPD feature capability to avoid false results and comparison with conventional methods

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PROCESS ANALYTICAL TECHNOLOGY (PAT)

PAT for real-time control in biosimilar manufacturing – a strategic advantage

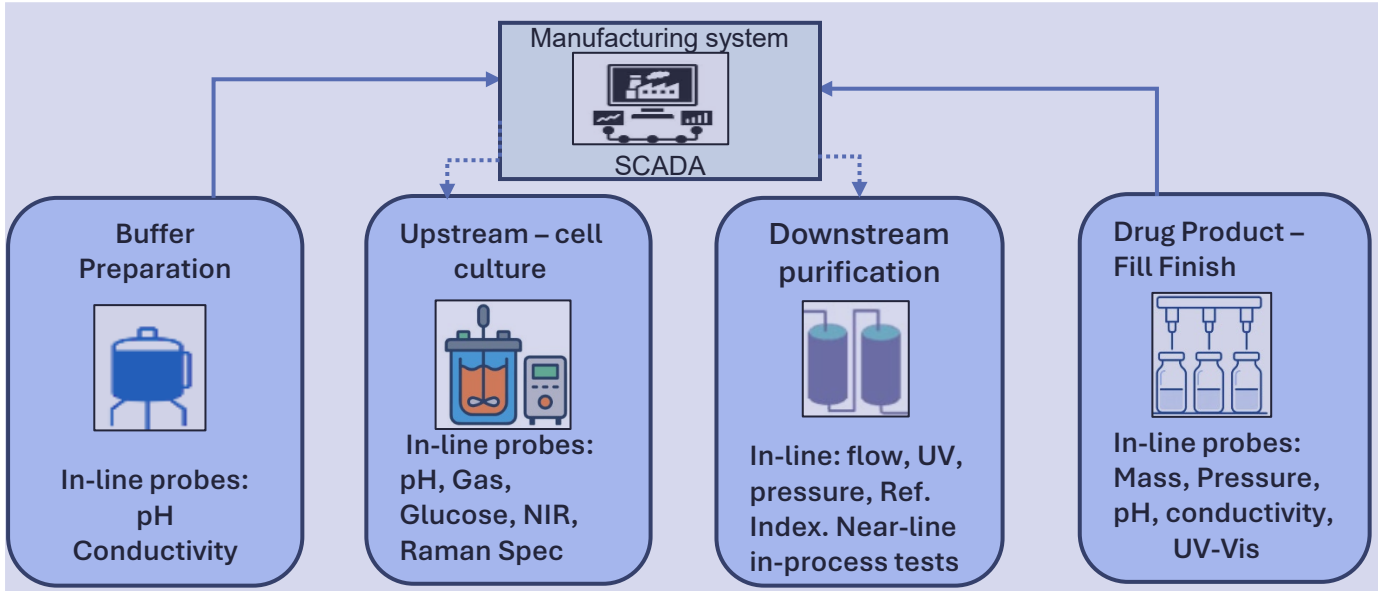
PROCESS ANALYTICAL TECHNOLOGY (PAT)

PAT- A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance as a mechanism to design, analyze, and control pharmaceutical manufacturing process through the measurement of Critical Process Parameters (CPP) with Critical Quality Attributes (CQA) of the product.

COMPONENTS OF PAT:

- Definition of CQAs and CPPs for the process
- Selection of Analytical Tools: UV-Vis, Raman, NIR
- Real-time monitoring after validation of tools
- Multivariate analytics & process analyzers
- Process validation - Product quality assurance
- Continuous improvement (CPV, statistics, Life Cycle)

PAT – POTENTIAL APPLICATIONS IN BIOSIMILAR MANUFACTURING



POTENTIAL TO SUPPORT ACCELERATED BIOSIMILAR DEVELOPMENT

- Upstream process: **track cell line health, maintain optimal conditions with feedback loops, monitor titer, recovery and purification of biologics in batch / continuous manufacturing**
- PAT can support deep process understanding, control improved batch consistency, yield and operational agility
- Keeping pace with the dynamic landscape, proactive, real-time controls can help compress development timeline with distinct advantages for global tech transfer, scale up etc.

REFERENCE: 9

ADVANTAGES AND LIMITATIONS OF PROCESS ANALYTICAL TECHNOLOGY

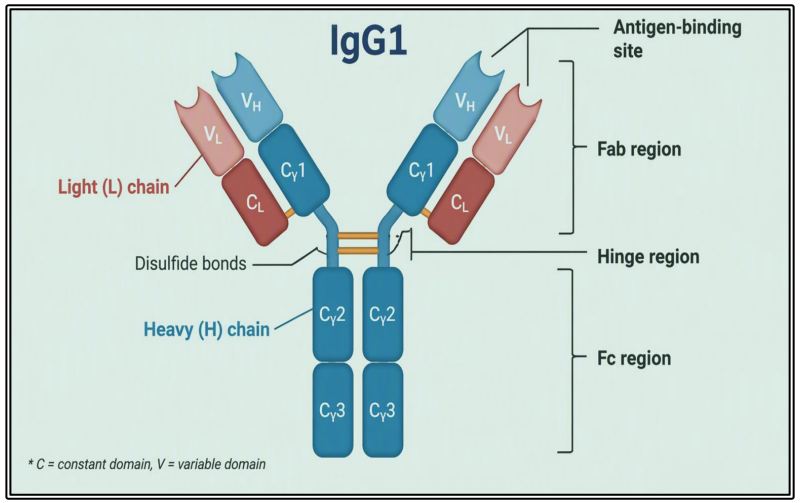
- **Enhanced Process Understanding:** Supports Quality by Design (QbD) development approach
- **Real-Time Quality Control:** Replaces slow offline laboratory testing with continuous, inline data streams where possible
- **Reduced Risk and Waste:** Detects process deviations immediately, preventing entire-batch failures and lowering rework costs
- **Higher Efficiency:** Speeds up cycle times and lays the groundwork for real-time release testing (RTRT)

❖ **High Initial Investment** – equipment and data management, technical and analytical complexity, integration hurdles into production lines, Validation and regulatory compliance requirements

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FUNCTIONAL CHARACTERIZATION

Comprehensive in vitro functional characterization- crucial for biosimilar development



- Functional characterization is critical for biosimilarity assessment and regulatory approval. Provides crucial information on structural interaction with the intended target and subsequent biological Mechanism of Action (MoA)
- State-of-the-art orthogonal analytical bioassays facilitate robust, MoA-driven functional characterization. With current advances and innovation, methodologies can be combined to build up an assay panel covering all potential MoAs
- Careful considerations are needed for method selection and to ensure reliability with phase appropriate qualification / validation.
- Highly controlled in vitro bioassays are often more specific and sensitive to detect differences between the biosimilar and the reference product than clinical trials (many variables).

Functional testing serves as more than a characterization exercise, providing the interpretation bridge between molecular characterization and clinical relevance. Some examples:

FcRn Kinetics is indicative of antibody clearance and hence the in vivo PK. Many PTMs (glycosylation, oxidation, c-terminal lysine etc.) can impact FcRn kinetics.

Functional assays support interpretation of structural differences. For example, If oxidation is observed near a binding region, antigen-binding or potency data will help assess its impact.

By establishing a robust "structure-function" data early on, residual uncertainties can be reduced / avoided and significant acceleration of Biosimilar development can be achieved.

Type	Assay	Analytical tool	Regulatory Importance
Fab binding / Function	Target antigen binding Cell based binding Cell-based reporter Cell proliferation / inhibition	SPR, BLI, ELISA Flow cytometry/FACS Reporter gene assays Cell based assays	Target binding and MoA - mirrors in vivo function
Fc mediated Effector function	FcY, C1q, and FcRn receptor binding, ADCC, CDC, ADCP etc	SPR, BLI, ELISA, AlphaLISA, Reporter gene assays, cell-based assays	potential safety, clearance, and overall efficacy risks

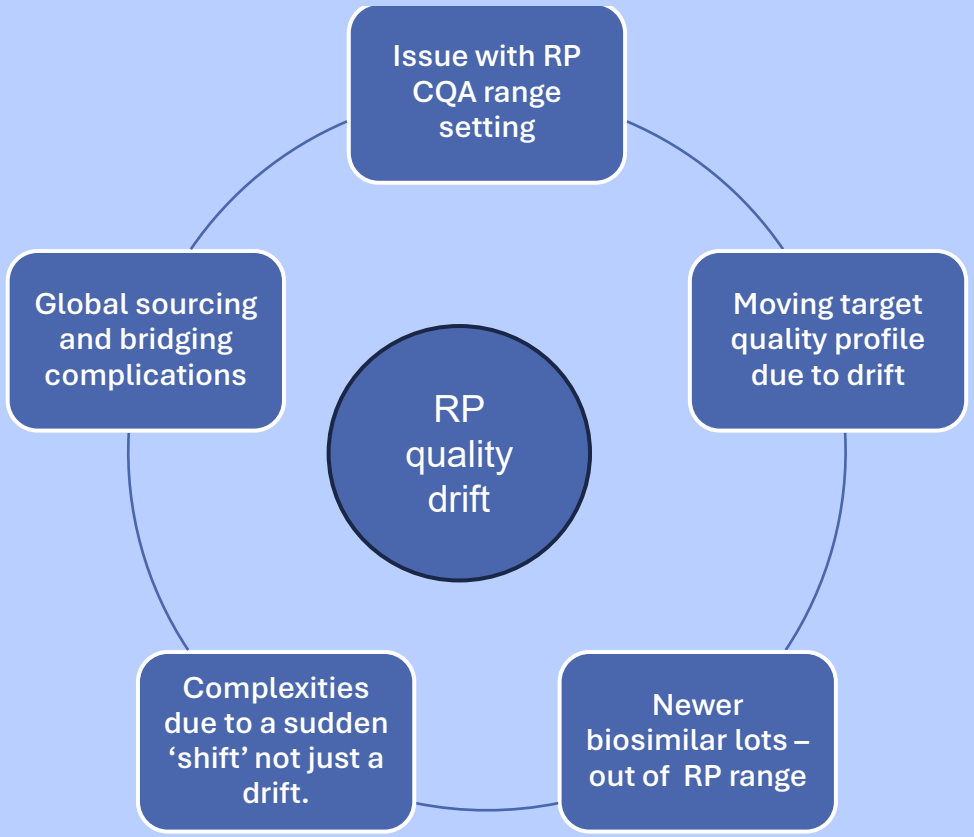
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REFERENCE PRODUCT (RP) QUALITY DRIFT

RP CQA ranges – beyond statistical boundaries towards clinical relevance

IMPACT ON BIOSIMILAR DEVELOPMENT

- Characterization of Reference Product lots during Biosimilar development is critical – for QTPP establishment and CAA
- RP quality drift may be observed for some of the CQAs due to process or site changes, formulation updates or lifecycle management



STRATEGIES TO MANAGE RP QUALITY DRIFT

- ✓ Analyze at least 10-15 RP batches spanning the shelf-life to establish a robust quality range
- ✓ Employ orthogonal methods to enhance CQA understanding
- ✓ Ascertain the difference is true – if it is real product variation (and not analytical variation)
- ✓ Assessment based on totality of analytical data and potential clinical impact of the attribute on the patient – not just by comparison with the RP CQA range or statistical limits
- ✓ In the case of a 'shift' in RP CQA – strategize excluding outlier lots based on risk

REGULATORY GUIDANCE AND PRINCIPLES

Major regulatory authorities FDA, EMA and HC have framework in place based on comparative science and life-cycle management principles:

- ❑ Totality of evidence approach: similarity is established through comprehensive comparability, minor variations not impacting safety and efficacy do not invalidate similarity
- ❑ Post approval, no re-alignment to RP CQA changes needed for biosimilar (both independently maintain their compliance within the framework)
- ❑ Both RP and Biosimilar governed by ICH Q5E for life cycle changes ensuring manufacturing changes do not impact safety, quality, efficacy.

ADDRESSING ANALYTICAL DIFFERENCES

Managing Analytical Differences Through a Totality-of-Evidence Approach

CORE STRATEGIES TO ADDRESS DIFFERENCES

To address analytical differences between a proposed biosimilar and its reference product, manufacturers rely on a structured, science-based approach anchored in extensive physicochemical and functional characterization

- ❑ **Apply Orthogonal Methods** to confirm CQA differences and minimize any method-specific bias
- ❑ **Characterize multiple RP Lots** over its shelf-life to fully capture natural batch-to-batch and age-related heterogeneity
- ❑ **Risk-Based Attribute Evaluation** Assess observed differences based on clinical relevance, mechanism of action, and potential impact on product performance
- ❑ **Demonstrate Functional Relevance** Use in vitro functional and biological assays (e.g., potency, receptor binding) to show that residual structural differences do not affect safety, purity, or potency.
- ❑ **Refine Manufacturing When Needed** If functional differences correlate with structural differences, implement upstream and/or downstream process changes to align CQAs with the reference product.

Some example scenarios and evaluation approach

CQA	Criticality	Clinical impact	Justification / mitigation
Afucosylation difference	High risk for oncology Mabs with ADCC as MoA	Impacts FcγRIIIa binding and ADCC - MoA	Evaluate ADCC – justifiable if within RP range
Methionine Oxidation difference	High risk if located in CDR/Fab region Medium risk if located in Fc region	Impacts target binding Impacts FcRn binding and clearance in vivo	Potency loss to be managed with process and / formulation adjustment Evaluate FcRn binding – justifiable if within RP range
C-terminal Lysine difference (higher basic variants)	Low risk - in Fc and will not impact function	Rapidly cleared in vivo	Carboxypeptidase digestion: basic to main peak. No impact on functional assays

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