Analytical CMC Requirements for Biotech and Biosimilar Products: More Than Meets the Eye!

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Why do biotechnology products have different CMC requirements from traditional chemical products for comparability, specifications, expiration dating, and the development of ‘generic’ forms?
Biological/Biotechnological vs. Chemical Pharmaceutical Products

Chemical Products

Protein Products
Biological/Biotechnological vs. Chemical Pharmaceutical Products

Significant Differences In:
✓ Raw Materials
✓ Production Processes
✓ Handling Conditions
✓ Physiochemical Characteristics
✓ Formulations
✓ Methods of Analysis
✓ Reference Standards
✓ Stability Profile
✓ Storage Conditions
✓ Expiration Dating
✓ Specifications

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Why is this more risky for Biopharms?

Oral Dosage Route (Chemical)
Not Destroyed with Ingestion

Oral Dosage Route (Protein)
Gut = Food!

Parenteral Delivery
Why are impurities and contaminants more risky for Biopharms?

Oral Dosage Route (Chemical)

5 second rule?

Parenteral Dosage Route (Biotech)

Impurities (e.g. host cell proteins)
Contaminants (e.g. microbials)
Why is degradation more risky for Biopharms?

Oral Dosage Route (Chemical)
Decrease in Active Moiety = Less Efficacy

Parenteral Dosage Route (Biotech)
Increase in Degradants = Less Safety

BODY’S MEMORY = IMMUNOGENICITY
“Due to the complexity of biological/biotechnology-derived products, the generic approach is scientifically not appropriate for these products. The “similar biological medicinal products” approach, based on a comparability exercise, will then have to be followed.” CHMP/437/04 Guideline on Similar Biological Medicinal Products Oct 2005
What world-wide regulations detail CMC analytical study requirements for biotech/biological products?
Where is it Written?
**U.S. Regulatory Documents** *(www.fda.gov)*

- **Code of Federal Regulation (CFR)**
  U.S. FDA quality practices approved by Congress and enforceable by law ("thou shalt...").

- **Guidance for Industry (GFI)**
  Information from FDA on recommended activities to satisfy specific CFR requirements ("how to...").

- **Compliance Policy Guidance (CPG)**
  Guidance to FDA reviewers/inspectors on what to look for in product submissions and manufacturing operations ("did they...").
Where is it Written?

International Regulatory Documents (www.ich.org)

International Conference on Harmonization:
U.S., E.U., Japan (Canada, Australia)

- Stability (Q1A, Q1B, Q1C, Q1D, Q1E, Q1F)
- Test Method Validation (Q2 R1)
- Product and Process Impurities (Q3A, Q3B, Q3C)
- Test Procedures and Acceptance Criteria (Q6A, Q6B)
- GMP for Active Pharmaceutical Ingredients (Q7A)
- Pharmaceutical Development (Q8)
- Quality Risk Management (Q9)
- Pharmaceutical Quality System (Q10)
- Development and Manufacture of Drug Substances (Q11)
- Content and Format of the Common Technical Document (M4Q; Module 3 “Quality”)

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Where is it Written?

**ICH Q5 Series = Biotech/Biologic**

- **Q5A** = Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin
- **Q5B** = Quality of Biotechnological Products: Analysis of Expression Construct in Cells Used for Production of r-DNA Derived Protein Products
- **Q5C** = Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products
- **Q5D** = Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products
- **Q5E** = Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process

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Current Regulatory Guidance Documents Relevant to Designated Biotech Product Types

✓ Monoclonal Antibodies
✓ Recombinant Proteins
✓ Naturally-Derived Products (Allergenic extracts)
✓ Vaccine Products (Protein, DNA, Conjugate, etc...)
✓ Cellular and Tissue-Derived Products
✓ Gene Therapy Products
✓ Plasma-Derived Biopharmaceuticals
✓ Plant-Derived Biopharmaceuticals
✓ Transgenically-Derived Biopharmaceuticals
✓ Synthetic Peptides (Redacted in 2006, but principles still relevant)
✓ Biosimilars
Is there one place where it is written where all of the required CMC studies and data to be presented in each regulatory dossier for biotechnology products?
ICH Common Technical Document (CTD)

ICH Guidance M4Q (August 2001)
  + ICH M4Q Q&A (July 2003)
  + ICH M4Q Q&A (December 2004)

Harmonized NDA/BLA content and format for regulatory reviews in US, EU, Japan, Canada, and Australia

Module 1: Administrative Information and Prescribing Information
Module 2: CTD Module Summaries
Module 3: Quality (CMC Sections)
Module 4: Nonclinical Study Reports
Module 5: Clinical Study Reports
3.2.S. Drug Substance

1. General Information
   1.1 Nomenclature
   1.2 Structure
   1.3 General Properties

2. Manufacture
   2.1 Manufacturer
   2.2 Manufacturing Process and Controls
   2.3 Control of Materials
   2.4 Control of Critical Steps and Intermediates
   2.5 Process Validation
   2.6 Process Development

3. Characterization
   3.1 Structure and Characteristics
   3.2 Product and Process Impurities

4. Control
   4.1 Specifications
   4.2 Analytical Procedures
   4.3 Validation of Analytical Procedures
   4.4 Batch Analyses
   4.5 Justification of Specification

5. Reference Standards
6. Container Closure System
7. Stability
3.2.P. Drug Product

1. Description and Composition
2. Pharmaceutical Development
   2.1 Components
   2.2 Drug Product
   2.3 Manufacturing Process Development
   2.4 Container Closure System
   2.5 Microbiological Attributes
   2.6 Compatibility
3. Manufacture
   3.1 Manufacturer
   3.2 Batch Formula
   3.3 Description of Process and Process Controls
   3.4 Control of Critical Steps and Intermediates
   3.5 Process Validation
4. Control of Excipients
5. Control of Drug Product
   4.1 Specifications
   4.2 Analytical Procedures
   4.3 Validation of Analytical Procedures
   4.4 Batch Analyses
   4.5 Justification of Specification
6. Reference Standards
7. Container Closure System
8. Stability
3.2.A Appendices
   A.1 Facilities and Equipment
   A.2 Adventitious Agents Safety Evaluation
   A.3 Novel Excipients

3.2.R Regional Information
   - Executed Batch Records (USA only)
   - Method Validation Packages (USA and ASEAN)
   - Comparability Protocols (USA only)
   - Process Validation Scheme for Drug Product (EU only)
   - Medical Device (EU only)

**Biosimilar Dossier: Quality and Safety**
3.2.S. Drug Substance

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   1.3 General Properties

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   2.2 Manufacturing Process and Controls
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   2.5 Process Validation
   2.6 Process Development

3. Characterization
   3.1 Structure and Characteristics
   3.2 Product and Process Impurities

4. Control
   4.1 Specifications
   4.2 Analytical Procedures
   4.3 Validation of Analytical Procedures
   4.4 Batch Analyses
   4.5 Justification of Specification

5. Reference Standards

6. Container Closure System

7. Stability
### 3.2.P. Drug Product

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   - 2.2 Drug Product
   - **2.3 Manufacturing Process Development**
   - 2.4 Container Closure System
   - 2.5 Microbiological Attributes
   - 2.6 Compatibility
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   - 3.2 Batch Formula
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   - 3.5 Process Validation
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5. Control of Drug Product
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   - 4.2 Analytical Procedures
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Biosimilar Dossier: Quality and Safety?
What CMC characterization, comparability, reference standards, release specification and stability data packages are required for a biotechnology-based product throughout product development and at time of commercial approval?
CMC (Chemistry, Manufacturing, and Controls) Data Packages for Biopharmaceutics

1. Product physiochemical profile (compositional and structural analysis)
2. Reference material characterization (establish product gold standard)
3. Test method qualification (linearity, precision, accuracy, specificity)
4. Test method validation (robustness, stability-indicating capability)
5. Lot release testing (manufacturing consistency and product quality)
6. Formulation screening (excipient selection, formulation stability)
7. Degradation evaluation (forced degradation to assess product, methods)
8. Stability testing (accelerated and ongoing real-time/real condition)
9. Extractable/Leachable studies (in parallel with ICH stability studies)
10. Comparability assessment (pre and post approval process/product changes)
11. Comparability – Biosimilar products (comparisons to reference licensed drug)
How should the required CMC analytical and stability studies be staged during the product development lifecycle?
CLINICAL STUDY TIMELINES

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Pre-Clinical

- SELECT CHAR-COMP DS and DP METHODS
- SELECT QC DS and DP METHODS*
- INITIAL PRODUCT CHARACTERIZATION
- PRELIMINARY FORMULATION TOX LOTS
- DS STABILITY TOX LOTS
- *VALIDATE QC SAFETY METHODS

Initial Clin

- QUALIFY METHODS*
- FORCED DEGRADE DRUG SUBSTANCE
- 1ST REFERENCE STD CHARACTERIZATION
- FORMULATION SCREENING STUDY
- STABILITY – INITIAL FORMULATION
- STABILITY – DS CLIN LOTS
- LOT RELEASE – DS LOTS
- LOT RELEASE – DP LOTS
- TOX - CLIN LOT COMPARABILITY
- ANALYTICAL SIMILARITY ORIGINATOR

Add’ I Clin

- VALIDATE QC METHODS
- VALIDATE STABILITY METHODS
- NEW REFERENCE LOT CERTIFICATION
- STABILITY – FINAL FORMULATION
- EXT & LEACH STUDY
- STABILITY – DS CLIN LOTS
- LOT RELEASE – DS LOTS
- LOT RELEASE – DP LOTS
- TOC-CLIN LOT COMPARABILITY
- ANALYTICAL SIMILARITY ORIGINATOR

Commercial

- RE-VALIDATE QC METHODS
- RE-VALIDATE STABILITY METHODS
- NEW REFERENCE LOT CERTIFICATION
- STABILITY - DP 1 ANNUAL LOT
- FORM or C/C CHANGE RE-DO E&L
- STABILITY - DS 1 ANNUAL LOT
- LOT RELEASE - DS EACH BATCH
- LOT RELEASE - DS EACH BATCH
- LOT RELEASE - DP EACH BATCH
- PROCESS CHANGE COMPARABILITY
- INTERCHANGEABILITY

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What four main elements are critical for establishing reliable, meaningful product specifications?
Biotech Product Specifications and Acceptance Criteria must be derived from:

1. Capabilities of the manufacturing process
2. Stability of the bulk substance and final product
3. Nature of batches used in pre-clinical and clinical studies
4. Capabilities of the analytical methods
Biotech Product Development CMC Activities

OBJECTIVE:
Establishment of Meaningful, Supportable Specifications

Specifications are proposed by sponsors and accepted by regulatory authorities as conditions of their approval to use the product in humans.

Product Specifications are Your CONTRACTS with the Regulatory Authorities

ANALYTICAL METHODS play a KEY ROLE in this system
CHEMICALS VS BIOLOGICALS
= DIFFERENT ANIMALS!

DRUG
Statin
MW 405

BIOLOGIC
IgG
MW 150,000

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“All of you are right. The reason every one of you is telling it differently is because each one of you touched the different part of the elephant. Actually the elephant has all the features you mentioned.”
Protein Biomolecular Analysis

Primary
http://psychology.wikia.com/wiki/Amino_acids

Secondary
Cozzone, A. J. Proteins: fundamental chemical properties. in Enc. of Life Sciences (Nature Publ Group, London, 2001)

Tertiary
Protein Tertiary Structure Prediction; D Xu, Y Xu; in Current Protocols in Protein Science (May, 2001)

Quaternary
P. Rudd, et. al; J. Immunology, 2004, 173: 6831-6840

Complex Structure
Linda Stannard, Dept of Medical Microbiology, U of Cape Town
Albrecht Dürer, “Rhinoceros” (1514)
Analytical Test Method
“TOOL KITS”

- STABILITY METHODS (S)
- BATCH RELEASE METHODS (R)
- CHARACTERIZATION COMPARABILITY METHODS (C)
What are the major analytical technologies used to assess physiochemical attributes of biotechnology products?
# Orthogonal Methods of Analysis

## Used with Biopharmaceutical Products

<table>
<thead>
<tr>
<th>METHOD</th>
<th>USE</th>
<th>ATTRIBUTES</th>
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<tbody>
<tr>
<td>pH (liquid)</td>
<td>R, S, C/C</td>
<td>Solution hydrogen ion, stability</td>
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<td>Karl Fisher (lyophilized)</td>
<td>R, S, C/C</td>
<td>Moisture, stability</td>
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<tr>
<td>Appearance (liquid, lyophilized)</td>
<td>R, S, C/C</td>
<td>Physical quality, stability</td>
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<td>Light Obscuration, MFI</td>
<td>R, S, C/C</td>
<td>Subvisible Particles, Leachates</td>
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<td>UV Absorbance</td>
<td>R, S, C/C</td>
<td>Concentration</td>
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<tr>
<td>SDS-PAGE (R/NR)</td>
<td>R, S, C/C</td>
<td>Identity, purity, stability (aggregation, hydrolysis)</td>
</tr>
<tr>
<td>SEC-HPLC/UPLC</td>
<td>R, S, C/C</td>
<td>Identity, purity, stability (aggregation, hydrolysis)</td>
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<tr>
<td>RP-HPLC, HIC-HPLC/UPLC</td>
<td>R, S, C/C</td>
<td>Identity, stability (deamidation, oxidation, disulfide scrambling)</td>
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<td>Peptide Mapping</td>
<td>R, S, C/C</td>
<td>Identity, stability (deamidation, oxidation, DS, hydrolysis)</td>
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<td>IEF, CIEF, iCE, IEX-HPLC/UPLC</td>
<td>R, S, C/C</td>
<td>Identity, stability (hydrolysis, desialylation)</td>
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<td>CE-SDS (R/NR)</td>
<td>R, S, C/C</td>
<td>Identity, stability (aggregation, hydrolysis)</td>
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<td>Immunoassay, ECL</td>
<td>R, C/C</td>
<td>Process Residuals (proteins)</td>
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<tr>
<td>qPCR</td>
<td>R, C/C</td>
<td>Process Residuals (nucleic acids)</td>
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<tr>
<td>Ligand Binding Assay</td>
<td>R, S, C/C</td>
<td>BiolIdentity, potency, stability</td>
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<tr>
<td>Cell-Based Bioassay</td>
<td>R, S, C/C</td>
<td>BiolIdentity, potency, stability</td>
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<td>N terminal Sequencing</td>
<td>C/C</td>
<td>Identity, heterogeneity</td>
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<tr>
<td>C-terminal Sequencing</td>
<td>C/C</td>
<td>Identity, heterogeneity</td>
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<td>Amino Acid Analysis</td>
<td>C/C</td>
<td>Identity, composition, concentration</td>
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<td>Monosaccharides (HPAE-PAD)</td>
<td>C/C</td>
<td>Identity, composition</td>
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<td>Oligosaccharide (HILIC/CLIF)</td>
<td>C/C</td>
<td>Identity, composition, stability (deglycosylation)</td>
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<tr>
<td>Sialic Acid (HPAE-PAD)</td>
<td>C/C</td>
<td>Identity, composition, stability (desialylation)</td>
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<td>Mass Spectrometry</td>
<td>C/C</td>
<td>Identity, purity, stability (oxidation, hydrolysis, glycation, DS)</td>
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<td>Circular Dichroism</td>
<td>C/C</td>
<td>Conformation</td>
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<td>FTIR</td>
<td>C/C</td>
<td>Impurities (Aggregates)</td>
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<td>AUC</td>
<td>C/C</td>
<td>Structure</td>
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<tr>
<td>NMR</td>
<td>C/C</td>
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</table>

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Current Analytical Benchmarks – Monoclonal Antibody Products

Analysis and Structure Characterization of Monoclonal Antibodies

BioProcess International (Feb 2004)

Mark A. Schenerman, Brooks R. Sunday, Steve Kozlowski, Keith Webber, Hélène Gazzano-Santoro, and Anthony Mire-Sluis
Current Analytical Benchmarks – Viral Vaccines and Gene Therapy Products

Lot Release and Characterization Testing of Live-Virus-Based Vaccines and Gene Therapy Products:

Part 1: Factors Influencing Assay Choices
BioProcess International (April 2006)

Part 2: Case Studies

Jim Gombold, Keith Peden, Denise Gavin, Ziping Wei, Khandan Baradaran, Anthony Mire-Sluis, and Mark Schenerman
What are the key parameters for release and stability testing of biotech products?
Key Product Parameters (ICH Q6B)

**Product-related substances:**
Molecular variants of the desired product formed during manufacture and/or storage which are active and have no deleterious effects on the safety and efficacy of the product.

Many (most) proteins have defined degree of heterogeneity that is expected; the process must generate consistent patterns of heterogeneity.

**Product-related impurities:**
Aggregates, truncated forms, other modified forms (deamidated, isomerized, oxidized, etc...).

Degradation products are considered product-related impurities; they change in nature and abundance over time on storage so could affect the safety and efficacy of the product.
Key Product Parameters (ICH Q6B)

**Process-related impurities:**

Host cell proteins, host cell nucleic acids, cell culture derived components (e.g. serum, antibiotics), downstream process additives (e.g. enzymes, reducing agents, ligands). Process impurities must be cleared by process steps to consistently low levels; specification limits are based on levels present in clinical trial batches.

**Process / product contaminants:**

Adventitious agents (viruses, prions), environmental microbes (bacteria, fungi), microbial fragments (endotoxins/pyrogens), equipment contact surfaces (glass, metal, fibers, solvents), container or closure leachates or flakes (glass, rubber, lubricants, elastomeric closure compounds).

Raw materials, equipment, and facilities must be kept as free of contaminants as possible at all points in production.
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Publication</th>
</tr>
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</table>
What are the specification considerations for acceptable amounts of heterogeneity in biotechnology/biosimilar products?
Expected (Allowed) Molecular Heterogeneity of Biopharmaceutical Products

“Define the pattern of heterogeneity of the desired product, and demonstrate consistency with that of the lots used in preclinical and clinical studies.” ICHQ6B

Each batch can have all of the heterogeneity you can measure, monitor, and confirm stays within its established ranges throughout shelf life.
Risks of Aberrant Molecular Heterogeneities

“When process changes and degradation products result in heterogeneity patterns that differ from those observed in the material used during preclinical and clinical development, the significance of these alterations should be evaluated.” ICHQ6B
Biotech Products Comparability Guiding Principles: Nature of the Process Change

- The more upstream of the manufacturing process the change is made, the greater the potential for impact of the change, and the greater the body of data required to demonstrate comparability.

- For each step in manufacturing that is changed, all claims made for that step (e.g., removal of a specific impurity) must be confirmed.

- *Biosimilar products present the ultimate process change:* An entirely new expression system, with limited ability for direct analytical comparability to the original process.
Comparability Requirements

- Demonstrate no clinically meaningful differences in safety, purity and potency between products
  - Analytical data
  - Animal testing
  - One or more clinical studies, unless the regulatory body deems this not necessary

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Comparability of Product-Related Substances

Process/Product A

Process/Product B
Comparability of Product-Related Impurities and Process-Related Substances

Differences In Minor Species?

Some species below LOD of analytical methods, BUT -

May not be below LOD of the human body

“Fingerprint” Methods
BioQuality

BioSimilars - all Grown-up with Grown-Up Problems
Interchangeability and Global References for Approval

The worst nightmare for all of us in the room is an undetected difference between the reference and the biosimilar which causes a clinical safety difference.

Emily Shacter, CDER, FDA
## Typical Comparability Analytical Test Plan for Glycoprotein

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Method</th>
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<td>Product-related substances/impurities</td>
<td>SDS-PAGE reduced and nonreduced, Coomassie stain;</td>
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<td>SDS-PAGE reduced and nonreduced Silver stain,</td>
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© 2015 N. Ritter, Ph.D.
Side-by-Side Chem-Phys Forced Degradation to Demonstrate Comparability

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**Illustration of Biotech Forced Degradation “Matrix” Study for Stability-Indicating Methods**

A = Appearance   B= pH   C = SDS-PAGE   D= SEC-HPLC  
E = RP-HPLC     F = IEF     G = Peptide Map     H = Potency

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**Agitation**


**Freeze-Thaw**


**Photodegradation**


**Hydrolysis**


**Oxidation**


**Deamidation**


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# Hypothetical Stability-Indicating Method Validation Results

A = Appearance    B = pH    C = SDS-PAGE    D = SEC-HPLC    E = RP-HPLC    F = IEF    G = Peptide Map    H = Potency

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Comparable PATTERNS of Force-Degraded Materials

Hydrolysis

Freeze-Thaw

Photolysis

© 2015 N. Ritter, Ph.D.
What are some of the current CMC ‘hot buttons’ for biotechnology analytical and stability studies that are of increasing regulatory concern for chronic deficiencies in product dossiers?
Current CMC ‘Hot Buttons’
Analytical and Stability

- Appropriate characterization and comparability of Host Cell Proteins (HCP); validation of specificity for the host cell proteome of HCP ELISAs
- Characterization and quantitation of subvisible particulates in protein solutions; semi-quantitative visual particle test
- Comprehensive, systematic experimental validation via FD of analytical methods that are claimed to be ‘stability-indicating’
- Establishment of the stability profile using appropriate orthogonal stability-indicating methods
- Sufficient assessment of container-closure interactions (extractables/leachates) under long-term, real-time conditions
- Development and validation of methods for immunogenicity screening for anti-HCP antibodies = CBER plasma products
Current CMC ‘Hot Buttons’

Analytical and Stability

- Tiered strategy for in-house reference standards; calibrating standards for potency assays; bridging and stability studies for reference standards

- Adequate, complete, traceable Method “Life Cycle” packages - CTD “method history” requirements; ICHQ10 “Product Knowledge”

- Quality practices in academic and R&D labs; control and documentation of the analytical methods used by non-GMP laboratories only for characterization and comparability

- Establishing allowable ranges of product characteristics for an analytical determination of biosimilarity; selection of regional reference licensed product; number of batches to include in analytical comparisons
What are some useful resources to help remain current on biotech/biosimilar CMC issues?