Develop a Roadmap to Analytical Method Validation Phase

Part 1: Method Validation Process

IVT Conference
September 2014

Amir Malek, Ph.D.
Analytical Development and Quality Control
Process for Method Validation

- Regulations and method validation.
- What are the phase appropriate activities for development and validation of analytical methods?
- What are the Method categories?
- Documentation for method validation at various clinical stages?
• Method Validation ICH Q2 (R1):

  • The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

  • Validation per ICH Q2 (R1) is required for commercial and Phase IV non-compendial methods. Commercial includes late Phase III clinical production with the intention of using the analytical methods for a registration of an NDA/BLA and/or future commercial sale. Phase IV is defined as the use of a previously commercially approved product in a clinical trial.

  • The level of validation (or qualification) required for each method is based upon the nature of the method, the intended use of the method, the phase of product development, and the stage of production (e.g., in-process vs. finished product).
Some Key Governing Documents

• **ICH:**
  – ICH Q2 (R1): Guideline on Validation of Analytical Procedures.
  – ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.

• **Pharmacopoeias:**
  – Japanese Pharmacopoeia (JP)
  – United States Pharmacopoeia (USP)
  – European Pharmacopoeia (EP)
Method Life Cycle

Trouble Shooting (In Unlikely event!) Investigation / Corrective Action Revisions / Change Control

- Method Development/Characterization
- Validation for Clinical Use
- Method Transfer for Clinical Testing
- Validation for Commercial Use (ICH)
- Method Transfer for Commercial Testing
- Routine Use (Monitoring)
- Maintenance of Validated Status
- Method Retire or Replacement

Commercial Activities

Clinical Activities
QS Elements Required for a QC Method

Prove Suitability for Intended Use

Validation Testing & System Suitability Determination

Draft Test Method

Reference Materials

Final Test Procedure for Testing

Trained & Qualified Personnel

Data Traceability, Integrity

Equipment Qualification

Software Qualification
**Method Validation Planning: Method Validation per ICH Q2 (R1)**

### Validation Characteristics Required for the Different Types of Methods

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Identification</th>
<th>Testing for Impurities</th>
<th>Assay (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Test Method:</td>
<td></td>
<td>Quantitative Test</td>
<td>Limit Test</td>
</tr>
<tr>
<td>Specificity (^b)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Linearity</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Range</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Accuracy</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Precision:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>−</td>
<td>+(^c)</td>
<td>−</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>−</td>
<td>−(^d)</td>
<td>+</td>
</tr>
<tr>
<td>Quantitation Limit</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

**Note:** 
+ = normally required; − = not required.

\(^a\) Including dissolution, content, potency.

\(^b\) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).

\(^c\) In cases where reproducibility has been performed, intermediate precision is not needed.

\(^d\) May be needed in some cases.

---

**The level of validation required for each method is based:**

- Upon the nature of the method (HPLC/CE vs BioAssay)
- The intended use of the method (Qual vs Quant.)
- The phase of product development (e.g., clinical vs. commercial)
- The stage of production (e.g., in-process vs. finished product)
• **Front-end loaded validation**
  - Qualification in early phases followed by validation before Process validation
  - Most commonly practiced by traditional pharmaceutical companies.
  - Less resource use at the beginning / more at the end.

• **Phase appropriate validation (Iterative)**
  - Phase appropriate validation
  - Clinical validation vs. commercial validation
  - Less resource at the beginning but increase as move toward BLA.

• **Back-end loaded validation**
  - Brief qualification followed by validation in all early clinical phase
  - Methods not requiring full validation (i.e. characterization)
  - More resource use early on.

Phase Appropriate Approach for Method Validation

Development Lab

Tox Assessment

Phase I Method Validation
> Preliminary Validation
  *Est. Ref. Material*

Phase II Method Validation
  *Est. Ref. Material (if r’qd)*
  *Formulation Change*

Phase III Method Validation
  *Est. Ref. Material*
  *Process Change*
  *Initial Method Lock*

BLA Method Validation
> Validation based upon ICH Q2 (R1)
  *Final Method Lock*
<table>
<thead>
<tr>
<th>Activity</th>
<th>Clinical</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Evaluation</td>
<td>Qualification</td>
<td>ICH Validation</td>
</tr>
<tr>
<td></td>
<td>Clinical Validation</td>
<td></td>
</tr>
<tr>
<td>Method Type</td>
<td>Generic</td>
<td>Product Specific</td>
</tr>
<tr>
<td>Training</td>
<td>Technology Specific</td>
<td>Method Specific</td>
</tr>
<tr>
<td>Document approval</td>
<td>Single approval</td>
<td>Multi-Departmental Approval</td>
</tr>
<tr>
<td>Method Transfer</td>
<td>Informal (local)</td>
<td>Formal Co-validation, Transfer Protocol</td>
</tr>
<tr>
<td>Component</td>
<td>Drug Substance/ Drug Product</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Quality</td>
<td>Appearance: COC</td>
<td></td>
</tr>
<tr>
<td>Identity</td>
<td>Physiochemical: CZE, MALDI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunological: Potency</td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td>Aggregates: SEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Size Based: CE-SDS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Charge Based: IEC/ iCiEF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endotoxin: LAL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other Purity Tests</td>
<td></td>
</tr>
<tr>
<td>Strength</td>
<td>UV Absorbance</td>
<td></td>
</tr>
<tr>
<td>Composition</td>
<td>pH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Osmolality</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polysorbate 20</td>
<td></td>
</tr>
<tr>
<td>Potency</td>
<td>Potency</td>
<td></td>
</tr>
<tr>
<td>Safety</td>
<td>Sterility, Particulates</td>
<td></td>
</tr>
</tbody>
</table>
**Biologics QC Method Categories**

**Compendial:**
- Based on compendial reference (USP, EP)
- Validation not required. Suitability to be demonstrated
  - CAC, Osmolality, LAL, VIC, COC, pH, Particulates, Sterility

**Generic/Multiproduct:**
- Limited Validation is required for each product usage
  - CZE, UV-VIS Spec Scan, Polysorbate determination, Optical Rotation, Protein A titer, PCR, Nonhost contamination, Bacteriophage, Protein A ELISA, CHOP ELISA, ECP ELISA, Container closure, FTIR, Bioburden, Virus detection and mycoplasma detection

**Product Specific:**
- Validation is required
  - IEX, Peptide Map, SEC, CE-SDS, LC-MS, Potency
## Comparison of Platform methods and Product Specific

<table>
<thead>
<tr>
<th>Activity</th>
<th>Specific</th>
<th>Generic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Development</td>
<td>Extensive</td>
<td>Minimal (Suitability)</td>
</tr>
<tr>
<td>Method Validation</td>
<td>Needed for each product</td>
<td>Reduced validation or assessment</td>
</tr>
<tr>
<td>Documentation</td>
<td>Product specific protocols</td>
<td>Generic protocols</td>
</tr>
<tr>
<td>Transfer</td>
<td>Product specific training</td>
<td>Not needed or reduced training</td>
</tr>
<tr>
<td>Testing and Release</td>
<td>Various reagent, Column equipments</td>
<td>Uniform materials and equipments</td>
</tr>
</tbody>
</table>
Streamlining Strategies for Method Validation

• **Optimization of current methodology**
  - Creation of Platform Methods i.e. CE-SDS, SEC

• **Incorporation of Innovative and efficient technology**
  - iCIEF, MALDI-PMF, pH Gradient IEC

• **Phase appropriate validation and leveraging the past information**
  - Risk based approach and utilizing pre-established method acceptance criteria
Streamlining Our Processes: Evolution of Platform Methodologies

Category 1
Optimization of Method

- Product Specific SEC
- Platform Specific SEC
- SDS Page
- CE-SDS
- Optimized CE-SDS

Category 2
Innovation

- CZE/Peptide Map Immunologic
- Combination ID
- MALDI-PMF

Category 3
Innovation/Method Optimization

- IEC
- CIEF
- CIEF or pH Gradient

Multi-Product Methods
Comparison of IEC (Product specific) and iCIEF (Generic) Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Analysis Time (min.)</th>
<th>Acidic Region</th>
<th>Main Peak</th>
<th>Basic Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEC</td>
<td>60</td>
<td>26.4</td>
<td>65.0</td>
<td>8.7</td>
</tr>
<tr>
<td>ICIEF</td>
<td>10</td>
<td>27.0</td>
<td>67.7</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Run time for iCIEF is shorter than traditional IEC.

While iCIEF produces comparable results to that seen for IEC, iCIEF is less precise than IEC.
## Comparison of IEC vs. ICIEF

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IEC</th>
<th>iCIEF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method Development</strong></td>
<td>Product specific development</td>
<td>Generic conditions</td>
</tr>
<tr>
<td><strong>Development Time</strong></td>
<td>1 – 4 month</td>
<td>2 – 3 weeks</td>
</tr>
<tr>
<td><strong>Validation Time</strong></td>
<td>8 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td></td>
<td>Extensive Robustness</td>
<td>Minimal robustness</td>
</tr>
<tr>
<td><strong>Reagent Consumption</strong></td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Training and Transfer</strong></td>
<td>Training required, HPLCs are commonly used and available</td>
<td>Training required (Operator familiarity with technique needed)</td>
</tr>
<tr>
<td><strong>Testing and Maintenance</strong></td>
<td>Long run time and routine maintenance issue</td>
<td>Short run time and minimal maintenance issues</td>
</tr>
</tbody>
</table>
Peptide Mass Fingerprinting (PMF)

Technique for identification of proteins by a **probability based matching** of observed **set** of peptide masses with theoretical ones generated by in-silico digest of protein database **entries**
Purpose:
- To develop a rapid, accurate, reliable method for unique identification of closely related therapeutic antibodies by Peptide Mass Fingerprinting (PMF)

Methodology:
- We developed a method that is:
  - Simple: Tryptic digestion without cysteine alkylation
  - MALDI spotting without the need of sample desalting
  - Rapid: Data acquisition and peak list generation
  - Quick Mascot database search
  - Specific: High quality MALDI-TOF data combined with MASCOT search
  - Consistent positive identity with high score
  - Universal: Applicable to multiple products including antibody and non-antibody proteins
  - Robust: Reproducible
Leveraging Past Information: Method Acceptance Criteria for Platform Methods

**Input**

- Establish GMP Transfer
- Establish NME Assessment Criteria
- Establish Method Acceptance Criteria
- Establish Platform Methods

**Activity Type**

- GMP
- R&D

**Output**

- GMP: TP figure, Spec target (if applicable)
- R&D: Gating criteria to Show Platform Method is Suitable for New Molecule
- GMP: Uniform and validated Method Acceptance criteria With Generic Standard
- R&D: Uniform Method Parameters e.g. SEC, CE-SDS, ICIEF
Validation Tool Box

- **Equipments**
  - Qualified equipment
  - Calibrated devices

- **Protocols/ Procedures**
  - Method parameters with identified method ranges
  - Fully signed Protocol

- **Personnel**
  - Documented Technique or method based training

- **Samples**
  - Reference Materials or representative materials.
  - Stress samples

- **Documentation Systems**
  - Lab note book, electronic notebook, Binder, etc.
CLINICAL/COMMERCIAL METHOD VALIDATION REPORT

Procedure: Size-Exclusion Chromatography of Clinical Products

Procedure No.: XXXX

Document No.: MVR-XXXX

Written by: John Scientist, Associate Scientist
Analytical Development

Technical Review by: Sarah Supervisor, Scientist
Analytical Development

Compliance Review by: Amy Quality, Compliance Specialist
Quality

Approved by: David Director, Director
Analytical Development
Documentation: Review Process for Method Validation Reports

- Data analysis and Notebook Completion
  - Analyst

- Technical Review of the Notebook
  - Peer Analyst/SME

- Draft Validation Report
  - Analyst

- Technical Review of Report
  - Supervisor

- Compliance Review of Report
  - Quality

- Management Approval of Report
  - Functional Head(s) Including QA

- Report Approved
9.3.5 Acceptable System Suitability Range for Reference Lot XXX

<table>
<thead>
<tr>
<th>Component ID</th>
<th>Monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acceptable range of % peak area</td>
</tr>
</tbody>
</table>

NOTE: The range was based on the mean ± 3SD. The mean and RSD were obtained from validation studies (n = XX).

7.0 SYSTEM SUITABILITY CRITERIA

NOTE: Perform on all bracketing material injections. Integrate all peaks attributed to protein. Do not include any peaks which are present in the reagent blank chromatogram.

7.1 Refer to product-specific instructions for example calculations and acceptance criteria.
Develop a Roadmap to Analytical Method Validation

Phase

Part 2: Systematic Method Validation

IVT Conference
September 2014

Amir Malek, Ph.D.
Analytical Development and Quality Control
Systematic Method Validation

• Selection of the appropriate method based on a QBD approach.

• How to decide on the extent of the validation and parameters for your experiments?

• Devising the proper method control strategy for your testing.

• Getting ready to use your method for testing.
How to select the appropriate method e.g. purity?

• Selection of appropriate method for an Antibody or any other biologics starts with understanding of the structure and the process.

• Next step is to Define the characteristics of the Analytical Method Desired to measure the product or process attributes (ATP).

• Example Analytical Target Profile:

  The Method must be able to accurately quantitative an in process impurity from 80-120% on the intended concentration with accuracy and precision such that measurements fall within $\pm 3\%$ of the true value, with 95% probability.
Systematic Approach for Development and Validation of Analytical Methods

Input
- Molecule Characteristics
- Process Needs
- CQAs
- Risk Assessment

Output
- Method Attributes
- Method Parameters
- Method Ranges
- Confirmation of Method Operational ranges

1. Define ATP and Identification of Method
2. Method Selection and Development
3. DOE and Identification of Method Parameters (effect screening)
4. Validation Design and Execution of Experiments
5. Method Performance and Failure Probability
6. Define Method Control Strategy
7. Method Monitoring
Biologics are complex with multiple sites for possible modification.

- **N-Terminal**
  - Glu→PyroGlu
  - VHS

- **Inter Disulfate Bridges**
  - Glutathionylation
  - Cysteinlyation

- **Anywhere**
  - Deamidation
  - Glycation
  - succinimide from Asp

- **C-Terminal**
  - Lys Variants
  - Amidation

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- **C-Terminal**
  - Lys Variants
  - Amidation

- **Acidic - Red**
- **Basic - Blue**
- **Neutral or uncertain - Green**
Each step has specific analytical technology requirements.

In Process control testing is an integral part of the control system, combined with CoA and Process validation.
Upstream

Cell Culture

Harvest

In Process CCF
Titer (g/L)
Prod. Quality

Harvested CC
Titer (g/L)
Prod. Quality
Downstream

**Affinity Chrom**

**Chrom #1**

**Viral Filtration**

**Chrom #2**

**Pool**
- Impurity
- Prod. Conc.

**Pool**
- Prod. Conc.
- Prod. Quality

**Pool**
- Prod. Conc.
- Prod. Quality

**Pool**
- Prod. Conc.
- Prod. Quality

*Technology is used in Process Development only*
Concentration and Formulation

UFDF → Filtration of DS → Freezing

Pool
Prod. Conc.
CHOP*

Pool
Prod. Conc.
Peptide Map
HPIEC
CE-SDS
Molecular Size

Pool
Prod. Conc.
Critical Quality Attributes (CQAs): Physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality (Annex to ICH Q8 R1)

- Desired product quality = patient safety and product efficacy
- Not linked to process capability

Example attributes:
- CQA: aggregates (immunogenic)
- Non-CQA: Mabs with C-terminal lysine processing (found on circulating antibodies, no impact on potency)
1. Identify CQAs
2. **Set acceptance criteria**
3. Perform process characterization studies
4. Identify CPPs and acceptable ranges
5. Define Design Space (combination of CPPs)
6. Establish Control Strategy

Based on in vitro, nonclinical and clinical information

- **Design Space**
  - Control Space
  - Optimal process performance: **6.9 - 7.1**
  - Delivers product quality (e.g., acidic variants within acceptance criterion)
  - Cell culture pH: **6.6 - 7.4**

Adapted from BioPharm International, Apr 1, 2007
Anurag S. Rathore, Ron Branning, and Doug Cecchini
CQA identification risk ranking and filtering tool

- Based on Impact and Uncertainty
- Standardizes judgment and documents rationale
- Process capabilities or historical ranges not considered

CQAs during development

- Identify attributes for further study
- Guide process characterization/validation
- Establish control system

CQAs ≠ Assays on specification

- Some controlled through process capability
- Multiple CQAs monitored by single assay
Method Development Strategy: Ion Exchange Chromatography

What are typical Parameters to evaluate during Method Development?

- **Column**: Column selection and size e.g. Dionex (4×250 mm), column temperature evaluation e.g. 35 °C to 55 °C (avoid ambient)
- **Mobile Phase**: Buffer selection based on molecule characteristics, buffer counter ion
- **Gradient**: Hold time, Gradient Curve
- **Instrument**: Dionex, Agilent and Waters, etc. with ≥6 independent runs
- **Sample**: Sample stability at 2-8 °C ≥48 hrs, Develop IEC for both native and CpB-treated samples
- **Performance**: Precision (n=6), column temp ± 4 °C target, pH ± 0.1 of target, different column resin lot, ± 20% of target CpB at ± 4 °C of digest temp, Stability indication
- **Stability Indication**: Ability to resolve sample degradants
Resolution as a function of pH and gradient at constant temperature

Factorial Design (screening)
Central Composite Design (optimization)

DOE experiments are completed to establish ranges for acceptable method performance

Space Filling Design (robustness)

Knowledge Space
Average Performance Acceptable
Design Space
Example for Establishing Acceptable Method Ranges

- Narrower range
- Acceptable variability
- Specified in TP as operational range

Practical Range
- Target Value

Developmental Range
- Wider range
- Larger variability
- Used to determine Practical Range During method development

Column temp is Set in Test Procedure
38 °C to 42 °C

Column temp is Evaluated 35 °C to 45 °C
Case Study for Validation: Capillary Electrophoresis - Sodium Dodecylsulfate Nongel Sieving (CE-SDS)

- Capillary filled with hydrophilic sieving polymer solution
- Replaceable polymer improves reproducibility and prevents carryover

- Negatively charged SDS-protein complexes are introduced into the capillary by electrokinetic injection

- Separation based on hydrodynamic size
- Proteins migrate across the detector in order of increasing size
• **CE conditions**: Utilize generic procedure and optimize voltage, temp. and time if needed

• **Protein Derivatization**: Determine protein recovery and optimize protein/Dye ration and excess dye removal on solid extraction column

• **SDS-Protein Complexation**: Optimize generic conditions for SDS, Alkylating and reducing agent.

• **Sample**: Sample stability at 20 °C and for 0 and 24 hrs

• **Performance**: Precision (n=6) for each reduced and non-reduced, Targets RSDs: Main peak/LC/HC ≤ 2%, NGHC ≤ 5%, Minor peaks (1%) ≤ 20%, Stability indication

• **Stability Indication**: Ability to resolve sample degradants
Method Validation Planning: Method Validation per ICH Q2 (R1)

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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Range</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Accuracy</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Precision:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>-</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>-</td>
<td>-&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>+</td>
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  - The intended use of the method (Qual vs Quant.)
  - The phase of product development (e.g., clinical vs. commercial)
  - The stage of production (e.g., in-process vs. finished product)
Validation Experiments: Specificity

Acceptance Criteria: No interference from buffer, blank or other components
Validation Experiments: Precision (Repeatability and Intermediate Precision)

### Sample Prep Repeatability

<table>
<thead>
<tr>
<th>Sample</th>
<th>Migration Time (min)</th>
<th>Corrected Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nonreduced</td>
<td>reduced</td>
</tr>
<tr>
<td></td>
<td>main peak</td>
<td>light chain</td>
</tr>
<tr>
<td>Sample 1</td>
<td>10.9</td>
<td>6.41</td>
</tr>
<tr>
<td>Sample 2</td>
<td>10.9</td>
<td>6.42</td>
</tr>
<tr>
<td>Sample 3</td>
<td>11.0</td>
<td>6.63</td>
</tr>
<tr>
<td>Sample 4</td>
<td>11.0</td>
<td>6.61</td>
</tr>
<tr>
<td>Sample 5</td>
<td>11.0</td>
<td>6.58</td>
</tr>
<tr>
<td>Sample 6</td>
<td>11.1</td>
<td>6.58</td>
</tr>
<tr>
<td>Mean</td>
<td>11.0</td>
<td>6.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.5</td>
<td>1.5</td>
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</table>

### Sample Injection Repeatability

<table>
<thead>
<tr>
<th>Sample</th>
<th>Migration Time (min)</th>
<th>Corrected Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nonreduced</td>
<td>reduced</td>
</tr>
<tr>
<td></td>
<td>main peak</td>
<td>light chain</td>
</tr>
<tr>
<td>Sample 1</td>
<td>10.8</td>
<td>6.24</td>
</tr>
<tr>
<td>Sample 1-2</td>
<td>10.9</td>
<td>6.25</td>
</tr>
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<td>Sample 1-3</td>
<td>10.9</td>
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<td>Sample 1-5</td>
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<td>6.28</td>
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</tr>
<tr>
<td>Mean</td>
<td>10.9</td>
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<tr>
<td>SD</td>
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<td>0.0</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.3</td>
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### Intermediate Precision

<table>
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<tr>
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<th>Migration Time (min)</th>
<th>Corrected Peak Area (%)</th>
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<td></td>
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<td>reduced</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>light chain</td>
</tr>
<tr>
<td>Analyst 1</td>
<td>Day 1</td>
<td>11.8</td>
<td>6.7</td>
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<td>Analyst 2</td>
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<td>Analyst 1</td>
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<tr>
<td>SD</td>
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<tr>
<td>RSD (%)</td>
<td>6.2</td>
<td>5.4</td>
<td>5.7</td>
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### Acceptance Criteria: RSD ≤ 10.0 %
### Validation Experiments: Accuracy (CE-SDS)

#### Non-Reduced CE-SDS

<table>
<thead>
<tr>
<th>Percent Recovery</th>
<th>Total CPA</th>
<th>Theoretical CPA</th>
<th>Experimental CPA</th>
<th>Percent Recovery</th>
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<td></td>
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<tr>
<td></td>
<td>1640.5</td>
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</tr>
<tr>
<td></td>
<td>2432.1</td>
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</tr>
<tr>
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<td>2449.2</td>
<td>2570.9</td>
<td>2456.4</td>
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<td>2487.9</td>
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</tr>
<tr>
<td></td>
<td>3471.7</td>
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</tr>
<tr>
<td>100%</td>
<td>3313.2</td>
<td>3427.8</td>
<td>3427.8</td>
<td>-</td>
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<tr>
<td></td>
<td>3498.6</td>
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</tr>
<tr>
<td></td>
<td>3916.5</td>
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<td></td>
</tr>
<tr>
<td>125%</td>
<td>3790.6</td>
<td>4284.8</td>
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<td></td>
<td>6021.4</td>
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<tr>
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Mean: 98.4

#### Reduced CE-SDS

<table>
<thead>
<tr>
<th>Percent Recovery</th>
<th>Total CPA</th>
<th>Theoretical CPA</th>
<th>Experimental CPA</th>
<th>Percent Recovery</th>
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<tbody>
<tr>
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<td></td>
<td>1968.0</td>
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<tr>
<td></td>
<td>2928.4</td>
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<tr>
<td>75%</td>
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<td>2989.3</td>
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</tr>
<tr>
<td></td>
<td>2937.2</td>
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</tr>
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<td></td>
<td>4020.0</td>
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<tr>
<td>100%</td>
<td>4032.1</td>
<td>3985.8</td>
<td>3985.8</td>
<td>-</td>
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<tr>
<td></td>
<td>3905.2</td>
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</tr>
<tr>
<td></td>
<td>4953.5</td>
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</tr>
<tr>
<td>125%</td>
<td>5093.3</td>
<td>4982.2</td>
<td>4887.7</td>
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<td></td>
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<tr>
<td></td>
<td>5697.5</td>
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<tr>
<td>150%</td>
<td>5550.9</td>
<td>5978.7</td>
<td>5697.8</td>
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</tr>
<tr>
<td></td>
<td>5845.2</td>
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<td></td>
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</tbody>
</table>

Mean: 97.3

RSD%: 1.4

#### Acceptance Criteria: Recovery 80-120%
Validation Experiments: Linearity CE-SDS Reduced and non-Reduced

CE-SDS Non-Reduced

\[ y = 558220x - 11056 \]
\[ R^2 = 0.997 \]
\[ r = 0.9990 \]

CE-SDS Reduced

\[ y = 381979x - 1689.8 \]
\[ R^2 = 0.9977 \]
\[ r = 0.9990 \]

Acceptance Criteria: correlation coefficient, \( r \geq 0.99 \)
Validation Experiments: LOD CE-SDS for Process Impurities

Spike of selected protein impurities covering a large MW range

Data analysis criteria: Consistent with Reference e.g. No new peak detected (new peak defined as >1% of total peak area)
The method must be sufficiently accurate and precise as to provide a high degree of assurance that product with acceptable characteristics will meet the (tentative) release specifications.

To do this we need to have:

- Data obtained from method validation studies (Intermediate precision SD) need to be used to evaluate the method performance.
- Statistical means for evaluating the method variability relative to the release specifications include calculating a process capability index (Cp, Cpk)
- Proper method acceptance criteria e.g. system suitability need to be established for achieving proper method performance during testing.
Method Performance calculation allows the Analytical Scientist to evaluate performance of the method in relation to specification and process variability.

Calculation need to be performed before final lock of the method to determine achieving the desired analytical Target Profile.
Method control strategy: System suitability

- **System Suitability (ICH):**
  - **ICH:** System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure **depend on the type of procedure being validated.**

  - **USP:** ”System Suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are **adequate for the analysis to be done**…….”

- **Examples of S.S:**
  - Calibration Standards, Reference Materials, Equipment parameter based, System parameters i.e. plate count, resolution, etc.

**What we want to know:** Is the method performing as expected?
Pertinent Documents for SST

- JP 2.01, Liquid Chromatography
- EP 2.2.46. Chromatography
- EP 2.2.47. Electrophoresis
- USP <621> Chromatography
- USP <1053> Electrophoresis
- ICH Q2(R1), Validation of Analytical Procedure
Foundations for Setting of System Suitability

System Suitability (SS)
Acceptance criteria

Method Validation
ICH Q2(R1)

Equipment Qualification
DQ/IQ/OQ/PQ

Consistent Method Performance (Valid Data)

Establish Method Performance (Define Performance)

Proper Equipment Performance
Other Definitions

• **System Suitability Criteria:** System Suitability criteria are established based on method properties where one or more property can be used to assure the performance of the test method at the time of analysis e.g. Percent area of main peak and HMWS in a size method. Selection of the method and thus criteria are dependent on the product quality attributes which are being reported.

• **System Suitability Controls:** Controls are selected based on the attributes of the product being analyzed during sample testing. Characteristics of the control must be defined. As an example, for a purity method for drug product containing critical species for which resolution is to be determined, a control sample must have similar species to be resolved to ensure the test system is capable of accurately detecting the desired peaks.
Desired approach to System Suitability (Phase Appropriate)

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal Robustness Studies</td>
<td></td>
<td>Method Change SS re-evaluation</td>
<td>Full Robustness Studies</td>
<td>Commercial Method Performance Criteria</td>
</tr>
<tr>
<td>Range (e.g. 5SD)</td>
<td>Experience Period</td>
<td>Range (e.g. 3SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI = X ± K SD</td>
<td></td>
<td>TI = X ± K SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Minimal Data
- Wider SS
- Generic SS
- Extensive Data
- Tighter SS
- Additional SS Criteria
• Method parameter lock is achieved
• Material for SST (e.g. Reference Standard, Assay Control) needs to be established and stable.
• Pertinent data is used for calculation of the criteria
• Minimum data points that capture the anticipated variation to be defined.
  • Less data points for early phases and more for later phases
Licensure Activities: SST Life-cycle

- Identification of Method Parameters
- Define Method Ranges (DOE)
- Initial Method Lock
- Phase III Validation
- Calculate initial Cp

Phase III Campaign
- RS COA
- Preliminary SST for BLA Validation

Phase III Ref. Std. Characterization
- BLA Validation
- Confirm Performance (Cp)
- Establish Final SST for Commercial

Licensure Campaign
- Annual Method Monitoring
- Periodic Method Review

BLA/MAA
- Final SST used for testing

Product Retirement
- Ref Std/Control
- Replenishment
- Re-establish SST

Development Activities

Commercial Activities
<table>
<thead>
<tr>
<th>Definition</th>
<th>Example</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative quantitative methods</strong></td>
<td>• Purity by SEC</td>
<td>• Percent Corrected Peak Area</td>
</tr>
<tr>
<td>Methods that determine a sample result based on the comparison of the peak areas within a single injection.</td>
<td>• Purity by SEC, IEC, RP</td>
<td>• Area Percent</td>
</tr>
<tr>
<td><strong>Absolute quantitative methods</strong></td>
<td>• Content of Polysorbate (PS20)</td>
<td>• Concentration mg/mL</td>
</tr>
<tr>
<td>Methods that calculate the sample concentration based on independent sample and standard injections.</td>
<td>• Content of Protein by RP</td>
<td></td>
</tr>
<tr>
<td><strong>Qualitative / Semi-Quantitative methods</strong></td>
<td>• Identity by Peptide Map</td>
<td>• Positive identity</td>
</tr>
<tr>
<td>Methods which compares the sample profile to that of a representative master profile via visual or a ratio assessment.</td>
<td>• Purity by CE-SDS</td>
<td>• Consistent to the master profile</td>
</tr>
<tr>
<td><strong>Direct Measurement Methods</strong></td>
<td>• Protein content by UV</td>
<td>• Concentration mg/mL</td>
</tr>
<tr>
<td>Methods that calculate a sample result based solely on measurement of a sample.</td>
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<td></td>
</tr>
<tr>
<td>Relative quantitative methods</td>
<td>Precision</td>
<td>Accuracy</td>
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<tr>
<td>-------------------------------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>• Bracketing controls</td>
<td>• %-area peak range for bracketing controls</td>
<td>• %-area peak range for bracketing controls</td>
</tr>
<tr>
<td>• %-area peak range for bracketing controls</td>
<td>• Define number of samples</td>
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<table>
<thead>
<tr>
<th>Absolute quantitative methods</th>
<th>Precision</th>
<th>Accuracy</th>
<th>Linearity</th>
<th>Resolution</th>
<th>S/N (LOQ)</th>
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<tbody>
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<td>• Precision criteria for bracketing controls</td>
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<td>• Precision criteria for replicate sample injections</td>
<td>• Define number of samples</td>
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<td>Precision</td>
<td>Accuracy</td>
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Summary SST Criteria
Examples of SST for biologics Testing IEC: Relative Quantitation Method

- % Main, Acidic and Basic peaks of bracketing RS are within ± x target value percentage.

- Resolution criteria based on product specific or external standard.

- Bracketing RS to be visually comparable to the example as shown in the test procedure.
Additional QC activities

Method transfer

- Validated QC methods are transferred to other laboratories for use using pre-approved Transfer Protocols. Additional validation is not required as part of method transfer unless relevant technical differences, such as method instrumentation that may affect method performance, are noted by the recipient laboratory.

Method validation status maintenance

- Methods for commercial products should be assessed periodically to ensure that the methods are operating according to their original validation characteristics. Maintenance activities include monitoring of method performance and assessment of change control, corrective actions, and discrepancies.
Considerations for Clinical Testing

First: Test Method need to be suitable for the intended purpose

- **Method robustness / ruggedness**
  - Different column lots
  - Different Instrument
  - Different Labs

- **Method efficiency**
  - Analysis time
  - Operator time and sample preparation

- **Vendor Support**
  - Instrument Setup and maintenance

- **Training**
  - Analysts familiarity
  - Equipment for method need in place
Commercial Activities for SST

- **Maintenance**
  - Re-assessment and potentially re-establishing system suitability criteria if significant change to the method.
  - SST data must be assessed at a minimum annually (trending, etc.)

- **Re-establishing of SST criteria**
  - Re-evaluate SST if a new lot of control is established. e.g. Ref. Mat.
  - Activity for re-establishment of new SST Criteria must be documented. e.g. validation report
Develop a Roadmap to Analytical Method Validation Phase


IVT Conference
September 2014

Amir Malek, Ph.D.
Analytical Development and Quality Control
### Example of System Suitability Data

<table>
<thead>
<tr>
<th></th>
<th>Acidic</th>
<th>Basic</th>
<th>Main</th>
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<td>27.5837</td>
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### System Suitability Criteria

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<td>Mean+3SD</td>
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<td>Mean-5SD</td>
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<tr>
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</table>
Example Questions 1

• Based on ATP a product acceptance criteria of 65% - 75% is needed for the main peak in an IEC method.
• What is the Cp value?
• What is acceptable SST range for this method?
• Based on ATP a product acceptance criteria of 6.6% - 6.8% is needed for the basic peak in an IEC Method.
• What is the Cp value?
• What is acceptable SST range for this method?
Appendix

Amir Malek, Ph.D.
Analytical Development and Quality Control
• **Type of reports**
  
  • Tabular (Early clinical)
  
  • Detail (Late stage/BLA)
Validation Report Examples: Short Report (Tabular Format)

- **Title:** Protocol number, Procedure title and number, Product Name

- **Summary:**
  - Parameters evaluated (Accuracy, Intermediate Precision, LOQ, etc.)
  - Acceptance Criteria
  - Result e.g. Pass, fail, numerical values

- **System Suitability:**
  - Results from intermediate precision
  - Ranges based on 3-5 SD

- **Robustness:**
  - Parameters evaluated e.g. Column temp, sample stability
  - Results: Pass or fail

- **Stress Sample:**
  - Parameters evaluated e.g. Heat, oxidation
  - Results: Is method stability indicating

- **Pertinent Records:** Deviation, Sample lot used, change control, etc.

- **Figures:** Representative figures (full and expanded) and captions
Validation Report Examples: Long Report (Text and Table Format)

- **Title**: Protocol number, Procedure title and number, Product Name

- **Summary**:
  - Brief summary of the method validation and intended purpose
  - **Summary Table**: Parameters evaluated (Accuracy, Intermediate Precision, LOQ, etc., Acceptance Criteria, Result e.g. Pass, fail, numerical values

- **Introduction**:
  - Background on the method and development summary.

- **Materials and Methods**:
  - List of methods and materials used for validation.

- **Results and Discussions**:
  - Detail discussion of results and findings along with reference to pertinent figures and tables.

- **Deviations and change control**: List of deviations and change control based on the validation.

- **Conclusions**: Concluding remarks on method acceptance and highlight any items of importance from validation.

- **Tables and Figures**: Tables used for calculation of validation parameters and pertinent figures