The Complex Relationship between Evolving Analytical Technologies and the Appropriate Implementation Throughout the Product Lifecycle

Dwight Moore
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Overview

• Introduction

• Implementation of Product Lifecycle Management and Analytical Lifecycle

• Case study #1 post approval changes
  ➢ Implementation of LDS-CGE to replace SDS-PAGE
  ➢ Revision of IE-HPLC and SE-HPLC acceptance criteria

• Case study #2 post approval changes
  ➢ Implementation of LDS-CGE to replace SDS-PAGE
  ➢ Introduction of a new IE-HPLC method to replace legacy method using a discontinued column

• Case study #3 pre-approval changes
  ➢ 8 method changes via implementation of new technologies and/or method optimizations throughout the clinical lifecycle ahead of Phase 3

• Conclusion
– Historically R&D develops, optimizes and qualifies methods through preclinical and clinical development.

– By Phase 3 all release and stability methods should be risk assessed, validated and transferred to commercial manufacturing (Global Manufacturing and Supply or GMS at GSK).

– Previously, once the methods were validated and transferred GMS owned the methods with limited involvement from R&D.

  ➢ The same methods and acceptance criteria would be appropriate throughout the life of a commercial product.

– Job Done…..
Evolving to Incorporate the Concepts of Product Lifecycle Management

- A more fully integrated end to end approach for managing a product from discovery, through development, approval, production, end of product life.

- There is an obligation for R&D and GMS to work together in order to support the product through its lifecycle.

- PLM is especially important given an often complicated supply chain.
  - Use of CMO’s
  - Different sites for production of DS and/or DP
  - Different expectations for different markets

- Analytical lifecycle sits within the PLM.
  - The need to reassess acceptance criteria
  - Assess platform, new and emerging technologies
Transition to New Methods and Acceptance Criteria as Part of the Analytical Lifecycle

• GSK’s strategy for analytical method transitions specifies that any modification or replacement of an existing validated analytical method requires bridging studies to demonstrate the following:
  ➢ Performance parameters of the new assay should be the same or better than the existing assay.
  ➢ The stability indicating properties of the new assay should be the same or better than the existing assay.
  ➢ When differences are observed with a new method (e.g. improved peak resolution) those differences are characterized and impact on specifications assessed.
  ➢ Changes in analytical methods must consider the impact on the assurance of product safety and efficacy over the drug substance and drug product shelf-life.

• Standard practice for method replacement includes:
  ➢ CGE for SDS-PAGE
  ➢ cIEF or IE-HPLC for IEF

• Need to re-evaluate the specifications for existing methods as a manufacturer gains more experience with commercial manufacture
• Background
  – Commercial product approved in 2010.
  – Over the past 6 years 217 Bulk Drug Substance (BDS) batches and 63 Drug Product (DP) batches have been manufactured.
  – In 2016 a new manufacturing facility was being registered.
  – Used this opportunity to re-assess the robustness of our testing strategy.

  – Outcome of assessment
    ➢ Implement LDS-CGE as a replacement for the less sensitive and selective SDS-PAGE
    ➢ Revise SE-HPLC acceptance criteria to align with the current manufacturing and analytical capabilities
    ➢ Revise IE-HPLC acceptance criteria to align with the current manufacturing and analytical capabilities
Case Study #1
Implementation of CGE

• Why?
  - Limitations of the SDS-PAGE method: semi quantitative nature of the technique and lack of sensitivity and selectivity
  - Current approach to replacing legacy technologies

• How?
  - Bridging studies comparing purity data generated using LDS-CGE and SDS-PAGE
  - Validation of the method
  - Generation of specification acceptance criteria based on statistical analysis of all available data
Case Study #1
Implementation of CGE – Bridging studies

• 75 BDS batches and 42 DP batches were tested by both SDS-PAGE and LDS-CGE.

• On average a 1.5% difference in purity is observed between the two methods.

• This difference is attributed to the capability of the LDS-CGE method to resolve the non-glycosylated heavy chain variant and other fragments not resolved or detected by SDS-PAGE.

• Characterization – no new species

What impact does this have on product stability and EOSL?
Case Study #1
Implementation of CGE – Bridging study

- Samples from various stability time points representing 3 DP batches at recommended and accelerated storage conditions were evaluated using both methods.

- An increase in non-reducible cross-links (primarily H-L and H-H) and clips are observed, and these degradation products are detected by both LDS-CGE and SDS-PAGE.

- The differences observed in the results generated are consistent throughout the shelf life of the product at recommended storage conditions.
### Case Study #1
Implementation of CGE - Specification

- Data from 75 BDS and 42 DP batches was available for statistical analysis.
- A tolerance interval approach (99.9% of the population is captured by the interval with 95% confidence) was used which included the BDS and DP release and stability data:

<table>
<thead>
<tr>
<th></th>
<th>Current (LDS-CGE)</th>
<th>Legacy (SDS-PAGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Release</td>
<td>EOSL</td>
</tr>
<tr>
<td>BDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity:</td>
<td>≥ 96.7%</td>
<td>Same</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity:</td>
<td>≥ 96.7%</td>
<td>Purity:</td>
</tr>
<tr>
<td></td>
<td>≥ 94.9%</td>
<td>≥ 97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Same</td>
</tr>
</tbody>
</table>
Case Study #1
Adjustment to SE-HPLC Specification

• Why?
  ➢ Over the past 6 years 217 Bulk Drug Substance (BDS) batches and 63 Drug Product (DP) batches have been manufactured.
  ➢ Are the current specifications truly representative of the manufacturing and analytical capabilities and product knowledge?

• How?
  ➢ Statistical analysis of available data including DP stability trends
  ➢ Structure function analysis
Case Study #1
Studies to support adjusted SE-HPLC specification

• Statistical Analysis
  ➢ Non-parametric tolerance interval capturing 98.9% of the population with 95% confidence was used due to the data not being normally distributed

• Structure function studies
  ➢ Product isolated from In-vitro serum study
    ➢ 10% aggregate over the half life and 15% at 28 days
    ➢ Minimal impact on biological activity (86% relative potency after 28 days in serum)

  ➢ Size variants isolated from BDS stored at 40°C for 2 months were found to be primarily dimer and trimer - low immunogenicity risk
    ➢ Fraction X was 87% dimer and had a relative potency of 99%.
    ➢ Fraction Y was 58% multimer (primarily trimer) and had a relative potency of 87%.

  ➢ Aggregate from real time DP stability data at recommended storage (2-8°C) is comprised almost entirely of dimer, with only trace levels of trimer (<0.1%).
### Case Study #1
Adjustment to SE-HPLC Specification

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>Legacy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BDS</strong></td>
<td>Release</td>
<td>Release</td>
</tr>
<tr>
<td></td>
<td>Main Peak: ≥ 98.5%</td>
<td>Main Peak: ≥ 99.0%</td>
</tr>
<tr>
<td></td>
<td>Aggregate: ≤ 1.5%</td>
<td>Aggregate: ≤ 1.0%</td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td><strong>DP</strong></td>
<td>Main Peak: ≥ 98.5%</td>
<td>Main Peak: ≥ 99.0%</td>
</tr>
<tr>
<td></td>
<td>Aggregate: ≤ 1.5%</td>
<td>Aggregate: ≤ 1.0%</td>
</tr>
<tr>
<td></td>
<td>Main Peak: ≥ 97.0%</td>
<td>Main Peak: ≥ 97.0%</td>
</tr>
<tr>
<td></td>
<td>Aggregate: ≤ 3.0%</td>
<td>Aggregate: ≤ 3.0%</td>
</tr>
</tbody>
</table>

- Adjustment made to the BDS release and shelf life criteria to allow for process and analytical variability while maintaining the same shelf life criteria for DP.
Case Study #1
Adjustment to IE-HPLC Specification

• Why?
  ➢ Over the past 6 years 217 Bulk Drug Substance (BDS) batches and 63 Drug Product (DP) batches have been manufactured.
  ➢ Are the current specifications truly representative of the manufacturing and analytical capabilities and product knowledge?

• How?
  ➢ Statistical analysis of available data including DP stability trends
  ➢ Structure function analysis
Case Study #1

Studies to support adjusted IE-HPLC specification

• Statistical Analysis

- Applying a tolerance interval (with 95% confidence) to all the batch release data to date results in acceptance criteria values of 71.0% for main and 22.0% for acidic after rounding.

- The upper 95% confidence limit on the trend over time (slope) support maintaining the current lower limit for main peak and upper limit for acidic.
Case Study #1

Studies to support adjusted IE-HPLC specification

- Structure function studies

  - The acidic and basic variants detected in the IEC separation are well characterized and show minimal impact on safety and biological activity.
  
  - N-terminal pyroglutamate and c-terminal lysine heterogeneity are common post translation modifications and they pose no impact to safety or activity (not CQA's).
  
  - Deamidation is observed in low levels and not in the CDR.
  
  - Oxidation is not observed in the CDR but observed in the Fc region; it is well controlled through the manufacturing process and recommended storage conditions for DS and DP.
## Case Study #1
### Adjustment to IE-HPLC Specification

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>Legacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDS</td>
<td>Release</td>
<td>Release EOSL</td>
</tr>
<tr>
<td></td>
<td>Main Peak: ≥ 71.0%</td>
<td>Main Peak: 73.0-81.0%</td>
</tr>
<tr>
<td></td>
<td>Acidic: ≤ 22.0%</td>
<td>Acidic: ≤ 14.0-21.0%</td>
</tr>
<tr>
<td>BDS</td>
<td>Same Main Peak: ≥ 73.0-81.0%</td>
<td>Same</td>
</tr>
<tr>
<td></td>
<td>Acidic: ≤ 14.0-21.0%</td>
<td></td>
</tr>
<tr>
<td>BDS</td>
<td>Main Peak: ≥ 60.0%</td>
<td>Main Peak: 73.0-81.0%</td>
</tr>
<tr>
<td></td>
<td>Acidic: ≤ 34.0%</td>
<td>Acidic: ≤ 14.0-21.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>Main Peak: ≥ 71.0%</td>
<td>Main Peak: ≥ 60.0-81.0%</td>
</tr>
<tr>
<td></td>
<td>Acidic: ≤ 22.0%</td>
<td>Acidic: ≤ 34.0-21.0%</td>
</tr>
<tr>
<td></td>
<td>Main Peak: ≥ 60.0%</td>
<td>Main Peak: ≥ 60.0-81.0%</td>
</tr>
<tr>
<td></td>
<td>Acidic: ≤ 34.0-21.0%</td>
<td>Acidic: ≤ 34.0-21.0%</td>
</tr>
</tbody>
</table>
Case Study #2
mAB, multiple dose IV

• Background
  – Commercial Specifications approved in 2011.
  – Shortly after there was a need to develop and validate a new IE-HPLC method for measurement of charge variants.
    • Discontinuation of the original column by the supplier
  – Used this opportunity to re-assess the robustness of our testing strategy.
  – Outcome of assessment
    ➢ Establish a higher resolution IE-HPLC method
    ➢ Implement LDS-CGE as a replacement for the less sensitive and selective SDS-PAGE – Same as case study #1 so just focusing on IEC
Case Study #2
Implementation of optimized IE-HPLC

• Why?

• How?

- Identification of a comparable or improved IE separation
- Bridging studies comparing the charge variant profiles for each method
- Validation of the new method
- Generation of specification acceptance criteria based on the data generated
Case Study #2

Implementation - bridging

• Comparison of the charge variant profiles using the old and new methods was performed.

- Main acidic peak of the previous method (attributed primarily to deamidation) splits into 2 deamidation isoforms identified as Aspartic acid and Isoaspartic acid.
- A second lysine variant species that eluted under the main peak on the previous method can now be resolved by the updated method.
- A concomitant decrease in main peak percent is expected, given the improved resolution of the second lysine variant species.
Case Study #2
Implementation of new IE-HPLC - Specifications

- Assessment of all available DP and BDS stability data showed no degradation over the shelf life of the product at recommended storage conditions.
- No degradation was observed as a result of the DP manufacturing process, therefore, the BDS and DP specifications are aligned.
- 35 DP batches were statistically evaluated using a 3xSD approach.
# Case Study #2
Implementation of new IE-HPLC - Specifications

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<thead>
<tr>
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<tr>
<td>BDS</td>
<td>Release EOSL</td>
<td>Release EOSL</td>
</tr>
<tr>
<td>BDS</td>
<td>BDS Main Peak: ≥ 72.8%-84.6% Acidic: ≤ 8.9-16.0%</td>
<td>BDS Main Peak: ≥ 78.0%-89.6% Acidic: ≤ 7.0-17.0%</td>
</tr>
<tr>
<td>BDS</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>DP</td>
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<td>DP</td>
<td>Same</td>
<td>Same</td>
</tr>
</tbody>
</table>

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Case Study #3
Lyo product with IV administration

• Background
  – Product was in development and the clinic for an extended period of time, >15 years.
  – Given product development started in the 1990’s many of the original release and stability methods used what are now considered out of date technologies.
  – In addition, methods such as HCP utilized non optimal reagents that were not fit for commercial release.
  – As a result many of the legacy analytical methods were replaced with more robust, accurate, and precise methods.
  – The timings of these changes were driven by the initiation of the Phase 3 clinical studies.
## Case Study #3

Summary of updated methods implemented for Phase 3 Studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Legacy Method</th>
<th>Current Method</th>
<th>Rationale for Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>Protein Concentration by Fixed Pathlength UV/VIS</td>
<td>Protein concentration by Variable Pathlength UV/VIS</td>
<td>Improvement in method precision and overall assay performance</td>
</tr>
<tr>
<td>Charge Variants</td>
<td>Gel based Isoelectric Focusing (IEF)</td>
<td>Capillary Isoelectric Focusing (cIEF)</td>
<td>Superior precision and accuracy Reportable pl used as confirmation of Identity</td>
</tr>
<tr>
<td>Size Variants (Fragmentation)</td>
<td>Coomassie SDS-PAGE</td>
<td>Capillary Gel Electrophoresis (CGE)</td>
<td>Superior precision and accuracy</td>
</tr>
</tbody>
</table>
## Case Study #3
Summary of updated methods implemented for Phase 3 Studies

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<th>Current Method</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Antigen Binding</td>
<td>Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>Surface Plasmon Resonance (SPR)</td>
<td>Superior precision and accuracy</td>
</tr>
<tr>
<td>Residual DNA</td>
<td>Residual DNA by Threshold assay</td>
<td>Residual DNA by Quantitative Polymerase Chain Reaction (qPCR)</td>
<td>More robust Greater sensitivity and specificity</td>
</tr>
<tr>
<td>Residual HCP</td>
<td>Residual HCP by Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>Residual HCP by Optimized Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>Nearly a 3 fold improvement in coverage determined by 2D Gel/WB</td>
</tr>
<tr>
<td>Residual Protein A</td>
<td>Residual Protein A by Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>Residual Protein A by Optimized Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>Current method aligns with practice of using commercial buffer/reagents and eliminating the need for product specific components</td>
</tr>
</tbody>
</table>
Case Study #3
Bridging studies to support P3 clinical studies and registration

- In support of P3
  - For stability indicating methods approximately 30 samples were tested side by side using both the legacy and new methods to support the release of P3 supplies using the new methods.

- In support of registration
  - Approximately 10 BDS and DP samples from P3 manufacture and stability time points from 2 BDS and 2 DP were tested side by side.
  - In order to confirm the methods were capable of detecting the same species, forced degradation and eosl samples were also tested.
Conclusions

• Lessons Learned -
  ➢ Analytical development never ends!
  ➢ Analytical lifecycle requires a reassessment of methods and acceptance criteria

• Implementation of Product Lifecycle Management assures an appropriate level of oversight from an end to end perspective through integrating people (R&D and GMS), processes, business systems, and information.

• Case studies highlight this need to reassess criteria and implement new technologies/methods as a means of improving process control throughout clinical development as well as post approval.

• When the appropriate studies are conducted and the rationale for changes are clearly articulated in terms of CQA’s, structure function relationship, and patient safety and efficacy agencies have been generally supportive of the change.