Critical Raw Material Control in Drug Substance and its Impact on Drug Product Comparability

Emerging Strategies in Drug Product Comparability and Process Validation
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LP541 is a PEGylated Protein intended for Diabetes therapy.

**Presentation:**
- The drug substance is a frozen solution (≤ -10°C).
- The drug product is a refrigerated multi-use product (2-8°C).

**Program Overview**

- **Purified Protein Intermediate (Bulk Powder)**
- **PEGylation reagent (Bulk Powder)**
- **Compendial Excipients**
- **Drug Substance**
  - Purification and Isolation (Frozen Solution)
  - ~45 gram/Liter
- **Buffer Section**
- **Drug Product**
  - (Refrigerated) Cartridges & Vials
  - ~23 mg/mL
20 kDa PEG is a critical raw material

- 20 kDa PEG uses Paranitrophenyl carbonate activation
- Specific to a Lysine side chain residue
- Generates a stable Urethane bond

Structural Formula \((n = \text{approx. 450 subunits})\):

- It defines the time-action profile that the patient would experience
- To allow commercialization, 3 suppliers were needed
- However:
  - PEG is polymeric distribution
  - Each supplier manufactures PEG with slightly different quality attributes
PEG Mw (weight average molecular weight)

- Molecular weight (Mw) is a critical quality attribute of PEG
- Mw is defined as the weighted average
- PEG suppliers were using GCP to measure Mw

Mass analysis of 20 kD PEG sample

Mw (Weighted Average Molecular Weight)
PEG Polydispersity Index (PDI)

- The breadth of the distribution is also a Critical Quality Attribute
- The term for polymeric distribution is: Polydispersity Index (PDI)

![Normalized Mn Curves for 20 kDa Mw](image)

- PDI = 1.003 (Representative of all CT Materials) & PDI = 1.05 (GPC Spec Limit)
- PDI = 1.003 (Clinical Experience)
- PDI = 1.05 (PEG suppliers) Far removed from clinical experience
PEG Mw and PDI and its impact to PK

- Extreme Mw could effect PK (Rodent Model)
  - PEGylated samples with various PEG size
  - 5 kD and 10 kD mPEG significantly different
  - Supplier range acceptable: 18-22 kD
  - Combination (intentionally increasing PDI) acceptable in 18-22 kD range

Rat IV clearance study
Map of PEG of suppliers:

- Parameters with potential to impact Drug Product CQAs

<table>
<thead>
<tr>
<th>Test</th>
<th>Supplier 1 (Phase 1/2 and 3)</th>
<th>Supplier 2 (Phase 3)</th>
<th>Supplier 3 (Phase 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Average Molecular Weight (M_w, Da)</td>
<td>NMT 1.10</td>
<td>18,000 – 22,000</td>
<td>18,000 – 22,000</td>
</tr>
<tr>
<td>Number Average Molecular Weight (M_n, Da)</td>
<td>18,000 – 22,000</td>
<td>Report</td>
<td></td>
</tr>
<tr>
<td>Polydispersity Index</td>
<td>NMT 1.10</td>
<td>NMT 1.05</td>
<td>NMT 1.05</td>
</tr>
<tr>
<td>Bi-functional PEG (%)*</td>
<td>NMT 3</td>
<td>NMT 3</td>
<td>NMT 4</td>
</tr>
<tr>
<td>20 kDa Fraction (%)</td>
<td>NLT 90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional Activity (%)</td>
<td>NLT 80</td>
<td>NLT 80</td>
<td>NLT 90</td>
</tr>
<tr>
<td>Free p-Nitrophenol (ppm)</td>
<td>NMT 200</td>
<td>Report</td>
<td></td>
</tr>
</tbody>
</table>

* PNP-PEG-PEG-PNP  Bi-functional PEG – has the potential to generate covalent dimers
Phase 3 Mw Data

High resolution LC-TOF-MS data:
♦ Data collected on PEG used in Phase 3 supply
♦ Each Supplier is able to reproduce Mw
♦ However; statistically significant differences are observed between suppliers
High resolution LC-TOF-MS data:

- Data collected on PEG used in Phase 3 supply
- Each Supplier is able to control PDI
- Populations are similar amongst different suppliers
PEG attributes that may affect DP CQAs

LP541 Degradation Pathways

Could PEG properties influence these CQAs?

- De-PEGylation or PEG degradation
- Glutamine Deamidation (pH induced)
- Dimer and HMWP formation
- Disulfide Rearrangements (occurs mainly in API)
A comparability assessment was conducted at the start of Phase 3
- Examined PEG raw materials
- Multiple parameters related to drug substance and drug product

**Drug substance testing:**
1. Release testing comparison
2. Characterization and biophysical testing
3. Accelerated stability studies / cell based bioassay

**Drug product testing:**
1. In-process solution testing
2. Release testing comparison
3. Characterization testing
4. Accelerated stability studies / cell based bioassay
<table>
<thead>
<tr>
<th>SEC % HMWP</th>
<th>PEG</th>
<th>Storage condition</th>
<th>T=0</th>
<th>0.5 M</th>
<th>1M</th>
<th>2M</th>
<th>6M</th>
</tr>
</thead>
</table>
| Supplier 1 | 2-8 °C  
30°C/60%RH  
40°C/75% RH | 1.2 | 2.6 | 1.6 | 2.2 | 1.4 |
| Supplier 2 | 2-8 °C  
30°C/60%RH  
40°C/75% RH | 1.4 | 2.7 | 1.9 | 2.5 | 1.6 |
| Supplier 3 | 2-8 °C  
30°C/60%RH  
40°C/75% RH | 1.6 | 3.0 | 1.9 | 2.8 | 1.8 |

6 Month Stressed Drug Product Sample @ 30°C/65%RH
RP-HPLC Analysis / DP Demo Batches

### RP-HPLC Related Substances and Impurities:

<table>
<thead>
<tr>
<th>PEG</th>
<th>Storage Condition</th>
<th>T=0</th>
<th>0.5 M</th>
<th>1M</th>
<th>2M</th>
<th>6M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplier 1</td>
<td>2-8 °C 30°C/60%RH 40°C/75% RH</td>
<td>1.3</td>
<td>3.1</td>
<td>2.3</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Supplier 2</td>
<td>2-8 °C 30°C/60%RH 40°C/75% RH</td>
<td>1.4</td>
<td>3.2</td>
<td>2.3</td>
<td>2.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Supplier 3</td>
<td>2-8 °C 30°C/60%RH 40°C/75% RH</td>
<td>1.6</td>
<td>3.6</td>
<td>2.7</td>
<td>3.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**6 Month Stressed Drug Product Sample @ 30°C/65%RH**
SEC and RP-HPLC Analysis: Phase 3 DP

- Monitoring of comparability continues throughout program
- Phase 3 batches shown:
  - Lower Mw PEG (supplier 3), influences:
    - API purification parameters
    - Results in reduced purity (may be compensated by column load)
  - Stability performance remains constant.
PEG remains intact over the shelf-life

- Mass analysis of stressed drug substance and drug product

**DS Batch C065137**
- 9 weeks, 25°C, 60% RH

**DS Batch C065137**
- Initial

**DP Batch TP12089**
- 3 Months, 30°C, 65% RH

**DP Batch TP12089**
- Initial
PEG remains intact over the shelf-life

- Expanded Plot of \([M+5H]^5+\) TOF-MS Spectra
- Identical observations for all 3 suppliers
CEX Analysis

<table>
<thead>
<tr>
<th>CEX-HPLC Related Substances and Impurities: % Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PEG</strong></td>
</tr>
<tr>
<td>Supplier 1</td>
</tr>
<tr>
<td>Supplier 1</td>
</tr>
<tr>
<td>Supplier 1</td>
</tr>
<tr>
<td>Supplier 2</td>
</tr>
<tr>
<td>Supplier 2</td>
</tr>
<tr>
<td>Supplier 2</td>
</tr>
<tr>
<td>Supplier 3</td>
</tr>
<tr>
<td>Supplier 3</td>
</tr>
<tr>
<td>Supplier 3</td>
</tr>
</tbody>
</table>
♦ Monitoring of comparability continues throughout program
♦ Phase 3 batches shown
  • Stability performance remains constant
Glu-C peptide map characterization method
- PEGylated fragment is late eluting (not shown)
- Utilized to confirm CEX-HPLC observation
- Sensitive to deamidation and disulfide rearrangements
Bioassay as a potential CQA

Phase 3 API (PS + PV) Stress at 25°C:

- 3 PEG suppliers
- Purity decline observed by RP-HPLC
- Potency decline is difficult to detect beyond method variability
Phase 3 DP PS batches Stressed at 30°C:

- Each batch is different PEG supplier
- Purity decline observed by RP-HPLC
- Potency decline is difficult to detect beyond method variability
Phase 3 accumulated experience

- 13 commercial scale API batches (data shown)
- 11 drug product batches
- The data set allows applying statistic tools for comparability studies
Clinical Evaluation of PEG Suppliers

♦ Phase 1 PK/PD Study
  ♦ Healthy volunteers administered single injection of LP541 with each PEG supplier on 3 separate occasions followed by PK sampling
  ♦ CMC provided information on CT batches including the Mw of the PEG batches used
  ♦ No correlation was observed to PEG Mw
  ♦ No difference observed in PD

♦ Phase 3 Study (one of the global Phase 3 studies)
  ♦ Patient population
  ♦ Open label study, LP541 arm randomized to a single PEG supplier
  ♦ Subgroup analysis as a function of PEG supplier
  ♦ Different suppliers did not impact the PK and PD
  ♦ No difference in safety data
PEG Supplier Evaluation

**Clinical Efficacy/Safety**

**PK/PD (human)**

**Toxicology Studies**

**Additional Characterization Testing**

**Bioassay Testing**

**Release & Stability Testing**

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**Phase 2/3**

**Primary Stability:**
Statistical comparability compared to clinical experience to date (API site change)

**Start of Phase 3:**
Side by Side PEG Supplier comparability analytical data package

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**PV and BLA**

**Phase 3 Global Study**

**Phase 1 PK/PD Study**

**Mixed PEG:**
Analytical comparability demonstrated with mixed PEG suppliers to support commercial supply chain flexibility
Control Strategy for Commercial Supply

- **PEG**
  - Mw
  - PDI

- **Drug Substance**
  - RP-HPLC
  - CEX-HPLC
  - LC-TOF analysis for Mw and PDI control of the incoming PEG reagent
  - RP-HPLC and CEX-HPLC ensures that only one PEG is attached to protein and at the correct position

- **Drug Product**
  - RP-HPLC
  - RP-HPLC ensures that de-PEGylation does not occur over shelf-life and patient in-use period

- **For commercial supply:**
  - Drug substance was stored frozen in 50 L HDPE containers
  - Required a “floating batch” strategy for DP
  - The DP facility was intending on using a FIFO approach
Quality control of the drug product

CEX Chromatography of DP made with more than one source of PEG-API

- 1.7 kD MW difference - OK
  Lot H01020-121-A
  18.4 kD + 20.1 kD PEG

- 2.3 kD MW difference - OK
  Lot H01020-121-B
  20.1 kD + 22.4 kD PEG

- 3.1 kD MW difference
  Lot H01020-121-E
  18.4 kD + 21.5 kD PEG

- 4.0 kD MW difference
  Lot H01020-121-C
  18.4 kD + 22.4 kD PEG

- If two PEG batches are combined that are separated by more than 2.3 kD, failures in the analytical methods would occur.
- Also occurs in SEC & RP-HPLC.
- Controlling this by segregating PEG in DS manufacturing is complex.
- Note: 18.4kD and 22.4kD PEGs intentionally ordered from suppliers.
Statistical Model for Mw and applying a singular control limit across suppliers

<table>
<thead>
<tr>
<th>Supplier</th>
<th>2</th>
<th>3</th>
<th>1 (Phase 1/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=</td>
<td>17</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Average Mw</td>
<td>21727</td>
<td>20125</td>
<td>21484</td>
</tr>
<tr>
<td>Max Mw</td>
<td>21871</td>
<td>20324</td>
<td>21716</td>
</tr>
<tr>
<td>Min Mw</td>
<td>21585</td>
<td>19880</td>
<td>21335</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>88</td>
<td>145</td>
<td>74</td>
</tr>
<tr>
<td>± 6 Std. Dev (rounded)</td>
<td>21200 - 22260*</td>
<td>19260* - 20990</td>
<td>21040 - 21930</td>
</tr>
<tr>
<td>95/99.5 Tolerance levels</td>
<td>21364 - 22089</td>
<td>19299 - 20950</td>
<td>21199 - 21768</td>
</tr>
</tbody>
</table>

Predicted TOF-MS with ±6 Std. Dev.

TOF-MS specification 19,260-22,260 Dalton
## Proposed commercial PEG specifications

<table>
<thead>
<tr>
<th>Test</th>
<th>Procedure</th>
<th>Acceptance criteria</th>
<th>Critical parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Appearance</td>
<td>Visual Appearance</td>
<td>White to Off-white Powder</td>
<td></td>
</tr>
<tr>
<td>Identity</td>
<td>LC-TOF-MS</td>
<td>Retention time</td>
<td>cGMP requirement</td>
</tr>
<tr>
<td>Weight Average</td>
<td>LC-TOF-MS</td>
<td>NLT 19.3 kDa and NMT 22.3 kDa</td>
<td>Imparts the long-acting PK/PD profile</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydispersity Index</td>
<td>LC-TOF-MS</td>
<td>Not more than 1.01</td>
<td>Defines distribution of molecular weights</td>
</tr>
<tr>
<td>Functional Activity</td>
<td>RP-HPLC</td>
<td>Not less than 90 %</td>
<td>Impacts only at extreme conditions)</td>
</tr>
<tr>
<td>Bi-functional PEG</td>
<td>RP-HPLC</td>
<td>Not more than 4.0 %</td>
<td>Shown not to induce dimers</td>
</tr>
<tr>
<td>Water Content</td>
<td>Karl-Fischer</td>
<td>Not more than 1.0 %</td>
<td>Significant hydrolysis could impact reaction</td>
</tr>
<tr>
<td>Free p-Nitrophenol</td>
<td>RP-HPLC</td>
<td>Not more than 200 ppm</td>
<td>No known impact</td>
</tr>
<tr>
<td>Residual Solvents</td>
<td>GC</td>
<td>Review of Supplier Certificate of Analysis</td>
<td>Considerable process removal exists</td>
</tr>
</tbody>
</table>
Introducing mixed PEG supplier batches

♦ A statistical comparison of drug product accelerated stability data (30°C/65% RH) was conducted.
♦ Intent was to verify the comparability of drug product manufactured using different PEG suppliers versus mixed PEG suppliers.
♦ Acceptance criteria were applied to batch release and characterization data
♦ Acceptance criteria were applied to accelerated stability data.
♦ Mixed PEG source DP was generated from all 3 possible combinations:
  • Supplier 1 + Supplier 2
  • Supplier 1 + Supplier 3
  • Supplier 2 + Supplier 3
Introducing mixed PEG Supplier batches

♦ Common slope test:
  - A singular slope is provided for the mixed PEG supplier
  - When the p-value is greater than 0.05, no statistical differences exist
  - p-Value was 0.69
Introducing mixed PEG Supplier batches

♦ Common slope test:
♦ p-Value was 0.046 and required further assessment:
  • The mix PEG supplier data had a slightly lower degradation rate indicating that using mix PEG suppliers would be acceptable
  • As more data was collected, the apparent differences diminished
Introducing mixed PEG Supplier batches

♦ Common slope test:
  • A singular slope is provided for the mixed PEG supplier
  • When the p-value is greater than 0.05, no statistical differences exist
  • p-Value was 0.59
Summary

♦ Analytical comparability (including in-vitro data) was used to introduce two PEG suppliers into the Phase 3 program
♦ PEG methods and control parameters were refined to allow a viable commercial supply chain using 3 PEG suppliers
♦ Analytical comparability with statistical acceptance criteria was utilized to allow mixed PEG supplier batches (Drug Product)
♦ Clinical Comparability was demonstrated during the Phase 3 registration program
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