Quality Considerations in Solid Phase Peptide Synthesis: A Case Study with Liraglutide

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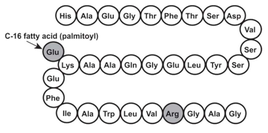
Abstract

Liraglutide injection (RLD: Victoza®) is a synthetic peptide drug to control blood sugar levels in adults with type 2 diabetes. Liraglutide API contains 31 amino acids with a C-16 fatty acid attached. In this case study, it is manufactured by Solid Phase Peptide Synthesis (SPPS). This manufacturing process is highly complex with numerous reaction and purification steps, which can collectively have considerable impact on the identity, strength and purity of the peptide. As a result, regulators often face significant challenges in determining the safety profile of the final peptides. In this poster, by using Liraglutide as a case study, we outline common quality considerations in SPPS that will aid regulators' manufacturing process assessment. This is achieved by providing examples of common manufacturing process deficiencies as well as recommended control strategies to mitigate the safety risk to patients. Quality considerations described herein will facilitate regulators in assessing critical aspects of SPPS manufacturing, thereby protecting the patients from increased safety risk as well as promoting quality peptide drugs for public health.

Introduction

RLD Victoza®

Liraglutide Peptide Sequence



Liraglutide injection (RLD: Victoza®) is a synthetic peptide drug to control blood sugar levels in adults with type 2 diabetes.

Liraglutide API contains 31 amino acids with a C-16 fatty acid. In this case study, it was manufactured by Solid Phase Peptide Synthesis (SPPS). This is a highly complex process with numerous reaction and purification steps, which can collectively have considerable impact on the identity and purity of the final peptides. As a result, assessors often face significant challenges in determining the safety profile of these peptides. In this poster, we use the assessment of a drug master file for Liraglutide, to outline several critical quality considerations in generic peptides manufactured by SPPS. Although not exhaustive in nature, these quality considerations will provide valuable insight to assessors in determining the adequacy of the proposed SPPS process.

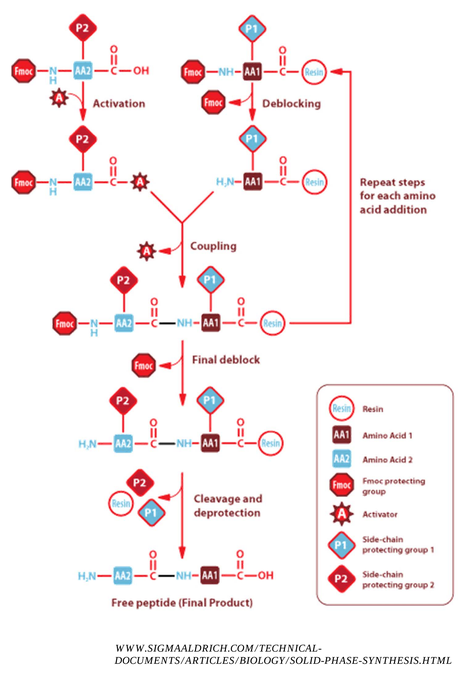
Materials and Methods

- This poster follows the SPPS process flow, from starting materials to final lyophilization.
- Process risks, representative examples, and recommended actions are described.
- Quality considerations in API sameness and related substances are also provided.

Results and Discussion

(1) PROCESS-RELATED QUALITY CONSIDERATIONS

Solid Phase Peptide Synthesis (SPPS)



WWW.SIGMAALDRICH.COM/TECHNICAL-DOCUMENTS/ARTICLES/BIOLOGY/SOLID-PHASE-SYNTHESIS.HTML

1. Starting Materials

Risks: Ambiguous specification, D-isomer content, amino acids with multiple chiral centers, uncommon peptides, resin ID tests

Example: The in-house Fmoc-Thr(tBu)-OH specification has a test for "Enantiomer" with an acceptance criterion of NMT 0.2%. However, it is unclear whether this enantiomer content includes all possible isomers such as Fmoc-D-Thr(tBu)OH, Fmoc-D-allo-Thr(tBu)OH and Fmoc-L-allo-Thr(tBu)OH.

Recommendation: Include a test and an acceptance limit for each individual enantiomer, as well as for total enantiomer content in specification.

2. Coupling

- ***First* Amino Acid Coupling**

Risks: Inadequate resin substitution (or loading)

Example: Process narrative suggests recoupling after first coupling step for Fmoc-Gly-OH, if the acceptance criteria "resin substitution > 0.29 mmol/g" is not met. However, the maximum allowable number of recoupling is not provided.

Recommendation: Propose a maximum number of times this recoupling can be repeated before meeting the acceptance criteria.

- ***Subsequent* Amino Acid Coupling**

Risks: Incomplete coupling, side reactions, capping

Example: Capping is performed only for select amino acids (AA1, AA25 and AA29). Except for AA1, there was no justification why capping is carried out for these amino acids but not others. In addition, capping reaction duration for AA1 is 6-24 hours, which is too wide and not supported by batch data.

Recommendation: Provide justification as to why certain amino acids require recoupling or capping. Consider tightening AA1 capping reaction time limit based on batch data.

3. Crude Peptide

Risks: Sub-batching, endpoint determination, inadequate precipitation control

Example: Regarding the precipitation of crude peptide, batch record indicates that the ether reaction will be carried out till the crude peptide precipitation is complete. It is unclear how the precipitation end-point is determined. Moreover, it is not clear how frequently the temperature is monitored during this temperature-controlled precipitation step at -20 ± 5 °C.

Recommendation: Revise master batch records to include end-point determination for precipitation, frequency of temperature monitoring as well as allocating space for operator to record temperatures.

4. Purification

Risks: Insufficient column/fraction details, inadequate testing of fractions

Example: Fraction volume, storage temperature and time for purified fractions were not specified in master batch records.

Recommendation: Provide the above information with development data, and revise master batch records accordingly.

5. Lyophilization

Risks: Inadequate lyophilization protocol, load volume, parameter optimization

Example: We are concerned about the optimization of the lyophilization process, as there is considerable variation in water content between the batches.

Recommendation: Provide data if any thermal analyses (e.g. DSC or freeze-dry microscopy) were performed to determine the primary drying temperature. Clarify how the end-point of primary drying is determined.

6. Additional Concerns

- **Inadequate Hold Time Limits:** Provide storage conditions and hold time limits for peptide-resin, crude peptide and purified peptide (prior to lyophilization), supported by data.
- **Inadequate Yield Limits: P**roposed synthesis yield, purification yield, and total yield do not have an upper limit. Furthermore, proposed total yield limit is NLT 6%, which is broader than what your batch data suggests. Yield should be calculated based on substitution level, yield limits should be proposed after each critical step, supported by data.
- **Insufficient Scale-Up Information:** There is a 1.5 times scale-up factor from validation batches of 800 mmol to commercial scale of 1200 mmol. Provide a tabular summary that compares validation and commercial batches in terms of equipment (make/model/capacity), as well as materials to be used and critical process parameters.

(2) API SAMENESS/RELATED SUBSTANCE QUALITY CONSIDERATIONS

7. Structural Elucidation

Risks: Inadequate primary/secondary/tertiary structure analysis (one-time study is acceptable)

Example: We acknowledge that you have included two identification (ID) tests: ID by MS and ID by amino acid analysis (AAA) in the drug substance release specification. However, a test to confirm the sequence of amino acids is not provided.

Recommendation: Include an additional ID test by peptide sequence mapping in the specification, to confirm the sequence of amino acids.

8. Impurities

Risks: Validation of reference standards and testing method, potential genotoxic impurities

Example: We acknowledge that you have rationally synthesized 78 possible impurities and used seven analytical techniques to demonstrate that these impurities can be separated from the main Liraglutide peak in liquid chromatography. However, you have not provided certificate of analyses (CoAs) for these in-house synthesized impurity reference standards. Moreover, method validation for these seven analytical methods has not been provided.

Recommendation: Provide flow chart/brief process description and CoAs for these impurity reference standards you synthesized, as well as partial method validation (including but not limited to, LOD and precision at LOQ) for all seven analytical methods.

Conclusion

Solid Phase Peptide Synthesis (SPPS) requires robust control strategies throughout the manufacturing process to produce consistently high-quality peptide drugs. Quality considerations described in this poster will facilitate assessors in identifying and assessing these critical aspects of generic peptide manufacturing, thereby protecting the patients from increased safety risk as well as promoting quality peptide drugs. These quality considerations encompass every manufacturing step including control of the starting materials, coupling, crude peptide, purification, and lyophilization. Scale-up strategy, hold time and yield limits should be based on development and batch data. API sameness with acceptable impurity levels should be demonstrated, preferably with multiple orthogonal analytical techniques.

Acknowledgement

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