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Control Strategies for Synthetic Therapeutic Peptide APIs - Part II: Raw Material Considerations

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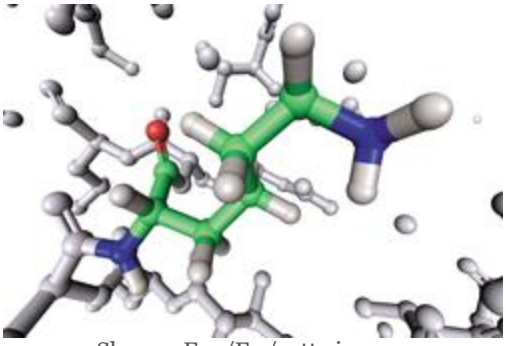
USP evaluates raw materials used in the chemical synthesis of peptides.



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The US Pharmacopeia (USP) Therapeutic Peptides Expert Panel was formed in 2013 to evaluate quality attributes for synthetic peptide APIs based on currently available regulatory guidance and expectations. This series of three articles by the panel explores the current manufacturing and regulatory landscape and provides a comprehensive overview of quality attributes to be considered for successful synthetic peptide API development from manufacturing to lot release. The first article covered analytical characterization methods, lot release tests and points to consider for synthetic peptide API manufacturers entering the market (1).

This second article discusses raw materials used in the chemical synthesis of peptides and their potential impact on the quality attributes of the final API. A special focus of this article will be on protected amino acid derivatives, setting their specifications and the types of quality control tests necessary to ensure consistent quality of the final API. The need for establishing close ties to manufacturers will also be discussed. Finally, the requirement for source verification of raw materials, in the context of transmissible spongiform encephalopathies (TSEs), will be presented.

Regulatory Considerations

The term “raw materials” encompasses the starting materials, reagents, and solvents used in the manufacture of an API. Of these, starting materials-defined as “a raw material, an intermediate or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API” (2)—are generally considered to be the most crucial, because they are actually incorporated into the final product during the manufacturing process. For the manufacture of peptide APIs, these “critical raw materials” are usually protected amino acid derivatives, although other substances may be proposed and justified.

The implementation of International Conference on Harmonization (ICH) Q11, which has been in effect since 2012 (3), has placed an increased emphasis on the control of raw materials, particularly the justification of starting material selection, with focus on the ability to detect and control impurities in starting materials, understand their fate and purge during the manufacturing process, and their relationship to the impurities in the final API.

Based on their justification, API manufacturers may define starting materials for synthetic peptides as amino acids, protected amino acid derivatives, or peptide fragments. The latter are typically assembled using the former; therefore, protected amino acid derivatives will be referred to as “critical

raw materials" in this article, because they are the most commonly designated starting materials and their quality may have a significant impact on the quality of the API.

Impurities in peptide APIs are generally classified as follows (4):

- Identified and qualified
- Identified
- Unidentified.

Depending on their levels in the final substance at the time of registration and the requirement to impose limits, impurities from the first two classes may be included in the final release specification as "specified impurities," together with limits for individual and total impurities from the third class.

The final impurity specifications for a peptide API are established on the basis of knowledge of the manufacturing process and also through "qualification" of individual impurities by virtue of their presence in the API during toxicological studies and clinical trials. In general, regulatory agencies are more willing to accept higher levels of qualified impurities (i.e., those in the first category), because their safety profile is known. To a lesser extent, the same is true of the second category of impurities, whose safety may be assessed through knowledge of their structure, even in the absence of toxicological data. In the case of the third category, however, where the structure of the impurity is unknown, allowed limits in the final substance must, necessarily, be lower. While ICH Q3A (5) limits such impurities to 0.1%, peptides are excluded from the guidance and, depending on the regulatory agency and the therapeutic dose of the API, higher limits of unidentified impurities may be permitted.

Quality Attributes for Raw Materials

The current expectation of regulatory agencies is that levels of peptide-related impurities, which may be derived directly from the starting materials through transformation of impurities in those materials, or from side reactions during the process used to manufacture the API, are strictly controlled. This discussion will focus on the impurities derived from critical raw materials, while those derived from the manufacturing process will be discussed in a subsequent article.

Critical Raw Materials

Virtually all of the likely impurities in critical raw materials (with the exception of non-amino acid residues from their manufacturing, such as most reagents and solvents) have the potential to react and, therefore, be incorporated as impurities during the manufacture of a peptide API. Such impurities can include:

- Free amino acids or amino acid derivatives (i.e., without the Nα-protecting group and/or the side-chain protecting group, if applicable, or containing an incorrect protecting group)
- Amino acid contaminants (i.e., amino acids other than the desired amino acid)
- Incorrect enantiomers
- Isomeric contaminants, such as isoleucine in place of leucine; or isomers of aspartic and glutamic acid derivatives with the protecting group on the α-carboxyl group instead of the side chain
- Dipeptides or oligopeptides in 9-fluorenylmethyloxycarbonyl- (Fmoc-) amino acid derivatives (6)
- β-alanine containing contaminants in Fmoc-amino acid derivatives (7).

While they are not related to the critical raw materials themselves, it is important to note that residues of carboxylic acids, such as acetic acid, in the starting material can also lead to impurities in the final substance, because they may react during the coupling of the critical raw material to the growing peptide chain. Because of the potential for impurities such as those described above to be

the origin of impurities in the final API, it is important that the specifications for these critical raw materials, including their impurity profiles, be tightly controlled. Methods used for their analysis should be validated.

Based on the considerations outlined above, the recommended minimum quality attributes for critical raw materials (protected amino acid derivatives) are summarized in **Table I**. Similar quality attributes are recommended for other types of starting materials.

Table I: Recommended minimum quality attributes for critical raw materials. HPLC is high-performance liquid chromatography. TLC is thin layer chromatography. GC-MS is gas chromatography-mass spectrometry.

Quality Attribute	Test Method	Comments
Appearance	Visual inspection	
Identification	Mass spectrometry or HPLC	
Purity	HPLC	
Related impurities		
• Free amino acid	TLC	
• Other amino acids	Amino acid analysis	Refers to amino acids other than the one contained in the amino acid derivative which is the subject of the specification
• Specified	HPLC	Identity (if applicable) and limits set on the basis of batch history and the outcome of process characterization and a risk assessment
Unidentified	HPLC	Same as for Specified
Enantiomer content	Chiral HPLC or Chiral GC-MS	Except for glycine derivatives
Assay	Titration	
Other components	As required	Includes potential reactive impurities, such as carboxylic acids determined during risk assessment

Limits for specified impurities, content of the incorrect enantiomer, free amino acid, or other amino acids can be justified through prior batch history, supported by process characterization and a process risk assessment. This assumes that analytical methods exist that can establish a correlation between the content of an impurity in the critical raw material and the content of a related impurity in the API; and that the process is well understood in terms of increasing or reducing or eliminating this impurity. Furthermore, the limit may be adjusted based on the process risk assessment.

For unidentified impurities, setting limits for acceptance criteria in the absence of data is based mainly on the process risk assessment. The API specification for unspecified impurities may be used as a starting point with an appropriate safety factor, which would include the following considerations:

- The peak area % by high-performance liquid chromatography (HPLC) may underestimate the true content of an impurity due to its potentially lower extinction coefficient
- The coupling rate of an impurity during synthesis may be higher than that of the targeted critical raw material, leading to its enrichment in the crude API
- The resulting peptide-related impurity may co-elute with the API during purification, thus making it difficult to reduce or eliminate
- The likelihood that a new, peptide-related impurity may not be detectable by routine API release methods.

Consequently, to minimize the potential impact on the API, the limits for this type of impurity should be very stringent.

It is important to note that an assay method based on titration is preferred, because the amino acid derivative may contain varying levels of carboxylate salt forms of the material (which may not be reactive in coupling steps), and other assay methods, such as those based on HPLC or elemental analysis, may not be sufficiently sensitive or accurate.

Other Raw Materials

Because all other raw materials, including the reagents and solvents used in the manufacturing process for peptides, are unlikely to be incorporated into the final product, there is generally less emphasis on their quality attributes. Thus, while the process risk assessment discussed previously should consider these raw materials, in general, most are considered to be non-critical, with only a very low risk of impacting the quality of the final product. Therefore, specifications are usually set based on those for the available materials used in the process. As a general rule, materials of the highest available grade, such as *United States Pharmacopeia (USP)* or *National Formulary (NF)* grade, should be used, unless materials of a lower grade are qualified through use in the validated manufacturing process.

An exception to this is the materials used in the final purification and isolation steps of the manufacturing process. In contrast to earlier steps in the process, where the raw materials are not considered to be final product contacting because downstream purification is performed, the materials used in the final steps of the process can be considered critical, because no further purification is performed. Furthermore, if the final manufacturing steps include concentration processes, such as evaporation or lyophilization, any impurities, especially those which are less volatile, may actually be concentrated in the final substance. Therefore, the solvents and reagents (e.g., acetonitrile, methanol, isopropanol, acetic acid, trifluoroacetic acid), which may typically be used during final, reverse-phase HPLC purification or precipitation steps of peptide APIs, should be identified as having a potentially high risk of impacting the quality of the final API. Quality attributes for these raw materials should take into account not only the identity and purity of the material but also, for example, tight limits for residue on evaporation.

Finally, according to *USP* < 231> (soon to be replaced by *USP* < 232> and < 233>) (8) and ICH Q3D, all APIs, including peptides, must be evaluated for their potential contamination by elemental impurities. While these may be introduced during the manufacturing process through use of metal catalysts or by leaching of metals from equipment, the possibility also exists for their introduction through the raw materials used in the process. As part of risk assessment, the manufacturing process must be evaluated for its ability to remove potential elemental impurities in raw materials through appropriate validation. If this cannot be demonstrated, appropriate limits for elemental impurities in raw materials must be established.

Analytical Control Strategies

Based on the risk assessment, a control strategy must be established that identifies the process steps that can impact the critical quality attributes of the final API. The ability of the manufacturing process to control impurities originating from the starting materials determines their purity requirements. Therefore, strict control of the quality of raw materials, especially critical raw materials, together with appropriate in-process controls, is necessary, to ensure consistency in the quality of the final API.

Supplier Relationships

In view of the increasing emphasis by regulatory agencies on raw material controls, it is essential to re-evaluate the approach to the control of the quality of critical raw materials. While ICH Q7 does not

require manufacture of critical raw materials under GMP conditions, it is recommended that suppliers manufacture them under, at a minimum, “GMP-like” conditions with appropriate systems and controls to assure quality, including implementation of stringent specifications for critical raw materials in the process (i.e., the starting amino acids, in particular), together with a system of batch-specific documentation and strict control of process changes, to ensure that manufacturing processes are both reliable and robust, resulting in critical raw materials of consistent quality.

It is also incumbent on the manufacturers of APIs to implement appropriate procedures to qualify suppliers of starting materials through auditing of their facilities and systems on an ongoing basis at regular intervals of time. During such audits, emphasis should be placed on systems that have been implemented to control product quality, including change control systems, as well as the manufacturer’s knowledge and depth of experience with the manufacture of the products. Such an audit can be a key factor in initiating the process of establishing a level of trust between the parties and building the relationship, with the goal of developing a true partnership.

It is also essential that the expectations and responsibilities between the two parties should be formalized in a “Quality Agreement” (often referred to as a “Technical Agreement”), to avoid misunderstandings. In particular, such an agreement should include an obligation for the supplier of the starting materials to inform the peptide manufacturer prior to the introduction of any significant changes in their manufacturing processes, such as a change of a recrystallization solvent, so that the impact of such changes on the manufacturing process for the final API may be assessed. Implementation of an appropriate confidentiality agreement is recommended to facilitate the exchange of confidential information. If multiple suppliers of starting materials are qualified, the same type of system and agreements should be implemented for each supplier.

While the requirements outlined previously for the tight control of quality may be difficult for some starting material manufacturers to comply with, the authors believe that they are an essential element of the control of the quality of the final peptide API. An assessment of whether a supplier is able to meet (or potentially is able to meet) these requirements should be an important element of the initial screening and eventual approval of potential suppliers.

Source Requirements

Current regulations in most countries require that all licensed pharmaceutical products must be evaluated for their potential risk of containing Transmissible Spongiform Encephalopathies (TSEs) (9). A risk assessment must be performed for all raw materials used in the manufacture of a peptide API to confirm the absence of TSEs. Raw materials, which are either directly derived from “TSE relevant animal species” or are manufactured using materials derived from such species should be identified and a certificate of suitability demonstrating TSE compliance obtained.

Conclusion

Based on the guidance provided by ICH Q11, it can be concluded that the starting materials used during the manufacture of peptide APIs play a crucial role in determining the quality of the final substance. This requires the establishment of detailed specifications for these materials, including limits for identified and unidentified related impurities, and the implementation of agreements with starting material suppliers, to minimize the impact of manufacturing process changes on the introduction of new impurities.

The USP hopes that, through the work of the Therapeutic Peptides Expert Panel and this series of articles, more consistent guidance may be provided, both for the minimum, acceptable quality attributes for peptide API monographs, and also to industry for the characterization and quality control testing of peptide APIs.

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