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Quality control in peptide manufacturing: specifications for GMP peptides

ABSTRACT

The analytical characterization and testing of peptides manufactured as active pharmaceutical ingredients according to GMP (Good Manufacturing Practice) is discussed. In particular, recommended methods and specifications for identity, purity and content are addressed.

INTRODUCTION

Although quality control can be viewed as all activities implemented to ensure adequate quality of manufactured products, in the pharmaceutical industry these processes are usually divided into two separate functions, namely Quality Assurance (QA), which oversees the entire manufacturing process, and Quality Control (QC), which is responsible for analytical testing and characterization of raw materials and finished products. In terms of GMP (Good Manufacturing Practice), Quality Control is also responsible for ensuring that analytical methods are developed and subsequently validated. The assessment of structural integrity and purity of the peptide¹ is critical during the development stages of a product. Without rigorous analytical characterization and evaluation of potential impurities at the start of each GMP manufacturing project, problems may be missed only to resurface at a later point in the process – sometimes with devastating consequences.

This paper discusses one aspect of Quality Control in GMP peptide manufacturing, namely the analytical characterization and testing of finished products.

GUIDELINES

Quality Control of pharmaceuticals is an integral part of manufacturing, and the approval of raw materials for production and release of products is not possible without extensively documented results showing compliance with predetermined specifications. For finished dosage forms and most active pharmaceutical ingredients (API), the guidelines published by regulatory bodies, such as ICH (International Conference on Harmonization), FDA (Food and Drug Administration) or EMEA (European Agency for the Evaluation of Medicinal Products) define both the type of testing that needs to be performed and the process of establishing specification criteria.

However, most current guidelines regarding the testing

and quality of pharmaceutical products specifically exclude peptides. Apart from general guidelines, such as outlined by the CFR (Code of Federal Regulations) or ICH Q7A ("Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients"), there is only one guideline specifically dedicated to peptides. Published in November 1994, the "Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances"² stipulates that the lot release specifications should be sufficient to ensure the identity, purity, strength and/or potency of the peptide and to demonstrate lot-to-lot consistency.

Quality Control must establish the exact methods and test limits that will be used to evaluate each lot of peptide. Also, the selection of reference standards, even those from official sources needs to be determined. The final specification for the peptide in question is built upon these three elements.

SPECIFICATION FOR IDENTITY

Even the first specification item "identity" presents a challenge. There is no simple, single test available (with the exception of NMR, which is expensive, time-consuming, and requires complex data interpretation) that would unequivocally establish the identity of a peptide with respect to amino acid composition, sequence and chirality. For this reason a combination of techniques is typically used, usually encompassing mass spectroscopy, amino acid analysis and HPLC. Each of these techniques is briefly discussed below.

MASS SPECTROSCOPY

The most appropriate characteristic of a peptide that can be established using mass spectroscopy is its monoisotopic mass (the mass of the isotopic peak whose elemental composition is composed of the most abundant isotopes of those elements). The accuracy of this determination should be better than ± 1 mass unit. Instruments that are only capable of determination of average molecular mass or have a resolution of less than ± 1 mass unit may not be able to differentiate between a peptide and its deamidated form and may not be suitable.

Mass spectroscopy can be utilized further, in order to confirm the sequence of amino acids in the peptide.

Mass fragmentometry has in many instances replaced the traditional method of Edman degradation, especially for peptides that are longer than 20 residues.

Sequencing is particularly useful for authenticating the identity of reference standards, although it is not necessary as a release criterion for each lot of peptide produced. Very long sequences may require the use of partial proteolytic digestion of the peptide using an enzyme selected to generate limited fragmentation, prior to separation of the fragments on HPLC and mass spectral analysis.

AMINO ACID ANALYSIS

Amino acid analysis, first introduced by Stein and Moore³, involves hydrolysis of the peptide (typically using acid conditions) to its individual amino acid residues, followed by chromatographic separation on a column and detection/quantitation. The classical method employing ion exchange chromatography and ninhydrin post-column derivatization is still the technique of choice despite the emergence of numerous pre-column derivatization methods, using reagents such as AQC⁴, OPA⁵, PITC⁶, etc., which typically offer increased sensitivity and accessibility to commonly available HPLC equipment. These more recently introduced methods are not as accurate and the derivatives are not always stable.

In order to fulfill the requirements for establishment of identity, the experimental findings for each amino acid should be $\pm 10\%$ of nominal, with the exception of Trp, Cys, Ser residues, for which the recovery may be lower due to their known instability under acid hydrolysis conditions. Amino acid analysis has two limitations: all the operations from sample hydrolysis through to analysis must be performed quantitatively, and an accurately quantitated reference standard must be employed. The latter task is not trivial considering that even the amino acid standard from NIST (Standard Reference Material 2389) claims an accuracy for the individual amino acids between $\pm 2.9\%$ (Ile) and $\pm 4.8\%$ (Arg).

The chirality of the individual amino acids may be checked in the hydrolyzate using Marfey's reagent followed by separation of diastereoisomers using HPLC⁷. This commonly used method is not very accurate however due to partial racemization of the sample during hydrolysis; this limitation may be overcome using hydrolysis in DCl/D₂O followed by derivatization of the amino acids to trifluoroacetyl n-propyl esters and subsequent determination of the enantiomers by GC-MS using a chiral column⁸.

PEPTIDE CONTENT/PEPTIDE ASSAY

The content (or assay) of the peptide can easily be determined from the results of amino acid analysis, by relating the molar amounts of each amino acid recovered to the total weight of the sample. If an adequately quantitated reference standard is available, the assay may be performed using HPLC, but it has to be noted that the standard itself is usually quantitated by amino acid analysis. Other methods include nitrogen determination through elemental analysis, Kjeldahl analysis or the use of a nitrogen specific detector in HPLC, but not being specific for amino acid derived nitrogen, these techniques are prone to systematic errors.

PURITY BY HPLC

The purity of the peptide is typically determined using HPLC, which is the most universally used method in quality control of peptides. Typically, for a first GMP lot, an HPLC purity specification greater than 97% with no single impurity greater than 1% will be set. However, a correctly developed chromatographic method must enable the separation and subsequent determination of the most common impurities in peptide products, such as enantiomers, deletion sequences, and products of deamidation or acetylation. Method development, however, is not a trivial task because it is difficult to determine which potential impurities should be included as separation targets. Usually, the different impurities are not readily available as separate compounds and may need to be synthesized separately at considerable cost. It is often necessary to perform the initial method development without access to these compounds. Thus, method development becomes an iterative process, in which the peptide product is screened using a variety of buffer systems based on different salts, pH values and columns. Typically, reverse-phase systems are attempted first, followed by ion exchange, ion-pairing or size exclusion methods. Under special circumstances HILIC (hydrophilic interaction chromatography), which is a variation of normal-phase chromatography using water as a mobile phase modifier, may be used. As impurities are revealed, isolated and identified, further optimization of the HPLC method can be undertaken. The best way forward is to synthesize the identified impurities and perform final method development using a well-defined mixture of peptides.

Another goal of HPLC method development is to ensure that the method is stability indicating, i.e. is capable of separating and determining degradation products of the peptide. This can be achieved by subjecting samples of the product to stress degradation, typically elevated temperatures (e.g. +70°C), either as dry powder or in solution (e.g. the solution in which final lyophilization is carried out). These samples should be included as part of the method development process described above.

COUNTERION AND MOISTURE DETERMINATION

The requirement to demonstrate lot-to-lot consistency cannot be fulfilled without showing that the remaining major components of the peptide, typically the counterion (e.g. acetate, hydrochloride or trifluoroacetate) and water (residual moisture remaining from the lyophilization procedure) are controlled. A variety of methods can be used for quantitation of the counterion – HPLC, ion-exchange and titration among the most usual ones. Water can be determined using any of the Karl Fischer titration methods, although the coulometric approach seems to be the most advantageous considering its accuracy, ease of use and small sample requirements.

MASS BALANCE

The results of assay, counterion and moisture content must add up to 100% (with a tolerance of $\pm 5\%$) in order to ensure that no significant quantities of inorganic salts or other unaccounted impurities from the purification process remain. Since most peptides are quite hygroscopic this requirement may not always be easy to

meet, and it is recommended that all weighing of peptide samples be carried out under conditions where both the temperature and moisture content of the environment are controlled.

OTHER TESTS

The above-described tests are considered mandatory components of a peptide specification. Quality Control must determine the need for other tests, such as appearance, solubility, residual solvents, specific optical rotation and tests for other residual materials. These decisions must be based on the regulatory status of the material, the validation status of the manufacturing process and knowledge of the synthesis and purification routes leading to the product. Most peptides are destined for use as parenterals and therefore should have a requirement for bioburden and endotoxin testing even if the finished dosage form is sterilized.

CONCLUSION

Quality Control in the pharmaceutical industry is frequently associated with mundane, repetitive testing of very well established compounds and their mixtures. In the context of peptides, however, it becomes a truly

challenging task for the analytical chemist, because of the complexity of the products and the processes that are used in their manufacture. In terms of GMP and peptide drug substances, it is an obligatory prerequisite to establishing a robust and reproducible manufacturing process.

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