

Improved peptide analytical technologies and the impact on process development

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The regulations controlling the use of synthetic peptides as therapeutics has changed significantly over the last number of years with peptides now being viewed as closer to small molecules in terms of process control, structural characterisation and individual impurity limits. In parallel to the increased regulatory requirements, advances in analytical technologies have led to an increased scrutiny of therapeutic peptides, making the understanding and control of individual impurities formed in the peptide manufacture process, critical. This can become complicated as peptides move through clinical development. If an analytical method is changed, this can result in previously co-eluting impurities being resolved and a change in the control strategy is required.

Peptide analytical development and peak purity assessment

In recent years there has been a huge increase in the development of technologies for the analysis of peptides. In particular, the use of U(H)PLC columns and systems is routine in all peptide analytical laboratories. Most manufacturers of analytical U(H)PLC columns offer technology in which the column chemistry is specifically designed to increase the separation of peptide impurities from the main component. Furthermore, there is an expectation that the analytical method is further scrutinised by LC-MS to confirm the main peak is pure, and no co-eluting impurities are present. This typically

involves an algorithmic assessment of the mass spectra of the main peak, coupled with further scrutiny of the data by an experienced mass spectrometrist to check for artefacts, e.g. multiply charged species. Where required, an orthogonal method is introduced to test for a specific impurity.

Analytical development continues to evolve and 2D LC is now becoming more common as a further means of improving the analysis of peptides, making the control of individual impurities in peptide manufacture increasingly important.

Regulatory requirements for synthetic peptides

The FDA issued and later withdrew guidance on synthetic peptides, before a guidance on ANDAs for peptides produced synthetically was introduced [1]. In Europe, the expectation for control of individual impurities in peptides is based on the information in the EP [2], as summarised in the Table 1.

Reporting Threshold	Identification	Qualification Threshold
>0.1%	>0.5%	>1.0%

Table 1: Limits for impurities in synthetic peptides as listed in EP (Table 2034.2)

In general terms, control of impurities should increase through clinical development. Specifically, individual impurities should not exceed their values as qualified in the Tox batch, or be greater than 1.0%, whichever is higher.

Impact of analytical method on specification

Naturally, the impurity profile and hence specification of a peptide is related to the analytical method used at that time. As additional development is performed, an analytical method may change which can have a significant impact on the impurity profile and hence specification limits of impurities that a process has to deliver against.

In the example shown in Figure 1, two impurities were present at a level >1.0% area when this peptide underwent pivotal toxicology studies, and hence the limit for impurities according to the EP guideline was set at a level equal to or lower than the values shown in any future batches intended for clinical studies.

However, as illustrated in Figure 2, this same batch of peptide had a different impurity profile

when a newly developed analytical method was used, where now it was noted that more impurities were resolved, and the reported levels of impurities was lower. This meant that the process had to be developed such that their levels were kept below 1.0%.

LC-MS is the common technique used to identify impurities, and in this case, LC-MS analysis determined that the impurities originally reported as >1.0% were mixtures of isomeric impurities. Isomeric impurities can be harder to characterise and chiral amino acid analysis is typically used to aid characterisation after enrichment of the impurity via preparative chromatography, followed by synthesis of the specific isomeric impurity if more than one of the amino acids is present in the sequence.

Process Development and Impurity Control

The classic model for peptide manufacture involves three stages. The types of impurities that are generated in each stage and the potential controls that can be investigated during process development are shown.

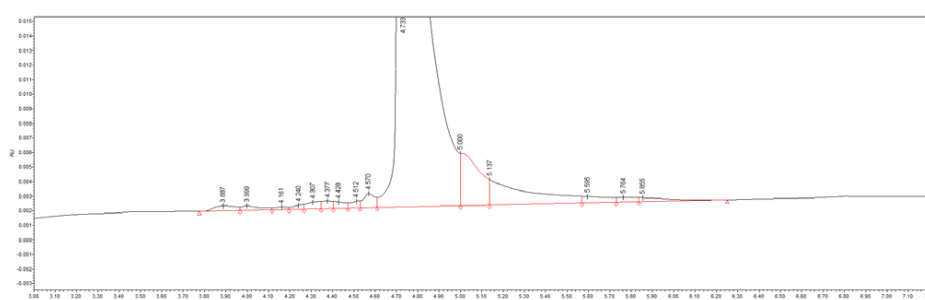


Figure 1: U(H)PLC analysis of peptide using first generation analytical method

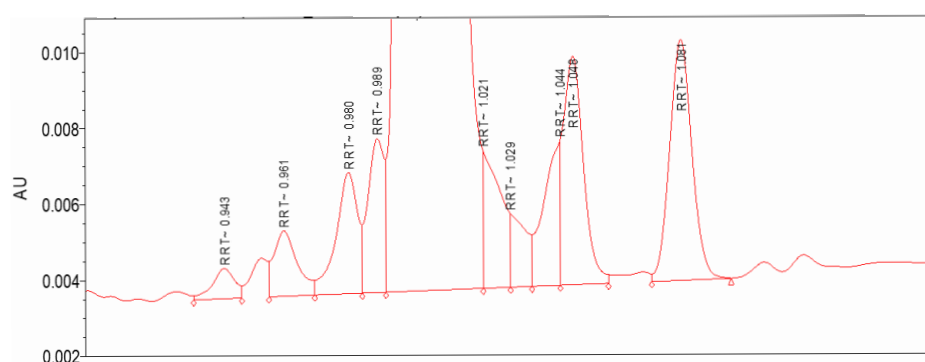


Figure 2: U(H)PLC analysis of peptide using developed analytical method

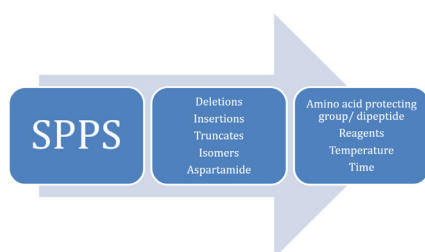


Figure 3: Solid-phase synthesis: Typical impurities that form and potential controls

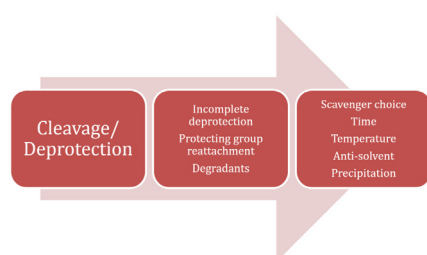


Figure 4: Cleavage/ Deprotection: Typical impurities that form and potential controls

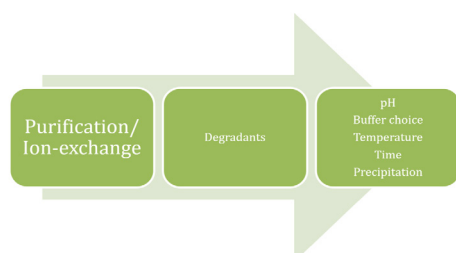


Figure 5: Purification/ Ion-exchange: Typical impurities that form and potential controls

Whilst the specification of a peptide is dependent on finalisation of the analytical method, clearly so is process development. Chemists performing process development must test development samples using the finalised analytical method. Ideally, the final analytical method should be compatible to allow direct analysis of crude peptide and preparative HPLC fractions in a suitable time-frame, so that individual impurities can be tracked and an assessment of impurities that are difficult to control through purification alone can be performed. This assessment leads to a better understanding of which impurities must be controlled at source. An example where this has been performed is shown in Figure 6 below.

Classifying the source of impurities allows for the process development team to focus on the impurities that are difficult to control during purification and hence must be controlled upstream. Impurities identified as degradants are those that form during the downstream process (e.g. deamidation) and control through careful pH and temperature in the process is typically employed.

Bespoke solutions are usually required to control specific impurities formed during synthesis. Such solutions may be through the judicious choice of

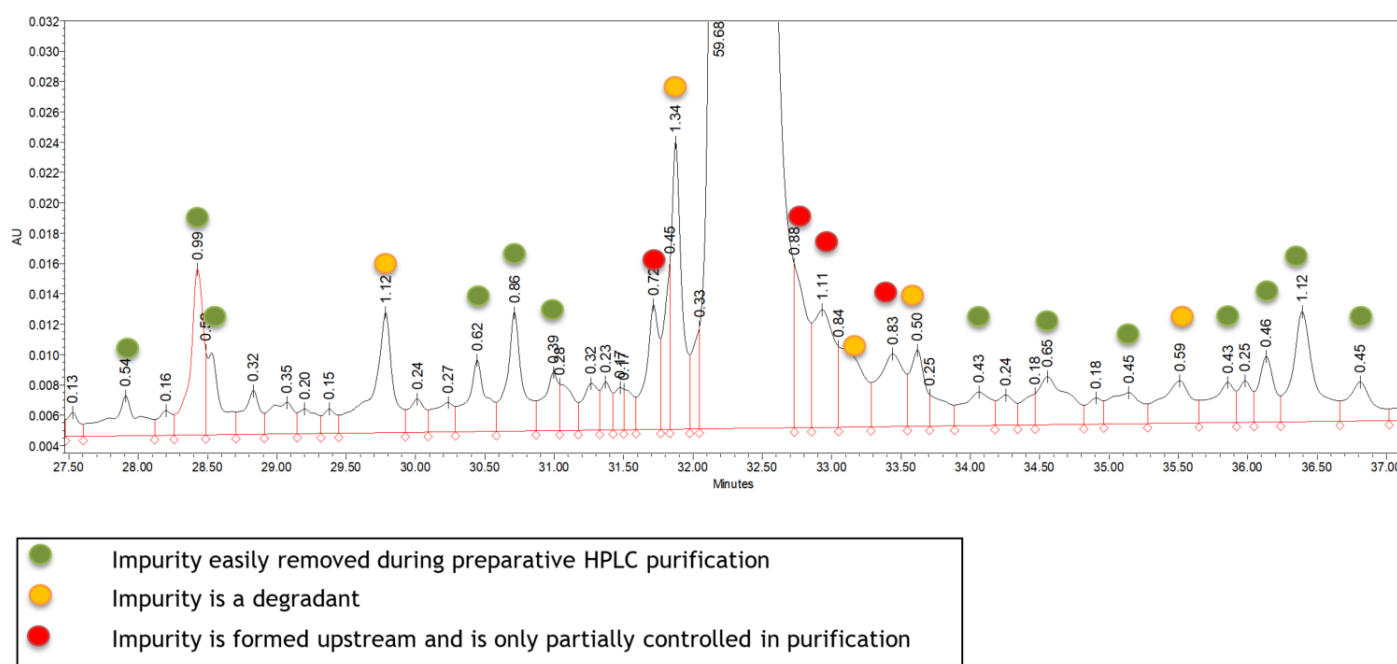


Figure 6: Crude peptide chromatogram with impurities 0.3% highlighted and characterised

coupling/deprotection conditions/inclusion of a dipeptide or choice of scavengers used in the cleavage/deprotection step. In other examples, control of an isomeric impurity is possible by changing the conditions used for coupling of a specific amino acid. In other cases, impurities that have formed under cleavage conditions have been controlled simply by the addition of an alternative scavenger.

Conclusions

The use of peptides as therapeutics is now an embedded technology in the pharmaceutical industry, with the majority being manufactured using fully synthetic processes. The chemistry used to make peptides is well established and has resulted in regulatory bodies viewing peptides as quite different from biologics with an increased expectation of control of the process and impurity profiles. This coupled with increased scrutiny of peptides through advances in analytical technologies has led to the requirement for process development teams to understand how individual impurities are formed and how these may be controlled in the manufacture at an earlier stage of clinical development. As analytical development continues, separation of previously co-eluting impurities can change the specification requirements for a peptide, which means the process development team must have a range of tools available to ensure control of impurities throughout the manufacture process.

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